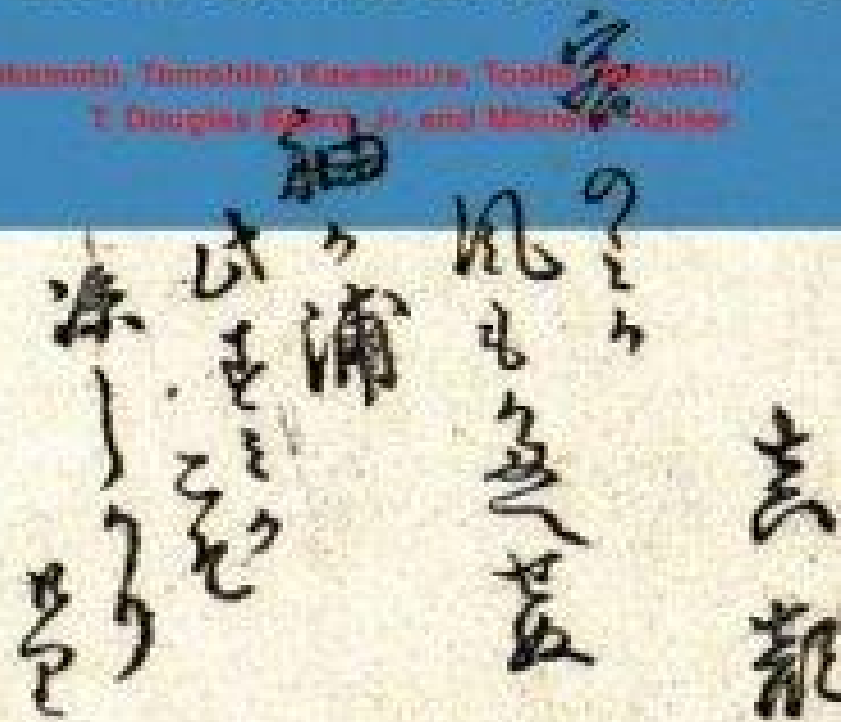




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Fisheries for Global Welfare and Environment

Memorial book of the 5th World Fisheries Congress 2008

Edited by

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TERRAPUB, Tokyo

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Preface

In the last 100 years, humans have confronted a crisis larger than any faced since we first walked the Earth. The ever-expanding human population and increasing demands for natural resources have caused turbulence in the global economy and disruptions in the food supply. As our population continues to grow in the coming years, food shortages together with environmental pollution and habitat destruction will continue to confront us. Human activity appears to be causing changes in climate that affect both the atmosphere and the oceans. Because of increased pressures on terrestrial food production in an uncertain climate with fluctuating rainfall, continued reliance on marine and freshwater food resources will be necessary.

Wise utilization of food resources in the sea could help alleviate the crisis currently facing humanity by providing sustainable fisheries resources. However, over-exploitation and destruction of marine environments are reducing the potential of the ocean to provide food resources and are impacting the overall resilience of marine systems. Scientific knowledge can help mitigate the negative effects of humans on the global ocean, so fisheries scientists need to provide the leadership necessary to provide the natural resources for future human welfare.

The old proverb in Japan “*sui gyo no majiwari*” speaks of “the friendship of water and fish” as being a very close and inseparable relationship. However, now we must consider ourselves as one more member of this ancient relationship between fish and their environment who has a great responsibility to look after both the fish and the water they live in. As fisheries scientists, we must lead the way to make this “*majiwari*” continue long into the future.

Fisheries science began as an applied science that studied fisheries and fisheries-related industries. From its beginning, fisheries science has greatly expanded into a wide range of aquatic sciences including disciplines such as fish biology, aquaculture, biotechnology, biodiversity, ecosystems, and environmental research, as well as socio-economics and post-harvest technology.

Thus, we can define fisheries science as an integrated science that studies the entire aquatic environment. Aquatic resources are not merely sea-food, but include biotic and abiotic resources such as medicine, genetic resources, water, minerals, and energy, as well as landscapes and tourism that also have aesthetic and cultural value. To sustainably utilize all these resources, we need to integrate all aspects of fisheries science and apply this information to policy-making.

The Japanese Society of Fisheries Science, established in 1932 with a 76 year history, hosted the Fifth World Fisheries Congress in Yokohama in October 2008. This congress was the largest meeting on fisheries science held to date. The Congress had nine sessions and 50 subsessions covering almost every discipline related to fisheries science. The steering committee of the Congress decided to publish a book of papers that represented the full range of subjects covered by the plenary speakers and invited keynote speakers from all regions of the world.

The objective of this book is to commemorate the subjects covered by the Congress and, at the same time, to help provide a guideline for world fisheries and fisheries science in the future, with the hope of helping to improve world human welfare. Therefore, the editors of the book urged the contributors to express their ideas and opinions about the problems and future perspectives in fisheries science together with a scientific review of their own field of research. We hope the book will be useful for policy-makers as well as students and researchers of fisheries science. We express our sincere thanks to all the authors for their precious contributions and to the referees from around the world for their valuable suggestions and constructive comments that helped to make the book a reality.

July 2008

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Preface

In the last 100 years, humans have confronted a crisis larger than any faced since we first walked the Earth. The ever-expanding human population and increasing demands for natural resources have caused turbulence in the global economy and disruptions in the food supply. As our population continues to grow in the coming years, food shortages together with environmental pollution and habitat destruction will continue to confront us. Human activity appears to be causing changes in climate that affect both the atmosphere and the oceans. Because of increased pressures on terrestrial food production in an uncertain climate with fluctuating rainfall, continued reliance on marine and freshwater food resources will be necessary.

Wise utilization of food resources in the sea could help alleviate the crisis currently facing humanity by providing sustainable fisheries resources. However, over-exploitation and destruction of marine environments are reducing the potential of the ocean to provide food resources and are impacting the overall resilience of marine systems. Scientific knowledge can help mitigate the negative effects of humans on the global ocean, so fisheries scientists need to provide the leadership necessary to provide the natural resources for future human welfare.

The old proverb in Japan “*sui gyo no majiwari*” speaks of “the friendship of water and fish” as being a very close and inseparable relationship. However, now we must consider ourselves as one more member of this ancient relationship between fish and their environment who has a great responsibility to look after both the fish and the water they live in. As fisheries scientists, we must lead the way to make this “*majiwari*” continue long into the future.

Fisheries science began as an applied science that studied fisheries and fisheries-related industries. From its beginning, fisheries science has greatly expanded into a wide range of aquatic sciences including disciplines such as fish biology, aquaculture, biotechnology, biodiversity, ecosystems, and environmental research, as well as socio-economics and post-harvest technology.

Thus, we can define fisheries science as an integrated science that studies the entire aquatic environment. Aquatic resources are not merely sea-food, but include biotic and abiotic resources such as medicine, genetic resources, water, minerals, and energy, as well as landscapes and tourism that also have aesthetic and cultural value. To sustainably utilize all these resources, we need to integrate all aspects of fisheries science and apply this information to policy-making.

The Japanese Society of Fisheries Science, established in 1932 with a 76 year history, hosted the Fifth World Fisheries Congress in Yokohama in October 2008. This congress was the largest meeting on fisheries science held to date. The Congress had nine sessions and 50 subsessions covering almost every discipline related to fisheries science. The steering committee of the Congress decided to publish a book of papers that represented the full range of subjects covered by the plenary speakers and invited keynote speakers from all regions of the world.

The objective of this book is to commemorate the subjects covered by the Congress and, at the same time, to help provide a guideline for world fisheries and fisheries science in the future, with the hope of helping to improve world human welfare. Therefore, the editors of the book urged the contributors to express their ideas and opinions about the problems and future perspectives in fisheries science together with a scientific review of their own field of research. We hope the book will be useful for policy-makers as well as students and researchers of fisheries science. We express our sincere thanks to all the authors for their precious contributions and to the referees from around the world for their valuable suggestions and constructive comments that helped to make the book a reality.

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Fisheries Management: Status and Challenges

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With fish providing at least 20% of animal protein intake for approximately 1.5 billion people or at least 15% for about 3.0 billion people in our world, it is critical that fisheries management is both efficient and effective. Yet, capture fisheries have reached their maximum productive capacity, and as we shift from the old productivism paradigm to one of responsible fisheries management, it is imperative that we balance viable utilization and conservation. The idea of inexhaustible fisheries must be replaced by the recognition that access to fisheries must be restricted if they are to sustainably generate wealth and to alleviate poverty.

This paper focuses on seven core challenges to implementing responsible and genuinely effective capture fisheries management: understanding fisheries and their environment, reconciling utilization and conservation, considering social and equity issues, assessing incentive structures, linking fisheries management with trade and with marketing standards, enhancing institutions and governance for management whilst embracing the vital concept of stakeholder participation, and engaging with developing countries. For each of these topics, the way forward is outlined—a way that, although challenging, can result in capture fisheries that create wealth, contribute to food security, and are part of sustainable patterns of consumption and production.

KEYWORDS capture fisheries management; governance; conservation; utilization; trade; incentives; developing countries; regional cooperation

* The views contained in this paper do not necessarily reflect the views of the Food and Agriculture Organization (FAO) of the United Nations.

1. Introduction

The range of fisheries and aquaculture issues that face the world is vast, complex, and potentially bewildering, and there are many issues worthy of inspection.

This paper focuses on seven core challenges to achieving genuinely effective capture fisheries management: understanding fisheries and their environment, reconciling utilization and conservation, considering social and equity issues, assessing incentive structures, linking fisheries management with trade and with marketing standards, enhancing institutions and governance for management whilst embracing the vital concept of stakeholder participation, and engaging with developing countries.

2. Setting the Scene: the Status of World Fisheries and Aquaculture

Aquaculture and capture fisheries currently supply the world with approximately 110 million tonnes of fish, or 16.7 kg per capita per year, for human consumption. World production from capture fisheries and aquaculture continues to rise, driven mainly by aquaculture production in China. Total

production amounted to 144 million tonnes in 2006 (Fig. 1).

Overall, fish now provides at least 20% of the animal protein intake for approximately 1.5 billion people or at least 15% of the animal protein intake for approximately 3.0 billion people— including those in low-income food-deficit countries— and 47% of the global production of food fish is now provided by aquaculture. Indeed, aquaculture is the fastest growing food sector, with total production growing at almost 9% per year over the period 1986 to 2006. China overwhelmingly dominates global aquaculture production with a share of 67% of total production, but there is growth in all regions of the world and diversification to new species, particularly crustaceans and marine fish. The outstanding question which remains is whether growth rates have peaked, either regionally or globally.

During the last three decades, the number of fishers and fish farmers has grown faster than the world's population, with an estimated 44 million people working as fishers and fish farmers in 2006. The vast majority of fishers and fish farmers are in developing countries, and most are in Asia, with China alone accounting for some 13 million. At the same time, the number of vessels in

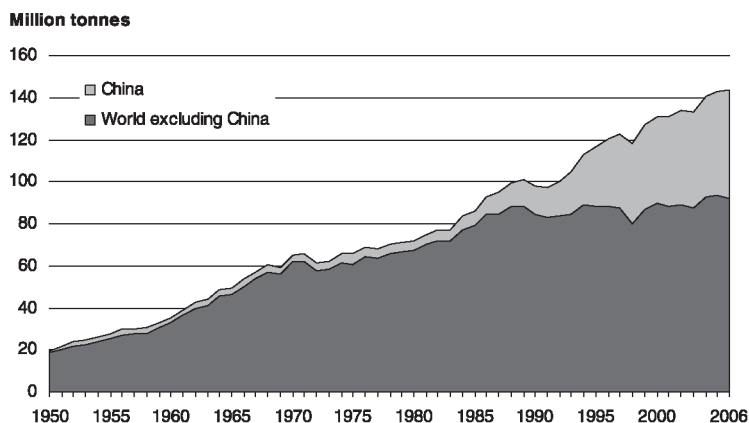


Fig. 1. World capture and aquaculture production (Source: FAO 2008¹).

the world fishing fleet has remained fairly constant for the last few years. In 2006, about 2.1 million were estimated as engine-powered fishing vessels, of which around 90% were small vessels less than 12 m in length. Almost 70% of them were flagged in Asia. There are many national fleet reduction policies in place, although reductions in effective fishing power are often less than those in number of vessels. Moreover, although the number of full time fishers has declined, the number of part-time fishers has grown rapidly—again, particularly in Asia.

International trade of fishery commodities reached approximately US\$ 86 billion in 2006, a dramatic increase of more than 55% since 2000.² The contribution of fish to GDP has doubled in the last 25 years, and currently fish is one of the most highly traded food commodities with 37% of all production now exported. This phenomenon is particularly evident in developing countries: total fishery net exports (i.e., the total value of fish exports less the total value of fish imports) have shown a four-fold increase in 20 years and been reaching US\$ 25 billion in 2006.

Since 2002, China has been the world's largest exporter of fish and fishery products, with exports valued at US\$ 9 billion in 2006. China's fishery exports have increased significantly since the 1990s, not only due to increased production, but also due to the expansion of China's fish processing industry. Befitting from competitive labour and production costs, Chinese exports also include re-exports of fish imported as raw material and processed to add value in the country.

International cooperation in the management of marine fisheries exploiting shared, straddling or discrete high seas fish stocks is being facilitated by regional fisheries management organizations (RFMOs). And, although some RFMOs are finding it difficult to achieve sustainable fisheries, many RFMOs are taking steps towards implementing the ecosystem approach to fisheries, adopting the precautionary approach, promoting transparency, and enhancing compliance. In contrast, and with respect to inland fisheries, only approximately 44% of international water basins are the subject of agreements, and many of these agreements do not include fisheries (FAO 2007, pp. 54–58).

The overall state of exploitation of the world's marine fishery resources has remained relatively unchanged over the past 10–15 years (Fig. 2). The proportion of fully exploited fish stocks has remained fairly stable at approximately 52% in 2006 (the most recent year for which there is data), and the proportion of underexploited and moderately exploited stocks has continued to decrease (20% in 2006). And, whilst the proportion of overexploited and depleted stocks increased steadily during the 1970s and 1980s, the figure has remained around 25–27% during the last ten years.

Such global figures, inevitably, mask some more specific concerns. A recent FAO report indicated that the proportions of overexploited and depleted fish stocks are much higher in some (again, aggregate) categories of fish: for highly migratory tuna and tuna-like species, some 30% are in this category—as are some 55% of highly migratory

¹ Source: FAO Fisheries and Aquaculture Information and Statistics Service. 2008. Total production 1950–2006. FISHSTAT Plus—Universal software for fishery statistical time series [online or CD-ROM]. Food and Agriculture Organization of the United Nations. See: <http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp>

² Source: FAO Fisheries and Aquaculture Information and Statistics Service. 2008. Fisheries Commodities Production and Trade 1976–2006. FISHSTAT Plus—Universal software for fishery statistical time series [online or CD-ROM]. Food and Agriculture Organization of the United Nations. See: <http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp>

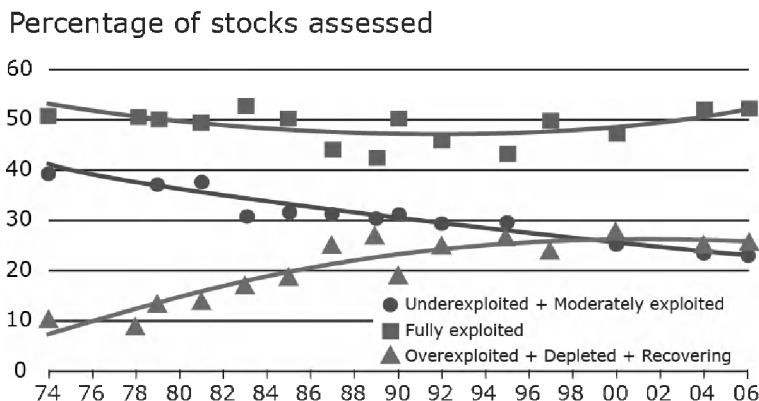


Fig. 2. Global trends in world marine fish stocks since 1974 (Source: FAO 2007, p. 32).

oceanic sharks and some 65% of straddling stocks and other high seas resources (FAO 2006: pp. 17, 30, 43). Moreover, there is no suitable stock assessment information for a large proportion of the world's fish stocks, highlighting the critical need for ongoing work such as the FAO Strategy for Improving Information on the Status and Trends of Capture Fisheries (FAO-STF Project).

With respect to inland fisheries, there is much evidence that many fisheries are overfished. Inland fishery resources are particularly vulnerable to habitat degradation resulting from pollution or use of water for irrigation and/or hydropower generation. Additionally, as with marine fisheries, there are particular challenges in protecting migratory species and those stocks shared among different jurisdictions.

In short, and as repeatedly stated by FAO, the overall maximum production potential from wild capture fisheries has been reached. The combination of further economic benefits and the sustainability of capture fisheries can only be achieved through fisheries management that is able to avoid overexploitation, to maintain (or rebuild) fishery resources, and to improve the commercial viability and generation of wealth

from capture fisheries.

3. Main Challenges and Ways Forward

The trends of the latter half of the 20th century—overfishing and the ineffectiveness of fisheries management—generated serious and widespread concerns amongst a range of agencies and governmental and non-governmental organizations, leading to the adoption of a range of legal instruments and calls for sustainable development, not the least of which was the United Nations Convention on the Law of the Sea of 10 December 1982 (UNCLOS).

Since then, the international community has spent much of the last decade extending UNCLOS and developing complementary international instruments for more effective fisheries management. At the 1992 United Nations Conference on Environment and Development, Chapter 17 of Agenda 21 called upon States to maintain or restore populations of marine species at productive levels, protect and restore endangered marine species, and preserve rare or fragile ecosystems and habitats. Three years later in 1995, the international community adopted the FAO Code of Conduct for Responsible Fisheries (CCRF) followed shortly thereafter

by the UN Fish Stocks Agreement³ for straddling and highly migratory fish stocks.

The international community has also worked with the FAO and within the framework of the CCRF⁴ to develop various International Plans of Action (IPOAs)—for seabirds, for sharks, for managing fishing capacity, and for illegal, unreported and unregulated (IUU) fishing—as well as to develop instruments regarding port State measures. New RFMOs have been established to fill the gaps in their coverage, and the mandate and the efficiency of RFMOs have been strengthened in response to technical and institutional audits.⁵ Concurrently, FAO has been contributing by developing complementary technical guidelines and guidance regarding specific aspects of the CCRF and these various related instruments and approaches.

The emphasis in addressing overfishing and the ineffectiveness of fisheries management has now shifted. Now, the priority is to implement the CCRF and related instruments. As expressed by the FAO Committee on Fisheries during its 26th Session in 2005, the period of 2005–2015 needs to be a “decade of implementation.” As part of doing so, countries will have to surmount seven core challenges.

3.1. Understanding fisheries and their environment

In addition to the many instruments and agreements mentioned above, the 2001 Reykjavik Declaration on Responsible Fisheries in the Marine Ecosystem and the 2002 Plan of Implementation of the World Summit on Sustainable Development established an ecosystem approach to fisheries (EAF). Consequently, whilst recognizing that the single-species approach to fisheries management frequently can be successful (Mace 2004; Hilborn 2007), these new developments reflect a desire to expand beyond the exclusive focus on the direct interactions between fishers and their target species and to consider the impacts of fishing in a significantly broader context.

3.1.1. Evolving knowledge and science

Fisheries interact with and within an ecosystem directly, affecting target species, bycatch species (whether retained or discarded), and critical or important habitats; however, fisheries also have indirect impacts which are typically transmitted through the food web. Heavy fishing of a prey species is likely to lead to a decline in the abundance and productivity of its predators which may be target species for other fisheries.

³ Agreement for the Implementation of the Provisions of the United Nations Convention on the Law of the Sea of 10 December 1982 relating to the Conservation and Management of Straddling Fish Stocks and Highly Migratory Fish Stocks.

⁴ The FAO CCRF (FAO, 1995) provides a comprehensive overview of responsible fisheries management. Some of the key requirements are that: conservation and management measures should aim for long-term sustainability and optimum utilization of resources and be based on the best scientific evidence available (Article 7.1.1, also 7.2); management authorities should identify and collaborate with the relevant stakeholders in a fishery (Articles 7.1.2–7.1.6); States should establish effective mechanisms for MCS (Article 7.1.7); excessive fishing capacity (i.e., overcapacity) should be eliminated and fishing effort should be commensurate with sustainable use of the fishery resources (Article 7.1.8); fisheries management and decision-making should be transparent (Article 7.1.9); and, authorities must ensure that the relevant laws and regulations are known and understood by all stakeholders (Article 7.1.10).

⁵ See the Chatman House report on RFMOs at <http://www.chathamhouse.org.uk/news/view/-/id/396> and http://www.chathamhouse.org.uk/files/10301/10301_rfmo0807.pdf.

Conversely, changes in the ecosystem can affect fisheries. Natural variability in temperature commonly leads to variability in the productivity and sustainable yield of a fishery. Anthropogenic factors such as land-based pollution and eutrophication, coastal habitat destruction, offshore mining and oil extraction can all lead to long-term changes in the ecosystem that will then have impacts on fisheries resources and the humans dependent on them for their livelihoods.

This is the extended environment in which fisheries scientists (of both the natural and human sciences), managers and stakeholders need to plan and manage peoples' activities. As a result, the information and advice supporting fisheries management must cover the same disciplines and dimensions—not only incorporating species population dynamics and ecosystem dynamics, but also economics, sociology, anthropology, and more.

3.1.2. Looking forward

Overall, a pragmatic approach to the EAF needs to use the best available information coupled with a reasonable application of the precautionary approach and a very strong emphasis on full stakeholder participation (e.g. FAO 2003, 2005c).⁶

High levels of scientific uncertainty are a significant obstacle to implementation of EAF in many cases, and this problem is most severe where the fisheries sector is diverse and dominated by small-scale, low value fisheries, where the biological resources are equally diverse, management and enforcement capabilities are frequently weak, and reliable scientific information is also scarce. To further compound the challenge, reliable stakeholder participation (and the information that stakeholders can provide) can be difficult to obtain, especially when stakeholders are physically scattered and have different cultural and educational backgrounds.

Despite these hurdles, the EAF has global relevance; hence, the real challenge for the future will be to develop and use:

- reliable, robust and cost-effective means of assessing and monitoring the status of ecosystems and their resources, and
- rapid means of detecting any undesirable and excessive impacts, from whatever source, that threaten sustainable use.

Then this sort of information can be used in determining and implementing suitable and effective fisheries management.

Decision makers will have to balance the need to make management decisions that are conservative enough to accommodate major uncertainties whilst not being so conservative as to unnecessarily forsake some of the potential yields from their fisheries and aquaculture operations that would be obtainable if there was less uncertainty.

3.2. Reconciling utilization and conservation

Until the final decades of the 20th century, fisheries science was dominated by theories and models based on single-species population dynamics and the concept of "maximum sustainable yield" (MSY).⁷

Subsequently, it has been recognized that the risks of overfishing are too high when MSY is used as a target reference point due to scientific uncertainty and natural variability, and that MSY should be used as an upper limit to be avoided. At the same time, due to the soaring catches and fishing power that characterized the second half of the 20th century, fishing began to have major impacts on many other marine species (e.g., sea turtles, some shark species, sea birds, etc.) as well as on some important marine habitats.

The result has been to recognize one of the fundamental realities of fisheries: because humans and their behaviour are at the heart of fisheries management, there is a serious need to reconcile commercial utilization (i.e., people's livelihoods) with conservation concerns.

Indeed, this can be seen in the FAO CCRF which aims at promoting responsible fishing and aquaculture operations whilst ensuring sustainable use of aquatic biodiversity—integrating the requirements of UNCLOS, the UN Fish Stocks Agreement and the Convention on Biological Diversity (CBD).

3.2.1. Finding a balance

To reconcile these issues, FAO and its Members, using the CCRF as their guide, and several other inter-governmental and non-governmental organizations have been seeking partnerships with a view to finding a workable balance between utilization and conservation; to implement the respective IPOAs for reducing the incidental catch of seabirds by fishers, for better managing sharks, for managing fishing capacity, and for combating illegal, unreported and unregulated (IUU) fishing; and to collaborate actively with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

For example, because the Parties to CITES take steps to regulate international trade of some species where such trade is considered to be a threat to the conservation status of the species, FAO and CITES have been working closely in recent years in relation to commercially-exploited aquatic species for which there are conservation concerns due to their trade. This cooperation has included (amongst other things) the development of listing criteria for CITES,

ensuring adequate consultations in the evaluation of proposals for listing commercially-exploited aquatic species in the CITES Appendices, as well as working on technical and legal issues relating to the listing of such species.⁸

At the level of global policy and practice, FAO's work on deep sea high seas fisheries and on the use of marine protected areas in fisheries management emphasize the need to balance technical solutions with strong training and capacity building activities. Similarly, EAF attempts to go beyond conventional fisheries management and to explicitly address the need for both the conservation and use of fisheries resources within a holistic framework that reflects a full set of all stakeholders' goals and objectives and the needs of future generations.

3.2.2. Looking forward

The WSSD Plan of Implementation identified two fundamental pre-requisites for sustainable development—namely, (i) the eradication of poverty and (ii) changing unsustainable patterns of consumption and production. Indeed, the current global suite of legally binding and non-binding instruments provides the policy framework necessary to ensure sustainable utilization and the conservation of biodiversity and ecosystem integrity.

The problem is that this policy framework is still not being adequately applied across the globe. Thus, the challenge to be

⁶ Good progress is being made in this way in Australia (e.g. Fletcher *et al.* 2005), the Alaska region of the USA (Fluharty 2005) and by Angola, Namibia and South Africa, the countries of the Benguela Current large marine ecosystem (Cochrane *et al.* 2007).

⁷ Article 61 of the UNCLOS urges States to "... maintain or restore populations of harvested species at levels which can produce the maximum sustainable yield, as qualified by relevant environmental and economic factors," implying that MSY or, equivalently, the biomass that can produce MSY, should be a target.

⁸ For example, sea turtles and the sawfishes are presently listed in CITES Appendix I, under which commercial trade is banned, while seahorses, the Napoleon wrasse, shark species such as white and whale sharks, and other species are listed in Appendix II which requires strict regulation of trade in those species in order to avoid utilization incompatible with their survival.

overcome will be to effectively direct political will into reconciling utilization and conservation of aquatic resources and ecosystems. Then this duality should be achievable.

3.3. Considering social and equity issues

3.3.1. Social and equity considerations in fisheries management

Fisheries make important contributions to meeting the Millennium Development Goals on poverty and food security. They can also be a source of wealth creation, supporting national economic development. Indeed, in some communities fishers are not the most impoverished, but in other communities, fishers are amongst the poorest of the poor. In such situations, poverty, vulnerability and low levels of social development often seriously compromise the ability of small-scale fishers to adopt responsible fishing practices, to constructively participate in co-management and community-based fisheries management regimes, and to benefit from the wealth inherent in fisheries.

The diversity and differences amongst fishers (and, especially, amongst those in the small-scale sector) defies definition. Whilst the absence of clearer and more focused socio- and economic information about the on-the-ground realities of fishers and their fishing communities has frequently contributed to inaction, trying to define small-scale fishers and fisheries may lead to inaccurate and incorrect perceptions and—as a result—improper management decisions.

Hence, the human aspects of fishers, especially those participating in small-scale fisheries, have gained increasing attention, and there has been a shift away from the traditional narrow focus on fisheries biology, ecology and conservation towards a wider developmental perspective based on the principles of integrated resource management. As a result, social and equity considerations have become recurring topics when

discussing the challenges of fisheries management, particularly in light of:

- the inevitable complications and implications of having to limit access to fisheries if they are to be commercially and biologically sustainable, and
- the serious concerns that arise about the allocation implications of fisheries management decisions and how people will react to them.

3.3.2. The way forward

Socio-economic and demographic studies—which provide insights about the living conditions, relationships, governance, and institutions in a community—can provide information essential for the details of fisheries management programmes that are acceptable to the participants. Such information is also a critical part of monitoring the impacts of management measures on the socio-economic well-being of fishers, their families and fishing communities. Accurately assessing and addressing the vulnerability and social exclusion of small-scale fishers is also critical for designing and implementing the sorts of access and user rights systems that will improve the contribution of fisheries to poverty reduction and food security.

Hence, the way forward towards being able to manage fisheries so that they create wealth and contribute to food security will require greater, more systematic, widespread collection and use of socio-economic information about fishing communities.

3.4. Assessing incentive structures

Ironically, many of the incentives—the things that tend to motivate or even provoke fishermen to act—that fishers are faced with on a daily basis as they operate their businesses are inadequately considered in fisheries management. And, yet, assessing and understanding the incentives that are created by different fisheries management measures is absolutely fundamental for ensuring that

regulations, plans, and programmes actually achieve what they set out to achieve.

3.4.1. The incentives when fishing without clearly defined rights

Whether small or large, coastal or international, small-scale or industrial, the world's fishermen are all out there fishing in order to make a living, a profit, which enables them to put food on their tables and to take care of their families. They can do this in two ways: either they can fish to maximize their revenues by taking as many fish as possible, or they can fish to maximize their profits within sustainable limits.

In the former case, the scenario becomes one of "If I don't catch the fish first, someone else will and I won't be able to make my living." Thus, fishers work to catch as many fish as possible—either legally or, when desperate, illegally—and even if their costs of doing so are high. The typical scenario of overcapitalization, overfishing, illegal, unregulated, and unreported fishing, and overcapacity emerges as fishers compete against themselves and the system.

This is the situation of ill- or weakly defined user rights. Moreover, it means that fishers will want to provide as little information about their activities as possible because information sharing amounts to reducing one's competitive advantage relative to the others in the same fishery. This, in turn, makes it extremely difficult to get accurate, first-hand assessments of the status of the resource.

3.4.2. A range of rights-based management approaches

Maybe it is not property rights *per se* that people find so problematic, but the particular kind of property rights that are promulgated. —S. Jentoft (2007, p. 93).

It is frequently helpful to put the topic of rights-based systems into a bit of perspective. Contrary to much of the media coverage of the use of property rights in fisheries man-

agement, rights-based fisheries systems are not limited to individual transferable quota (ITQ) systems. Indeed, it is possible to say that all fisheries management systems are based on user rights. It is simply the degree to which the four characteristics (exclusivity, durability, security and transferability) are defined—i.e., the strength of the total bundle of rights—that distinguish fisheries management systems and the incentives they create for fishermen.

Systems based on licences (or some other definition of participants), whether they limit access or not, somehow determine who may participate in a fishery. However, when used alone, the generally competitive nature of individual fishermen (regardless of whether their boats are large or small) will cause them to invest in technologies (better paddles, sails, fishing gear, etc.) that help them increase their catches and revenues. As a result, these systems set up incentives to over-invest and overfish, resulting in conflicts and overcapacity. To avoid this, licensing and limited access systems in general need to be accompanied by regulations to allocate sustainable units of catch or effort amongst the different rights holders.

Customary access/tenure programs as well as various types of territorial and group user rights in fisheries define many of the questions of authority, rights, and rules and thereby set up positive incentive structures for participants. These systems encourage participants to take care of the resources, but only for the duration and the exclusivity of these rights. Moreover, if not supported by contemporary laws, customary programs are not always respected by people outside the customary system and are weakened when national, regional and global forces are brought to bear on the fishery, causing conflict and competition.

The category of most clearly defined rights-based systems is comprised of catch share systems for groups or communities (e.g., community fishing quotas) or individuals (e.g., individual share quota systems). In

fisheries where determining total allowable catches may be problematic, the shares can be in the form of individual transferable effort units—sometimes described in terms of a particular part of the fishing gear or other technological units—as a proxy for shares of a total allowable catch. The drawback of this effort-based share-system is that it will create incentives for the fishermen to invest in technology (“technology creep”) and require constant readjustment of the units to compensate for this. In fisheries where quantitative TACs are set, it is possible to develop individual transferable quota and share quota systems where the individual participants have clearly defined percentage-based shares. In this case, the system still needs to address the problem of discarding motivated by “high-grading” as well as possible concentration of shares to a small number of the rich at the exclusion of the poor.

Not surprisingly, there are common issues that are crucial and applicable for all of these different systems. One is the ability to monitor and to enforce catches effectively. Another is whether the system readily encourages fishers to address bycatch concerns in a cost effective manner. Finally, there is always the question of whether or not the system will encourage or discourage consolidation, particularly in fisheries where overcapacity is a problem.

3.4.3. The way forward

For fisheries to be both biologically and commercially viable, it is necessary to promote fisheries management systems that are not only implemented transparently and collaboratively with all relevant stakeholders, but also based on clearly-defined and legally defensible user rights. Such systems are the only ones that create the conditions and incentives for fishermen to work to maximize their profits on limited amounts of catch—and they do this by minimizing their costs, catching fewer fish, and becoming stewards of the resources they have the rights to use.

Indeed, with the new demands for the ecosystem approach to fisheries, clear and secure fishing rights are essential because they create the environment in which fishing behaviour, conservation objectives, and commercial forces reinforce each other.

Nonetheless, the challenge is that there is not one style of rights-based system to fit all fisheries. Rights-based management systems need to be designed to reflect and build upon the norms and governance structures that the participants and their communities consider legitimate and acceptable. Moreover, when rights-based systems are applied to fisheries where there is overcapacity and overfishing, it is critical to address the impacts of transitioning to rationalized fisheries, the impacts on livelihoods, and the consolidation that will occur. In doing so, fishers and their communities will be able to sustainably generate the wealth that fisheries have to offer.

3.5. Linking fisheries management with trade and with marketing standards

3.5.1. Government failures, market responses

Traditionally, managing fisheries has been totally in the hands of governments as fishery resources in most countries are defined for the “common good.” Yet, because fishers are not vested in these fish resources, overfishing and rent losses have become prevalent as fishers race for the fish.

In part to try to overcome fisheries governance failures and in part due to the corporate social responsibility movement and its social and environmental reporting requirements, the private sector has responded with the development of standards, ecolabels, and product traceability initiatives.⁹ Purchasing a commodity such as fish is no longer simply based on price. Instead, purchasing decisions for fish are now being based on a wider range of factors—including respecting the environment, the welfare

of their workforce, the humane treatment of animals, etc. —and corporate performance is being evaluated on this. Whilst these initiatives are not mandatory, compliance with these standards, the use of ecolabels to distinguish fish products from competitors' products, and product traceability are increasingly becoming industry norms.

In essence, ecolabels are based on market mechanisms. These programs allow retail buyers to source fish from sustainable sources that have positive environmental images. Buyers are assured of an ongoing (sustainable) supply of raw materials, and they are also assured that the market value of their fish products is not compromised by negative images of overfished fisheries and environmental degradation. At the same time, consumers are able to demonstrate their support for sustainable fisheries and/or fisheries that use environmentally friendly methods when producing fish (such as ensuring that tuna is not caught in a way that kills a number of dolphins or turtles) by purchasing fish products bearing ecolabels.¹⁰

There are several key elements for successful ecolabels. As described in the FAO Guidelines for the Ecolabelling of Fish and Fishery Products from Marine Capture Fisheries (FAO 2005b) there is the need for reliable, independent auditing, transparency of standard-setting and accountability, as well as the need for standards to be based on sound science. Moreover, the economic incentives (literally, the commercial paybacks) created by the label have to be strong enough for fishers to want to participate in the pro-

gram and for fishery managers to improve management so that a fishery can qualify for an ecolabel. These elements, in turn, can build necessary consumer confidence and trust.

3.5.2. The way forward

The linkages between fisheries trade, marketing standards, and fisheries that are managed so as to be sustainable are, without a doubt, gaining strength.

Ecolabels do mean that the producers—the fishermen in developing and developed countries alike—will need to be able to adapt to the resulting evolution in demand. And, whilst this new commercial economic environment is an additional challenge to accessing the global value chain, the developing world is now recognized by major ecolabels as a major participant in global markets. Hence, there is the will to harness the power of consumer demand for responsible, sustainable fisheries that demonstrate reduced risks to food security and livelihoods.

The development of ecolabels and private standards is helping to increase awareness about the failures of fisheries management and to lead the way towards better fisheries management practices. Although many ecolabels are being applied to fisheries that are already fairly well managed, even there the fishers and fisheries managers have had to make changes and improvements.

Public relations, awareness creation and educational activities that may accompany an ecolabelling programme can also influence the political arena and contribute to

⁹ Product traceability—stringent chain of custody documentation—complements ecolabel programs because it allows for products to be traced throughout the full production, distribution and marketing chain down to the retail level or, as more popularly described, from “deck to dish.”

¹⁰ Over a decade ago, the failures of governments to ensure the sustainability of fisheries, plus the increasing importance of sustainable fisheries, led corporate and conservation interests to create the Marine Stewardship Council and its ecolabel that signifies that fish have been sourced from certified well managed resources. Today, a plethora of ecolabels exists to meet perceived consumer demands for ethical business behaviour and to help consumers to make informed choices about what they are buying and to support responsible food production.

the political will required for society and politicians to bear the short-term costs of improving fisheries management for the longer-term good. However, it has been emphasized by retailers that ecolabels alone will not relieve governments of their duty to manage fishermen effectively and in accord with increasingly extensive environmental requirements.

3.6. Enhancing institutions and governance for management

Aside from adjusting laws and institutions to new international and national requirements of fisheries management, many countries also need to account for the changing nature of fisheries management by enhancing their institutions and governance of fisheries.

Typically, the extension of national jurisdiction in the 1970's was followed by two decades of rapid growth of the fishing industry. Public policy was characterized by a paradigm of "productivism" and the promotion of fleet expansion and increasing production. Frequently, this occurred quite independently of management measures that were aimed at conserving stocks.

The results of ineffective governance on a broad scale can be seen in the common failure of fisheries management to achieve its biological, ecological, economic and social objectives. Furthermore, in addition to the widespread absence of sufficient political will or political ability, the generally unsatisfactory state of fisheries governance is characterized by:

- i. the high levels of scientific uncertainty, often leading to poor or inappropriate management decisions;
- ii. an inherent conflict in fisheries between short-term economic and social objectives and the longer-term objectives of biological and economic sustainability, with the former usually being given priority;

- iii. poorly defined or inherently conflicting objectives in fisheries leading to reactive decisions and actions rather than proactive ones;
- iv. shortcomings in the institutional frameworks including the use of inappropriate systems of user rights and inadequate participation by stakeholders;
- v. inadequate human, physical, and/or financial resources in management agencies;
- vi. inadequate monitoring, control and surveillance (MCS) and penalties for fisheries infringements, and the existence of incentives to overharvest or otherwise ignore regulations (Cochrane and Doulman 2005).

The requirements for addressing these problems and for achieving effective management systems are well known and understood. FAO identified the main factors of unsustainability and overexploitation in fisheries as the lack "good governance" and "inappropriate incentives" and noted "the need to achieve sustainability by granting secure rights to resource users (individually or collectively)" to overcome these problems (Swan and Gréboval 2003: p. 8).

3.6.1. Institutional changes

With global catch levels flat and an increasing number of stocks showing signs of overexploitation, the last decade has seen signs of a transition. Since the mid-1990's, most countries have participated in the extensive debates on fisheries issues that led to the proliferation of new international instruments, and many countries have also initiated institutional adjustments on this basis. Quite simply, most countries have recognized the limited nature of fisheries resources in their policy framework and are giving more emphasis to fisheries management in lieu of sheer productivism.

However, few countries have undertaken reforms to completely break away from the old productivism paradigm to adopt a new

Table 1. Examples of “classical” and “emerging” topics in fisheries management and conservation.

Classical Topics	Emerging Topics
Natural oscillations	<i>Climate change</i>
Overfishing	<i>Precautionary Approach, extinction, CITES and FAO</i>
Bycatch and discards	<i>Biodiversity</i>
RFMOs and multi-level management	<i>Ocean policy development</i>
Overcapacity	<i>Ecosystem approach to fisheries</i>
Economic viability of fisheries	<i>Rights-based fisheries management systems</i>
Non-tariff barriers and trade	<i>Wealth generation from fisheries</i>
Labour standards	<i>Ecolabelling, traceability and catch certification</i>
Subsidies	<i>Ethics and fair trade</i>
Statistics and reporting	<i>Low cost and cost effective management</i>
Monitoring, control and surveillance (MCS)	<i>Stock recovery strategies</i>
Quality standards	<i>Decentralization and co-management</i>
Production maximization	<i>Port state measures and illegal, unreported, unregulated fishing</i>
—	<i>Deep sea fisheries in high seas</i>
—	<i>Spatial management, marine protected areas (MPAs)</i>
—	<i>Sustainable and ecosystem-based aquaculture</i>
—	<i>Use of fishmeal in aquaculture</i>
—	<i>Open ocean and capture-based aquaculture</i>
—	<i>Environmental impacts of aquaculture</i>

paradigm of responsible fisheries management. Major reforms are still needed in most countries to significantly change legislation, policies, and key institutions (research, administration, management, stakeholder organizations and their role, monitoring control and surveillance, extension services, etc.) so that their fisheries management can address emerging issues (Table 1).

3.6.2. Governance and participation

The key principles underpinning good governance are:

- i. **Openness.** Institutions should work in an open manner and actively communicate the decisions and actions taken, using accessible, understandable language.
- ii. **Participation.** The quality, relevance and effectiveness of policies depends on wide participation throughout the policy chain to increase confidence both in the end results and in the institutions which deliver policies;

iii. **Accountability.** Institutions must provide greater clarity and take responsibility for their own activities as well as for their respective roles of institutions working in a multi-level governance framework;

iv. **Effectiveness.** Policies must be effective and timely, delivering what is needed on the basis of clear objectives, evaluations of future impacts and, where available, of past experiences; and

v. **Coherence.** Policies and action must be coherent and easily understood, and this is all the more important as the range of management issues expands (Table 1).

Poorly designed industry participation actually has led to less effective management with immediate concerns prevailing over requirements for long-term sustainability, and adequate inclusion of conservation stakeholders has also proved difficult. Hence, it is vital to build these principles into fisheries institutions, governance, laws and management approaches.

3.6.3. The way forward: institutions, governance, and participation

Enhancing institutions and governance is not necessarily a simple step, but the use of auditing to assess principles of governance, institutions and management policies, and—together with institutional analysis—is increasingly proving extremely useful in guiding reforms for improved governance and management frameworks.

Fisheries authorities are beginning to organize and promote governance based on the systematic building-up of consensus around the idea of responsible fisheries, with the aim of facilitating decision making on fisheries management issues—especially on the sensitive topics of access limitation, user rights, and the effective management of fishing capacity.

The effectiveness of participation is very much linked to properly addressing these issues. Ensuring appropriate representation and managing fisheries with interest groups while ensuring that societal goals are met are fundamental components of responsible fisheries management. Effective participation can still be considered very much a learning and adaptive process.

In short, moving forward requires that governments and stakeholders work to ensure that their institutional and legal frameworks are able to implement the solutions to ineffective fisheries management and that human and other resources required to effect good governance are available.

3.7. Engaging with developing countries

Following the extension of national jurisdiction in the 1970's, developing countries have made very important efforts to develop their fisheries and to build up the institutional base required for such development.

From the mid-1970's to the early 1990's the international community of donors, development banks and specialized or-

ganizations provided massive support for productivist development in terms of equipment, infrastructures, training and institutional building. As a result most developing countries were able to increase the production from their fisheries. However, the longer term result is that they are now facing the same fisheries problems as those faced by developed countries—overexploitation, overcapacity and deteriorating economic performance.

Unfortunately, donor support to the fisheries sector has been significantly reduced since the late 1980's. As the limits of capture fisheries production were progressively reached, development banks and most donors rapidly redirected their focus on other sectors, including aquaculture. Donor support to capture fisheries was subsequently pursued on a far more limited scale and with more focus, e.g. on small scale fisheries, quality control, export promotion, basic landing infrastructures, conservation and general institutional building. Today, donor support for changes in fisheries management remains quite limited and largely insufficient, despite some signs of renewed interest from the part of some institutions like the World Bank.

3.7.1. The way forward

The need for legal and institutional reforms is vital for successful fisheries management, and the need for improved fisheries management is very much recognized in developing countries. Yet, developing countries have struggled to keep up with the fisheries management requirements of the CCRF and related instruments whilst trying to redirect their policy focus away from “productivism” and in favour of increasing the value of catches within sustainable production limits. To achieve this, the main ways forward include:

- strong national political will to support successful fisheries management;
- international cooperation that creates and develops the skills and means for

developing countries to implement appropriate, collaborative fisheries management on an enduring and self-perpetuating basis; and

- international cooperation that engages developing countries and expands regional cooperation for fisheries management.

4. Conclusion

Capture fisheries have reached their maximum productive capacity in the face of ever increasing demands for fishery products, and with fish providing at least 20% of animal protein intake for approximately 1.5 billion people or at least 15% for about 3.0 billion people in our world, it is all the more critical that fisheries management is both efficient and effective.

Global understanding of fisheries resources and their environment continues to expand, and the way in which the world thinks about fisheries resources is becoming increasingly complex, and our society is increasingly demanding that fisheries management be more sophisticated in conserving ecosystem integrity.

As the world shifts from the old productivism paradigm to one of responsible fisheries management, it is imperative that we balance between viable utilization and conservation. The "inexhaustibility" of the oceans is still a pervasive idea that needs to be replaced by the recognition that there is a need to severely restrict access to these resources if they are to sustainably generate wealth and to alleviate poverty.

Management of fishery resources have been highly problematic largely due to inef-

fective institutions and governance, conflicting objectives, and weak user rights. Nonetheless, we are beginning to meet the challenge of establishing adequate institutions and creating governance and management systems that can complement increasingly global market forces and the incentives that fishermen around the world face on a daily basis. The importance of clear fishing rights has been emphasized strongly in recent years, and countries now need to set in action the political process of resource allocation.

The way forward will be challenging, but it can result in fisheries that create wealth and contribute to food security and are part of sustainable patterns of consumption and production, and FAO will continue working to implement solutions for creating sustainable fisheries that are at peace with humanity's claims on them.

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Exploring the BOFFFF Hypothesis Using a Model of Southern African Deepwater Hake (*Merluccius paradoxus*)

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The Big Old Fat Fecund Female Fish (BOFFFF) hypothesis is explored quantitatively using an age-structured stock production model in which the usual stock:recruit relationship is replaced by a three-stage recruitment process based on maternal age. The recruitment process is decomposed into three age-dependent stages: 1) number of eggs spawned per batch per female, 2) number of batches spawned per year (related to the length of the spawning season), 3) increased survival of young stages with maternal age. The first two stages together give the stock recruitment potential and can be well justified by experimental observation in many fish species. The third stage is affected by both maternal age and also environmental conditions. The results indicate that the number of batches spawned per year is the most important component. They also indicate that a few old fish potentially contribute many times more recruits to the population than many younger fish. It is argued that the concept of spawner stock biomass (which is heavily weighted by the large numbers of young first time spawners, especially in heavily exploited populations) should be replaced by the concept of stock recruitment potential, which takes the age-structure of the population into account. The conclusion is that for many long-lived fish populations such as hake and cod, management should aim to maintain the age-structure of the population, rather than encouraging, or even targeting, exploitation of large, old fish.

KEYWORDS spawner biomass; stock recruitment potential; effects of maternal age; age-structured population model

1. Introduction

Marteinsdottir and Steinarsson (1998), Scott *et al.* (1999), Longhurst (2002), Berkeley *et al.* (2004a, b), Palumbi (2004), and Scott *et al.* (2006) have hypothesized that Big Old Fat Fecund Female Fish (BOFFFF) are essential for the successful maintenance of many long-lived fish populations. The essence of the argument is that fish longevity has evolved to cope with the variability in recruitment of young fish into populations whose young stages need to survive variable mid-latitude pelagic environments (Longhurst 2002). In these stocks it seems that successful recruitment occurs very seldom, say one year in 20, so some individual fish need to live for at least that period of time in order to reproduce successfully so that the population can be maintained.

It has long been known that large females produce many more eggs than smaller, younger females (e.g. Morita *et al.* 1999; Osborne *et al.* 1999). In spite of this knowledge, most traditional management models are based on stock–recruit curves that assume that all female fish contribute equally (per unit biomass) to future recruitment. Many management strategies, moreover, are based on the assumption that after fish have spawned once they can be fished, thus targeting older, larger fish and reducing the age-structure of the population to younger age classes under the age of an arbitrary (often 50%) age of sexual maturity. Recent findings, however, indicate that not only do large older females spawn exponentially more eggs than smaller females, but that often these eggs are larger and contain more oil (Morita *et al.* 1999), and that this in turn leads to 60–80% better survival rates than the offspring of younger, smaller females (Ojanguren *et al.* 1996; Berkeley *et al.* 2004a). The size and number of eggs spawned are functions of the individual mother's biomass, which increases with age, and therefore maternal

age is referred to hereafter, rather than maternal size.

The truncation of the distribution of age-structure associated with targeting older fish may result in a number of negative impacts on fish spawning. These negative impacts include: a shortening and change in timing of the spawning season (Wieland *et al.* 2000; Berkeley *et al.* 2004b; Scott *et al.* 2006), a decrease in the production of eggs and larvae (Berkeley *et al.* 2004a, b; Cardinale and Svedäng 2004; Birkeland and Dayton 2005; Macchi *et al.* 2005), a reduction in the probability that favourable conditions will be encountered by at least some larvae, a decrease in the average survival potential of larvae (Conover and Munch 2002; Berkeley *et al.* 2004b), and a reduction in genetic heterogeneity (Law 2000). These factors affect the chances of recovery of populations that have been overexploited (Law 2000; Birkeland and Dayton 2005) and together suggest that the effects of sustained heavy exploitation may not be reversible.

This paper sets out to explore quantitatively the implications of the BOFFFF hypothesis for recruitment, using Southern African deepwater hake as an example of a fairly long-lived fish (about 25 years maximum age-span; R. Leslie, MCM, pers. comm.) that inhabits a mid-latitude environment on the west coast of Namibia and South Africa from about 16–36°S.

2. The Fishery

Cape hakes have been the target of major trawl fisheries since the start of the South African trawl fishery in about 1900, with *Merluccius capensis* being fished in shallower water (ca. 50–350 m) and *M. paradoxus* (also known as deep-water hake) in deeper water (from 250–600 m). *M. paradoxus* inhabits the outer continental shelf from the Agulhas Bank in the south (ca. 36°S) to northern Namibia in the north (16°S). Both species have been managed as a single stock

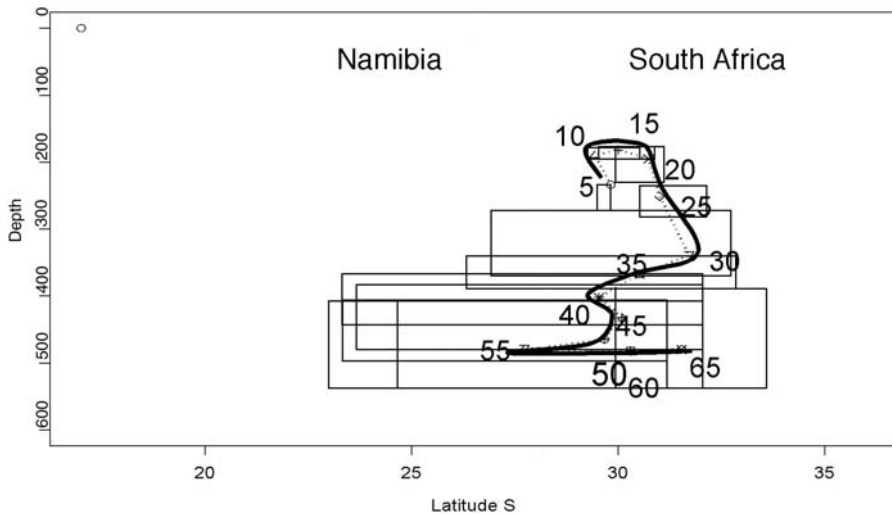


Fig. 1. Diagram depicting the changing centres of distribution of different size-classes of *Merluccius paradoxus* in 2006 off the coasts of South Africa and Namibia. Latitude is given on the x-axis and depth on the y-axis. Size classes are numbered according to their upper limits (cm), with 50 percentile distribution boxes for each size class (From Stroemme *et al.* in prep.).

until very recently, largely because of the difficulty in distinguishing the two species commercially, although the Namibian fishery has been managed separately from the South African one. The hake fisheries have been relatively stable except during the period of 1960–1976 when international fleets heavily exploited both species in international waters beyond the 12 n. mile limit. South Africa declared a 200 n. mile exclusive economic zone in 1976 and introduced strict controls on the local fleet in order to rebuild the stocks, but in Namibian waters hakes continued to be heavily exploited by international fleets until Namibia's independence in 1990, when stricter controls were introduced there too. Since the 1990s the heavy demand for large fresh hake in top condition in Europe, encouraged the development of long line fisheries in both countries. This has the effect of targeting larger fish and exploiting fish on rocky ground that bottom trawlers traditionally avoided. At the same time it encouraged trawlers to explore deeper waters for large hake, waters

that had previously been less economic to fish than shallower water. This had the double effect of exploiting previous refugia for larger, older fish of both species on rocky ground and in deep water (deeper than about 600 m depth) for *M. paradoxus*.

The life history of Cape hakes has been described by Botha (1986) and Payne (1989) who noted that juveniles are found inshore of the adults and that the size of hake increases with depth. Juveniles feed largely on euphausiids and other crustacea, and the diet becomes more piscivorous as the fish grow. Large *M. paradoxus* feed on smaller *M. capensis* (pseudo-cannibalism) where their distributions overlap, and there is also a strong element of cannibalism in both species. Until very recently, little work had been done on the early life history of the hakes. Stroemme *et al.* (in prep.) have shown that *M. paradoxus* tends to spawn in the south (ca. 34°S), recruit inshore on the west coast of South Africa and then migrates north and offshore as they grow larger (Fig. 1).

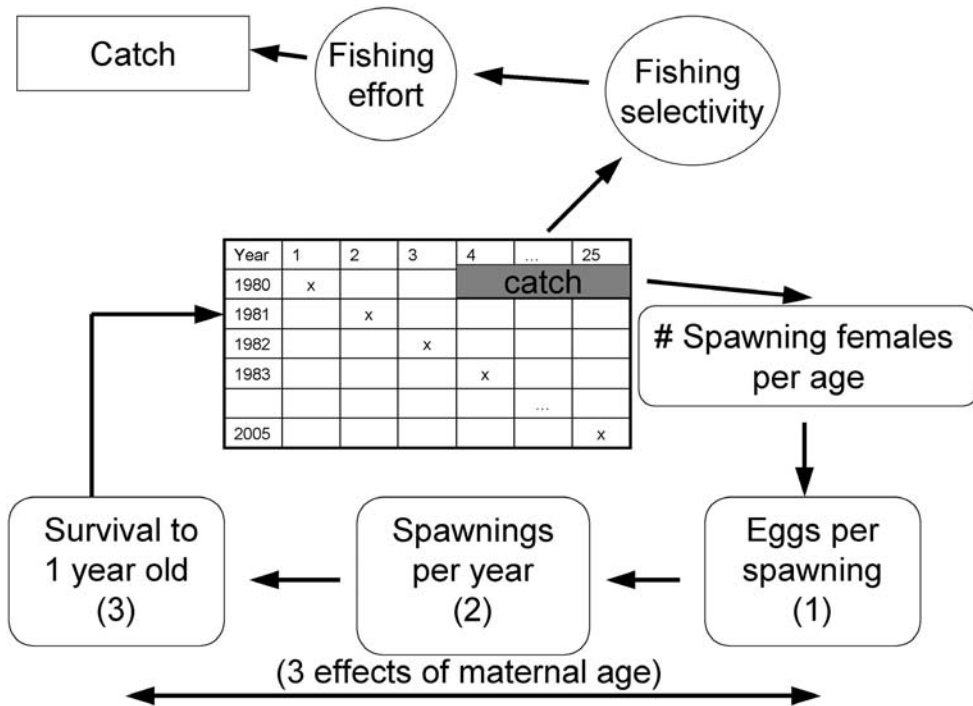


Fig. 2. Diagram depicting the age-structured population model for *M. paradoxus*. Recruitment is depicted as the product of three processes dependent upon maternal age ("3 maternal effects").

3. The Model

We use an age-structured production model (ASPM) on a simple spreadsheet (Fig. 2). In a conventional ASPM, recruitment is modelled using a stock–recruit curve. We substitute three "maternal effects of age" for the stock–recruit curve. The first maternal effect is the increasing batch fecundity with age (and size). Our assumption is based on the work of Osborne *et al.* (1999) on *M. paradoxus*, but extrapolated beyond the size and age range of that work, since the size range of the fished population had already been somewhat truncated by then, with very few large, old females in the research survey catches.

The number of females is assumed to be half the numbers in each age class (Botha 1986), and $(\text{Eggs\_per\_female})_a$ is the number

of eggs spawned in a batch per female at age a , which depends on fish size (Osborne *et al.* 1999):

$$(\text{Eggs\_per\_female})_a = 8.02L_a^{2.67} \quad (1)$$

where L_a is the length at age a .

The second maternal effect is the number of batches spawned per season. There have been no studies of the spawning frequency of Cape hakes, but spawning frequency is positively related to the maternal age or size of most fish species, including Argentine hake *Merluccius hubbsi* (Murua *et al.* 1998) and European hake *Merluccius merluccius* (Macchi *et al.* 2004). Based on the above hake studies we assume that the spawning frequency increases linearly from once per year at age 2 to 14 times per year at age 15, and remains constant at 14 batch spawnings per year thereafter (Fig. 2).

The third maternal effect is increased survival rate with maternal age. Here we make the assumption that survival rate ranges linearly from 30% below the mean survival rate to 30% above the mean over the range of maternal ages (4–25 years). This is based on studies of two unrelated groups of fish, black rockfish *Sebastes melanops* (Berkeley *et al.* 2004a) who found an increase of 60% in survival rate with age, and brown trout *Salmo trutta* (Ojanguren *et al.* 1996) who found an 80% increase in fry fork length and survival rate with maternal size. The mean survival rate was estimated by running the model of the unfished population to steady state.

Recruitment was calculated by multiplying the number of females per age class by batch fecundity, by the number of batches spawned per year (eggs_per_female), by the survival rate for eggs spawned by each age; these were then summed over all the ages:

$$\text{Recruits} = \sum_{a=2}^{25} \left\{ (\# \text{ females})_a \times (\text{eggs\_per\_female})_a \times (\text{batches})_a \times (\text{survival})_a \right\} \quad (2)$$

where a is maternal age, batches is the number of spawnings per year, and survival rate ranges from 0–1. The subscript a denotes age-dependence.

The standard average von Bertalanffy growth model was used to calculate the size of fish as they grow into the next age class, based on the parameters for South African hake of Punt and Leslie (1991). Similarly, fish populations decrease exponentially in numbers according to the sum of natural and fishing mortality. The stock was assumed to have been lightly fished until 1950, so this year was used as the base for starting the model, using population parameters given by Rademeyer (2003). Density dependence was implicitly modelled by capping recruitment at a maximum value of 300 million recruits per year. This does not take account of can-

nibalism after recruitment at age 0, which is included as a density-independent value for natural mortality used to tune the model. The maximum number of recruits was estimated by running the model of the unfished population to steady state. The model was run for 50 years and tuned by varying fishing and natural mortality to give realistic population age distributions and yields at steady state.

4. Results and Discussion

Figure 1 gives 50% percentiles of the distribution of *M. paradoxus* from research surveys in 2006. It is interesting to note that these limits are much narrower for the early stages in the life cycle than the older stages, indicating fairly restricted survival of eggs and larvae coupled with tight schooling behaviour of these early stage survivors to specific nursery localities. This lends support for the hypothesis that only a few of the batches spawned find windows of opportunity in an environment favourable for survival. It also lends credence to the idea that some predatory young fish survive best by adopting tight schooling behaviour in which the fastest growing juveniles may become cannibalistic on their slower growing cohorts, further enhancing the growth and survival of some in the school at the expense of the slow growing ones. This appears to be a reproductive strategy favouring fecundity in numbers of eggs at the expense of producing fewer larger eggs (Nishimura and Hoshino 1999). As the fish grow older they disperse more widely, presumably reducing the incidence of cannibalism and extending their diet to crustacea such as copepods and euphausiids, before becoming more piscivorous at a size of about 20 cm (Pillar and Wilkinson 1995). The older fish migrate to deeper water, with centres of distribution that also tend to move northwards into Namibian waters, before returning south to spawn again.

Figure 3 shows the cumulative results of each of the 3 maternal effects of age on

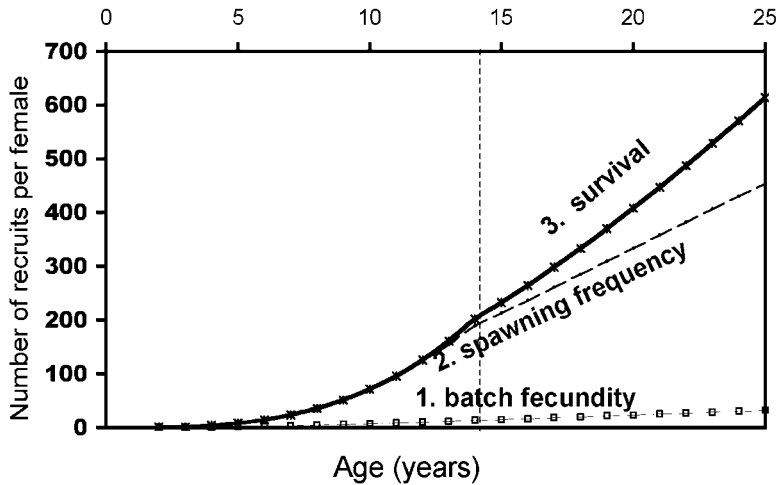


Fig. 3. Cumulative contributions of the 3 maternal effects to recruitment: 1. Effect of increasing batch fecundity with age; 2. Increasing spawning frequency with age; 3. Increasing survival to recruitment with age of mother. Y-axis gives potential number of recruits per female, x-axis the age of female (years). Note that the major effect is given by the increased number of spawnings per year.

potential recruitment. The first effect of maternal age (increasing batch fecundity) has a relatively small effect on potential recruitment, the second effect (increased spawning frequency) is largest, and the third effect (survival) adds to the overall contribution. It is notable that increased spawning frequency is not only important quantitatively, but also translates into increased duration (and often timing, Wieland *et al.* 2003) of the spawning season. This is particularly important in a variable pelagic environment, as it opens more windows of opportunity provided by favourable conditions, such as the timing of plankton blooms in spring or after upwelling events. The first two maternal effects are well founded on data in the literature for hakes, the third effect is not as firmly founded and there are no published data for hake on egg size or oil content relative to maternal size or age. Nevertheless, the first two effects alone are sufficient to cause us to rethink management strategies based on stock–recruit curves that assume equal contributions of all females per unit biomass, to

recruitment. The point of inflection of the cumulative potential recruitment curves at 14 years reflects the assumption that spawning frequency increases linearly from 2–14 years of age and then remains constant.

An interesting outcome of the study suggests that each really old female produces up to 300–600 potential recruits. Initially this may seem high, but these values need to be considered in the light of the high mortality rates of young fish as they grow through the age classes to an age when they are likely to make a real contribution to the future population. It is also likely that there are strongly density-dependent effects caused by cannibalism of younger fish, in addition to other predation. These have not been explicitly modelled in our initial model, which introduces density dependence by putting a maximum value to recruitment.

Figure 4 shows the steady state age structure of the model hake population when unfished compared to the recent levels of fishing ($F = 0.3$) and selectivity from age 2 caused by minimum mesh size regulations

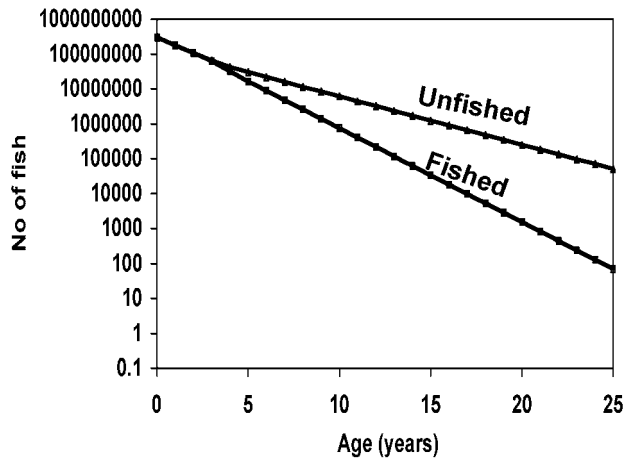


Fig. 4. Age structure of model unfished population of *M. paradoxus* compared to the population fished according to present trawl harvesting strategy and fishing mortality ($F = 0.3$), with increasing selectivity of fish aged 2+ and full selectivity from 4+. Note the logarithmic y-axis.

for the commercial trawl cod end. It can be seen that there is exponential decline in the age structures of both fished and unfished populations, but the decline is much steeper in fished populations, even without modelling the increased targeting of older fish that is believed to occur now. The model result shown is based on trawl fishing only with constant selectivity from age 4 years. This is very conservative.

5. Conclusion

This study replaces the commonly used stock:recruit curves with three model assumptions based on known biological relationships that vary with maternal age, the “three effects of maternal age”: 1) number of eggs per batch spawned, 2) number of batches spawned per year and 3) increased survivorship of eggs and larvae of older fish. It is shown that number of eggs per batch alone has a moderate effect on recruitment with increasing maternal age. Increasing survival of larvae with maternal age also has a moderate effect, but the number of batches spawned per year has a major effect on re-

cruitment. This is probably particularly significant because the increase in number of batches spawned per year with age is manifest mainly by an increase in the duration of the spawning season. This is likely to result in exposure of more batches of eggs spawned to “windows of opportunity” created by environmental conditions favourable for recruitment in the variable pelagic environment created by a pulsed upwelling system in the southern Benguela region where the hake spawn. Furthermore, new results suggest that there is tight schooling of young hake into specific nursery grounds inshore on the west coast of South Africa, and this may lead to cannibalism of slow growing individuals by their faster growing cohorts, enhancing the growth and survival of fast growing young. As they get older the distribution range of deep water hake widens and they migrate to deeper water, with centres of distribution that tend to move northwards into Namibian waters (See Fig. 1).

The concept of spawner biomass used in stock:recruit relationships is heavily weighted in favour of young fish in stocks that have truncated size distributions, simply

because of the large numbers of small, young fish. This is commonly the case in heavily fished stocks. We suggest that it would be much more accurate to adopt the concept of Stock Recruitment Potential (SRP) (Solemdal 1997; Scott *et al.* 1999; Scott *et al.* 2000; Jarre-Teichmann *et al.* 2000), which takes into account the number of eggs spawned by different size- or age-classes. Thus in this paper the SRP is based on the first two effects of maternal age: eggs per batch and batches per year. These are both strongly age- and size-dependent. The third maternal effect is the survival rate. This is also likely to be dependent upon maternal age (Ojanguren *et al.* 1996; Berkeley *et al.* 2004a), and depends very heavily upon environmental conditions, but no more so than the variability found in stock:recruit relationships. By basing the first two stages on sound biological observations, we are moving a step closer to understanding the complex relationships between environment and recruitment, that will ultimately allow improved management of fisheries.

It has long been known that small young fish have much higher natural mortality rates than larger older fish, because as fish grow larger, they have fewer fish bigger than themselves to prey on them. Natural selection favours fast growing fish that quickly grow out of the predator pit into larger size classes. There is some genetic evidence for this in cod, indicating that moderate selective effects act mainly through size-specific mortality and fecundity (Case *et al.* 2006). If we

catch more young small fish, we add to the high natural mortality of such ages. By targeting large fish, humans tend to reverse natural selection by targeting fast-growing fish, possibly altering the gene pool permanently, and certainly reducing the recruitment potential of the stock, as shown by the results of this model. If we target large old fish, we act against nature. Of course there is a caveat, in catching young fish we must leave enough fish in the water to survive and grow into large, old ones.

The final conclusion is that, for many long-lived fish species, fisheries management measures need to take into account the exponentially greater importance to recruitment of Big, Old, Fat, Fecund Female Fish (BOFFFF). Perhaps the best management strategy for such fairly long-lived fish as cod and hake is to manage these fish stocks in such a way as to maintain the age-structure of the population (Longhurst 2002; Berkeley *et al.* 2004b; Birkeland and Dayton 2005). This may be achieved through discouraging the targeting of big fish. This is not easy, but is probably best achieved through marine protected areas that prohibit fishing in habitats frequented by the large old fish, if they are not highly migratory.

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Effects of Fishing on Inter and Intra Stock Diversity of Marine Resources

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Maintenance of phenotypic, and in particular genetic, diversity between and within stocks is one of the most critical goals of fisheries management and conservation. Fishing has been held responsible for the extinction and depletion of a number of stocks, and the majority of those remaining are threatened by over-exploitation. Fishing mortality often exceeds that which is natural and in addition fishing activities tend to be highly selective. Accumulating evidence suggests that selection pressures exerted through harvesting that is selective for size, age, sex, maturity and/or certain behavioural/genetic components have influenced the structure, plasticity, production, sustainability and recovery potential of a growing number of exploited stocks. In this paper we review the known effects that fishing has had on inter- and intra-stock diversity, and the potential consequences for fish stocks in terms of alteration of genetic and phenotypic properties.

KEYWORDS phenotypic, genetic diversity; fishery management; conservation; population richness

1. Introduction

Commercial fishing has had extensive effects on exploited fish stocks. For many stocks, fishing is the main source of mortality and may exceed natural mortality by more than 400% (Mertz and Myers 1998). In all stocks,

declines have been very rapid with catch rates often falling dramatically during the first 10 years of exploitation (Myers and Worm 2003). Greatest changes have been among predator species, and it has been estimated that more than 90% of large predatory fishes have been lost from the global oceans (Myers and Worm 2003). Through

their removal, not only global catches but also the mean trophic level of catches have consistently declined (Pauly *et al.* 1998; Myers and Worm 2003).

Due to their highly selective nature, most fishing practices not only affect fish communities at the inter-stock level, but also composition and diversity at the intra-stock level. Changes in age and size structure due to excessive removal of large and old individuals have been reported for many exploited fish stocks (Trippel *et al.* 1997; Marteinsdóttir and Thorarinsson 1998; Marteinsdóttir *et al.* 2005; Ottersen *et al.* 2006). In recent years, evidence that fishing not only affects populations demographically but also changes their genetic composition has accumulated (Heino and Godø 2002). Evolutionary pressures are exerted through fishing that is most frequently selective with respect to size, maturity status, morphology or behaviour (Jørgensen *et al.* 2007). Changes in life-history traits, particularly age and size at maturation, have been reported in an increasing number of commercially exploited fish stocks (see reviews by Trippel 1995; Dieckmann and Heino 2007; Jørgensen *et al.* 2007). In the majority of cases, such trends are unlikely to be explained by environmental influences alone, and fisheries-induced evolution has repeatedly been found to offer the most parsimonious explanation (Jørgensen *et al.* 2007).

These changes in the characteristics of the stocks range from being easily observable and measurable (size and age structures), to those that are not as easily determined due to interacting and confounding effects of density dependence and the environment. Still to be discovered are potential changes in genetic diversity that are undoubtedly ongoing, but are largely concealed due to our lack of knowledge of population structures.

Traditionally in fisheries science, the term 'stock' has described an arbitrary group of individuals belonging to a fish species,

numerous enough to be self-reproducing, relatively homogenous in life-history and demography, and occupying a sub-area of the geographic range of the species (Hilborn and Walters 1992; see Begg and Waldman 1999 for a review). The terms 'stock' and 'population' are frequently used interchangeably, although, at times confusingly, a population may be a unit recognised by taxonomists whereas a stock is not (Cushing 1968; Begg and Waldman 1999). However, regardless of the criteria used to define a particular stock (Waples and Gaggiotti 2006), the 'stock concept' is essentially based on the interaction between an exploited species and the management of that exploitation (Begg and Waldman 1999), and is therefore fundamental to fisheries science and management. These management units (stocks) are commonly geographically based, assuming the existence of a single stock within management regions. This spatial definition is thus infrequently based on concise information on stock structures. Today however, many commercially important fish stocks, for example Atlantic cod, halibut, turbot, and herring have been found to consist of smaller sub-units which vary in fitness-related traits, behaviour, and in some cases are genetically distinct (Ruzzante *et al.* 1999, 2000, 2006; Imsland *et al.* 2000; Jonassen *et al.* 2000; Hutchinson *et al.* 2001; Purchase and Brown 2001; Knutsen *et al.* 2003; McIntyre and Hutchings 2003; Salvanes *et al.* 2004; Neat *et al.* 2006; Jónsdóttir *et al.* 2006a, b; Pampoulie *et al.* 2006).

Maintaining diversity between and within stocks is one of the critical aspects of conservation. All major international agreements on conduct for fisheries adopted during the last two decades emphasise the need for adoption of an ecosystem approach to fisheries (EAF). Explicit sub-goals of ecosystem based management are to assure sustainability and conserve genetic, species and ecosystem diversity (FAO 2003). While management advice regarding many of our most important fish stocks is often either

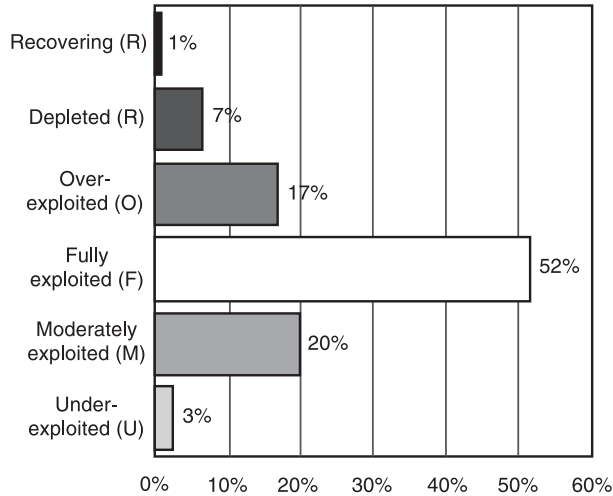


Fig. 1. State of world fish stocks in 2004 (from Garcia *et al.* 2005).

ignored or only partially implemented, recent developments in fisheries research have revealed immediate dangers due to potential erosion of stock properties.

In this overview, we focus on changes that have occurred at both the inter- and intra-stock levels due to fishing. Our objective is to provide up to date information on the potential consequences and importance that these changes have for the general fitness, productivity and sustainability of the stocks. Where applicable, examples are provided involving commercially exploited fish stocks.

2. Inter-Stock Diversity

2.1. Effect of fishing on inter-stock diversity

To date, a single fish species has been documented extinct on a global scale as a result of fishing, and two more due to habitat loss or other causes (Dulvy *et al.* 2003). Furthermore, fishing has been held responsible for the extinction of a number of marine and anadromous stocks, including several stocks of Pacific salmon, the beluga sturgeon in the Adriatic Sea (see overview and references

in Kenchington 2001) and the Icelandic spring spawning herring (Jakobsson 1980a). Additionally, a high number of marine stocks are considered severely overfished and depleted (Musick *et al.* 2000; Dankel *et al.* 2007). According to global statistics compiled by the United Nations' Food and Agriculture Organization (FAO) in Rome, 52% of the world's fish stocks were fully exploited, 17% overexploited and 7% depleted in 2004 (Fig. 1; Garcia *et al.* 2005). At a regional scale, more than 80% of the exploited fish stocks in the North Atlantic are overfished (ICES 2004). Of these, 34% are considered to be in danger of collapse and under ICES advice should be closed to all fishing activities (ICES 2004).

Exploited stocks commonly originate from more than one spawning component, with these spawning aggregations contributing unevenly to the harvestable stock (Begg *et al.* 1999). If these spawning aggregations consist mainly of philopatric individuals, genetic differentiation between the spawning components may be expected (Wootton 1998). Tagging studies have provided evidence of the presence of natal homing and spawning site fidelity within a

number of marine fish stocks (Godø 1984; Thorsteinsson and Marteinsdóttir 1993; Jónsson 1996; Thorsteinsson *et al.* 1998; Robichaud and Rose 2001, 2004).

Features of the environment which influence the biological and demographic characteristics of a fish stock are rarely distributed uniformly across the geographic range of that stock. Fish which reside in, or return to, particular habitats for significant parts of the year tend to develop phenotypic characteristics reflecting local adaptation. This picture of patchy distribution becomes more complex when there is ontogenetic (e.g. between spawning, nursery and adult areas) and/or seasonal (e.g. between feeding and spawning areas) changes in habitat occupancy. As a result, many exploited fish stocks do not represent single spawning stocks, but complex aggregations of sub-units that have variable or limited interaction. The degree of mixing between individuals within different habitats will determine whether a stock is truly a single panmictic population or a combination of sub-components that have limited interaction across their geographic range (Metcalf 2006).

Depletion of inter-stock diversity is likely to be manifested as an overall reduction in genetic diversity, less optimal use of habitat space and reduction in species' ability to contend with stochastic environmental conditions. What's more, the magnitude of stock erosion that has been observed may be only a fraction of the actual depletion that has taken place. Over the last decades, new and improved discrimination techniques have enabled us to identify stock structure that is often considerably more complex than was previously assumed (see examples below).

2.2. Examples of inter-stock diversity

Of all marine species, few have been studied more than Atlantic cod. During the last decade, an increasing number of studies have provided evidence of complex stock struc-

ture at relatively small scales. In the NW Atlantic, genetic differences have been reported between most of the major population complexes (NE Newfoundland Shelf, Grand Banks, Flemish Cap, Scotian Shelf, Georges Bank). At even smaller scales, genetic differences have been detected between inshore and offshore components of the Northern cod stock (Ruzzante *et al.* 1996, 1997) and between migratory cod that spawn in the Gulf of St. Lawrence and cod that reside and spawn outside of this area (Ruzzante *et al.* 2000). In the NE Atlantic, genetic differences have been established between North East Arctic cod and coastal cod (Fevolden and Pogson 1997; Sarvas and Fevolden 2005; Westgaard and Fevoldin 2007), as well as among coastal populations inhabiting different fjords along the Norwegian coast (Knutsen *et al.* 2003; Westgaard and Fevoldin 2007 and references therein). Evidence for gradual transition between putative populations and an existence of a hybrid zone was established for the North Sea and Baltic cod (Nielsen *et al.* 2003). West of Scotland and in the northern North Sea, the existence of resident spawning groups were suggested based on limited home range and exchange between spawning areas up to 200 km apart. In Icelandic waters, spatial analysis of molecular variance revealed three potential populations; two in the southwest separated by depth and one in the northeast region (Pampoulie *et al.* 2006). These findings were supported by tagging data and discrimination based on otolith shape and elemental fingerprints (Jónsdóttir *et al.* 2006a, b; Pampoulie *et al.* 2006).

Similarly, a number of genetically distinct population complexes have been described in herring. Ruzzante *et al.* (2006) demonstrated genetic differences ($F_{ST} = 0.027$) among herring from three regions: a) North Sea autumn spawners, English Channel winter spawners and Norwegian spring spawners b) Skagerrak spring spawners and c) the Western Baltic. This

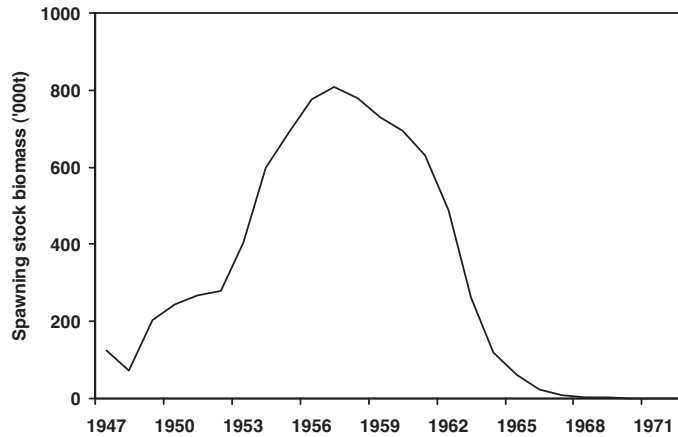


Fig. 2. Estimated spawning stock biomass of Icelandic spring-spawning herring in 1947–1972 (Jakobsson 1980a).

variation was supported by differences in life history strategies including migratory behaviour and spawning time and location, despite mixing of offspring during early life history stages (Ruzzante *et al.* 2006).

2.3. Examples of loss of inter-stock diversity

Of the three stocks of herring that have been identified in Icelandic waters; Icelandic summer-, Norwegian spring-, and Icelandic spring-spawners, only two are still present today. The Icelandic spring-spawning stock, which provided catches of 50–270 thousand tonnes in the 1940–1960's, collapsed in the late 1960's (Jakobsson 1980a) (Fig. 2). The collapse of this stock was not solely due to over-exploitation. Environmental conditions were difficult in Icelandic waters at this time due to low salinity and cold temperatures associated with the “Great Salinity Anomaly” of the late 1960's (Dickson *et al.* 1988). Resulting declines in primary and secondary production (Thórdardóttir 1976; Jakobsson 1980b) were held responsible for the sharp reduction in recruitment in 1965 (Jakobsson 1980a). Despite harsh environmental conditions, Jakobsson (1980a) demonstrated that

the stock could potentially have been saved if fishing effort had been kept at very low levels during the 1960's. Unfortunately, reduced recruitment levels and the decreasing stock size were not detected early enough due to the high efficiency of the purse-seine fleet (Jakobsson 1980a).

The northern cod was once one of the largest groundfish resources in the Northwest Atlantic. The stock responded to increasing fishing pressure in the 1960's–1970's with a rapid decline in abundance; the stock declined from a maximum of 3 million to less than 500,000 tonnes (Lilly and Carscadden 2002; Lilly *et al.* 2004; Lilly 2005). Following the extension of the fisheries jurisdiction to 200 miles in 1977, there was some improvement with the fishable stock exceeding 1 million tonnes in the 1980's. However, stock levels decreased rapidly in the early 1990's and a moratorium on fishing was declared in 1992. Although the causes are under ongoing debate, most scientists agree that overfishing was one of the most important factors leading to the decline and later collapse of this commercially and biologically important fish stock.

In the North Sea, genetic evidence suggests that one of four cod populations, the

Flamborough Head population, has disappeared since the 1950's (Hutchinson *et al.* 2003). This population became depleted twice in the period between 1954 and 1970. Microsatellite data indicated a significant reduction in genetic diversity during this time, followed by a recovery between 1970 and 1998; although this was characterized by a significant change in allele frequencies. The results were consistent with a period of prolonged genetic drift, accompanied by replacement of the Flamborough Head population with others nearby, most likely from the Aberdeen ground and German Bight.

In the 1970s, the Barents Sea capelin stock was one of Europe's largest single species fisheries (Hjermann *et al.* 2004a). Since then it has undergone two collapses during which biomass fell by >95%. The role of harvesting as the main cause of these collapses has been a matter of debate (Gjøsaeter 1998; Ushakov and Prozorkevich 2002). Several studies have demonstrated the importance of larval predation, particularly by herring, for subsequent capelin stock dynamics (Gjøsaeter 1998; Hamre 1994; Hjermann *et al.* 2004b). Hjermann *et al.* (2004a, b) described the additive nature of several mortality factors (harvesting, herring predation, cod predation and climate) and emphasised that one factor is unlikely to be counteracted by a decrease in another. Instead, overharvesting will tend to render a population more susceptible and sensitive to predation, leading to potentially large and abrupt changes in biomass.

Lastly, many Atlantic salmon stocks have declined dramatically, particularly within the southern range of the species (Parrish *et al.* 1998). As with capelin, the causes of these declines are of diverse origin. Impacts of climate, overfishing, aquaculture and oceanic conditions are likely to have had additive effects (Friedland *et al.* 2003; Dempson *et al.* 2004; Jonsson and Jonsson 2004, 2006).

3. Intra-Stock Diversity

3.1. Effects of fishing on intra-stock diversity

The first and most obvious response of a fish stock to exploitation is a decline in abundance. Severely reduced stock sizes will lead to fragmentation and shrinkage in overall distribution as well as spawning areas (Frank and Brickmann 2000; Hutchings 2000; Kenchington 2001; Smedbol and Stephenson 2001 and references therein). Associated with this are declines in intra-stock diversity, with respect to both phenotypic and genotypic variation.

Changes in phenotypic variation may result from size selective harvesting, along with unequal exploitation pressure on a particular sex or distinct life history units, leading to excessive removal of certain stock components (see further below). Such selection will naturally lead to changes in genetic diversity of the stocks, and may in some cases remove certain genetic components responsible for variation or plasticity in essential life history traits.

Evidence for size selective harvesting is plentiful (Trippel 1995; Fenberg and Roy 2008). Typically, fishing gear removes the largest (and fastest growing) individuals of a cohort at the time they enter the harvested part of the stock. Commonly, large individuals are also specifically targeted to maximize yield per unit effort. Even if size selective harvesting is not intentional, intensive exploitation will always lead to truncation of age and size structures simply because members of a cohort are not allowed to survive and attain a relatively old age. Indeed, one of the first indications of overharvesting is truncation in the age structure of a stock, leading to a decline in age diversity (Martensdóttir and Thorarinnsson 1998).

Most, if not all, temporally persistent changes in phenotypic diversity are likely to

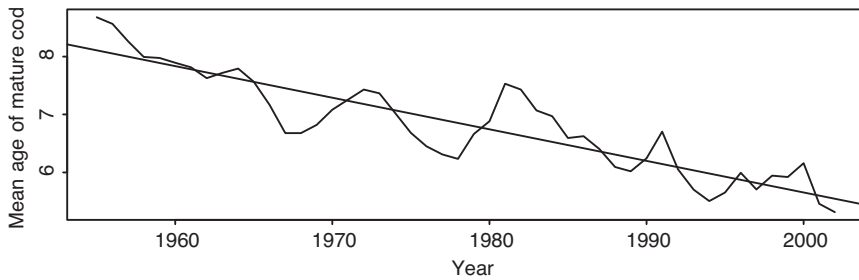


Fig. 3. Mean age of mature cod in Icelandic waters during 1955–2002 based on VPA results (Anon 2006).

be accompanied by changes in genetic diversity. Our knowledge of heritabilities associated with life history traits is somewhat limited. However, breeding studies have demonstrated that the heritability of characteristics such as growth and age at maturity are high enough to facilitate a rapid response to directional selection (Gjedrem 1983, 2000; Weigensberg and Roff 1996; Roff 2002; Henryon *et al.* 2002; Kause *et al.* 2003; Thrower *et al.* 2004; Reznick and Ghalambor 2005; Wang *et al.* 2006), and laboratory experiments have shown that size selective harvesting can cause evolution of growth rates and multiple other traits including fecundity, offspring size and food consumption rates (Conover and Munch 2002; Walsh *et al.* 2006).

3.2. Examples demonstrating importance of maintaining intra-stock diversity

3.2.1. Age and size structures

Declines in average age and size of spawners have been reported for many commercially exploited fish stocks. In the Arcto-Norwegian cod stock, average age and length of spawners decreased by more than 3 years (from 10–11 years to 7–8 years) and 10 cm (from 90–80 cm) between 1949 and 1990 (Ottersen *et al.* 2006). In the Icelandic cod stock, average age of spawners has also de-

creased by around 3 years (from approximately 8 to nearly 5 years) during the last 50 years (Fig. 3). In these stocks, as well as most other cod stocks in the North Atlantic, the number of age classes contributing to the spawning stock has decreased in a similar manner (Marteinsdóttir and Thorarinsson 1998; Marteinsdóttir *et al.* 2005; Ottersen *et al.* 2006). The importance of maintaining intra-stock diversity has been demonstrated for both these stocks because of the link between high age diversity, successful recruitment and greater resilience to environmental fluctuations (Marteinsdóttir and Thorarinsson 1998; Ottersen *et al.* 2006).

Long-term temporal changes in body size have also been reported for salmon (Ricker 1981), bluegill, black crappie and yellow perch (Beard and Kampa 1999), a variety of species on the Scotial Shelf (Zwanenburg 2000), several rockfishes in the Northeast Pacific (Harvey *et al.* 2006) and herring (Power and Iles 2001).

3.2.2. Reproductive potential

Changes in age and size structures will inevitably have large effects on the reproductive potential of stocks.

Many studies, addressing a variety of species, have provided ample evidence for the relationship between size and spawning experience of spawners and the number, quality, duration and time of offspring

Table 1. Relative fecundity (number of eggs/g) of small, medium and large: cod (50, 80, 110 cm), Icelandic herring (25, 30, 35 cm) and Irish Sea plaice (200, 600 1000 g).

Species	Spawner size			Citation
	Small	Medium	Large	
Icelandic cod (<i>Gadus morhua</i> L.)	525	628	781	Marteinsdóttir and Begg (2002)
Herring, Icelandic summer spawners (<i>Clupea harengus</i> L.)	200	480	565	Óskarsson and Taggart (2006)
Irish Sea plaice (<i>Pleuronectes platessa</i> L.)	186	264	310	Kennedy <i>et al.</i> (2007)

production (Solemdal 1997; Trippel *et al.* 1997; Trippel 1998; Kjesbu *et al.* 1996, 1998; Marteinsdóttir and Björnsson 1999; Marteinsdóttir and Begg 2002; Berkley *et al.* 2004). The impact of these relationships cannot be easily evaluated in nature (see below however) but results from laboratory and modelling studies indicate that only small changes in age or size distributions can have large effects on the immediate productivity of the stocks (Marshall *et al.* 2000; Trippel *et al.* 1997; O'Brien *et al.* 2003). Indeed just by looking at a simple measure like fecundity, it has been demonstrated that by decreasing or eliminating the number of large spawners in a population, potential egg production will be lowered by as much as 50% (Table 1). The amplitude of these changes, in terms of stock productivity, is likely to be much larger when other factors such as atresia, egg and larval size and quality, spawning time and duration are also considered.

In addition to the above studies on Icelandic and Arcto-Norwegian cod, that by O'Brien *et al.* (2003) on Georges Bank cod is one of the few empirical studies to actually provide evidence for the effects of maternal size on offspring abundance or survival in nature; it was found that egg survival was positively related to wider egg distribution and age diversity of spawners (Fig. 4).

Declines in population size due to exploitation are expected to be compensated for by increased fecundity at maturity. This has been found to be true among many Gadiformes and Pleuronectiformes including North Sea sole and plaice (Rochet *et al.* 2000 and references therein). However this does not apply to some other taxa, including Clupeiformes and Salmoniformes. Clupeiformes do not compensate for high adult mortality by increasing their current reproduction. Increased fecundity associated with decreased density does not occur in the smaller size-classes, but by steeper increases in size-dependent fecundity (Armstrong *et al.* 1989; Rochet *et al.* 2000). Similarly, in Salmonids and Osmeridae, fecundity has been found to be only weakly related to changes in density (Rochet *et al.* 2000 and references therein).

Reproductive potential can also be impaired due to disproportionate selection of the sexes and consequential changes in the operational sex ratio (Rowe and Hutchings 2003). In crab and lobster fisheries, size selective harvesting tends to remove large males in greater numbers than smaller females (see review in Fenberg and Roy 2008). In gadoids, skewed sex ratios in catches of spawning cod have been suggested to result from earlier arrival and greater activity of male compared to female spawners (Morgan and Trippel 1996). Robichaud and Rose

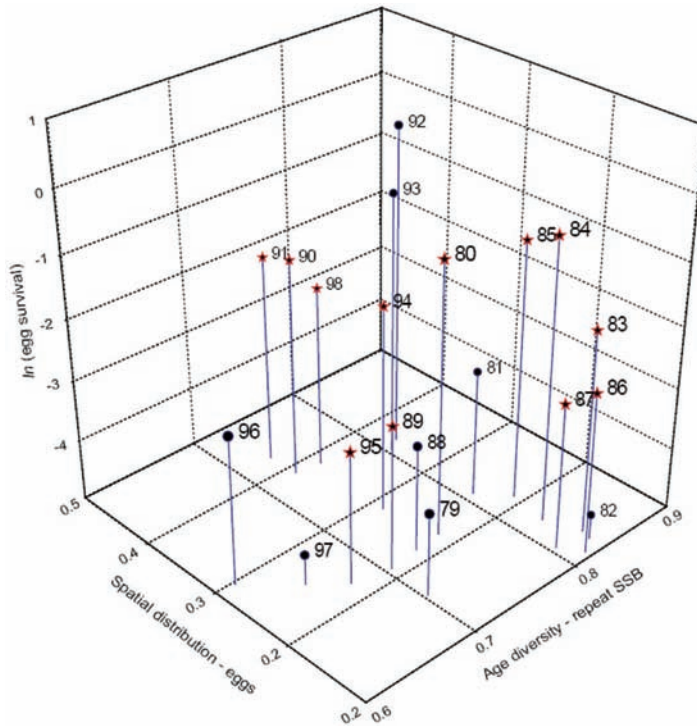


Fig. 4. Georges Bank cod: Age diversity of repeat spawner biomass, spatial distribution of eggs, egg survival rate and bottom temperature anomaly (circle = cold years, stars = warm years) during 1979–1998 (O'Brien *et al.* 2003).

(2003) lacked information on arrival of spawning cod, but showed that male spawners departed earlier than female spawners. Therefore, depending on when harvesting takes place with respect to spawning, and the magnitude of differentiation in characteristics which affect vulnerability to exploitation such as growth and behaviour (Rowe and Hutchings 2003), fishing activities targeting spawners are likely to remove the different sexes in an unequal manner.

The effect of size selective harvesting and its impact on behavioural ecology of fish species is unclear, and available information is painfully scarce. In cod, courtship rituals and vocalization are likely to be size related (Brawn 1961; Hutchings *et al.* 1999). Larger males were found to possess larger drumming bladder muscles, and the size of these

muscles was positively related to condition and fertilization potential (Rowe and Hutchings 2004). Size has also been shown to play a role in migration of spawning cod (Rose 1993) where large spawning migrations consisting of age structured aggregations were lead by large “scouts”. Similar phenomenon has also been reported for groupers for which it was observed that the young joined spawning migrations lead by more experienced adults (Birkeland and Dayton 2005).

The potential implications of harvest mortality, particularly that which is size selective, for age and size at maturation of exploited fish are both better understood and documented. According to life-history theory, increased mortality favours maturation at younger ages and smaller sizes. If in addition this mortality is size selective, those

evolutionary pressures will be stronger. Such changes in age and size at maturation have been observed in a number of commercially exploited fish stocks (see reviews by Trippel 1995; Dieckmann and Heino 2007; Jørgensen *et al.* 2007). Fisheries-induced evolution in these life-history traits has repeatedly been found to offer the most parsimonious explanation for these trends (Jørgensen *et al.* 2007). However, establishing that these changes are definitely of a genetic basis before such methods and data become available is highly problematic because of the confounding effects of phenotypic plasticity. Density-dependent changes in growth and condition as stocks are reduced in size, along with direct effects of other stochastic environmental factors such as temperature on maturation schedules, offer alternative hypotheses for these observed trends (Marshall and McAdam 2007; Wright 2007).

3.2.3. Population structure

Studies on small-scale population differentiation have suffered from the widely held perception that marine fish represent large, high gene flow populations, which are limited only by geographical boundaries of relatively large scales, i.e. continental shelf or ocean basins (De Woody and Avise 2000; Hutchings and Baum 2005). Not only have recent discoveries shown that many of our commercial fish stocks are composed of a multitude of genetically distinct populations (Hutchinson *et al.* 2001; Knutsen *et al.* 2003; Pampoulie *et al.* 2006; Ruzzante *et al.* 2006), but also that structure in terms of genetic properties linked with behavioural ecology is, in some cases, much more complicated than previously believed. For example, in Icelandic cod a number of different behavioural types have been recognized (Pálsson and Thorsteinsson 2003). Two of these, a shallow water type foraging in coastal waters and a deep water type migrating and foraging in temperature fronts, have been shown to differ in terms of gene frequencies at the

Pan I loci (Pampoulie *et al.* 2008). Regardless of whether the cod spawned at a southerly or northerly located spawning ground, those that undertook the deep water migrations were dominantly of the *Pan* I^{BB} genotype, while those that remained in shelf waters after spawning were more likely to carry the *Pan* I^{AA} genotype. Cod of the *Pan* I^{AB} genotype displayed either of the two behaviours.

One of the implications of these divergent behavioural patterns is that fishing pressure may vary between the different types. Those cod that migrate into the frontal areas undergo intense vertical migration throughout the year (Pálsson and Thorsteinsson 2003; Pampoulie *et al.* 2008) and do not appear to spend much time at the sea floor or in the vicinity of bottom trawls (Thorsteinsson unpublished data). Conversely, those cod that stay in shelf waters (shallow migrating cod) are likely to spend more time at the bottom and thus be exposed to traditional gear types. Therefore, harvesting may differentially remove the different behavioural types and thus influence the overall genetic diversity of the stock.

Selection can be linked with behaviour in various ways and is likely to be happening at a much larger scale than is presently recognized. For example, in a study on rainbow trout, genotypes with high intrinsic growth rate and bold behavioural traits were more vulnerable to simulated commercial fishing than slow-growing and shy genotypes (Biro and Post 2008). This was true even in the absence of directional size selective harvesting.

Genetic differences have also been detected over small spatial scales in herring. McPherson *et al.* (2003) detected genetic divergence between different spawning waves of herring at Devastation Shoal on the Scotian shelf ($F_{ST} = 0.005$), while at three other locations in the vicinity of Nova Scotia, no significant differences were detected among spawning waves. Similar results were

obtained for herring spawning at Rügen in the West Baltic, while at Gdansk Bay, differences between spawning waves were not significant (Jørgensen *et al.* 2005). In this respect, it should be noted that estimation of genetic divergence may result in a positive bias, especially among large gene flow species such as many marine fish. Kitada *et al.* (2007) have demonstrated a way to overcome this problem by using a Bayes procedure to estimate locus-specific pairwise F_{ST} 's.

For North Sea cod found in waters off west Scotland; despite the fact that genetic tools have not been useful in discriminating among populations, tag-recapture studies have provided evidence for resident adult populations in this region (Wright *et al.* 2006; Neat *et al.* 2006). Evidence for re-colonisation at one North Sea spawning area has also been found from a recent study of long-term genetic variation (Hutchinson *et al.* 2003). Furthermore, differences in population dynamics within spawning groups in this region, as well as limited exchange between these sites during the early life history period have been verified (Wright *et al.* 2006; Gibb *et al.* 2007). These studies illustrate that even if we are unable to discriminate among local populations using the genetic tools presently available, population structure may still exist.

Consequently, failure to account for such population richness in fisheries management may lead to the depletion of stock components and intra-stock diversity.

4. Future Goals of Fisheries Management

It is undisputable that harvesting of marine resources is selective by nature (Law 2001; Fenberg and Roy 2008). Selection occurs through unbalanced removal of fish in terms of size, age, growth, maturity, sex and behaviour. As discussed earlier, such strong, directional selection for heritable traits can induce evolutionary responses that may even

occur on decadal time scales. Theoretically, the concept of fisheries-induced evolution appears sound, and indeed the increasing number of studies reporting changes in life history traits in commercially exploited fish stocks are providing supporting evidence for the presence of contemporary evolution (Olsen *et al.* 2004; Walsh *et al.* 2006; Jørgensen *et al.* 2007; Biro and Post 2008 and references therein).

Of additional concern for management is the message provided by those studies that demonstrate potential anthropogenic selection of different behavioural types (Pampoulie *et al.* 2008; Biro and Post 2008). There is urgent need for more information on behavioural and evolutionary ecology of targeted species, and as pointed out by Myers and Worm (2003); managers may not always be aware of the true magnitude of change in the marine ecosystem as the majority of stock declines happened during the first years of exploitation, or prior to present day assessments. Furthermore, due to lack of knowledge on behavioural ecology of fish, harvesting is without doubt causing a number of changes to our stocks that present-day management and stock assessment methods are incapable of detecting.

Many authors have expressed the need for an evolutionary perspective to fisheries management (e.g. Conover 2000; Law 2000; Olsen *et al.* 2004; Williams and Shertzer 2005; Jørgensen *et al.* 2007; Kuparinen and Merilä 2007), but fewer have suggested or provided solutions for how to avoid fisheries-induced evolution. Management suggestions have included establishment of Marine Protected Areas (MPAs) (Baskett *et al.* 2005), use of minimum or maximum size restrictions (Conover and Munch 2002; Ernande *et al.* 2004), alternation of size at first capture between years (Kenchington 2001), avoidance of "derby" style harvests (Williams and Shertzer 2005) and maintenance of wide age distributions; particularly through the preservation of old and large

individuals in the stock (Martensdóttir and Thorarinnsson 1998; Birkeland and Dayton 2005; Ottersen *et al.* 2006; Law 2007; Hutchings and Fraser 2008).

The design and implementation of any management strategy that is intended to prevent the loss of phenotypic or genetic diversity requires knowledge of the life history, ecological and behavioural traits of the exploited species. Even for the most intensely studied species such as cod, such detailed information is often lacking. The recent emergence of evidence of stock complexity and behavioural vulnerability indicates that we are still far from comprehending the total variation that is built into these stocks. What is urgently needed, for any commercially exploited species, is a complete inventory of phenotypic plasticity and

as many genetically linked traits as possible. Fisheries data should be collected with high spatial resolution in order to enable identification of potential sub-division that may later be found to be important for management. Due to the potentially rapid rate of erosion of genetic diversity from these stocks, and because such changes are likely to be hard to reverse (Law 2000), this needs to be achieved at a much faster pace than has been previously realised. Until then, stocks should be managed at the smallest possible scale (spawning and/or behavioural units) in order that the loss of important, and perhaps unknown, components may be prevented; a strategy that is in compliance with the precautionary approach to fisheries management.

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Knowledge on How To Achieve Sustainable Fisheries

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I review the state of current knowledge with respect to the requirements for achieving sustainable fisheries. I consider the range of objectives for fisheries and identify conflicting objectives as a major issue in achieving sustainability. Next I review historical and current practice in allocation of fish resources and regulation of harvest and highlight existing knowledge. Evidence suggests that both restriction of access and maintenance of biological productivity are necessary conditions to achieve biological, economic and social sustainability. However, the tools appropriate to achieve these differ greatly across fisheries and societies, and for both elements of fisheries management local solutions are needed in most cases. Attempts to impose standardized solutions to either issue frequently result in ineffective solutions. Evidence also suggests that involvement of consumptive users through appropriate incentives is an essential element in achieving sustainability.

KEYWORDS sustainable fisheries; lessons learned; allocation; harvest strategies; incentives

1. Introduction

As we survey the world of fisheries management in 2008 we can find many notes of optimism and hope, and many of despair. Depending on where we look and the perspective of the viewer, we can find fisheries improving in biological abundance, economic performance and contribution to communities (Hilborn 2007b). Similarly in other

places we find fish stocks continuing to decline in abundance, economic performance is poor and declining, and communities are in severe trouble (Pauly 2007).

Even the same fishery, viewed with different disciplinary lenses, may appear to be headed in different directions. For instance, the commercial salmon fisheries of Alaska are widely regarded as examples of well managed fisheries whose biological sustainability remains unthreatened and

produces at or near the biological maximum. Yet many of these same fisheries are suffering economically and socially. Economic returns to fishermen poor, many individuals have left the fishery and communities are suffering for loss of income and tax base (Hilborn 2006).

The purpose of this paper is to explore what has been learned about how to manage fisheries well and where this evolution of fisheries management is leading. The question I ask is do we now have enough knowledge to move the world's fisheries to an era of sustainable management? I begin by first exploring many of the conflicts in the definition of "well managed." Can we even move to better fisheries management if we have conflicts in our basic objectives. Then I consider the elements of fisheries management with my emphasis being that fisheries management consists of a broad range of activities, many of which are often overlooked by outsiders. Then I discuss the evolution and lessons from fisheries management in industrial fisheries and small-scale fisheries. Finally I consider what has been learned, and the extent to which we know how to move forwards to more sustainable fisheries and communities.

2. Objectives and Defining "Well Managed"

Before we can consider moving towards a better fisheries future and having most fisheries being well managed, we must recognize the range of perspectives regarding the objectives of fisheries management and what would constitute success or being "well managed." There are a wide range of "stakeholders" who are involved in fisheries, including industrial, artisanal, subsistence and recreational fishermen, suppliers and workers in allied industries, managers, scientists, environmentalists, economists, politicians and the general public (Hilborn 2007a). Each of these groups has an interest

in particular outcomes from fisheries and the outcomes that are considered desirable by one stakeholder may be undesirable to another group.

For example, economist often cite the use of individual transferable quotas (ITQ's) and related tools (Pearse 1992; Norse *et al.* 2003) as the key to making fisheries profitable and "successful." Others consider the outcome of ITQ systems that often lead to profitability and wealth for ITQ holders as undesirable, and indeed recently the UN Commission on Human Rights has called the ITQ system in Iceland "unfair" and the Icelandic Green Party has called for abandonment of the county's ITQ system.

Other obvious conflicts in objectives occur between fishing groups who are interested in sustainable exploitation, and conservation groups interested in protecting ecosystems from disturbance. The current controversies over establishment of Marine Protected Areas closed to fishing is the most obvious sign of this conflict. Other examples occur over by-catch of non-target species, where there is no intrinsic interest for fishermen to protect species of little or no value to them, but these species are often highly valued by conservation stakeholders.

Is the purpose of a fishery to make money or to provide jobs? In many places the best way to maximize profit from a fishery appears to be to sell access rights to foreign fleets, or to arrange charter/lease agreements with foreign fleets. In these fisheries the country or quota holders achieve profitability but there is little if any employment generated for the domestic economy.

Within fishing groups there are frequent and long-standing battles over allocation—within the U.S. and in many other countries, recreational fishing groups have repeatedly attempted to have commercial fishing restricted to allow greater access to fish for their groups.

Thus there is substantial disagreement on what we want from fisheries and what

would constitute successful management. However, I do believe there are many common elements of success across most stakeholders, including primarily biological and economic sustainability.

2.1. Biological sustainability

The long-term persistence of a fishery depends upon maintaining the productivity capacity of the stocks being exploited. No stakeholder's objectives can be achieved by extirpating the resource. Almost all of the criteria that are currently applied in various forms of fisheries certification, such as the Marine Stewardship Council (Phillips *et al.* 2003), focus on the biological sustainability of the management system. Within the realm of biological sustainability, one does find conflicting objectives when we consider other elements of the ecosystem. For example, in the New Zealand hoki fishery, which is largely mid-water and bottom trawl, there is an incidental catch of fur-seals. To the fishermen the by-catch of seals is unintentional, but of no real consequence, they come up dead and the deck and are thrown overboard. To conservationists this by-catch is a concern, and have repeatedly argued that further efforts should be taken to restrict the by-catch of seals. Several groups objected to the certification of the NZ Hoki fishery by the MSC with one of the issues raised being the by-catch of fur-seals.

Another area of conflict is around the level of ecosystem modification considered appropriate. Fishing often transforms ecosystems. At the very least fishing reduces the abundance of the target species, in the extreme fishing can totally transform the nature of the ecosystem. For example, the overfishing of groundfish in a number of ecosystems has led to major increases in abundance and catch of several invertebrate species such as shrimp, crabs and lobster (Worm and Myers 2003). These ecosystems can be thought of as being transformed from a groundfish dominated, to invertebrate

dominated. The economic value of the invertebrates is often higher than the groundfish they replaced. While it is not clear that these transformed ecosystems are sustainable in the long term, they may be, and if they are then there is an obvious conflict between the economic value of these fisheries and the fishermen that depend on them, and the traditional objectives of maintaining ecosystem structure and function.

2.2. Economically viability

The economic viability of fishing fleets and communities would be accepted as a desired outcome by almost all stakeholders. Most environmental NGOs say that they support fishing communities. Thus we could evaluate any fishery where the fishing fleets are bankrupt and communities in distress as not successful or well managed. Again however, as soon as we look deeper there are considerable conflicts between stakeholders. Community-oriented groups tend to see any form of large-scale industrial fishing and large firms as an anathema, and their vision of economic viability is small-scale community-based fisheries. Some countries such as New Zealand have made clear decisions to favor economic profitability, and if that profitability is found by a few large firms harvesting the majority of the catch, that is considered consistent with national fisheries objectives.

There is similar conflict on the role of subsidies to individuals and communities. In some places such as eastern Canada, subsidies in the form of unemployment insurance are a major element in the fisheries policy to maintain communities, yet in many other countries (Australia, New Zealand) such approaches would be considered inconsistent with good economic management of fisheries.

3. Elements of Management

In most popular and scientific discussions fisheries management has become almost

synonymous with the regulation of harvest and maintenance of stock levels. When one considers whether fisheries management has succeeded or failed, the focus is almost invariably on whether catches have been too high and how and what are the trends in the abundance of the stock.

However, fisheries management is a much more diverse activity, and the other elements of fisheries management are often neglected, or at least undervalued. However, if we wish to consider the success of fisheries management, and indeed the trends in fisheries management in different parts of the world, we must look to a broader range of fisheries management activities. If we look to what fisheries management agencies actually do, the activities they perform, I can identify six types of activity; (1) access to fishing, (2) regulation of harvest, (3) data collection and research, (4) enforcement and compliance, (5) artificial propagation, and (6) habitat protection. Due to limitation of space I am only going to consider the first two elements of fisheries management.

Access to fishing and gear—who fishes with what and where: Fisheries management agencies, by design or default, determine who gets to fish, what gears they can use, and when and where they can fish. We generally call this process allocation, and distinguish between allocating the catch to alternative users, and the regulation of catch levels.

Regulation of catch: Agencies frequently attempt to regulate the levels of catch by a variety of means usually either direct regulation of the total allowable catch (TAC), or restricting the amount of fishing effort, the efficiency of the gear, or the time and space opportunities for fishing.

4. Historical Evolution of Management Practice

In this section I wish to review trends in fisheries management practice comparing indus-

trial fisheries with small-scale fisheries. The emphasis will be on what has been learned.

4.1. Industrial fisheries

This group of fisheries constitutes the high-volume fisheries of the world and includes fisheries in western countries, international waters and some Asian and African countries. The common characteristic of these fisheries is that they involve many vessels, catch large volumes of fish, and are of enough economic value that management agencies can often devote considerable resources to their management. The industrial fisheries of the North Atlantic formed the basis for the modern “theory of fishing” as codified in the classic Beverton and Holt book from the 1950’s (Beverton and Holt 1957) and described in Smith (1994). A very large portion of the world’s fish catch comes from these fisheries, and most discussion of “state of the worlds fisheries” (Hilborn *et al.* 2003) would focus on these fisheries. However, we recognize that most of the people in the world who earn their livings fishing are in Asia and Africa and most of them depend on the small-scale fisheries described in the next section.

4.1.1. Access to fishing

Almost all industrial fisheries began as open access, where anyone with the ability to purchase a vessel could participate. Within the last 50 years a wide range of mechanisms for providing access have been tried and much has been learned. Open access fisheries have almost uniformly resulted in development of excess fishing capacity to the point where profitability of fishing becomes close to zero. This problem is especially acute when there are cycles in price and abundance. During the periods of high profitability, more participants enter the fishery, and when there is a downturn in abundance, or price, or an increase in costs, most of the vessels operate at a loss.

A common step beyond open access is limited entry, where the number of licenses or vessels is fixed, but the ability of the license-holders to increase their catching power is usually not. In such systems the result is commonly the same as with open access, a highly overcapitalized fishery with poor economic performance. The exception is in fisheries where there are ways to effectively constrain the ability of increased investment to increase the catching power of vessels. In these cases, if the fleet size is capped before it is too large, the tendency of capital to increase catching power and ultimately reduce profits may be controlled and fleets may be profitable for considerable periods of time.

A rarely used alternative to granting of permanent or long-term fishing rights is the sale of short-term fishing rights, either to domestic fishermen or to foreign fleets. Such systems are rare in industrial fisheries, and are generally unpopular with domestic fishermen who almost uniformly argue for fishing rights to be given to them at little if any cost. However, where implemented, such mechanisms have proven to provide considerable revenue to states or management agencies and have avoided the problem of development of excess fishing capacity.

A method that is growing in use is the granting of catch shares to individuals or vessels in systems commonly called Individual Transferable Quotas (ITQ) or Individual Vessel Quotas (IVQ). New Zealand and Iceland have adopted these systems for most of their fisheries, and within the U.S., Canada, Australia, South Africa and Namibia they are now in common use. ITQ systems have long been advocated by economists as a way to prevent, or to reduce overcapitalization. ITQs do this by eliminating any incentives for increased catching power: if a vessel has a fixed share of the catch it cannot increase its catch share by technological means. In ITQ systems the incentives are to reduce the cost of fishing and to increase the

quality and price of the product, leading to increased profitability. Another common result of ITQ systems is a reduction in total fleet size, effectively concentrating the harvest to a smaller number of vessels. Such concentration is frequently decried by sociologists and anthropologists because of the loss of jobs and concentration of catch, while economists generally view this outcome as a desirable aspect of economic efficiency. Others object to the fact that ITQ systems often generate considerable wealth in the quota holders, and since the common practice has been to allocate ITQ to vessels largely based on their catch histories, many decry this practice as socially inappropriate.

Another approach is sector allocation, in which a fixed share of the catch is assigned not to individual vessels but to groups of vessels. Perhaps the most common application of sector allocation is in international fishing agreements where nations are granted a certain share of the total allowable catch. Alternatively, in some cases specific fishing fleets in national fisheries are allocated a fixed share. An example of this is the factory trawler fleet for pollock in the Bering Sea (Mansfield 2004). This fleet has a fixed share, and the companies who own the vessels have worked out an agreement on how much of the allowable catch each vessel is allowed to catch, effectively making the system like an ITQ system. The consequences of sector allocation differ markedly. When the size of the sector is small and they have been able to cooperate in how the catch is internally allocated, sector allocation has proven to promote considerable economic efficiency. When the sectors who receive allocation are large, or not well organized, there often remains a competitive race within the sector members and the outcome is not dissimilar to open access.

Experience with industrial fisheries has taught us that the economic performance of the fishing fleet is largely determined by the method used to allocate access to the fishery.

Open access and limited entry have generally resulted in poor economic performance, while ITQ/IVQ systems, and state sale of short-term fishing rights generally result in much more profitable fisheries. Sector allocation has a very mixed track record, but when the groups are small and working together, sector allocation can achieve economic profitability.

The biological sustainability of the resource is much less directly influenced by the allocation method. Numerous fisheries have achieved biological sustainability while remaining in open access or limited entry. The biological success depends much more on the management agencies' choice of harvest levels and their ability to enforce these limits.

Discussion of access in industrial fisheries does always lead to a direct confrontation of the tradeoff between economic profitability, and employment and equity. If you want to make fisheries profitable you always want to use fewer boats and people than would naturally happen in open access.

4.1.2. Regulation of catch

The level of knowledge regarding harvest levels in industrial fisheries has evolved over the last 100 years, beginning with initial debates about whether overharvesting of such fisheries was even possible. Thomas Huxley's viewed these fisheries as one of unending surplus. "... that the cod fishery, the herring fishery, the pilchard fishery, the mackerel fishery, and probably all the great sea-fisheries, are inexhaustible; that is to say that nothing we do seriously affects the number of fish. And any attempt to regulate these fisheries seems consequently... to be useless" (Huxley 1884).

Since Huxley's time much has been learned and it is now widely accepted that there are limits to the potential harvest of all fisheries in the ocean, and that catch levels in most fisheries need to be regulated. There has now evolved in most managed industrial

fisheries a process of management that often occurs on an annual basis. The elements of this cycle are (1) data collection and monitoring of the fishery and the stock, (2) assessment of stock status from the data available usually using population dynamics models, (3) determination of catch limits or other regulations for the next year or period of the management cycle, an (4) implementation of the regulations and pursuit of the fishery (Hilborn 2003). Almost all international RFMOs, and governments in Europe, North America, Namibia, South Africa, Peru, Chile, Argentina, Australia and New Zealand, all have processes with these elements for most of their most significant industrial fisheries.

The first two elements of the cycle are highly fishery specific and I won't go into any details. However, in determining the catch limits there are some near-universal themes. In the second phase of the cycle the data are used to estimate the stock size and productivity, from that assessment the catch limits are set usually based on "reference points." One kind of reference points are "targets" which may be as simple as a target exploitation rate (Hilborn 2002; Koeller 2003). From the assessment, one obtains an estimate of the total population size, that is multiplied times the target exploitation rate to obtain the annual allowable catch. In other fisheries, the targets are stock abundance levels, and if the stock is below the target abundance, catches are reduced, if abundance is above the target it might be increased.

A second type of reference point is "limits." Limit reference points are levels beyond which management does not wish to operate. For instance a common limit reference point is a minimum stock size, and if this limit is exceeded, then more drastic management action is taken, either the fishery is closed, or a new set of harvest rules are considered to assure stock rebuilding. There are also exploitation rate limit reference points, exploitation rates which the agency does not want to exceed.

Figure 1 shows a typical harvest rule adopted by the Pacific Fisheries Management Council known as the 40:10 rule. The target population size is 40% of a theoretical “unfished population size” and the limit population size, below which any directed fishing is stopped is 10% of the unfished population size.

A refinement on the cycle of management described above are more formal “management procedures” in which not only is there a fixed harvest rule, but the assessment process is also completely specified. In most management agencies the assessment procedure evolves from year to year as new methods or assumptions are tried, new data become available or staff turns over or staff changes. This can lead to dramatic “revisions” in stock assessments, where estimated abundance changes as much as 2–3 fold from year to year (Parma 2002). In management procedures, the assumptions are fixed for significant periods of time such as 5 years. A major advantage of management procedures is they allow computerized simulation testing of the entire procedure to determine the consequences of using that procedure across a range of assumptions about the true stock biology. Much more extensive discussion of the experience with management procedures is found in a paper in this book by Butterworth (2008).

There are significant exceptions to the annual cycle of management described above. This process applies primarily to industrial fisheries where an annual allowable harvest is specified. In some fisheries, such as the Maine lobster fishery (Acheson and Gardner 2004), there are no catch limits, but limits on the minimum size, season length, type and amount of fishing gear. These regulatory rules have proved sustainable over many decades and indeed in the Maine fishery yields have risen dramatically in the last 20 years. Systems regulated by effort rather than catch are theoretically more robust to uncertainty in stock size, although they are

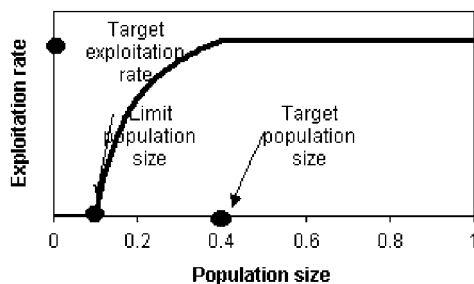


Fig. 1. The 40:10 harvest rule of the Pacific Fisheries Management Council.

vulnerable to increasing technological efficiency leading to growing exploitation rates.

The primary lessons that have been learned in harvest levels are first that overexploitation is possible for almost all stocks and we now have a considerable body of knowledge on what levels of fishing mortality and stock size lead to loss of potential yield (Myers *et al.* 1994). Secondly, when managing by annual catch limits, a feedback management system is required and updates in the allowable catch need to be made in a timely fashion consistent with the biology of the species. I believe there is a broad consensus that application of harvest control rules without implementation in a management procedure leads to highly uncertain outcomes, and harvest control rules should be evaluated in the context of a management procedure.

An outstanding issue is what quantity to have on the X-axis of the harvest control rules. The U.S. has been at the forefront of the kind of harvest control rules as described, and commonly the X-axis is the stock size relative to the estimated unfished level. This often leads to great complications as the unfished level is often difficult to estimate, and as the importance of systematic environmental changes to stock productivity is more broadly accepted, we recognize that the unfished level in one production regime is different from the unfished level in another

production regime. There is considerable active research on how to find robust strategies across production regimes.

A further area of considerable discussion is the appropriate spatial scale of management. The traditional assumption has been the regulation of catch on the basis of the "unit stock" (Hilborn and Walters 1992), a theoretical self-contained population. As we have learned more about the biology of fish stocks we discover more and more structure within stocks that leads to the potential to try to manage dozens, or even hundreds of stock units separately. Management agencies rarely would have the resources or ability to control fishing on such small spatial scales. The best approach with such spatially structured stocks is one of considerable debate, but one promising solution is to think of such fisheries not as large-scale industrial fisheries, but rather as many small-scale fisheries, and move to management methods appropriate for such stocks as discussed in the next major section.

Most of the basic theory of industrial fisheries management has been derived from management of single stocks. Many of the industrial fisheries of the world harvest a mix of stocks, and even many of the large single species fisheries, catch significantly amounts of other species. There remains considerable discussion and debate about how best to manage the non-target species in such fisheries.

4.2. Small-scale and community-based management

At the other end of technology, spatial scale and volume are the many small-scale fisheries of the world. These fisheries employ the majority of the people in the world engaged in fishing, and are the backbone of fishing communities throughout the world, often including industrialized countries. These fisheries have seen an evolution of fisheries management systems very different from the industrial fisheries of the world, and many

argue that many of the lessons learned in these fisheries should be applied much more broadly. My personal experience is primarily in industrial fisheries, and I will draw on lessons learned in small-scale fisheries primarily from the Chilean artisanal fisheries (González 1996; Castilla and Fernández 1998; Castilla *et al.* 1998), South Pacific Island nations (Johannes 1978; Johannes 1981; Johannes 2002), and Japanese coastal fisheries (Akimichi 1984; Akimichi and Ruddle 1984; Ruddle 1989, 1994, 1998a, b, c).

4.2.1. Access to fishing

The most important lesson from the small-scale fisheries of the world is that limiting access, usually to local participants, is the key to biological, economic and social sustainability. It is now almost 30 years since Johannes wrote "The Words of the Lagoon" and the lessons he described ring more truly every day.

The most important form of marine conservation used in Palau, and in many other Pacific islands, was reef and lagoon tenure. The method is so simple that its virtues went almost unnoticed by Westerners. Yet it is probably the most valuable fisheries management measure ever devised. Quite simply, the right to fish in an area is controlled and no outsiders are allowed to fish without permission.

(Johannes 1981)

Where such tenure of marine fishing grounds exists it is in the best interest of those who control it not to overfish . . . Self-interest thus dictates conservation. In contrast, where such resources are public property, as is the general case in Western countries, it is in the best interest of the fisherman to catch all he can. Because he cannot control the fishery, the fish he refrains from catching will most likely be caught by someone else. (Johannes 1981)

In his last paper on fisheries management practices in the Pacific (Johannes 2002) Johannes describes how many of the traditional

village-based practices that had been abolished under Colonial governments were re-emerging and leading to the re-establishment of village-based tenure and rebuilding of local fish stocks that had been depleted when access was not regulated.

In the Japanese coastal fishery, the access is limited to members in the local cooperatives. Similarly in the Chilean artisanal fisheries, control of the near-shore resources has been granted to local community cooperatives and they can exclude any others from fishing in their areas. Local tenure is not a sufficient condition to ensure sustainability, local people can for various reasons over-exploit or in other ways not effectively use their resources. However, it does appear that for small-scale resources' local control, at least to the extent of being able to exclude others, is a necessary condition for biological, economic or social sustainability.

In Chile, prior to the advent of the local control system, the major fishery for "loco" a valuable snail, was regulated with the traditional methods of industrial fisheries, size limits, season limits and allowable catches. This system totally failed, primarily due to the inability to enforce any of the regulations, and the fishery was legally closed in an attempt to rebuild the resource. Again enforcement of this ban was ineffective, and the system of local control was established which has, in general, been very effective and providing for much better biological and economic performance.

Within small communities across the three areas discussed above, and around the world, there are a multitude of methods used to allocate fish within communities, and I know of no summaries of the relative success and failure of these methods.

4.3. Other elements of management

It is impossible to summarize or describe the other elements of management across the range of small-scale fisheries around the world. Perhaps the most important lesson is

that there is no single solution, and what is appropriate in one community for a specific species may be totally different for another species or community. A characteristic of small-scale fisheries around the world is the reliance on local communities for the actions of management such as setting harvest levels, data collection, and enforcement. The top-down approach developed and applied in industrial fisheries simply cannot work at small spatial scales and these systems are by their nature dependent on either local management or co-management where the state organizations simply have a small role assuring that some guidelines are met by the local communities.

Similarly, few of the models of assessment and regulation from industrial fisheries apply to small-scale fisheries. Complex computer models have little role and can be replaced by simple rules of thumb. In small-scale fisheries there is considerable potential for good catch monitoring and fishery independent surveys of abundance to provide the information for harvest rules, but these rules would need to be data based rather than model based.

5. Discussion

I believe the most important lesson learned in fisheries management is that any attempts to achieve biological, economic and social sustainability require limiting access to the resource. This is as true for the large industrial fisheries of Europe as it is for the small-scale fisheries of Oceania. This is true regardless of the objectives of the stakeholder. The appropriate mechanism for limiting access will differ enormously across societies and objectives. A society may choose to have a few individuals take most of the catch of a species, or spread the catch broadly over many users. If economic efficiency is the social goal, then the answer will almost always be for a small number of individuals specializing in their gear and talents for that

species. The limitation on access can take the form of legal mandates, or social conventions.

What remains uncertain is how best to achieve limiting access in a wide range of societies given the constraints of their infrastructure and governance. Restricting access always involves winners and losers, and it is often difficult to find a mechanism within a society that can achieve a socially acceptable result. We know we need to restrict access, what we have not yet worked out is how to achieve this in different societies. There is no single solution, and even in the same country we will find a method working well in some fisheries and failing in others. A second lesson is that every fishery needs to be understood as unique and access solutions crafted for the special circumstances of that fishery. We now know of a large "toolkit" of methods for limiting access, and what is needed is a broader systematic understanding of how these work in local circumstances.

An obvious reason for the need for local adaptation of the tools of restricting access is the difference in objectives. There can be no global solutions because there are no global objectives. This is the reason I devoted significant space to objectives at the beginning of this paper. Recognizing the diversity in objectives we must then seek a diversity in solutions.

The second most important lesson is the need to maintain the biological productivity of resources. The use of target and limit reference points as essential elements of a harvest strategy has emerged from the North Atlantic fisheries and is rapidly being adopted in a range of countries, often as a legal requirement. However, these fisheries are in many ways a special case, being some of the most over-exploited and most intensively researched fisheries in the world. This means that there is usually a good idea of what the former biomass was—because the exploitation rates were so high the caught all the fish. There is also both in Europe and Atlantic

North America a long history of research surveys. These two conditions are unusual in the world scene, where research surveys are much less common, and exploitation rates may be lower or catches undocumented so we don't know historical biomass. The result is that I doubt that the "reference points linked to harvest strategies" approach is broadly applicable to most of the world's fisheries. This is especially true when the reference points are defined in terms of an unfished stock size, and it is assumed we know stock size and exploitation rate well enough to base our rules on these quantities.

However, there are many fisheries where this approach is being applied, at least in theory, and one must ask why many of these fisheries remain overexploited. The answer is simply that the consumptive stakeholders have failed to accept the process and resist it through their political power, and through non-compliance. In Europe and New England it has been common for the political process to set the catch limits higher than management strategies would suggest, and in Europe at least, overcatch of quota is common.

Achieving maintenance of the productive capacity of the resource appears to require enough cooperation with consumptive users that they accept and cooperate with the management process. This implies a level of local adjustment of the management process to the circumstances of the local fishery. The measures used to track abundance need to be consistent with the knowledge of the local fishery and accepted by them.

While maintaining biological productivity is essential for sustainability, there is a wide range of ways that productivity can be maintained. Should the ecosystem be managed to maintain a large groundfish population, or should we allow continued heavy fishing on groundfish in order to maintain larger invertebrate populations? Should marine mammals be exploited to reduce their predation on valuable fish? Should large

areas of habitat be devoted to various forms of marine aquaculture?

In summary, we have accumulated a great deal of knowledge around the world about how to achieve successful fisheries management. The lessons I see are that access needs to be restricted and biological productivity of the stocks maintained. The best way to do either of these tasks depends greatly upon the local circumstances, particularly the specific objectives of the society associated with the fishery. We have a broad range of tools for achieving limited access and biological productivity and we these tools need to be crafted for local solutions.

In all aspects of fisheries management, including the areas I have not had space to

consider, data collection and research, enforcement and compliance, artificial propagation and habitat protection, we see cooperation with local stakeholders as almost universally essential to success. I and others have written extensively about the role of incentives in leading to successful fisheries (Hilborn *et al.* 2005; Grafton *et al.* 2006) so I will not elaborate on this further here. However, if you look around the world for well managed fisheries that are biologically, economically and socially successful you will almost always find these three elements, restricted access, maintenance of biological productivity, and cooperation of stakeholders.

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The Role of Docosaehaenoic and Arachidonic Acids as Determinants of Evolution and Hominid Brain Development

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Lipids played a major, as yet unrecognised, role as determinants in evolution. Life originated 3 billion years ago during which time there was ample opportunity for DNA modification. Yet there was little change in the life forms for the first 2.5 billion years. It was not until about 600 million years ago when the oxygen tension rose to a point where air breathing life forms became thermodynamically possible, that a major change is seen in the fossil record. The sudden appearance of the 32 phyla in the Cambrian fossil record which flowed from this environmental change is referred to as the “Cambrian Explosion”. It was also associated with the appearance of intracellular detail and cell differentiation. That detail was provided by cell membranes in which the lipids were structural essentials. Thus not just oxygen but also the lipids were drivers in the Cambrian explosion. Docosaehaenoic acid (DHA) provided the basic membrane backbone of the new photoreceptors that converted photons into electricity laying the foundation for the evolution of the nervous system and the brain. Although there are two closely related fatty acids with only one double bond different DHA was not replaced despite some 600 million years of genomic change. Whilst the marine food chain is rich in long chain omega 3 fatty acids, the land food web is dominated by omega 6 fatty acids. With the brain utilising omega 6 and 3 fatty acids in a ratio of between 1 to 1 and 2 to 1 the injection of the omega 6 through the

appearance of omega 6 rich protected seeds in the Cretaceous Period, would have played a critical role in the advance of brain evolution. This symbiosis between land and marine food chains, most likely created the condition that finally led to the cerebral expansion in human evolution. Lipids are still modifying the present evolutionary phase of our species with their contribution to a changing panorama of non communicable disease. The contemporary lipid malnutrition is most likely contributing to the rise in brain disorders which in the European Union has overtaken the cost of all other burdens if ill health at €386 billion for the 25 member states at 2004 price.

KEYWORDS evolution; genomics; lipids; docosahexaenoic; arachidonic; omega 6; omega 3; brain; vascular development; cerebral expansion; fish; sea food; oceans

1. Introduction: The challenge of the rise in brain disorders

Brain disorders now account for the highest cost in the burden of ill health in Europe (Andlin-Sobocki *et al.* 2005). It follows the rise in death from cardio-vascular disease as predicted by Crawford and Crawford (1972). The cause is most likely nutritional with a similar background in the change in dietary fats which adversely impacted on cardio and vascular health that would logically lead to disorders of brain development and function. The reason for linking heart disease and brain disorders is that during early development, the brain relies heavily on an efficient placental vascular and the fetal cardio-vascular system. The fetal brain uses 70% of the energy transferred to the fetus from the placenta. The placenta itself is a rapidly growing vascular system which needs to be in place ahead of the fetal brain growth thrust of the last trimester. This paper raises several questions about the role of DHA in the brain, its extreme conservation in signalling systems with its possible relevance to human evolution. Importantly it raises a question on how to meet the challenge of human mental health in face of the problems facing aquatic food resources.

2. Docosahexaenoic Acid

Docosahexaenoic acid (all-*cis*-docosa-4,7,10,13,16,19-hexaenoic acid—C22:6 ω 3, DHA) is a major, essential fatty acid constituent of the brain (Crawford and Sinclair 1972). DHA or its precursors have

to be provided in the diet, hence the balance between the ω 6 and 3 fatty acids is important. There is a paucity of DHA in the land food chain which also contains competing fats. The brain first evolved using the marine food web some 500–600 million years ago and the richest source of DHA is the marine food chain. The movement in the 20th and 21st centuries away from historical use of sea foods and fish with an emphasis on land based food supply, is a likely cause in the rise in brain disorders now apparent (Hibbeln 1998). A better understanding of DHA and its function could help to motivate the required policy changes needed to meet this challenge.

Neural cells have a particularly high membrane content of DHA. In different mammalian species the profile with arachidonic acid and DHA does not vary: it is brain size that varies (Crawford *et al.* 1976, 1993) suggesting a high degree of evolutionary conservation of the neural lipid profile (Fig. 1). DHA is rapidly and selectively incorporated in neural membranes and is concentrated at synaptic signalling sites (Suzuki *et al.* 1997). It is the most unsaturated of cell membrane fatty acids (Jump 2002). DHA is synthesised from α -linolenic acid. However, the process is rate limited (Sprecher 1993; Sprecher *et al.* 1999) and moreover α -linolenic acid is oxidised at a rapid rate (Leyton *et al.* 1967).

In 1972 Crawford and Sinclair first published evidence that DHA itself, was an independent determinant of brain growth and evolution¹ (Broadhurst *et al.* 2002). Deficiency studies in rodents (Sinclair and

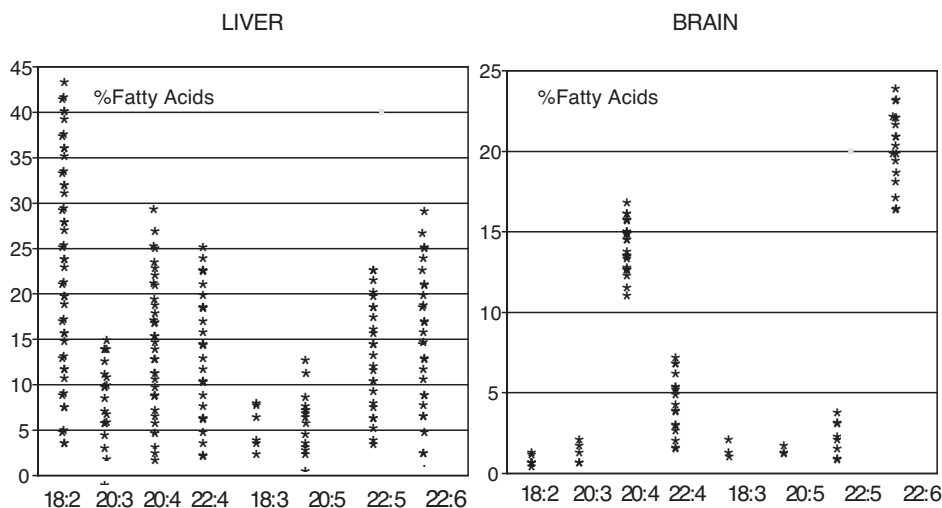


Fig. 1. 42 Species: inner membrane lipid varies widely in the liver but not the brain (Data from Crawford *et al.* 1976 and subsequent papers).

Crawford 1972; Benolken *et al.* 1973; Galli and Socini 1983; Weisinger *et al.* 1999; Catalan *et al.* 2002), chickens (Budowski *et al.* 1987), primates (Fiennes *et al.* 1973; Neuringer *et al.* 1986) and visual and cognitive trials in human infants (Carlson and Werkman 1996; Martinez and Vazquez 1998; Birch *et al.* 2000) have indicated that DHA is essential to brain development and function. Moreover, collaboration with the Hebrew University of Jerusalem (HUJ) we described competition existing between $\omega 6/\omega 3$ fatty acids and showed that their balance is critical for brain development and structural integrity (Budowski and Crawford 1985).

3. DHA Function—a question of liquidity?

Whilst the significance of DHA to brain function, is now recognised its mechanism of action is unknown. We have speculated that its unique, six methylene interrupted *cis*-double bond sequence may be responsible for its mechanism of action and conservation in neural tissues (Bloom *et al.* 1999).

The conventional view is that DHA provides for the high degree of liquidity needed by the brain. However, the notion that it is “needed” is teleological. In 1999 Bloom *et al.* discarded the idea of liquidity as an ex-

planation for its striking conservation in neural systems, on the grounds that the difference in liquidity between the $\omega 3$ -docosapentaenoic acid (all-*cis*-docosa-7,10,13,16,19-pentaenoic acid C22:5 $\omega 3$, $\omega 3$ DPA) and DHA was marginal yet the $\omega 3$ DPA being more readily synthesized, less difficult to obtain from the food chain and less vulnerable to oxidative damage, does not seem to have replaced DHA in the visual and neural systems in the teleosts, elasmobranchs, cephalopods, fish, amphibia, reptiles, birds or mammals. The $\omega 6$ DPA, which also differs from DHA again by the absence of one double bond (between carbons 19–20) does not replace DHA except under extreme, artificial deficiency conditions in the laboratory and then the replacement is only partial and function is depressed. Nature’s preference for DHA in the brain is strikingly demonstrated in large, vegetarian land mammals, in which DPA is the dominant $\omega 3$ metabolite found in non neural tissues and thus abundantly available (Crawford *et al.* 1969). Yet neural membranes even in these mammals still conserved the DHA-rich composition. During the evolution of the land mammals,

¹ See *Science* 2002; **296** (April 12): 233, 340.

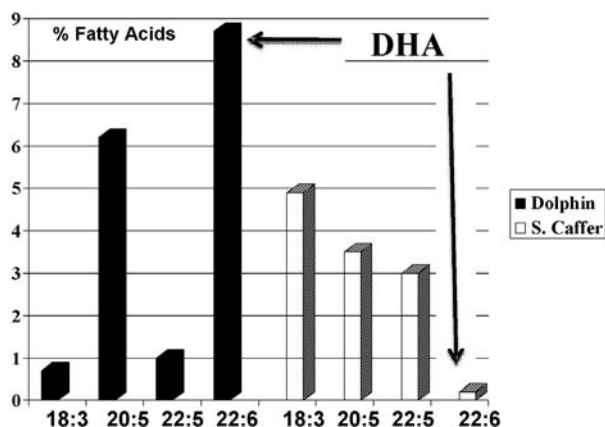


Fig. 2. Liver ethanolamine phosphoglyceride from *Syncerus caffer* (Cape buffalo) and *Tursiops truncatus* (dolphin).

this retention of composition in land mammals was associated with economy in brain size with a logarithmic reduction in relative brain size as they evolved larger bodies (Crawford *et al.* 1993).

4. Evolution of *Homo sapiens*

Certain mammals left the land to radiate into the marine habitat starting about 50 million years ago. With unlimited access to the DHA food web, the marine mammals retained a far better brain body weight harmony than is seen on the large land mammals. The dolphin for example has 1.8 kg brain which compares to little more than 350 g in a zebra which has a similar body weight and is also a non-ruminant (Fig. 2). A coastal ecological niche would have provided the rich source of DHA, iodine and other trace elements essential to the brain and in poor supply on land. Such a niche would have offered the evolutionary advantage compared to that of the land food chain and so avoid the loss of relative brain capacity on land.

The presence of DHA's full complement of six double bonds is for some reason an important priority in neural membranes and from the evolutionary record would seem to have been conserved in this capacity for 600 million years. The striking conservation of DHA in signalling systems implies that

biology is highly sensitive to the slight difference of the one double bond between DHA and DPA molecules. The reality is that all land mammals lost brain size as they evolved into larger body sizes demonstrating that different principles are involved in body as opposed to brain growth. In Fig. 3 we plot the approximate arithmetic decline in some land mammals.

Because of this discrepancy between body size and brain size some have used logarithmic plots to obtain straight lines to explore the relationship. This strategy of course means that one of the parameters is varying logarithmically to the other. In this case brain size diminishes logarithmically with body size. Even so *H. sapiens* and the marine mammals do not fall on the straight line. Of the large mammals, the Dolphin with about 1% of its body size, comes the closest to *H. sapiens*. At just under 2%,² *H. sapiens* has a brain body weight ratio which would be totally exceptional if considered as a land based mammal. Interestingly, *H. sapiens* has a smaller brain to body weight ratio than the squirrel. Indeed all the very small mammals have brain bodyweight ratios similar to or greater than *H. sapiens*.

The conclusion is that evolution on land resulted in diminishing relative brain size, a feature readily explained by the lack of DHA

² 70 kg is considered a standard for men and at 1.3 kg brain the ratio is 1.86%, at 1.4 kg it is 2% (Blinkov 1968).

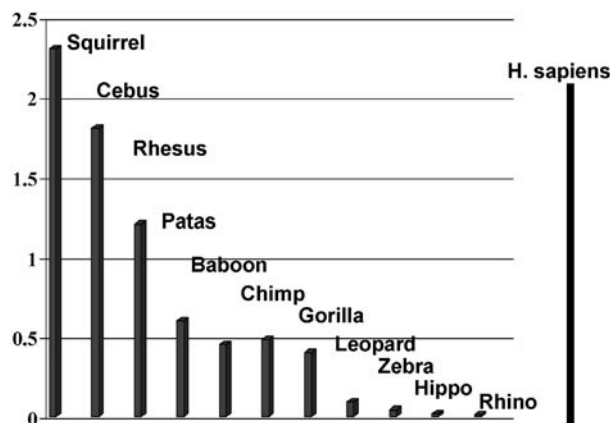


Fig. 3. Approximate brain weight as a proportion of body weight declines as body weight increases.

in the land food web together with the rate limited synthesis being outstripped by the velocity of protein accretion and body growth. Note in Fig. 2 the buffalo liver lipid is quite rich in α -linolenic acid, EPA and even the ω 3 DPA but despite this wealth of precursor, fails synthesise significant DHA. The contrast with the Dolphin lipids in this respect is striking.

However, both arachidonic and DHA are needed for the growth and development of the brain and its function (Crawford and Sinclair 1972). The difficulty the Dolphin and other marine mammals have is in obtaining arachidonic acid from the marine food chain to serve the brain. Hence arachidonic acid supply would be a constraint on brain evolution in the marine habitat although assumedly still required for mammalian reproduction (Williams and Crawford 1987). A littoral ecosystem would have provided and evolving primate with access to both arachidonic acid and DHA and hence would have had the best of both worlds.

This evidence puts the evolution of *H. sapiens* firmly at the marine and lacustrine coastlines with access to preformed DHA from the aquatic resources. Indeed, the concept that *Homo sapiens* actually went through an aqueous phase was put forward by Sir Alistair Hardy (1960) and followed up in several books written by Elaine Morgan (1995, 1997). For example, the loss of DHA

in the land based food web would lead to decline in relative brain capacity which in fact has happened. Conversely, the marine food web would be more likely to support brain capacity than that of the land which is also in evidence from the high relative brain capacity of the marine mammals versus the corresponding size in land mammals (Williams and Crawford 1987; Crawford *et al.* 1999; Broadhurst *et al.* 2002).

The evolution of humans at a coastal rather than a land based hunting system is now well explained by the evidence on omega 3 fatty acids and in particular on DHA in neural gene expression (Barcelo Coblijn *et al.* 2003a, b; Puskas *et al.* 2004; Kitajka *et al.* 2002, 2004). DHA is the dominant omega 3 fatty acid in the brain (Crawford *et al.* 1976). It has a far superior biological activity for brain growth, compared to its synthesis from plant fatty acids, even in rodents (Sinclair 1975). This evidence provides a simple mechanism and explanation as to why DHA and a marine food web would have supported brain evolution in an upward direction rather than downward.

Chris Stringer (2000) has suggested that *H. sapiens* populated the planet by migrating "out of Africa" around the coastlines. A coastal route would have certainly meant use of the marine food chain. There is fossil evidence of incontrovertible use of the marine food web dated to a time close to the biological

emergence of modern humans (Broadhurst *et al.* 2002; Marean *et al.* 2007). There is also contemporary evidence of fishing people in the Rift Valley of Africa with healthier cardio-vascular profiles than their inland cousins (Pauleto *et al.* 1996; Crawford *et al.* 1999), and contemporary evidence of the Moken and other sea dwellers living around the coast of Asia with a healthy, life style, possibly as a remnant of this migration (Gislén *et al.* 2003).

5. DHA in Neural Signalling Systems

Nuclear magnetic resonance (NMR) and fluorescence studies have attempted to differentiate the membrane properties conferred by PUFAs. Some of the constraints of such approaches have been discussed previously by Bloom *et al.* (1999). NMR investigations of the effects of polyunsaturation on lipid acyl chain orientational order, revealed significant changes as the number of double bonds increased from one to three (Holte *et al.* 1995; Soubias *et al.* 2006). Ehringer *et al.* (1990) directly compared the effects of 18:3 and 22:6 on membrane physical properties, and observed considerably higher permeability and perhaps vesicle fusability in the samples containing DHA. But again the differences are not of the order one would expect to be responsible for DHA to be chosen over 600 million years. A powerful reason is needed to explain why DHA was chosen and not its immediate precursor with only one double bond less.

Klaus Gawrisch and his colleagues have so far made the best attempt using solid-state NMR measurements and molecular simulations they portray an image of DHA (22:6 ω 3) as a highly flexible molecule with rapid transitions between large numbers of conformers on the time scale from picoseconds to hundreds of nanoseconds. The low barriers to torsional rotation about C–C bonds that link the *cis*-locked double bonds with the methylene carbons between them are responsible for this unusual flexibility. Both the amplitude and frequency of motion increase toward the terminal methyl group of DHA (Gawrisch *et al.* 2003; Mihailescu and Gawrisch 2006).

5.1. A special case for DHA as a receptor domain as targets for psychotropic drugs

Solid-state magic angle spinning (MAS) ^{13}C -NMR produces sharp resonances of C-atoms in the solid state, such as glycerophospholipids in dried liposomes (Underhaug Gjerde *et al.* 2004). Chlorpromazine (CPZ) is a cationic, amphiphilic psychotropic drug of the phenothiazine group that was the first drug used to treat schizophrenia and other psychiatric disease. When CPZ was included in liposomes of pure dipalmitoyl-phosphatidylcholine (DPPC), no alteration of the CH_2 resonances relative to liposomes without CPZ was found with MAS ^{13}C -NMR (Nerdal *et al.* 2000). However, when the liposome contained 30 mol-% pig brain phosphatidylserine (PBPS) together with DPPC, CPZ caused a large ($\approx 30\%$) low-field shift of the CH_2 resonances of 5–15 ppm at 37°C. This was interpreted as interdigitation of CPZ among the acyl chains of PBPS. This commercial phospholipid (from Sigma) was subjected to reverse phase HPLC that gave the separation of the molecular species, which revealed that the major species was 18:0/18:1 (63%) while the 18:0/22:6 species was next (24%). Later studies (Underhaug Gjerde *et al.* 2004) on liposomes with ^{31}P -NMR showed that CPZ interacted electrostatically with both the negative phosphate and carboxyl groups of PS and that PBPS strongly enhanced this interaction. Recently, similar studies (Chen *et al.* 2005) with pure 18:0/22:6n-3-PS in the DPPC-containing liposomes showed the same effects of CPZ with PBPS as described above, and T_1 relaxation measurements showed that CPZ reduced the mobility of the C_4 and C_5 atoms in DHA, which are attached to each other with a double bond.

It is therefore reasonable to assume that:

- 1) CPZ does not intercalate in liposomes containing the neutral PC.
- 2) CPZ intercalates in liposomes containing the acidic PS and neural PS is especially enriched with DHA.
- 3) The cationic CPZ binds electrostatically to the negative phosphate and carboxyl groups of PS.

- 4) The intercalation of CPZ in PS is affected by the unsaturatedness of acyls in PS with little intercalation in monounsaturated acyl and large intercalation in polyunsaturated acyls.
- 5) The double bond between C₄ and C₅ in DHA seems to be crucial for the strong intercalation of CPZ in DHA containing PS liposomes.

The small intercalation of CPZ in 18:0/18:1-PS (SOPS) and great intercalation in 18:0/22:6n-3-PS (SDPS) are important for psychotropic drug-membrane interaction. CPZ binds electrostatically to the negatively charged phosphate and carboxyl groups in SOPS through the positively charged tail amino group, but the phenothiazine moiety of CPZ is not intercalated among the acyl chains. In contrast, CPZ binds electrostatically to SDPS in the same way as in SOPS, but the lipophilic phenothiazine group is completely intercalated among the acyl groups and adjacent to the C₄=C₅ double bond. The apparent importance of this double bond should be tested in the future with 18:0/22:5n-3-PS, which should intercalate CPZ much less than SDPS.

The intercalation of psychotropic drugs into glycerophospholipid liposomes is not restricted to CPZ. Recently it was shown that the modern drug olanzapine also intercalates in both PC and PS liposomes (Song and Nerdal 2008), but these liposomes did not contain DHA. The intercalation of bulky molecules like CPZ in mono- or bilayers of phospholipids leads to increase of the intermolecular distances between the phospholipid molecules and alteration of the membrane structure. PS is exclusively in the inner leaflet of biological membranes. However, all psychotropic drugs distribute between membranes and water with distribution coefficients in the range of 10,000 to 20,000 (for references, see Oruch *et al.* 2008). This suggests that the drugs will enter the membranes through the outer leaflet and diffuse through the acyl layer and be able to interact with the PS in the inner leaflet. One would assume that the structural changes caused by CPZ will affect the positioning of the proteins, such as membrane-

bound enzymes and receptors and thereby alter their functions. Thus, in addition to act as antagonists for receptors, the drugs may also alter membrane protein activities.

DHA is very concentrated in nervous tissues, and in rat brain PS the major molecular species is 18:0/22:6n-3 (Bakken *et al.* 2006). Thus, brain PS may be the target for at least CPZ, and perhaps for other psychotropic drugs. This type of drug has been developed as brain receptor agonists. According to the discussion above, the drugs have membrane distorting activities in which DHA may play a central role. It has moreover practical relevance as it may be possible to modulate the DHA in the receptor-lipid domain in nerve cell membranes and so alter the efficacy of psychotropic drugs and neural membrane function despite its strong protection against external influences.

Added to this evidence on DHA-PS, Hee-Yong Kim has shown that neuronal apoptosis under adverse conditions is prevented by DHA enrichment in a PS-dependent manner. Moreover, the protective role of DHA enriched PS is not similar when DHA is deficient and there is an increase in the ω 6DPA (Kim *et al.* 2003). They have also shown that DHA activates neurite outgrowth at low micromolar concentrations with a remarkable effect on morphological differentiation of hippocampal neurons which is achieved by increasing the population of neurons with more branches and longer neurites. This effect does not seem to be mediated by the expected nuclear receptor (retinoid X receptor) and may be achieved by some function of DHA itself (Calderon and Kim 2007) again pointing to the significance of neural DHA rich PS and DHA itself.

5.2. Docosanoids

The example above is an entirely new role for DHA as a mediator of a receptor which is likely to be more widespread than just this example. The serine phosphoglycerides are known to be especially rich in DHA and are closely associated with membrane proteins. Added to this physico-chemical role of DHA in a receptor domain, Nicholas Bazan has discovered a striking anti-oxidant effect of derivative docosanoids from DHA: the

Neuroprotectins (NDP1). They claim that NDP1 acts against apoptosis mediated by A2E, a by product of phototransduction that becomes toxic when it accumulates in aging retinal pigment epithelial (RPE) cells. With DHA being selectively rich in neural systems, its neuroprotectins also protects against neural cell damage, most likely those associated with ageing, and Alzheimer's Disease (Lukiw *et al.* 2005; Bazan 2008). The design of DHA the polyenoic fatty acid most susceptible to peroxidation and located in regions of the most intense oxygen use, is a remarkable feat of Nature.

In addition to this neuroprotection role for DHA metabolites there is new evidence on the resolution of inflammation which has been shown by Charlie Serhan *et al.* (2008) to resolve inflammation through the action of biochemical processes that enable inflamed tissues to return to homeostasis. Following tissue injury it has been long thought that tissue injury, followed by inflammation, then repairs in good time, spontaneously. However, it now seems as though fatty acid derivatives marshal the actors in the process of resolution and damage repair. Although it seems that resolvins can be derived from both EPA and DHA it is worth noting that human tissues, there is little EPA and the omega 3 family is mainly represented by DHA and some small amount of ω 3 docosapentaenoic acid. This focus by cell systems on DHA is especially pronounced in the brain and the testes.

5.3. The extreme conservation of DHA in neural signalling systems

A number of studies have been conducted on the physical effects of polyunsaturation on membranes, in which DHA has been compared to a range of other unsaturated chains having from one to five double bonds. Thus far, however, all differences that have been measured have been matters of degree, and none provide a compelling explanation for the striking specificity with which DHA is selected for membranes of the eye and brain over 600 million years of genomic change and evolution.

Where, then, can we hope to find an explanation of DHA's preferred status in neural membranes since the beginning of animal evolution in the Cambrian Era? An obvi-

ous starting point is that membrane protein interacts with the lipid in some way in which DHA favourably merges with the stereo and electro-chemistry of the protein of which the CPZ discussion above is an example. Such an effect could conceivably involve either an interaction with specific lipid molecular species, or modulation of bulk properties of the bilayer.

The conventional portrayal of proteins in lipid bilayers is of the lipid represented by a double row of soldier, standing to attention and the protein slipping in between them, so to speak, dissolved in the membrane. This cannot be a correct portrayal as otherwise lipid chemists would not need to use acid in extraction procedures.

Some believe specific binding interactions between lipid and protein molecules in a biological membrane are unlikely, since the membrane's fluid state means that individual lipid molecules will be undergoing rapid translational diffusion within the bilayer, and thus will never be in prolonged contact with any one protein. Furthermore, Brown's studies (1994) on the rod photoreceptor outer segment membrane revealed that specific chemical-type interactions could not be the cause of DHA's established role in supporting rhodopsin function. It was found that full rhodopsin efficiency could be obtained by substituting other lipid mixtures designed to mimic the bulk mechanical properties of the physiological, DHA-rich membrane. This gave rise to a model in which DHA's role was to promote mechanical conditions in the membrane suitable to stabilize certain critical conformational changes undergone by rhodopsin in the course of photoactivation. These models do not fully reconstitute the structure of the photoreceptor cell and its synaptic function, the ten thousand fold adaptive capability of which is still unexplained. However, should this model be valid to conditions *in vivo* it could potentially be extended to other G-protein systems elsewhere in the central nervous system (CNS).

6. A Hypothesis on the Molecular Dynamics and π -Electron Function in DHA

A more speculative, possibility is that DHA *in vivo* plays a more direct role in neuronal

signalling, in which some special properties conferred on the membrane by DHA chains exert an influence on membrane electrical phenomena (Bloom *et al.* 1999). These might include distinctive dielectric or polarizability properties arising from the unique periodic and symmetric arrangement of double bonds in the DHA chain. This arrangement is disrupted with the loss of the $\Delta 4$ double bond when the first seven carbons can occupy many more conformers than with the more ordered structure of the full sequence of six methylene interrupted double bonds.

It is conceivable that some polarization of π -electron clouds might occur in the DHA structure, and perhaps even be transmitted from one double bond to another, either within a given chain, or between neighbouring chains in the membrane. Our molecular dynamic calculations reveal that the π -electrons could come closer together in adjacent molecules than they are in the chain of a DHA molecule itself. In a similar vein, Penrose (1990, 2001) has postulated that some brain functionality may arise due to quantum coherence in the microtubules of neurones; it may be worthwhile to look for a similar phenomenon in signalling membranes containing DHA.

6.1. Nuclear overhauser enhancement

As a first step we have tested the possibility of electrical properties of DHA by examining its response to a magnetic field. In our Nuclear Magnetic Resonance (NMR) experiment, the magnetic moment is flipped [e.g. for ^{13}C] perpendicular to the magnetic field of the NMR magnet. The energy released to return to alignment with the magnetic field is then measured. The Nuclear Overhauser Enhancement (NOE) makes this process go significantly slower. If there is 2 s or 20 s between scans, a uniform molecule sees the same sea of magnetic moments in each scan. Spatially unequal concentration of magnetic moments as in $\text{CH}=\text{CH}-\text{CH}_2-$ or $-\text{N}-(\text{CH}_3)_3$ concentrates the magnetic polarization at these specific sites which is then detectable. Any response by any section of the molecule is then seen in a difference between the scans. Note that the proton magnetic moment four times greater than that of ^{13}C (Fig. 4).

It is important to note that polarization built up at the terminal methyl group as this would have potential to interact with adjacent molecules in the vertical plane of the bilayer.

These NMR experiments, which will be reported in detail elsewhere, demonstrate that the DHA molecule is subject to polarization in a magnetic field which is a signal of its potential to be electrically active. The final evidence comes from the expected polarization of the polar head group which contains a strong dipole moments. These are candidates for interaction with the aqueous phase and its ions.

6.2. The brain as an electrical machine

The conclusion from these studies is that DHA has the potential to act electrically. Further studies will be required to define the extent of this activity. However, molecular dynamic studies of the 2D electron distribution gives a clue as the special significance of DHA. It can be seen that the electron density map spreads across the whole molecule and even involved the aliphatic groups. This property is confirmed by the NMR experiment described above (Fig. 5).

In 1941 Albert Szent-Györgi wanted to know why electrons wandered from enzyme to enzyme in the electron transfer process of mitochondria. Hence the concept of electron involvement in biological processes is not new. Oleic acid in the membrane phosphoglycerides can increase membrane conductance, allowing the use of a voltage-clamp technique. Brunaldi *et al.* (2005) suggest that certain FAs increase proton transport across the lipid bilayer. In such studies, the membrane-unspecific or leak conductance contributes importantly to the measured conductance and constitutes a major source of indeterminacy.

Neural signalling is associated with the development of a potential difference across a lipid bilayer with subsequent depolarization. Whilst the action is considered to be down to large ions (e.g. sodium, potassium and calcium), it is difficult to imagine that the electrons are oblivious to the potential difference. The dumbbell shape of the π -electron clouds

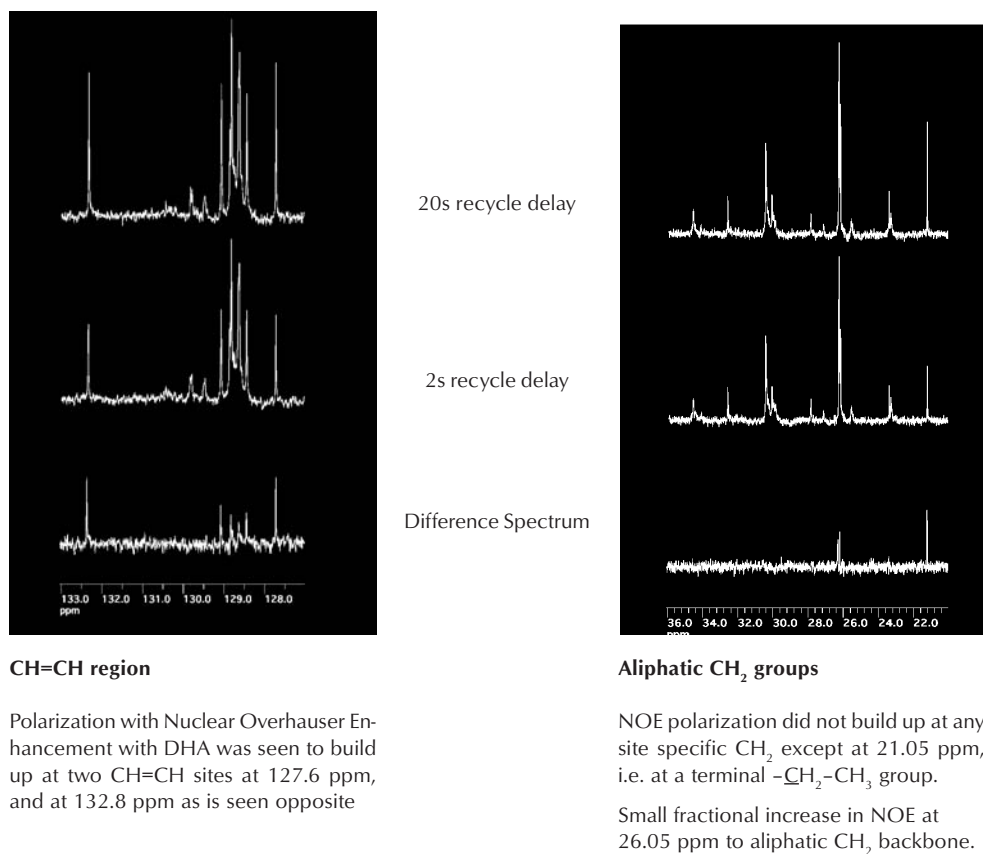


Fig. 4. Alignment of DHA in a magnetic field: Nuclear Overhauser Enhancement.

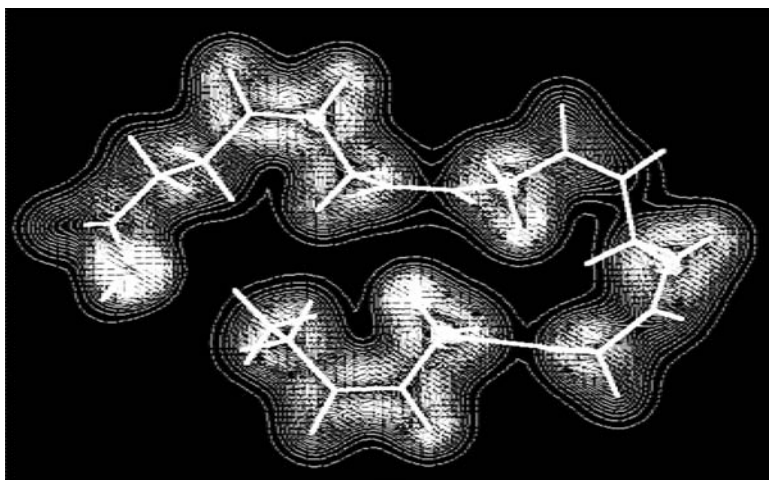


Fig. 5. The 2D-charge density (3 double bonds coplanar).

would lean towards the positive charge and in consequence set up a differential charge across each double bond. With six in a row it would seem plausible that this arrangement would hold the potential for special conductivity.

Electron tunnelling (ET) would be one such mechanism whereby such conductivity could occur. It is precisely determined by quantum mechanics. Electron tunnelling is known to occur on proteins of the electron transport system (Yue *et al.* 2006; Moser *et al.* 2006). Rhodopsin is one of the best characterized trans membrane proteins involved at a site where large potential differences are created with subsequent depolarization to effect signal transduction in response to activation by a single photon which isomerizes the retinal receptor. Jin *et al.* (2006) have detected current flow through the (retinal-free) apo-membrane of bacteria rhodopsin (bR) which is approximately three orders of magnitude lower than was observed with native bR membranes. This result supports the idea that current flows dominantly through the bR proteins and that the retinal with its conjugated sequence of double bonds serves as a current transporter. Furthermore, the photo-effect observed with the native bR-containing membranes can be ascribed to the retinal rhodopsins.

Jin *et al.* (2006) conclude that transmembrane electron transport occurs essentially only *via* bR and not *via* the lipid bilayer and requires the presence of retinal or a similar conjugated π -electron system in the protein. The contribution of light-driven proton ejection to the steady-state photocurrents is negligible. Their result suggests that π -electron system in the retinal conjugated double bond sequence is acting as a copper wire with isomerisation acting as a switch to disconnect electron flow. So the dark current flows until stopped by a photon isomerising the retinal and changing its position relative to the aromatic amino acids from conduction to non conduction. They are concerned and ask why such π -electron transfer systems have not been described—are the “biological processes ... hidden so well that we have not found them.”

Whilst proteins contain aromatic rings ideal for electron transfer there is no such identity for the lipid bilayer. However, the calculated charge density of DHA is sugges-

tive of a system which unlike retinal will not conduct electrons except under the special circumstance such as tunnelling. In tunnelling systems, transfer ability diminishes exponentially with distance. Hopfield estimated that an 8 Å edge to edge distance between ET parameters was about the limit. The distances in methylene interruption is <6 Å. In the case of the ω 3 terminal end the distance for the first π -electron cloud is about 4.2 Å within the tunnelling range. However, remove one double bond and the distance jumps to over 8 Å implying that tunnelling would not work in the ω 3DPA. This virtually complete uniformity of electron density in DHA is destroyed if one double bond was missing at either end of the molecule (Fig. 6).

Extended Huckel calculations based on the least occupied orbitals for DHA show as discussed above that the π -bonds have + and – lobes and that the + and – signs of orbitals of the two different hydrogens on the CH₂ groups also have + and – signs related to (typically opposite to) the signs of the adjacent π -bonds. This is a simple mechanism to explain electron coherence over a large distance, even though the double bonds are not extended resonance structures across a sequence of carbons with only single hydrogens.

This full, electron coherence does not seem to work with DPA which has five methylene groups following the double bond sequence. In DHA there are only two methylene groups sandwiched between the sequence and the carboxyl attached to the polar end group permitting cohesion throughout the whole molecule.

6.3. Is DHA a quantum gate to control transmission of electrical information?

The methylene groups present an energy barrier to π -electrons. However, if one electron is removed from the end of the cohesive sequence in DHA it is then possible for an adjacent π -electron to tunnel through to the vacated level and so on down the sequence.

But electron tunnelling will only occur at the precise energy level vacated. This mechanism would provide an *absolutely precise*, quantum gate which would only open at a specific energy condition and then only permit a precise quantum of energy transfer (Table 1).

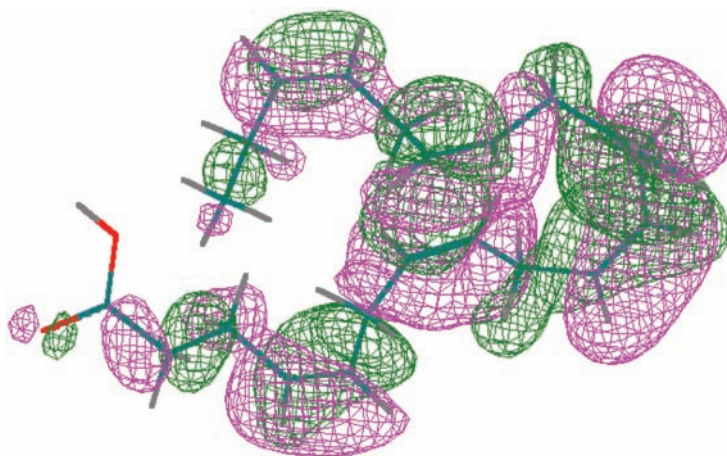


Fig. 6. Electron distribution around the DHA molecule high and low alternating densities. (π -bond energy different above and below planes; green lower, mauve higher energy).

Table 1. Electron tunneling edge to edge distances.

Vertical distances:			
• Docosapentaenoic 22:5,n-6			
CH ₃ ∖∨∨ [∞]	8.4 Å	∞∖∨CO-	4.2 Å
• Docosahexaenoic 22:6,n-3			
CH ₃ ∖∧ _∞	4.2 Å	∞∖∨CO-	4.2 Å
• Docosapentaenoic 22:5,n-3			
CH ₃ ∖∧ _∞	4.2 Å	∞∖∨∨∨CO-	8.4 Å
Horizontal minima distance between DHA double bonds			
4 Å for 3 planar double bonds			
Horizontal minima distance between DPA double bonds			
7.6 Å for 2 planar double bonds			

This precision is evident in photoreception but is so far difficult to explain (Rieke and Baylor 1996; Field and Reke 2002; Dunn and Rieke 2008). Although photoreception is one of the best described signalling systems, it is plausible that within it, DHA may be acting as a semi-conductor that will only allow electrons to pass at a specific energy level to contribute or control the depolarisation. This event would be consistent with the precision seen when the photoreceptor is activated by a single proton yielding the same energy regardless of energy input. The speculation is that as Jin *et al.* (2006) describe, the dark current would use retinal, isomerisation to disconnect the transmission of the dark current by the conjugated double bonds

of the retinal, stop electron transfer leading to a build up of a potential difference. The ET properties of the DHA would ensure the potential difference continues to accrete until it reaches the level required for tunnelling at which point depolarization would follow. This speculation is not inconsistent with the present concept of photo transduction. The same principle could operate in synaptic transmission. It is already described in the electron transfer in the proteins of the cytochrome system (Gray and Winkler 2003). This potential is supported by the NOE studies indicating DHA will polarize in a magnetic field. As seen below with the edge to edge maximum distance for electron tunnelling to occur (about 8 Å) is exceeded if one double bond is removed from the DHA making such a process less likely.

Tunnelling may also operate in a cohesive manner collaborating with adjacent molecules in the bilayer with the potential for the very long chain (>26 carbons) contributing to connecting the outer and inner leaflets of the membrane. The coherence of DHA-ET is on the lines suggested for neural function by Penrose (1990, 2001).

7. A 600 Million Year Track Record in Neural Signalling

DHA was the only molecule so selectively used over 600 million years of evolution in

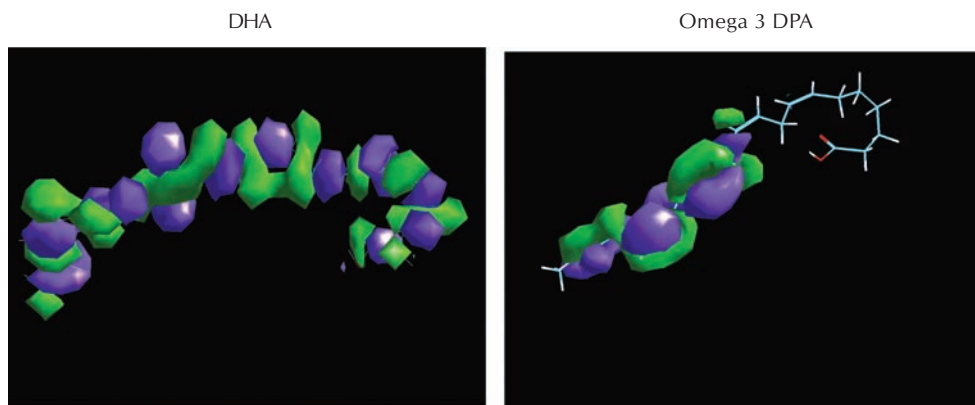


Fig. 7. Electron density of higher energy state as might be expected with a strong positive negative charge across the membrane. The response of the electrons in DPA would make electron tunnelling unlikely along the full length of the molecule. The full length with alternating polarities is feasible with DHA.

the photoreceptor and synaptic junction: locations where there is the most intense oxygen utilization, a threat to such a highly, unsaturated molecule.

The precision of the energy output of the photoreceptor is not explained by an unquantized effect on the number of G-protein molecules activated. That does not mean to say that they do not contribute to the transduction process and signal amplification. However, photo-transduction has precision, which is a hall mark of quantum mechanics. Precision can be explained by tunnelling which can only take place at the precise energy and state of the first electron removed from the DHA methylene interrupted sequence.

Moreover, the same closeness in space that allows DHA orbitals to co-relate also enable them to co-relate in phospholipids. The results are remarkably simple: DHA-DHA allows at least 3 double bond pairs on each DHA molecule to be close enough in space to align with 3 on the other chain. And where does one find the Di-DHA phosphoglycerides but in the photoreceptor. Ergo, activation energy now is even simpler: head to foot/head to foot association in low energy [ground] state together with alignment of polarization head to head and foot to foot on individual chains.

In response to closure of the dark current and or the build up of a powerful poten-

tial difference across the lipid bilayer, which would create a relative negative-positive-negative-positive arrangement as seen in the LUMO molecular dynamic figures. The π -dipole can be visualised as a bar magnet in each of the DHA molecules in the phospholipid. So: in the ground state, the bar magnets align head to foot, head to foot. On activation by a signal (light in the case of the photoreceptor) in the presence of an appropriate, potential difference, the bar magnets align resulting in a force double the π - π force in each of the DHA molecules. Since the phospholipids align along the membrane: again, the dipoles align longer and longer, producing a greater and greater signal. On closure of the signal the dipoles slowly flop, back to head to foot, head to foot, no less the wear for their rearrangement (Fig. 7).

Independent support for an electron function for DHA comes from the early studies of Robert (Gene) Anderson on photo-transduction (Benolken *et al.* 1973). He had discovered that the rod outer segments of several species contained about >50% of its fatty acids as DHA. He raised rats on a fat free diet and observed significant alterations in the electrinoctinogram (ERG) indicative of reduced A and B wave function.

The amount of rhodopsin and the shape of the absorption spectra and general bleaching characteristics were the same for rhodopsin

from EFA deficient and control groups of eyes. The density and packing of rods appeared normal in the deficient, test animals, and the ultrastructure of rod outer segments from these animals was preserved and indistinguishable from the ultrastructure of rod outer segments from control.

The A-wave of the ERG is a photoreceptor response function, while the B-wave is generated by electrical activity in other neural layers of the retina. The DHA content of the rod outer segments fell from 45.2% of the fatty acids to 19.0% in the EFA deficient rats. The loss of DHA was partially replaced by docosapentaenoic acid in the $\omega 6$ family. This means the altered ERG was a function of the specific loss of membrane DHA. That in turn means that DHA is itself involved in the electrical response of photo transduction. The mechanism we propose here would enable DHA to act in a manner similar to a semi-conductor providing a quantum gate.

8. DHA and Neural Pathways?

A key characteristic of $\omega 3$ deficiency is reduced learning capacity and behavioural pathology. We were the first to describe the behavioural pathology in an $\omega 3$ deficient primate which is seen in Dr. Joseph Hibbeln's work at the NIH USA (Hibbeln *et al.* 2005, 2007). Suzuki *et al.* (1997) demonstrated the selective uptake by the synapse for DHADHA somewhat similar to that shown by Bazan and Anderson for the photoreceptor. The brain turns over its constituents rather than relying on imports. No recycling process is 100% efficient. Hence there will be continual loss which has to be replaced by some import.

Let us assume the letter A is seen on a teach-yourself typing screen. The response of putting the left hand's small finger on the second end key on the left of the 4th row of the PC keyboard requires the correct visualisation of A, its recognition as requiring a motor response, transmission to a motor section of the brain, identification of the hand and then the small finger, left hand and then the transmission of the message in 3D to the small finger. That neural pathway has to be learnt so that when the photoreceptors and then the brain calls for the letter A, the correct response is elicited. Learning requires repetition. In the rep-

etition process the synapses fire and reconstitute. With selective uptake of DHA the synapses in the pathway will be enriched. The more enriched the synapse the better its function which is the converse of the $\omega 3$ deficiency experiment which depresses learning ability. Repetition will enrich a pathway and just as water takes the path of least resistance when flowing down a hill, so the signal A from the photoreceptors will take the least resistance DHA enriched pathway to the small finger on the left hand in time and space.

This concept of memory is not independent of other similar concepts of protein activation except the evidence on memory is mostly published with respect to $\omega 3$ deficiency. One would expect the proteins which are encoded by DNA to be robustly built to the same specification. However, the lipids and lipid composition is subject to environmental inputs and variation. This proposed function of DHA would facilitate conduction of a signal and the establishment and function of a neural pathway.

9. Darwin and Conditions of Existence

Darwin in *The Origin of Species* (1868) stated there were two forces in evolution, *natural selection* and the *conditions of existence*. Of the two, he said, *the latter was the most powerful*.

However, Weismann (1893) rejected this view in the all sufficiency based on experiments in which he cut off the tails of breeding rodents and observed that subsequent generations still produced tails. That set in train the present paradigm of the *modern synthesis*, and genomic determinism within which the DNA is seen as the sole dictator of difference and evolution, and to the notion of the "Selfish Gene". Darwin spent much of the later part of his life searching for what he called "Pangenes" that were responsible for translated environmental influences. His failure served the all sufficiency of natural selection and excluded the conditions of existence. However, Darwin's "Pangenes" are now evident in the response of plasma membrane receptors responding to nutrients influencing gene expression (Chawla *et al.* 2001; Puskas *et al.* 2003, 2004; Anderle *et al.* 2004) and *vice versa* (Corella *et al.* 2005). Epigenetic effects consequent on manipulation of gene expression during early development,

were in evidence in the follow up of the Dutch food shortage in World War II. Low birthweight was transmitted to a second generation (Stein *et al.* 2006). Another example is prenatal programming (Barker 2004) resulting in adult risk to heart disease diabetes and stroke from poor maternal/fetal nutrition.

Darwin's original view is on conditions of existence are consistent with the remarkable conservation of DHA in signaling systems over 600–500 million years. That is despite wide ranging changes in the genetic code and the great evolutionary changes, DHA has been rigorously conserved. It is as though DHA has been instructing the genes to do its bidding rather than the conventional view which is the other way round. Apart from vindicating Darwin's concept and his superiority over Weismann and his all sufficiency, it raises basic questions in biology enhancing our understanding of the relationship between environment, the genes and function. The functionality of natural selection is readily identified in animal systems. It has one drawback in that it does not fit with degeneration. The loss of relative brain size in all land based mammals as they evolved larger bodies (Crawford *et al.* 1993) does not sit well with their loss of brain power and lack of ability to survive as witnessed in recent time. More importantly it does not predict as it is based on randomness. Darwin's conditions of existence offer predictive value which is a hall mark of science.

The evidence on omega 3 marine food consumption in pregnancy affecting childhood intelligence and behavior measured at 8 year of age acts as a reminder that *H. sapiens* is also subject to Darwin's conditions of existence (Hibbeln *et al.* 2007).

10. Reason for Concern on the Food System and the Brain

The 600 million year track record of DHA in neural systems is compelling evidence for its absolute requirement. We now wish to return to the evolutionary implications. The evidence for the evolution of *Homo sapiens* as a coastal dweller, utilising the marine food

chain is now very persuasive. The brain first evolved in the marine environment utilising marine nutrients of which clearly DHA was a key for neural systems. It still utilises DHA today. As Philip Tobias said at a lecture in London³ "Wherever humans evolved, they had to have water to drink."

In human fetal growth the priority is brain development which receives 70% of the growth energy delivered from the mother. Maternal nutrition before and during pregnancy is an independent risk factor for low birthweight and poor pregnancy outcome (Doyle *et al.* 1989; Wynn *et al.* 1994; House 2000; Carlson 2001; Rees *et al.* 2005). Post-natal nutrition is also a priority to ensure good maternal nutrition for herself and for milk (Birch *et al.* 2007). Although the outcomes of supplementing preterm infants is accepted as beneficial to cognitive development, there is some variation in the human studies of term infants (Simmer 2000). However, in the human species, most brain cells divide pre-natally and the studies in preterm infants have been consistently positive (Fleith and Clandinin 2005). This variation is likely to arise from supplement type, dose and background diets. None the less, tissue DHA levels have consistently correlated with outcomes (Gibson and Makrides 2001).

Hence, poor maternal health and nutrition before and during pregnancy disadvantages fetal development with permanent mental and cognitive deficits (Litt *et al.* 2005) and behavioural dysfunction (McNamara and Carlson 2006; Hibbeln *et al.* 2007) with a risk of heart disease, diabetes and stroke in later life (Barker 2007). Poor neurodevelopment restricts the individual's capacity to acquire numeracy and technical skills (Birch and Gussow 1970). In addition, to the $\omega 3$ fatty acid protection against sudden death from heart disease (Marchioli 2002) evidence has accumulated on the competitive effect of fatty acids with the $\omega 3$ fatty acids inducing behavioural pathology (Fiennes *et al.* 1973; Hibbeln *et al.* 2004). Deficits of marine fats have been linked to manic depression (Saugstad 2001, 2007; Young *et al.* 2005). Depressed $\omega 3$ status has also been linked with

³ McCarrison Society "A New Light on Human Origins," at the Zoological Society of London, NW1 4RY, 22nd September 2000.

Alzheimer's disease (Beydoun *et al.* 2007).

11. Implications

There are 1.6 billion people at risk to iodine deficiency, a sure progenitor for mental retardation but seldom seen in the fishing communities. In Europe, brain disorders have now overtaken all other burdens of ill health (Andlin-Sobocki *et al.* 2005) and mental ill health is predicted by the Global Forum of Health (www.globalforumhealth.org) to be in the top three burdens of ill health world wide by 2020. There is compelling evidence that the reasons are related to the loss of sea foods and their replacement by land foods (Hibbeln *et al.* 2002, 2004, 2007). To solve this problem and prevent further rise in disorders of the brain may well require a new paradigm in food with a focus on the nutritional requirements for the brain. This may well mean agriculturalising the oceans and enhancing the development, use and consumption of sea foods worldwide.

12. Conclusion

The use of DHA in neural signalling systems over a 600 million year stretch of evolution is compelling evidence for its essentiality. It is now known to be involved in neural receptor domains, gene expression with derivatives providing protection from oxidative stress in the brain and resolution of injury. We speculate that DHA uniquely contributes π -electrons to signal transmission providing

quantum mechanical provision for precise, signal control and an explanation for the uniqueness of DHA in signalling systems.

The DHA is the most limiting biosynthetically of the brain specific fatty acids. It therefore needs to be best obtained preformed for human nutrition, especially during pregnancy and lactation when the new fetal and infant's brain is forming at high velocity. The DHA is poorly represented in the land food chain. The richest source is the marine food web where the brain first evolved. The implication of the conclusion on its essentiality, if correct, is important to the future of humanity.

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Genomics and the Genome Duplication in Salmonids

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Salmonids have been classified into nine genera and roughly sixty-eight closely related species. Considerable basic biological knowledge of trout and salmon has been developed as a result of their widespread use in scientific research, as an environmental sentinel species and their use as a food and sport fish. More is known about the biology of salmonids than nearly any other fish group. More recently genomic studies have contributed gene lists and genetic and physical maps as well as new technologies to integrate gene expression patterns with traditional ecological, evolutionary and physiological studies. Of particular interest is that the common ancestor of salmonids purportedly experienced a whole genome duplication event between 25 and 100 MYA. Given that gene duplication may one of the most important mechanisms of generating molecular and physiological diversity, the study of salmonid genomes provides an excellent opportunity to examine genetic and ultimately species diversity. Here we review some of the recent genomic data and suggest areas of further research.

KEYWORDS Salmonids; genomics; gene duplication; diversity; evolution; repeats; ESTs

1. Introduction

Our long-term objectives are to study the impact of genome duplication on the modes and rates of change in gene number, gene function and evolution particularly in the

purported genome duplication in salmonids. This entails the study of loss, dosage effects, neofunctionalization and subfunctionalization of gene functions. It also entails the study of mechanisms of genome structure reestablishment after genome duplication and the potential role of interspersed repetitive elements

in restructuring the genome through rearrangements, insertions and deletions. This should be relatively straight-forward but recent EST data suggest a much more complicated history than can be explained by a single genome duplication in salmonids. Given the large volume of work done in salmonids and that “gene duplication is probably the most important mechanism for generating new genes and new biochemical processes that have facilitated the evolution of complex organisms from primitive ones,” (Li 1983) the first and most important objective of our studies is to test and verify an ancestral salmonid genome duplication and then, if our initial data are correct, document the extent to which very recent segmental gene duplications have occurred. Genome duplications are thought to be defining genetic events in the evolution of complex vertebrates. In our studies we have focused efforts on documenting and characterizing; i) global versus regional duplication patterns, ii) gene family duplications (immune systems), and iii) the role of interspersed repeats in genome stabilization. The purpose of this chapter is review some of the broad and very general interpretations of recent data pertaining to the purported genome duplication in salmonids and suggest areas of further research.

The Salmonidae family includes; whitefish and ciscos (subfamily Coregoninae); graylings (Thymallinae); and trout, salmon and charr (Salmoninae), Fig. 1.

These fish have been further classified into nine genera and roughly sixty-eight species (Nelson 2006) that are more than 92% similar at the DNA level. They are native to the cooler climates of the Northern Hemisphere but have been widely introduced around the world.

Considerable basic biological knowledge of trout and salmon has been developed as a result of their widespread use in scientific research, as an environmental sentinel species and their use as a food and sport fish. More is known about the physiology and biology of salmonids than any other fish group. In the past 20 years there have been over 20,000 reports on the ecology, behavior, physiology and genetics of these species (Thorgaard *et al.* 2002). These studies provide data from an economically important and phylogenetically distinct group of fish. Recent genome studies have targeted Ostariophysi (zebrafish, catfish, flathead minnow, etc), or Acanthopterygii (cod, cichlids, fugu, medaka, sticklebacks, rockfish) euteleostei lineages that have been separated from Protacanthopterygii (salmonids) 200–300 million years ago [MYA] (Ichiguro *et al.* 2003; Nelson 2006; Yamanoue *et al.* 2006; Inoue *et al.* 2006). Salmonids, with their genome duplication and wealth of biological data, are excellent model organisms for studying comparative genomics, evolutionary processes, fates of duplicate genes and genetic architecture of complex phenotypes, as well as carcinogenesis, toxicology,

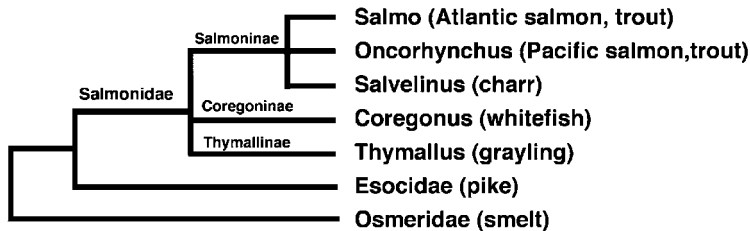


Fig. 1. General relationships of the major salmonid and closest sister groups (Ichiguro *et al.* 2003; Nelson 2006; Yamanoue *et al.* 2006; Inoue *et al.* 2006).

comparative immunology, disease, ecology, physiology, nutrition and many other genetic and physiological processes (Thorgaard *et al.* 2002).

2. Genome Duplications

The common ancestor of salmonids purportedly experienced a whole genome duplication event between 25 and 100 MYA (Ohno 1970; Allendorf and Thorgaard 1984). Extant species would therefore be the result of reversion back to a stable diploid state. Evidence for the ancestral salmonid autotetraploid genome duplication includes; multivalent chromosome formation during meiosis and evidence for tetrasome segregation at some loci; one of the largest euteleost genome sizes (3–4.5 pg) with double that of sister orders Esociformes (0.8–1.8 pg, pike) and Osmeriformes (0.7 pg, smelt) (Gregory 2005); homeologous chromosomal segments based on recent genetic maps and comparative studies using microsatellite markers (Leder *et al.* 2004), and duplicated gene family studies such as Hox, MHC, growth hormone, many allozymes and others (Thorgaard *et al.* 2002; McKay *et al.* 2004; Moghadam *et al.* 2005; Hoegg and Meyer 2005; Shiina *et al.* 2005).

The genome duplication in salmonids is just the most recent of a series of genome duplications in this lineage. There is good evidence primarily from sequenced zebrafish, and pufferfish genome sequences for tetraploidization/rediploidization early in the ray-finned fish lineage (350–400 MYA) (Hoegg *et al.* 2004; Volff 2004; Steinke *et al.* 2006; Crow *et al.* 2006). In addition, several studies have supported Ohno's original 2R hypothesis that one and possibly two rounds of genome duplication occurred in early ancestral vertebrates (~600 MYA) (Taylor and Raes 2004). The 2R hypothesis (1 to 2 to 4 gene rule) is the prevalent model that is used to explain the evolution of gene families and vertebrate genomes. The 3R

hypothesis further explains gene families in ray-finned fish (1–2–4–8). While evidence from Hox, sodium channels, glycolytic enzymes and other genes (Novak *et al.* 2006) support the 3R hypothesis, debate continues (Hughes 1999) and it may be impossible to deduce events that happened so long ago using current methods. Interestingly, in salmonids, 14 Hox clusters have been recently identified thus supporting a 4R hypothesis (1–2–4–8–16 with 2 losses) (Allendorf and Thorgaard 1984).

The importance of understanding the role of genome duplications lies in that vertebrate species diversity and body plan diversity have commonly been linked to genome duplications although there is some debate on how well we can draw these conclusions based on the very old genome duplications commonly studied (Donoghue 2006). The number of salmonid species and the relatively recent genome duplication make salmonids ideal for examining recent events that could have played such a pivotal role in generating gene diversity and possible species diversity found in modern vertebrates.

The most commonly discussed mechanism by which organisms acquire new functions has been Ohno's classical model (Ohno 1970) which is based on the principle that duplication events provide gene redundancy upon which natural selection may be relaxed and new functions evolve. This model predicts that one of the genes remains under conservative selection while the most likely fate of the other gene duplicate is non-functionalization via mutations. However, occasionally, rare beneficial mutations at the redundant loci followed by positive natural selection may give rise to a gene with a novel function (neo-functionalization), thus preserving both duplicates (Ohno 1970). This model predicts that retention of duplicates is a rare event. However, what is observed in the case of many genes is that duplicated genes are commonly retained (Hughes 1999).

Previous estimates of 30–70% duplicate retention occur in salmonids (est. time of duplication is 25–100 MYA), 20% retention in teleost duplicates (~350 MYA), 50% retention in xenopus duplicates (30 MYA) and 72% retention in rice (11 MYA). To account for this high level of duplicate retention, Hughes (1999) suggested that gene duplications that lead to functionally distinct proteins are ordinarily preceded by a period of gene sharing: that is, a period in which a single generalist gene performs two or more distinct functions, perhaps in different tissues or in different developmental stages. Upon gene duplication, these functions are able to take on separate specialist functions through positive selective forces. This model is used to explain some of the very large immunoglobulin, olfactory receptor, and defensin gene family structures in which selection for sub-functionalization, or genes expressed differently in various tissues or developmental stages appears to precede gene duplication. More recently, the duplication/degeneration/complementation (DDC) model (Force *et al.* 1999; Lynch 2000; Hughes 2005) proposes partitioning of the expression patterns of the original gene between the duplicates (sub-functionalization) via complementary degenerative mutations, particularly in regulatory regions. This does not require positive selection pressure to preserve both copies in the genome, only complementary mutations in the duplicates. Larger numbers of small populations are particularly important variables in this model. Salmonids offer particularly valuable life histories in this regard.

3. Expressed Sequence Data

One very puzzling observation arising from preliminary EST (expressed sequence tag) analysis of the Atlantic salmon (436,000 Atlantic salmon ESTs; GenBank, September 2007) is that the number of expressed duplicate transcripts (presumed paralogues) de-

creases more regularly with respect to percent divergence than expected (see Fig. 2).

Over 81,000 contigs derived from 436,000 ESTs (cGRASP) were compared to each other by BLAST analysis and the number of alignment pairs with *e*-values < 1e-25 and lengths > 200 bp were plotted against % identity. A similar analysis of Atlantic salmon EST contigs compared with rainbow trout EST contigs showed a peak at approximately 94% identity and 92% when compared to lake whitefish ESTs (unpublished data, Brown 2008). Within this overall context, the data from Fig. 2 suggest that many of the Atlantic salmon contig consensus sequences are more similar to each other than Atlantic salmon contigs consensus sequences were to rainbow trout or whitefish consensus sequences. These preliminary data indicate that there may be more gene duplicates of recent origin than expected from a single ancestral genome duplication in the common ancestor of salmonids. These observations raise very intriguing questions, i) why are there so few paralogous alignment pairs among expressed genes and ii) why is there no paralogous peak at ~80–90% similarity?

The distinguishing feature between multiple segmental genome duplications and a genome duplication is that a whole genome duplication affects all genes at the same time and predicts similar levels of divergence among duplicates; within the bounds of differential selection pressures. Multiple segmental duplications on the other hand affect only portions of the genome and several may occur over a broader time period. In the later instance we expect a broader range of divergence values between duplicates, perhaps similar to the pattern shown in Fig. 2. Extended genomic sequence data are only beginning to become available and the problems associated with resolving extensive duplications requires diligence. Some regions clearly show an 80–90% divergence in noncoding sequences [MHC class I regions;

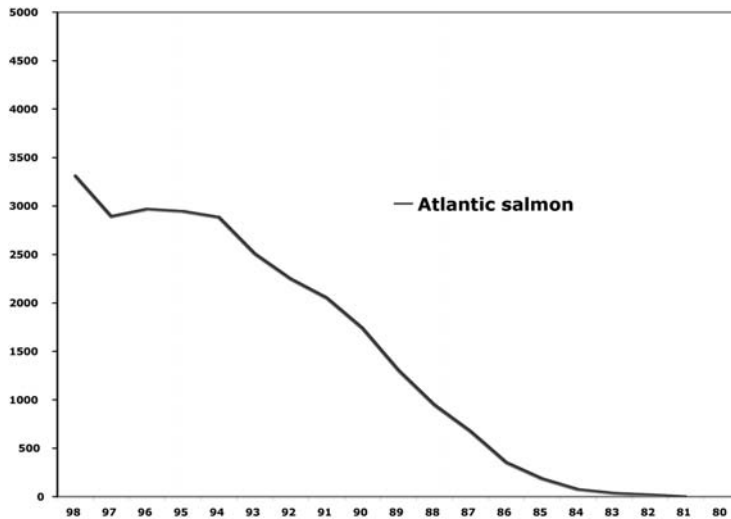


Fig. 2. The number of Atlantic salmon EST contigs that have a BLAST hit (E-value < $1e-25$; length > 200 bp) against another Atlantic salmon EST contig is plotted with the percent identity (x-axis). Modified from GD Brown thesis (Brown 2008).

>500 kb comparisons (Shiina *et al.* 2005; Lukacs *et al.* 2007) and others (TCRG regions; >250 kb comparisons (Yazawa *et al.* 2008)] show no similarity at all outside of coding regions. However since most gene targets were originally *a-priori* chosen to verify the ancestral salmonid genome duplication hypothesis, we need to reexamine our initial assumptions and look for examples of other gene/region duplications. Specifically we need to;

- i) Identify more duplicate gene sets that are 92–96% similar in inter-genic regions, isolate and characterize corresponding BAC clones and use FISH analysis to test for segmental duplications (on the same chromosome) or a hallmark of a genome duplication (on different chromosomes).
- ii) Examine all genes in duplicated genomic regions (existing plus new regions) for changes in rates of evolution among duplicates (Ka/Ks ratios), determine frequency of gene loss, and identify levels of altered transcription in tissues and

developmental life stages. This will enable us to more accurately identify patterns of duplicate death, dosage effects, rates of gene evolution and possible sub/neo-functionalization.

- iii) Use the total EST data from rainbow trout, chinook, sockeye, brook trout, lake whitefish, grayling, northern pike and rainbow smelt to; a) build contigs and consensus transcript sequences from all species b) identify bins of similar transcripts, c) for each bin, generate common alignments and identify largest common alignment regions, d) for each bin generate phylogenies and analyze them for species relationships and gene duplications, e) examine common patterns that support or refute the ancestral salmonid duplication hypothesis. The use of species relationships will enable us to clearly identify gene duplications in ancestral salmonids and those occurring more recently.
- iv) Continue building a full-length salmonid gene database. This will be done using

a combination of EST assemblies and sequenced full-length cDNA clones. Efforts to identify 8,000–10,000 full-length cDNAs will need to expand and the results made available for all salmonid researchers.

4. Repeated Regions

A second puzzling observation arises from an analysis of salmonid transposons. We initially hypothesized that subsequent to an ancestral salmonid genome duplication and during the rediploidization process, that we would expect an increase in transposable element (TE) activity to facilitate the restabilization of the salmonid genome back to a diploid state required for successful cell replication. Transposable elements (TEs) are sequences capable of integrating into new sites within the genome and are classified into retrotransposons (use RNA intermediates and reverse transcription) or transposons (no reverse transcription). TEs can alter or disrupt gene expression depending on specific insertion locations. Several observations that have been made on TEs specifically in fish are: i) the diversity of TEs is higher in fish than mammals; ii) there tends to be a higher turnover of TEs in teleosts; and iii) TEs tend to be localized especially to heterochromatic areas of the chromosomes. It was hypothesized that subsequent to an ancestral salmonid genome duplication, during the rediploidization process, that there would be an increase in TE activity to facilitate the restabilization of the salmonid genome. For non-coding TE DNA, this would correspond to sequence divergence values greater than that between salmon/trout and whitefish (~8%). While the specific role of transposable elements in speciation, genome duplication and subsequent genome restabilization remains uncertain (Kazazian 2004; Sverdlov 2000) there is no doubt that TEs can be very important drivers of genome evolution. Certainly the enormous impact of

TE's is evident in the more than 30% composition of the total human genome.

When we did a phylogenetic comparison of several hundred TEs (avg. 1.5 kb in length and consisting of over 30% of the 7 MB of genomic DNA thus far analyzed; Fig. 3), we found that one of the waves of TE activity (comprising ~one third of the total) roughly corresponded to the time of the *Salmo/Oncorhynchus* speciation period and another wave (again one third of the total) seems to represent very recent and ongoing TE activity (de Boer *et al.* 2007). the majority of new TE families correspond to recent waves of activity (~6–8% and 2–4% divergence) that correspond to times and levels of divergence seen among salmonid species. We had expected the majority of transposon activity to have occurred immediately after an ancestral genome duplication and during a restabilization period (or and estimated 10–20% divergence). These observations also raise an intriguing question. As transposons are both an indicator and facilitator of extensive genome-wide changes, why do so few transposon duplications appear to occur pre-salmonid speciation during which a purported genome duplication and subsequent rediploidization/restabilization process occurred?

What we see from our initial data is that one of the waves of TE activity roughly corresponds to the time of the *Salmo/Oncorhynchus* speciation period (corresponding to 94% identity or 6% difference) and another wave seems to represent extensive ongoing TE activity. It is not clear whether the observed TE activity is associated with speciation processes, or is involved in ongoing restabilization efforts to eliminate occasional tetravalent structures evident during cell division. What we need to do is to obtain better estimates of when and where bursts of TE activity occurred by expanding the number of Tc1-like elements in not only Atlantic salmon but also other salmonid species. We also need to test whether the same

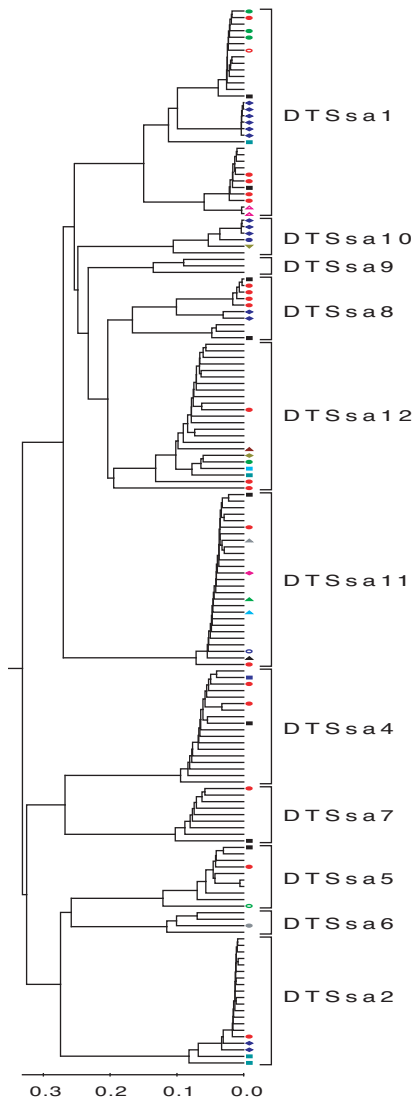


Fig. 3. Recent ancestry of salmonid transposons. Salmon DNA transposons, SALT1 and Tss sequences, transposons obtained from approximately 5 Mbp of in-house *Salmo salar* BAC clone sequences and sequences recovered from GenBank searches were aligned and a UPGMA phylogenetic tree was generated. The absence of a marker on terminal branch links indicates *Salmo salar* repeat elements and colored markers indicated repeats from other fish species (see de Boer *et al.* 2007 for details). Distances (%) are indicated by the bottom bar. Figure modified from deBoer *et al.* (2007).

pattern is found in other repeat families. Initial observations from SINE and LINE elements suggest a similar pattern. One of the benefits of these studies will be a repeat library that will be essential in genome sequence analysis and resolution of repeated regions.

Still another intriguing result comes about from the identification of *Xenopus*, catfish, and lamprey Tc1 transposons all within genomic BAC sequences that show 95–99% similarity to *Salmo salar* Tc1 transposons (de Boer *et al.* 2007). Additional GenBank searches further identified >97% identity to several partial (480 bp) Tc1-like transposons in EST clones of *Schistosoma japonicum* (Melamed *et al.* 2004). This exciting observation strongly suggests that there may be horizontal transfer of these elements. Testing this idea will require confirming the initial observations by obtaining much broader TE sequence representation in salmonids as well as different fish species. Extensive phylogenetic studies will be needed to determine the spread and timing of elements within species. In addition, two open reading frames with high similarity to transposase have been found, so we will try to confirm RNA and protein expression. This will be very important in the further development of vertebrate gene transfer biotechnologies, like the Sleeping Beauty vector (Wadman *et al.* 2005). The potential for horizontal DNA transfer between frogs, lampreys, catfish and salmonids is particularly intriguing in developing our understanding the role of repeat elements in times and regions of genomic stress.

5. Discussion

Salmon, trout and charr comprise a group of fish that are of great economic and societal importance to coastal, rural and aboriginal communities of many northern countries. Although Atlantic salmon is the main aquaculture species, there are also vibrant

commercial fisheries for wild salmonids. As salmonid aquaculture continues to develop and expand, it must find ways of minimizing its impact on wild fisheries and the environment. There is a real need for domesticated broodstocks that maximize disease resistance, optimize adaptation to local environments and minimize escape viability and impact on wild populations. A better understanding of how natural populations of salmonids adapt to local conditions will benefit agencies that have to make management decisions concerning stock assessment and harvesting plans. The aquaculture industry and enhancement schemes can also take advantage of this knowledge so that all commercial activities relating to salmonids can develop in a complementary manner.

One of our objectives is to demonstrate the power of genomics to conduct scientifically exciting research that will yield practical benefits for salmonid production and provide sound advice for managing wild stocks and the environment. Expansion of existing genomic research efforts of Canada, Norway, the United States, Chile and the United Kingdom will: (i) expand genomic resources for Atlantic salmon and rainbow trout; (ii) extend genomic resources to other salmonids including, chinook, sockeye, whitefish, grayling, and brook charr; and (iii) use the existing and expanded genomic resources as tools to answer questions that are of biological, economic and social importance to aquaculture, conservation, and the environment.

Physical map resources, gene identification (ESTs) and BAC end sequencing provide the starting resources for many genomic activities. Genomic efforts have built a physical genomic framework for Atlantic salmon consisting of approximately 4,200 contigs (Ng *et al.* 2005), and have provided key BAC libraries for both Atlantic salmon, rainbow trout and rainbow smelt (cGRASP; Ng *et al.* 2005; von Schalburg *et al.* 2008a). Contig BAC end sequences and end sequences from more than 100,000 Atlantic salmon BACs

enable genome characterization, and provide resources for consolidating the physical map and integrating it with the linkage map. Completion of over 700,000 ESTs from Atlantic salmon, rainbow trout, chinook, sockeye, brook trout, Arctic whitefish, Arctic grayling, northern pike and rainbow smelt provide an excellent foundation for the identification of genes and polymorphic variation in genic regions (cGRASP). Atlantic salmon and rainbow trout have the 17th and 27th largest EST representation of any species to date (October, 2007). This foundation facilitates the identification of full-length coding sequences of genes. These resources provide an excellent gene representation for application tools such as microarrays, and provide tens of thousands of potential polymorphic markers for genetic maps. The distribution of more than 3,000 16K microarray (cGRASP; von Schalburg *et al.* 2008b) slides in the last year to over 40 laboratories around the world points to the impact of these resources on the fish community. These arrays facilitate unsurpassed assessment of gene expression of thousands of genes and provide whole new avenues of studying duplicated gene families. The completion of several megabases of finished genomic has enabled a more thorough understanding of key functions such as repeat families, immunity (MHC, TCR, IL-2) and growth (GH1, GH2) as well as suggesting candidate genes for sex determination and upper temperature tolerance.

At sequence divergences corresponding to a purported ancestral salmonid genome duplication we find fewer gene duplicates and less transposon activity than expected. Conversely, we find greater gene duplication and greater transposon activity at sequence divergences corresponding to salmonid speciation events and more recent times. Given that an ancestral salmonid genome duplication is assumed in thousands of independent studies and is considered a fundamental tenant in our current understanding of salmonid genetics, physiology and

biology, these two puzzling observations need to be carefully examined.

Genomic and EST sequences have also provided key insights into the nature of the genome duplication in salmonids. As a result, we now have a much better understanding of the enormous complexity and reorganization that occurred in salmonid genomes

during the rediploization process (e.g., extensive segmental duplication, transposons/ repeat element expansion). We also have a much better appreciation of its impact on the generation of new species and their variation in response to environmental conditions, pathogens and disease.

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A Tale of Two Initiatives: Integrated Coastal Management in Xiamen and Batangas Bay Region

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Two parallel initiatives on the application of Integrated Coastal Management approach in Xiamen (PR China) and the Batangas Bay Region (Philippines) for achieving sustainable coastal development were assessed in relation to Integrated Coastal Management (ICM) applicability, effectiveness, replicability and scaling up in two different political, socio-economic, ecological and cultural settings. The paper analyzed the conditions under which various policy and management interventions were made and how they contributed to policy reforms, integration of sectoral policies and interagency functions to reduce policy conflicts and overlapping responsibilities. The importance of stakeholder participation and creating an informed public are stressed in relation to strengthening political commitments and building enabling environment for management actions.

The results obtained over a span of 14 years suggest that the ICM approach is doable and effective in facilitating policy reforms, improving interagency and multisector coordination. ICM is also as efficient through its management framework, planning and implementing processes as well as its interagency, multi-stakeholders consultative platform for the implementation of various coastal programs. The paper draws conclusions on coastal governance from the lessons learned and identifies essential elements that are instrumental to effective implementation of the ICM program as well as room for improvement.

Finally, the paper describes current efforts in codifying the ICM framework. Further, it outlines an improved ICM program development and implementation process using international standards for quality and environmental management and appropriate documentation through the use of a “State of the Coasts” reporting format. A complete ICM system has now evolved, the application of which enables one to document the required processes and actions so that the outputs and status can be effectively measured and assessed against its set objectives.

KEYWORDS integrated coastal management; coastal governance; sustainable development; coastal planning; coastal zoning

1. Introduction

Integrated Coastal Management (ICM) is defined as “a natural resource and environmental management system which employs an integrative, holistic management approach and an interactive planning process in addressing the complex management issues of the coastal areas” (Clark 1996; Cicin-Sain and Knecht 1998; Chua 2008). ICM integrates planning and management of the watershed, river basin and coastal waters; addresses the consequence of interactions between the ecosystems and human activities on land and sea; administers policy and management actions that would balance human activities and protection of the functional integrity of the terrestrial and marine ecosystems.

In a broad sense, the ultimate goal of ICM is sustainable coastal development. Achieving ICM is a long and complicated process but is necessary considering that over half the world population will be located in urban coastal centers by 2015. Pressure from human activities (such as human settlement, food supply, water use, sanitation, livelihood and other socioeconomic activities) on coastal ecosystems will increase as a result of coastal population movement (Chua 2004, 2006).

2. Initiatives at the Local Level

In 1993, both Xiamen and the Batangas Bay Region were selected as ICM demonstration sites (Fig. 1) under a regional programme co-financed by the Global Environmental Facility (GEF). The sites were to test and verify the effectiveness of the ICM approach and potential for ICM demonstration.

The two ICM initiatives being implemented by the local government of Xiamen (PR China) and that of Batangas Province (Philippines) are guided by the ICM Project Development and Implementation Cycle (commonly known as the ICM cycle), which consists of a cyclical process (Fig. 2) involving various stages of planning and implementation of an ICM program: preparing, initiating, developing, adopting, implementing, refining and consolidating (Chua and Scura 1992; Chua 2006, 2008). Both are areas where tiny fishing villages once abound. They share similar development and management problems during the early stages of the ICM initiatives: (a) both became progressively urbanized and were (and still are) national centers for industrial development; both local governments placed great efforts to attract international investment to boost a diversified economy, however, less effort was paid on environmental concerns; (b) the per capita income of both areas compared to their national average were rated as moderate, but the pressures for economic development



Fig. 1. Location of the two PEMSEA ICM demonstration sites.

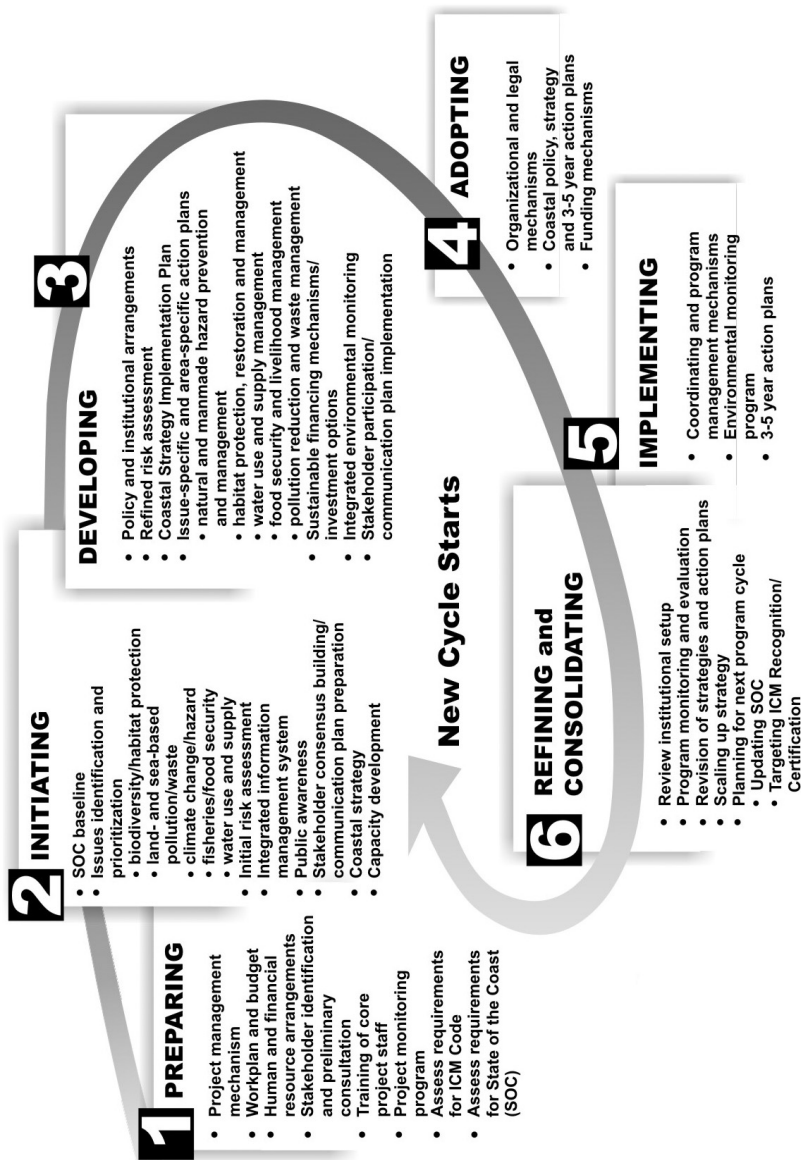


Fig. 2. ICM project development and implementation cycle (Source: Chua 2008).

were high; (c) both practiced sectoral planning (e.g. fisheries, transport, mining, port and harbor), mostly on land use and very little or none on sea-use; (d) their line agencies (e.g. transport, fisheries, mining, environment, enforcement, planning) had (and still have) overlapping functions related to coastal management; (e) extensive multiple uses of coastal lowland and adjacent waters began to create negative environmental impacts and posed serious threats to habitats and natural resources.

However there are differences between the two areas, namely:

1. They are operating in different political, social and cultural systems. Xiamen operates in a socialist system—centrally controlled, but with certain degree of autonomy as one of the five autonomous economic centers of China. Batangas, on the other hand, is democratic, with a high degree of decentralization.
2. The governance structure in Xiamen consists of a government machinery led by the mayor and a party machinery led by a Communist Party Secretary. Government operation must be led by Party principles and decisions. The mayor is generally a Deputy Secretary of the Party. In the case of Batangas Province, the elected governor is the administrative head of the local government and has the legislative right to make decisions based on the Local Government Code. Municipalities and cities are headed by elected mayors.
3. Religious influence in Batangas Province is predominantly Roman Catholic, while there is no religious influence in Xiamen.

The ICM projects were launched in early 1994. Each project was able to complete the first ICM Development and Implementation Cycle in about six years. Essential information on the elements of coastal governance (policy, strategic action programs, legislation, institutional arrangement, financing,

stakeholder consultation, information management and capacity development) were acquired and ICM program formulated. During the first ICM cycle, GEF's contribution to total project funds was about half to two-thirds of the total amount allocated for the two projects. The remaining amount came from the local governments and counterpart contributions from the private sector, as in the case of Batangas Bay.

From 2000 to 2007, a second program cycle was started to implement activities identified in their action plans. In this stage, the local government covered almost 90 percent of the total costs while GEF's contribution was used mainly to support incremental cost such as preparation of case studies and to support the development of ICM training centers.. A third cycle will begin in 2008 and focuses on geographical and functional scaling up of ICM practices.

3. Assessment of Key Activities and Achievements

The ICM initiatives at both study sites were able to produce the needed outputs as determined by each stage of the ICM program development process such as coastal strategy, issue- or site-specific action plans, communication plans, risk assessment reports, ICM policy, local ordinances, etc. The timely delivery and quality of outputs, however, are dependent on available technical and management capacity of the local governments. Both sites were able to secure the needed expertise from national and local universities and research institutions (PEMSEA 2006a, b; Chua 2006).

While the initiatives in the two sites differed in terms of level of inputs (such as budget, time, expertise), delivery of outputs (e.g. governance framework, action plans, studies), effectiveness of enforcement of action plans and measurable impacts, they did share the same vision for sustainable development and environmental concerns, as

well as the approaches and strategies. More importantly, they were able to follow the same path (stages of ICM cycle) towards sustainable coastal development amid political, socioeconomic and ecological differences and complexities. These initiatives essentially tell the same tale.

Assessment of the key activities and accomplishments related to coastal governance and the implementation of strategic action plans of the two initiatives are given in Tables 1 and 2 respectively.

3.1. Implementing activities to strengthen coastal governance

Under the context of governance, key activities undertaken at the two sites (Table 1) focused on: (a) creating a shared vision to build and synergize interagency and stakeholder cooperation for policy and management priority; (b) formulating local coastal sustainable development policy that could streamline sectoral policies (such as fisheries, marine transport, port, tourism) in line with the sustainable development principles; (c) establishing a coordinating mechanism for integrated planning and management; (d) creating enabling legislation to strengthen law enforcement; (e) promoting stakeholder involvement and participation within and across sectors to reduce interagency and sectoral conflicts; (f) reducing multiple use conflicts especially through science-based sea-use zoning plans and permit systems; (g) sharing database and information within and across sectors to increase management effectiveness and cost-efficiency; (h) creating an informed public to exert public pressure against policy or management decisions harmful to the environment; (i) integrating capacity development into ICM development process through encouraging horizontal and vertical learning so as to prepare a critical mass of local expertise; (j) financing and sustaining the ICM

program using local resources by integrating ICM into the local economic development agenda and utilizing bilateral and multilateral assistance for large environmental improvement facilities (Chua 2006).

Significant progress has been made at both sites in the abovementioned activities related to governance. However, there are considerable policy and management deficiencies that need further improvement including those related to coastal reclamation, transboundary pollution control, response to climate change, effective implementation of international instruments, and local and national policy integration and coordination, as well as translating political commitments into financial allocation for management actions.

3.2. Implementing a long-term coastal strategy and action plans

Long-term strategic environmental management plans have been developed for both sites as part of the ICM initiatives (ITTXDP 1996; PG-ENRO 1996). The strategic plans normally considered a timeframe of at least 25 years and are guided by the shared vision. The plans were developed after a thorough profiling of the coastal areas, identification of key issues, risk assessments and extensive consultation with stakeholders to reach consensus on the priority areas for actions. The strategic action plans for Xiamen and Batangas Bay Region are being implemented by the two local governments although at different scales and speed (Table 2). The biggest challenge to both local governments, however, is to implement issue or site-specific action plans which require substantive financial and human resources.

Key activities undertaken by the two sites during the last 14 years, include implementing a waste management plan to address solid, hazardous, industrial and domestic wastes. The waste management plans have

Table 1. Assessment of the performance of ICM implementation related to the key objectives under the governance component of the ICM program.

	Xiamen, PR China	Batangas Bay Region, Philippines
Background: The need for ICM initiatives	Population growth from 1.25 m (1995) to 2.25 m (2005) largely due to migration from rural areas; designated as one of five national Special Economic Zones; GDP (1995) 25 billion RMB; 88 billion RMB (2004); rapid economic transformation from primary sector (fisheries and agriculture-based) to secondary and tertiary sector development (manufacturing and services); multiple use conflicts intensified, environmental damage severe; policy and management deficiency and lack of managerial capacity hinder sustainable development. Initiate ICM practice in 1994 till present.	Population: 0.825 m (1995), 0.964 m (2000) with annual birth rate of 2.2–3%; GDP (national): \$4200 (2002); one of key national economic development area: the Calabarzon. Transformation from primary sector (fisheries, agriculture) to mainly secondary sector (manufacturing industries, port, shipping); multiple use conflicts intensify, pollution and ecosystem damage severe; policy and management deficiency, poor enforcement and inadequate local technical, financial and management capacity threaten sustainable development. Initiate ICM practice in 1994 till present.
Creating a shared vision and a stakeholders' platform	Established shared vision: modernized, industrialized, port and garden city; interagency consultation mechanism established; inadequate consultation with other stakeholders	Established shared vision: environmentally sound port and maritime economy; interagency, multisectoral consultative mechanism established; periodic meetings of stakeholders
Formulating an ICM policy	• Local policy with clear direction; • Integrated sea use into land-use planning; • Strong political will and financial commitment secured; • Inadequate policy direction on land reclamation	• Local ordinance to implement ICM secured; • Moderate political will; • Financial commitment limited; • Inadequate policy on land reclamation, sea-use zoning scheme, fisheries management
Establishing a coordinating mechanism	• Legally instituted interagency coordinating mechanism established with an interdisciplinary expert group; • Chaired by mayor with concerned agencies as vice chairs and membership composition with representation from concerned agencies and committees;	• Initial Batangas Bay Coastal Resource Management Council reorganized into a Batangas Bay ICM Board, supported by a technical working group; • Each municipality forms its own Municipal ICM Board, chaired by its own mayors;

	• Undergone transformation in Phase II, membership reduced from 22 to 14 to increase efficiency and effectiveness; • PMO: Marine and Fisheries Bureau.	• New Batangas Environmental Protection Council established, with the provincial governor serving as chair; • BEPC directly linked with the Provincial Development Council so that environmental concerns and decisions can receive priority consideration by the PDC. The three-tiered organizational structure enables coordination of ICM activities at all levels of government.
Enabling legislation to strengthen enforcement	• Several environment and marine-related legislation and ordinances enacted by the Xiamen Municipal Government • Integrated law enforcement mechanism established, with law enforcers from major marine-related law enforcing agencies This is effective in reducing conflicts between enforcing agencies, removal of cages and rafts clogging the navigational channel and implementation of the sea-use zoning scheme.	• Modification or refinement of existing ordinances made at provincial and municipality level • Law enforcement requires stronger political will and capacity of the enforcing agencies to effectively regulate commercial trawling in municipal waters, illegal fishing, pollution from small and medium industries, and solid waste disposal.
Promoting stakeholder involvement and participation	• Scientific advice through the Interdisciplinary Expert Group but not the citizenry; • NGOs do exist but mostly in the form of government-controlled specialized associations; and • Public awareness raised through media and education.	• Public hearings done for major infrastructure projects; • Stakeholders represented in various ICM board; and • Stakeholder consultation appropriately organized.
Reducing multiple use conflicts through sea-use zoning scheme	• Sea-use zoning scheme established with appropriate legislation; • Effectively resolved conflicts between raft and cage culture and ship movements in the navigational channel, provided greater protection for endangered species by establishing conservation zone; • Effectively stopped sand mining and rehabilitation of a public swimming beach, removal of heavy industry outside the city limit.	• The zoning scheme ensures a clear navigational route for vessels entering and departing the Batangas port and oil refinery terminals; • The marine protected area in the Municipality of Mabini reduces competing use by law; • Zoning scheme has no legal personality.

Table 1. (cont.)

	Xiamen, PR China	Batangas Bay Region, Philippines
Sharing of database and information	• Integrated Information Management System for Coastal and Marine Environment (IIMS) established; • Integrated environmental quality monitoring program established to share data with participating agencies.	• IIMS established and data enriched through new sources; • Managed by the environmental office (ENRO) of the province.
Creating an informed public	• Media plays a major role in keeping the public aware and informed through local newspapers, radios and television; • ICM well advertised through research and educational institutions; • Beach cleanup campaigns.	• Regular radio program, programs on ICM on national television, features in newspapers; • Beach cleanup and tree planting campaigns; • Effective community platform.
Developing capacity through horizontal learning	The diversity of activities developed and implemented under an ICM program provides ample opportunity for the participating stakeholders to learn from each other. Wide range of topics under ICM include policy reforms, integrated planning, risk assessment, coastal zoning, legislation and ordinances, economic instruments, natural resource valuation, social analysis, financing sustainability, information management. Such informal learning through the ICM program has proven effective in developing a pool of local technical and management expertise.	
Financing an ICM program	• Regular city budget; • Substantial collection of permit fees through sea-use zoning scheme implementation has strengthened the financial position of the Bureau of Marine and Fisheries which is the ICM coordinating body; • BOT and BOO initiated through for infrastructure development, and public-private sector partnership (PPP) explored.	• Regular budget from provincial government; with contribution from the Batangas Bay Coastal Resource Foundation; • User fees collected from divers and tourists to the Mabini coral sanctuary helps to sustain local ICM program; • BOT and BOO explored, PPP tried but failed.

Sources: Chua 2006; ITTXDP 1996; MPP-EAS 1996; PEMSEA 2006a, b, c, 2007; PG-ENRO 1996

Table 2. Assessment of implementation of the strategic action plans of the two ICM initiatives.

	Xiamen, PR China	Batangas Bay Region, Philippines
Implementing a long-term coastal strategy and action plans	Long-term Xiamen Strategic Environmental Management Plans formulated; adopted by city government and being implemented; revision made for Phase II.	Batangas Bay Environmental Management Plans being implemented; revision made for Phase II.
Implementing waste management plan	Successful in reducing pollutant load into the coastal water arising from agriculture, industries and domestic wastes. • By 2003, seven sewage treatment facilities were established. The city was able to treat 72.8% of the 140.76 million tons of sewage. • Most industrial wastewaters are being treated, with more than 93% of industrial wastewater discharged meeting the government water quality control standard. • Established sanitary landfills which are able to accommodate more than 90% of solid wastes.	Despite the integrated waste management plan, the scorecard has yet to be improved. • Most solid wastes are disposed in open dumps despite national regulation to close all dumpsites two years ago; • Waste recycling is widely practiced and greatly promoted; • No sewage treatment facilities; • No effective controls over agricultural waste discharge. • Industrial wastes are comparatively better managed. Most large industries are equipped with treatment facilities and oil terminals with oil reception facilities; small- and medium-sized industries have difficulty complying.
Implementing oil spill preparedness and response plan	• Appropriate oil spill preparedness and response plan is available and implemented by the Maritime Transport Bureau and the Xiamen Port Authority; • No major spills reported.	• Concerned oil companies have developed a tier-one response plan equipped with the appropriate booms and skimmers; • Regular exercises held in close cooperation with the coast guard and the Batangas Port Authority; • Readiness in addressing accidental spills; • No major spills reported.
Implementing a red tide response plan	Frequency of red tide occurrence has been greatly reduced. This is attributed by experts to the decrease in nutrient discharge due to sewage treatment. Red tide monitoring and surveillance is part of the seawater quality monitoring implemented through integrated water quality monitoring plan.	No red tide occurrence has been reported.

Table 2. (cont.)

	Xiamen, PR China	Batangas Bay Region, Philippines
Implementing conservation, ecosystem protection and restoration plan	• Rehabilitation of mangroves areas extended; conservation zone for lancelets and egrets; • Control over cruising speeds of vessels into the navigational channel where the endangered white dolphins are found; • Extensive landscaping has greatly increased vegetation cover.	• Efforts made in rehabilitating mangroves; stopping conversion of mangroves into fish ponds, and establishment of network of marine protected areas; • Vegetation cover is comparatively higher, as the rate of urbanization is comparatively slower than that of Xiamen.
Providing basic water services	While Xiamen is located in Southern China and has access to river water supply, the rate of population increased from 1.2 m in 1994 to 2 m in 2007 making freshwater supply an increasing threat to city development.	Despite being located in a tropical rainforest region and despite being fortunate to have plenty of freshwater sources, increasing contamination of river basins and overexploitation of underground water will soon pose a limit to province's future expansion. Long-term planning on water supply and use has yet to be developed.
Integrating fisheries and aquaculture management into ICM program	• Despite the lucrative market for fish and fishery products due to population increase and improved living standards in the city, fishing in Xiamen coastal waters has been increasingly difficult due to competing use conflicts. The once extensive eel fry gathering practice had to give way to the development of port and maritime transport. The western channel, once filled with fish cages, and oyster and mussel farms, is now cleared of these. More than 5,000 fishfarming households were either moved to new zoning sites or compensated by the city government to abandon the farming practice.	Fishing is no longer a lucrative industry and is limited to small-scale subsistence fishing in nearby coastal waters. As Batangas is getting more industrialized with increased varieties of industrial development along its bay areas, fisherfolks find it hard to compete with other maritime activities. Like Xiamen, Batangas Bay Region was once known for its small fishing "barangays" (villages). These are now gradually disappearing, being transformed as a result of urbanization-an unavoidable trend.

• With the aquaculture zoning scheme, a large area of water space has been allocated for the development of mariculture. A permit system is required to use this water space, allowing for orderly development of the industry and better environmental quality for farm operation. Higher demand for live food fish has considerably increased the income of fishfarmers. • Much effort has been made in open stocking of the commercially important finfish and shrimp fry to augment existing stocks. The Marine and Fisheries Bureau was able to breed some endangered species, such as horseshoe crab, for release in open waters to enhance fish stocks.	Managing natural disasters City government was able to reduce the adverse impacts of typhoons to very low fatality through a comprehensive risk reduction disaster management plan.	Natural disaster preparedness and response plan, including typhoons and earthquake
Sustaining livelihoods of coastal poor The persistent GDP growth has provided ample opportunity for employment, greatly contributing to Xiamen's ability to transform many poverty-stricken rural areas into modern towns and districts. Although poverty reduction in Xiamen is the result of government economic policy and political will of the local government to implement it, the application of ICM helps to reduce much of the adverse impacts of economic development.	The Provincial Government and the Batangas Bay Coastal Resource Foundation implemented livelihood projects to increase livelihood opportunities in coastal communities especially for the coastal poor.	

Sources: Chua 2006; ITTXDP 1996; MPP-EAS 1996; PEMSEA 2006a, b, c, 2007; PG-ENRO 1996.

had with varying degrees of success (Table 2, PEMSEA 2006b, c). While Xiamen had been rather successful in treating most of its wastes, it still faces considerable challenges in addressing non-point sources of pollution especially effluents arising from upstream and agricultural wastes. Batangas Bay Region, on the other hand, has yet to present a good scorecard for most of its solid wastes, agricultural and industrial wastes have yet to be effectively treated and monitored despite having a well-prepared comprehensive integrated waste management plan (MPP-EAS 1996).

Extra efforts have been placed in ensuring an efficient oil spill preparedness and response system as both port and shipping are instrumental to Batangas and Xiamen economy. In terms of the impacts of harmful algal blooms (or red tide), the efforts of Xiamen in reducing nutrient loads into the coastal water had been reported to be effective in reducing the frequency of red tides (PEMSEA 2006a, c).

Significant progress in implementing conservation, ecosystem protection and restoration plans have been made at both sites (Table 2). Neither site has placed sufficient attention on freshwater resource management, freshwater use and provision of water services. Freshwater services were initially not included in the ICM initiatives. The fact that freshwater use and supply issues were not identified as priority areas of concern site during the environmental profiling stage reflect: (a) the wrong impression that freshwater is always plenty in areas within tropical rainforests or near river basins; and (b) ignorance of the fast depletion of potable freshwater due to contamination and overextraction.

Integrating fisheries and aquaculture management into ICM programs is an important strategy for addressing: (a) the increasing transformation of rural coastal towns into urban centers; (b) increasing fish consumption and change in consumption

patterns and use in urban areas; (c) rapid deterioration of coastal waters near urban centers which might rendering aquaculture products less healthy for human consumption; and (d) overexploitation of fish stock in coastal waters. In most countries in Asia, fish consumption will increase in urban areas as rural people from the coasts move to settle in urban towns and cities. The consumption and use patterns of urban people are also different from the rural areas due to higher standard of living. However, aquaculture products (e.g. oysters and mussels) from nutrient-rich water in coastal bays and estuaries might be contaminated by bacteria and or accumulated high concentration of pesticides which render aquaculture products unfit for human consumption. The changing conditions described above require a paradigm shift in fishery management approach as coastal fishery management are directly related to changes and trends in coastal development and most of the management issues fall outside the fisheries sector. Fisheries, in the context of urban development in Xiamen and the Batangas Bay Region, had been marginalized! Thus it was an essential consideration to include impacts on the livelihoods of fishers and coastal communities in the equation of economic development for these two coastal areas.

Although natural and human-made disasters have been identified during the profiling stage, they were not considered as a major role of an ICM program and as such there were no concerted efforts on hazards management in the early phase despite the frequency of typhoons and earthquakes in the region. The 2004 Indian Ocean tsunami and the IPCC report on climate change have prompted the sites to take more proactive efforts. However, each hazard is being addressed individually, making it difficult for local governments to effectively cope with various hazards, both natural and man-made. An integrated natural and human-made disaster response and management plan is now being developed.

4. Conclusions Drawn from the Two Initiatives

The two ICM initiatives helped answer several questions and provided important conclusions that are instrumental to the sustainability of the ICM approach and the replication and scaling up of ICM practices (Chua 2006).

1) ICM approach is effective and ICM practices can be sustained locally.

The two ICM initiatives clearly tell a single story that the ICM approach has proven to be feasible in different political, socioeconomic and ecological conditions. They tell us that the ICM framework can: (a) allow local implementation of the ICM program within a geographically defined, administrative boundary to institute policy and management changes; (b) create a common stakeholder platform; and (c) operate through an interactive process that generates incremental management gains, the accumulation of which can lead to economic and environmental sustainability. The two initiatives have proven to be sustainable as the operation of ICM practices had been based largely on self-financing by the local governments. The two initiatives have provided testimony that ICM works both conceptually and practically, although the pace and level of achievements may vary according to the local political and socioeconomic conditions, as well as on local technical and management capacity.

Despite the lack of a legal personality, both ICM initiatives has survived several changes of local administration. Over a span of 14 years, local administration changed three times in Xiamen and four times in the Batangas Bay Region. The change of mayors and governors who head the ICM initiatives and who may come from different political affiliations, as in the case of Batangas Province, did not seem to deter the normal operations of the ICM activities.

2) Successful ICM has strong influence on national policy and strategies on coastal governance.

Implications of the two successful ICM initiatives have a strong bearing on the policy of the central government. The experience and success of the sea-use zoning system in Xiamen strengthened central government resolve to implement a nation-wide sea-use zoning program in China (PEMSEA 2006a). By 1997 a national sea-space utilization law was passed mandating all provincial, municipality, city and district governments to develop sea-use zoning plans. The Batangas Bay Region experience, however, has led to the scaling up of the ICM project in the Batangas Province and the signing of Executive Order 533 by the President of the Philippines, making ICM a national strategy. E.O. 533 also mandates the development of a national ICM program plan.

The two ICM initiatives serve as working models for national implementation of ICM programs in the countries, thereby contributing to replication and scaling up. Learning from the do's and don'ts helps in saving time, effort and resources.

3) The ICM Framework and process provide the essential scope and consultative platform for interactions with stakeholders.

The broadbased ICM framework enables coastal managers to develop a governance framework which promote policy reforms, build coordinating mechanism and enact local legislation or ordinances. The ICM framework also provides a stakeholder consultative platform that enable stakeholders to identify and prioritize environmental and sustainable development issues, understand the implication of government policy, and contribute to the development of action plans. Built into the long-term strategy is a system of information gathering and management so that stakeholders can utilize the available information for developing and executing their plans of action.

Of equal significance is the evidence of an integrated planning and management process that allows a systematic, interactive and incremental approach through a stepwise process of analyzing the status, identifying pressures, determining causes, selecting options and mobilizing interagency, intersectoral and interdisciplinary efforts for management interventions.

- 4) *Despite the availability of a broad ICM framework and process, the lack of standardization and documentation weakens timely delivery of outputs and measurable impacts.*

The current ICM approach and practices have no quality assurance in terms of meeting standard requirements pertaining to governance or environmental management. Successful application very much depends on the intuition and experience of coastal managers. Although adaptive management allows management flexibility, the inadequacies in quality assurance in the current ICM framework and process would continue to weaken the effectiveness of ICM practices and therefore slow down the process of replication and scaling up.

With the exception of Xiamen, current ICM initiatives tend to focus more on the development of the governance framework than on on-the-ground actions. This has resulted in lack of visible outcomes. The downside of these initiatives is that it is difficult to hold the interest and enthusiasm of stakeholders.

During the first phase of ICM in Xiamen, some on-the-ground actions have already taken place such as the Yuandang Lagoon cleanup, the removal of heavy industries in Gulangyu (Gulang Island), the reconstruction of the coastal ring road, the restoration of the public beach and the landscaping of the city.

The scope of activities of an ICM program should not only include the clear definition of a governance framework, but should also undertake implementation of

some strategic action programs within the first program cycle.

5. Codification of ICM

The outputs and outcomes of the two ICM initiatives (Tables 1 and 2) suggest the need to strengthen current ICM concepts and practices by: (a) consolidating the current elements of governance into a broad but comprehensive coastal governance framework to ensure these major elements are being included in all future ICM program development; (b) identifying key actions that are normally required in most coastal management for addressing commonly met issues; (c) codifying ICM planning and implementation using available international standards to ensure quality management; (d) designing a documentation and reporting format that enables periodic monitoring of outputs and outcomes.

In a recent review of ICM concept and practices, Chua (2008) proposed a governance framework that includes six areas of concerns (see below). Governance framework and strategic action programs are two major components that must be carefully and thoroughly addressed in all ICM programs (Chua 2008). The essential elements of both the framework and strategic action programs form the core of the ICM Code being developed by the Partnership in Environmental Management for the Seas of East Asia (PEMSEA 2007). However, adaptive management (an management approach that takes into consideration of scientific uncertainties and incomplete information) still remains a major fundamental principle of sustainable development and continues to play a key role in shaping ICM program implementation (Imperial and Hennessey 1993; Chua 2006).

5.1. Governance

The major elements of the governance framework for ICM closely match the basic requirements for quality management under

ISO 9001 standards (ISO 2005; Chua 2006). These elements include: (a) policy, strategies and action plans; (b) coordinating mechanisms; (c) legislation; (d) financial sustainability; (e) information management and dissemination; and (f) capacity development. A description of these six elements are given in PEMSEA (2007) and Chua (2008).

5.2. Strategic action programs

Almost all coastal areas have to address a host of common issues that affect biodiversity, water supply, food security and livelihood of the coastal poor. These issues are interrelated but should be addressed through a set of strategic management programs through which common root causes, such as poverty, policy deficiency and capacity disparity (in terms of financial and human resources), can be addressed under the governance framework. Broad areas of concern such as risk management, habitat protection, pollution, water use, etc., that must be addressed, though not necessarily simultaneous. These five strategic action programs arising from essential management needs of local governments are: (a) natural and human-made hazards prevention, response and management; (b) natural habitat and cultural heritage protection, restoration and management; (c) water-use and supply management; (d) food security and livelihood management; and (e) pollution reduction and waste management. Details on each program are given in PEMSEA (2007) and Chua (2008). The implementation of each strategic action program can be measured and evaluated against international standards of environmental management using ISO 14001.

5.3. ICM Code

The Code is designed to ensure that future ICM practices can follow a standard code of practice that can help achieve sustainable

development goals. Implementation of the Code also ensures a common standard and criteria at which progress can be measured and evaluated. Compliance to the Code is on a voluntary basis. Thus the Code has no legal personality unless it becomes obligatory by law.

5.4. Documentation and reporting

The State of the Coasts reporting is a new PEMSEA initiative designed to measure on a regular basis the changing conditions of the coastal areas brought about by economic development and environmental management measures (PEMSEA 2007). The purpose is to improve the process of documentation of management interventions and ecosystem responses. The SOC documents existing conditions and new interventions, and tracks changes in trends.

With the implementation of the SOC, information pertaining to severity of the environmental conditions or the lack or inadequacies of policy or management measures can be easily detected.

6. The Way Forward

The two ICM initiatives have contributed to the evolution of the ICM concept and practices and have provided valuable information and insights for moving from a trial-and-error approach of coastal management to a systematic and codified ICM system. The implementation of the ICM Code will standardize ICM practices, impose mandatory documentation of the activities and process, and monitor progress and changes. The outcomes of such practices ensure not only accountability but also replicability and scaling up of ICM practices throughout the coastline; certainly a positive step towards achieving the goal of Agenda 21 (UNCED 1992).

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Age-validated Longevity of Fishes: Its Importance for Sustainable Fisheries

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Marine fisheries have spread to the deep-sea because the fishes in many of the shallow-water marine habitats have been overexploited. Many studies have found that deep-sea fishes generally grow slowly and can achieve long life spans; however, most age estimates have not been validated. Several methods for validating age and growth estimates have been developed and applied to deep-water and other long-lived organisms. These include lead-radium and bomb radiocarbon dating of calcified structures. Application of these techniques has usually validated high lifespan estimates, some in excess of a century. In this paper, we discuss the applications and limitations of these techniques for difficult-to-age fishes. Because many fishery management techniques focus on biomass or yield and not as much on life history stages of fished populations, we specifically focus on the relevance of age validation to fishery management of deep-dwelling fishes, especially relative to slow growth, late age at maturity, long life spans, and the relative contribution of big, old, fecund females (BOFFs) and maternal-age-dependent larval survival to future generations via lifetime fecundity. These life history traits make their populations more vulnerable to fishing mortality, thus emphasizing the need to accurately assess longevity in fishes.

KEYWORDS age validation; bomb radiocarbon; carbon-14 ($\Delta^{14}\text{C}$); deep-water fishes; fecundity; fishery management; growth; life history; life span; lead-radium dating; longevity; maternal-age-dependent larval survival; mortality; vulnerability

1. Introduction

Many marine fisheries are reported as overexploited on a global scale (Pauly *et al.* 2003). This overexploitation appears to have influenced the relative abundance of predatory fishes in some marine ecosystems (Pauly 1995; Worm *et al.* 2005) and may have upset the balance of some major ecosystems (Pauly and Christensen 1995; Pauly *et al.* 1998, 2002, 2003; Christensen *et al.* 2003; Frank *et al.* 2005). There is argument about the extent and the causes of these fishery declines and ecological impacts (Pauly 1995; Pauly *et al.* 1998, 2002, 2003; Myers *et al.* 2003; Myers and Worm 2003, 2005; Walters 2003; Hutchings and Reynolds 2004; Safina *et al.* 2005; Sibert *et al.* 2006; Worm *et al.* 2005; Hilborn 2007). However, it is certain that many near-shore fisheries have been so highly overexploited that fishing effort has moved further offshore and into deeper water (Clark 2001; Gordon 2001; Haedrich *et al.* 2001; Roberts 2002; Stockley *et al.* 2005).

Fishery modeling and management practices have primarily focused on stock assessments, which mainly use biomass and maximum (MSY) or optimum (OSY) sustainable yield, to attempt to determine the level of exploitation or sustainability of a given fishery (Hilborn and Walters 1992; Gallucci *et al.* 1996; Berkeley 2006). In some cases, numerical abundance and/or age composition were taken into consideration, but even age-structured analyses often make major assumptions about age-driven biological quantities. Because the complexities of population age-structure are poorly understood, dynamic parameters that are an important consideration must be modeled or assumed to be fixed (e.g., Methot 1990; Millar and Methot 2002). Stock assessments are now commonly performed, but the concept of sustainability is still very much in question (Millar and Methot 2002; Methot and Piner 2002; Ralston *et al.* 2003; Beamish *et al.* 2006; Longhurst 2006).

Fishing practices in many cases have caused the size (and presumably age) structure of many populations to be fished down (Mason 1998; Pauly *et al.* 1998; Worm *et al.* 2005; Harvey *et al.* 2006), and this may have seriously impacted long-lived fishes like chondrichthyans and those in the deep sea. In nearshore environments along the west coast of the U.S. there is evidence of significant shifts downward in the size composition of rockfish landings (Mason 1998). This fished-down size and age structure can have numerous impacts and a few of the most significant are further discussed here.

First, it can cause a loss of genetic diversity or heterozygosity (e.g., Hauser *et al.* 2002). In addition, some fishes are genetically structured at the regional and sub-regional scales, as was demonstrated for Patagonian toothfish (*Dissostichus eleginoides*; Rogers *et al.* 2006) and further described in otolith elemental analyses (Ashford *et al.* 2007), which influences how fishing might affect the genetic structure of populations. Stockley *et al.* (2005) demonstrated that the deep-sea black-spot sea bream (*Pagellus bogaraveo*), which was heavily fished in recent years due to depletion of shallow-water fish stocks, may have undergone a severe bottleneck in its genetic population structure; low genetic variability in its populations was documented due to the small population size that resulted from fishing.

Second, removing the larger individuals can favor slower-growing and earlier-maturing fishes (Kenchington 2002; Ziegler *et al.* 2007). Thus heritable differences in yield and life-history traits can be caused by selective fishing which has been demonstrated for fishes such as the northern cod (*Gadus morhua*; Novikov *et al.* 2002; Hutchings 2004; Olsen *et al.* 2004).

Third, there can also be long term ecological and evolutionary implications of size-specific fishing mortality, resulting in a "dynamic tug-of-war between natural and harvest selection" (Edeline *et al.* 2007) in

terms of size and age at first reproduction and size- and age-specific reproductive output (Conover and Munch 2002; Berkeley *et al.* 2004a; Bobko and Berkeley 2004; Berkeley 2006; de Roos *et al.* 2006; Walsh *et al.* 2006; Conover 2007; Jorgensen *et al.* 2007).

Fourth, there can be ecosystem effects (Frank *et al.* 2005; Scheffer *et al.* 2005; Worm *et al.* 2005) that can be manifested as loss of top carnivores and changes in trophic relationships of ecosystems. One management technique that can help alleviate this problem, at least on a local scale, is the implementation of Marine Protected Areas (MPAs; National Research Council 2001). These are being seriously considered and implemented in several places around the world, especially along the west coast of the United States (Parker *et al.* 2000). Berkeley (2006), however, pointed out that MPAs do not seriously consider the life histories of fished populations, except as sources of larvae for export from mature females.

Fifth, size-selective fishing has been estimated to cause a subsequent reduction in lifetime production, measured by O'Farrell and Botsford (2005, 2006a, b) as lifetime egg production (LEP) in rockfishes (Genus *Sebastes*; Family Scorpaenidae) and by Harvey (2005) as age-specific egg production. In addition, it can reduce survivorship of larvae from "fit" or well-adapted adults. It has been shown for some species that the larvae from these older females in some rockfishes are more numerous and robust, being stronger and more likely to survive (Love *et al.* 2002; Berkeley *et al.* 2004a, b; Bobko and Berkeley 2004; Berkeley 2006). And those larger, most likely older, females not only are often more fecund but they can also exhibit maternal-age-dependent increased larval survival. This has caused some authors to contend that fisheries, in general, need to "leave the big ones" (Birkeland and Dayton 2005).

2. The Importance of Age Validation in Estimating Longevity (Lifespan)

We propose here that the life history parameters longevity (length of life) and/or lifespan (average time between birth and death) are very important to consider when determining how to manage exploited populations. Long-lived fishes have often had their ages and life spans underestimated, causing fishery management policies to be less effective. This is not a new concept because the importance of age validation was strongly emphasized more than two decades ago by Beamish and MacFarlane (1983) and more recently by Campana (2001), among many other authors. However, many age, growth and longevity studies have not provided validated age estimates leading to the parameters determined in their studies.

Traditional age validation methods (mark-and recapture, laboratory rearing, or marginal increment analysis) are often difficult or impractical to use for long-lived or deep-sea fishes (Campana 2001). There was little hope for such applications to deepwater fishes until recent developments provided some promise for tagging deep-sea species *in situ*. This was pioneered by Starr *et al.* (2002) for eastern Pacific deep-water rockfishes, and used in other deep-sea applications (e.g. Sigurdsson *et al.* 2006) in deep water (~500–800 m) off Iceland on redfish (*Sebastes mentella*).

In lieu of such applications, a naturally occurring radioisotope pair found in calcified structures of fishes, radium-226 (^{226}Ra) and its daughter product lead-210 (^{210}Pb), can act as a built-in chronometer. In addition to this radiometric technique, atmospheric testing of thermonuclear devices in the 1950s and 1960s caused a rise in radiocarbon ($\Delta^{14}\text{C}$) and produced a globally distributed signal that diffused into the ocean surface worldwide (Broecker and Peng 1982).

In recent years, many of the age estimates for deep sea fishes have been reevaluated and

in many cases were deemed drastically greater than previously suspected. A number of these age estimates, ones that were often met with disbelief because of the great longevity estimates, have been validated through use of lead-radium and bomb radiocarbon dating, some of which also included tag-recapture and oxytetracycline from field studies (e.g., Beamish and McFarlane 1983; Kastle *et al.* 1994). Application of these radiometric methods has been successful for deep-sea fishes (e.g. Andrews *et al.* 1999, 2007) and this is especially true for a numerous rockfishes (Andrews *et al.* 2002, 2005, 2007; Bennett *et al.* 1982; Campana *et al.* 1990; Kastle *et al.* 2000; Kastle and Kimura 2006; Kerr *et al.* 2004, 2005; Piner *et al.* 2005; Stevens *et al.* 2004; Stransky *et al.* 2005; Watters *et al.* 2006). In some cases, complications in what appears to be a system that was not conserved with time provided circumstances that violated the necessary assumptions for successful application (e.g., cartilage of chondrichthyans and sturgeon; Burton *et al.* 1999; Welden *et al.* 1987). For otolith material, however, numerous other bony fishes have been validated using lead-radium and bomb radiocarbon dating (e.g., Fenton *et al.* 1991; Smith *et al.* 1991, 1995; Campana 1997; Andrews *et al.* 2001; Kalish *et al.* 2001; Ewing *et al.* 2007).

In this paper, we return to Beamish and McFarlane's (1983) plea for age validation to become a more common element in fish age and growth studies. In doing so, we will demonstrate the importance of age-validated longevity to fish population dynamics, especially in exploited conditions. We will also incorporate the results and concepts initiated by Berkeley *et al.* (2004a, b), Bobko and Berkeley (2004), and Berkeley (2006), in which they demonstrated the importance of larger, fecund female rockfishes (recently coined as "Big Old Fecund Females" or BOFFs), using the black rockfish (*Sebastes melanops*) aged 50 years old by counting rings in sectioned otoliths.

O'Farrell and Botsford (2005, 2006a, b) used size structure of the same species (*S. melanops*), but also included five other rockfish species to demonstrate the effect of size-selective fishing on LEP. They assumed that size classes represented age classes, but no age validation has been accomplished for these species. Similar studies have been done on rockfishes by Harvey (2005) and Harvey *et al.* (2006), assuming specific growth characteristics, not all of which were validated. Thus, it is important to note that the ages estimated or inferred from size-composition or otolith readings could leave some doubt about the reality of the BOFF and/or maternal-age-dependent larval survival, and perhaps the reduction in LEP. Hence, we emphasize use of age-validated species and that this perspective on age validation becomes more important when taking the previously stated factors into consideration.

3. The Importance of Lifetime Fecundity (Reproductive Output)

It is important to note that true reproductive output is a factor that can only be obtained by validating longevity or lifetime of a given species (Harvey 2005; O'Farrell and Botsford 2006a, b). In this paper, two terms will be used with regard to reproduction; fertility, the actual reproductive productivity or production of offspring, and fecundity, the physiological potential for reproduction or the age-specific state of being fertile. It appears that most fishes, including rockfishes, continue to reproduce after maturity, without evidence of senescence in all years following the age at first maturity (Cailliet *et al.* 2001). Because larger, and presumably older, fish often have higher fecundity and may have better adapted larvae, their lifetime fecundity becomes an important parameter in understanding and predicting population dynamics (Phillips 1964; Gunderson *et al.* 1980; Haldorson and Love 1991; Lea *et al.* 1999; Love *et al.* 1990, 2002; Berkeley

et al. 2004a, b; Bobko and Berkeley 2004; Harvey 2005; Berkeley 2006).

Summarized data on size-specific fecundity for rockfishes demonstrated that larger rockfishes typically produce more offspring (Love *et al.* 1990, 2002). Thus, fishing these larger size and older age classes heavily can truncate the number of reproductive years and thus reduce their ability to maintain viable populations. This has been demonstrated demographically by Ralston *et al.* (2003) for the shortbelly rockfish (*Sebastes jordani*) and by Ziegler *et al.* (2007) on the banded morwong (*Cheilodactylus spectabilis*). Also, environmental processes, like El Niño events, can also have a serious influence on reproductive output like egg production in fishes (Ven Tresca *et al.* 1995; Harvey 2005). We propose that this approach, using age-validated lifetime fecundity, should be a major research objective for all exploited fishes, including rockfishes.

Demographic approaches using either life table analyses or stage- and/or age-based matrices are good ways to produce predicted population dynamics, with and without exploitation (Cailliet 1992; Ebert 1999; Caswell 2001; Mollet and Cailliet 2002, 2003). In addition, sensitivity and elasticity analyses (Heppell *et al.* 2000; Frisk *et al.* 2005; Garcia *et al.* 2008) will allow the most ecologically important life stages to be identified and used in fishery management policy. However, it may be that sustainability is unachievable (Longhurst 2002; 2006), especially without knowing these age-specific life history traits.

4. Deep-water Rockfish Age Determination, Validation, and Longevity

For this paper, we have chosen a few examples of age validation research on rockfish populations with which we are familiar off the west coast of the United States (Andrews *et al.* 2002, 2005, 2007; Kerr *et al.* 2004; Piner *et al.* 2005). We will focus on three

species of rockfishes for which age has been validated, and life spans determined based on the maximum growth zone counts, for which one or both of the radiometric techniques (lead-radium or bomb radiocarbon dating) was used to support estimates of age.

Our bomb radiocarbon age-validation results on bocaccio (Andrews *et al.* 2005; *Sebastes paucispinis*) are useful relative to the papers cited above on black rockfish (*S. melanops*) because they are similar in terms of lifespan (~50 years), but perhaps not with regard to the other parameters as studied by Berkeley *et al.* (2004a, b) and Bobko and Berkeley (2004). We investigate both their size and age at maturity and their fecundity in this regard. We also present age-validated results for two other species on which we have published age-validation papers, the canary (*Sebastes pinniger*) and yelloweye (*Sebastes ruberrimus*) rockfishes, both of which have had their ages (and life spans) validated using lead-radium and bomb radiocarbon dating (Andrews *et al.* 2002, 2007; Kerr *et al.* 2004; Piner *et al.* 2005).

For these and other age estimation and validation studies, calcified structures such as otoliths, vertebrae, other bones, spines, and thorns have often proved useful and accurate. These structures are prepared for age determination in ways that enhance the readability of growth zones that are typically present. In age validation studies the use of core or growth zone specific material from adult structures and from various year classes have provided better age resolution than previously possible for both lead-radium and bomb radiocarbon dating.

Successful application of lead-radium and bomb radiocarbon dating to rockfishes (family Scorpaenidae) has a well developed history. The first application of lead-radium dating to otoliths in general was an age validation study on splitnose rockfish (*S. diploproa*) in which a 60 yr lifespan was supported from the growth-zone derived age estimation criteria (Bennett *et al.* 1982).

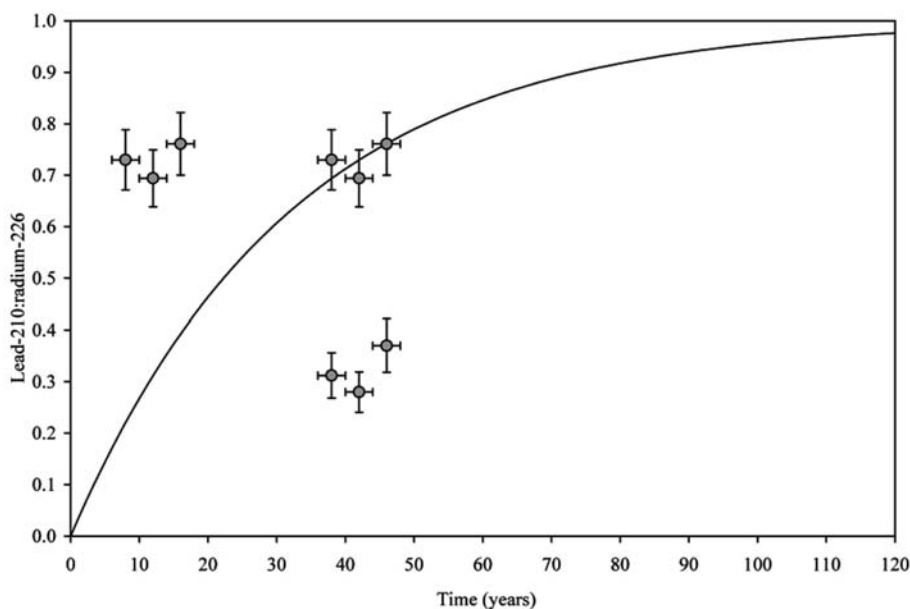


Fig. 1. Diagrammatic representation of the theory behind lead-radium dating. Represented is the ingrowth of lead-210 from radium-226 as the ingrowth curve in the figure. The clusters of hypothetical data points represent the various forms of potential age differentiation from lead-radium ratios measured from samples with estimated age. The clusters not in agreement with the ingrowth curve represent scenarios where age was either underestimated or overestimated by several decades. The margin of error for age is typical for an age distribution from a pooled group of otoliths. The margin of error for lead-radium ratios is approximate for lead-radium activities that would be somewhat ideal. See Fig. 2 for a different graphic analysis of these same theoretical data examples.

Since this pioneering application, the technique has been applied to several other rockfishes with varying degrees of age resolution (Burton *et al.* 1999; Campana *et al.* 1990; Kastelle *et al.* 2000; Andrews *et al.* 2002, 2005, 2007; Stevens *et al.* 2004; Watters *et al.* 2006). Bomb radiocarbon dating of fish otoliths began in the southern hemisphere (Kalish 1993, 2001), followed by a series of studies in the northern hemisphere (e.g., Campana 1997; Kalish *et al.* 2001).

Lead-radium dating relies on the incorporation of naturally occurring radium-226 from the environment into the otolith and its subsequent decay to lead-210. By measuring the disequilibria of these two radioiso-

topes in otolith core material (first few years of life), an independent estimate of age can be determined based on the known ingrowth rate of lead-210 from radium-226 (Fig. 1; Campana *et al.* 1990; Smith *et al.* 1991; Kimura and Kastelle 1995; Francis 2003). This technique works well as a tool for determining the validity of age interpretations that differ considerably, but its application is limited by relatively low resolution at ages approaching 100 years. Typically, the end result for this kind of study is support for, or refutation of, a given age estimation technique and establishment of an independent estimate of age or lifespan. For example, lead-radium dating performed on the yelloweye rockfish (*S. ruberrimus*) provided

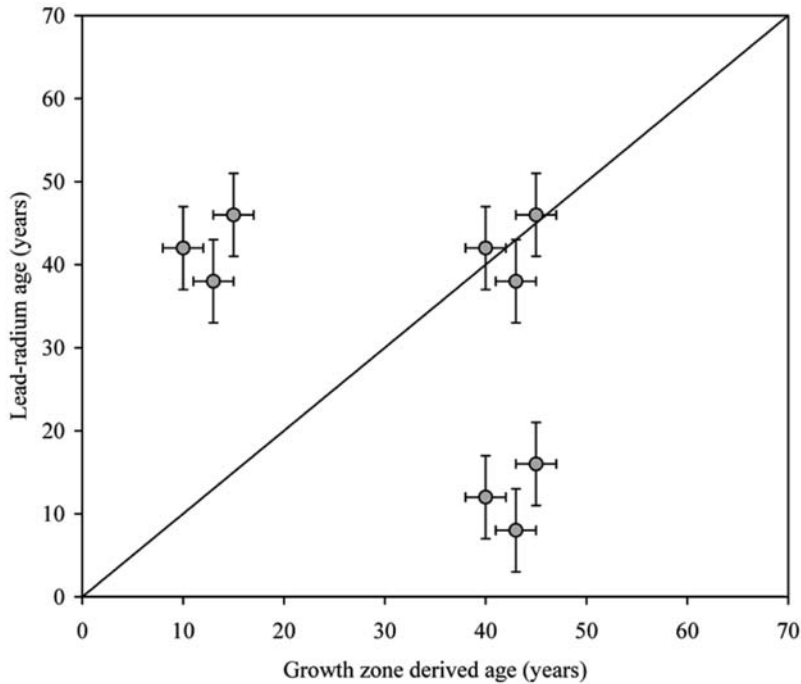


Fig. 2. Comparison of otolith-generated age estimate to radiometric age estimate using ratio of lead-210 to radium-226. The cluster of value on the line indicate that age estimates were confirmed by lead-radium age estimates, while those to the upper left mean that lead-radium ages were higher than growth zone derived ages. At the lower right the values indicate that otolith-derived ages were too high and lead-radium age estimates indicated that these samples were younger. See Fig. 1 for a different graphic analysis of these same theoretical data examples.

support for age exceeding ~100 yr (Andrews *et al.* 2002). The application refuted age estimates that were significantly younger and provided age validated support for transverse otolith ageing techniques, but this finding was the extent of the maximum age resolution because of technique limitations. These findings were similar for bocaccio rockfish for which a minimum age of 31 years was determined using lead-radium dating (Andrews *et al.* 2005).

Many papers now have been written on radiometric age determination of fishes since Bennett *et al.* (1982) first confirmed the longevity of the splitnose rockfish (*Sebastes diploproa*) using lead-radium dating. Such age validation studies usually involve two

graphical comparison techniques. The first involves plotting the predicted ingrowth curve over time, plotted with individual age estimates (usually from growth zone counts in otolith sections), to determine the agreement between measured and expected lead-radium ratios (Fig. 1). The second approach is to plot the growth zone derived age estimates versus the radiometric age estimate for each sample (Fig. 2). With this kind of comparison, it is possible to test for trends of agreement or disagreement based on the hypothesis that there is a one-one agreement that results from a regression fitted to the data. The one discrepancy in this process that is still a work in progress is taking into consideration the change of radiometric age uncertainty with increasing age.

Use of the bomb radiocarbon chronometer requires a series of individual otoliths for which the birth years, based on standardized growth-zone age estimates, range from a time prior to significant atmospheric testing of thermonuclear devices (pre-1957) to the post-bomb period. This approach can utilize both the 1) time of first rise in radiocarbon, and the 2) sloped period for the rise in radiocarbon, as a time-reference markers for age validation. It is the agreement of the bomb radiocarbon record, from the species with age in question, with a reference bomb radiocarbon time-series that provides a basis for age validation. Hence, the utility of this approach for determining age or lifespan is dependent upon the difference between the collection year and time of first rise in radiocarbon for the reference time series. For example, a minimum lifespan of 43 ± 3 years was determined for canary rockfish (*Sebastes pinniger*) based on the first sample to have measured radiocarbon levels that were pre-bomb (Andrews *et al.* 2007). In addition, age can be determined by projecting measured radiocarbon levels back to a reference time series (Campana 1997); a minimum life span of 37 ± 2 years was determined in this manner for bocaccio rockfish (Andrews *et al.* 2005). A thorough evaluation of age classes can also be undertaken with a series of samples with different collection years (Piner and Wischniowski 2004).

For each of the three species discussed here, age has been determined using both lead-radium and bomb radiocarbon dating. Lead-radium results for yelloweye rockfish provided strong support for the age estimation criteria used to determine ages that exceed 100 years and the consistency of fit to the ingrowth curve for younger age classes provided additional support. Based on the ease of otolith reading for this species using the same criteria, we considered a radiocarbon record from otoliths of yelloweye rockfish would provide a reference chronology for validation of other regional fish spe-

cies (e.g., Kerr *et al.* 2005). Some (e.g. Stewart and Piner 2007) have questioned this assumption and suggested that the follow up study using radiocarbon was instead further validation for the age estimate criteria and further support for the findings of the lead-radium study.

It seems there is an element of truth in both perspectives. Based on the findings of the combined studies, and depending on your perspective, the evidence that yelloweye rockfish is one of the longest lived rockfishes is robust. Lead-radium results for bocaccio rockfish provided only a minimum age for this species because of the remarkably low radium-226 levels; there was 95% confidence that the minimum age this species can attain is 31 years. This finding was well supported and exceeded with the application of bomb radiocarbon dating. Lead-radium and bomb radiocarbon results for canary rockfish worked well together by providing robust support for ages estimated in the Canadian canary rockfish fishery. Each application has had variable degrees of age validation based on the uncertainty or assumptions of the application, and there is still a need for developing a high-precision dating method.

Here, we stress the paramount importance that some form of validation or support be given for age estimation procedures, and ultimately estimates of longevity, before we can accurately understand the population dynamics of a population supporting a given fishery. Without determining the temporal periodicity of the growth zones in their otoliths, the variation in growth and longevity would not be fully understood for different species of fishes like bocaccio, canary and yelloweye rockfishes.

5. Deep-water Rockfish Age-Specific and Lifetime Reproductive Output

For the three species of interest here, we have provided age validated support using both lead-radium bomb radiocarbon dating (e.g.,

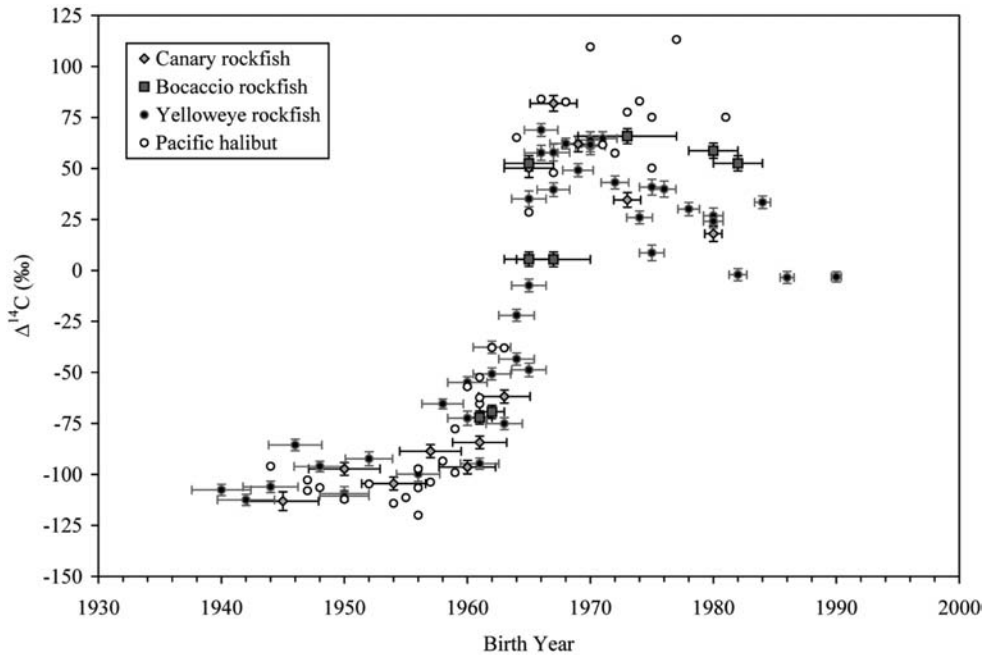


Fig. 3. Plot of measured bomb radiocarbon ($\Delta^{14}\text{C}$) levels in otolith cores, representing the first year of growth, from adult bocaccio (*S. paucispinis*; Andrews *et al.* 2005), canary (*Sebastes pinniger*; Andrews *et al.* 2007), and yelloweye (*S. ruberrimus*; Kerr *et al.* 2005) rockfishes, plotted with $\Delta^{14}\text{C}$ data from whole otoliths of juvenile Pacific halibut (*Hippoglossus stenolepis*; Piner and Wischniowski 2004). Birth year was estimated using growth zone counts for both canary and bocaccio rockfishes and measured $\Delta^{14}\text{C}$ levels from each aged core was compared to the chronological reference time series established for the Northeastern Pacific Ocean; the chronological time series was established for the region with the age-validated yelloweye rockfish (Andrews *et al.* 2002) and known age juvenile Pacific halibut otolith records. Error bars for the data represent the analytical uncertainty of the $\Delta^{14}\text{C}$ measurements and the potential range for the birth date based on the age estimate uncertainty.

Fig. 3). For bocaccio, canary, and yelloweye rockfishes, the age estimates from otolith growth zone counts produced age estimates that conformed well to known regional bomb radiocarbon levels. Thus, age estimation procedures are independently supported for the estimated life spans of these three fishes and it is this supported life span that we propose is an important factor in determining the sustainability of fisheries.

This allows us to also feel confident that the resulting growth curves (Fig. 4 from Love *et al.* 2002) provide evidence of a variety of growth characteristics and life spans among

many species of the genus *Sebastes*. Growth curves for 13 species of rockfishes, including yelloweye and canary rockfishes, are plotted in Fig. 4, but no growth curve was yet available for bocaccio when Love *et al.* (2002) published this figure. We now have a better idea of bocaccio growth, with best estimates, taken from MacCall (2007), of growth coefficients (K) ranging from 0.184–0.210, and asymptotic lengths (L_{∞}) being 75.9 and 65.6 cm TL, for females and males respectively. A life span estimate of ~50 years was used by Love *et al.* (2002). However, there is considerable variation in the value

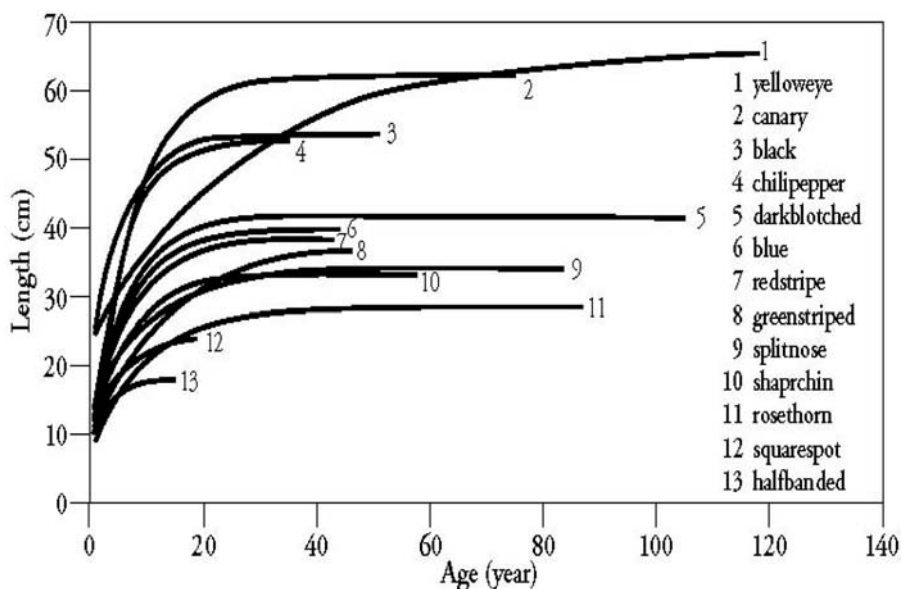


Fig. 4. A compilation of data from numerous studies where von Bertalanffy growth curves were fitted to female length-at-age data (otolith section age) for 13 rockfish species. In the context of this study, the bocaccio growth data were not plotted because at the time of publication, age data was not yet resolved (Data and figure from Figure 9.6, page 61, in Love *et al.* (2002) with permission to reprint).

used for maximum age. MacCall (2007) reduced his maximum age estimate to 27 from 45 years used in his original 2003 stock assessment. Our most recent work indicates 37 ± 2 years for the largest fish used in the study (Andrews *et al.* 2005). Therefore, we feel the best estimate would be ~ 37 years based on our age-validated results, the importance of which will be discussed further.

A synopsis (Love *et al.* 2002) of fecundity information gathered from numerous studies has produced evidence that larger, and presumably older, rockfish females can provide maximum fecundities (number of eggs or number of larvae produced) that increase exponentially with maximum length (Fig. 5). Thus, the deeper-dwelling species, which are often larger, may have increased fecundity coupled with their increased longevity (Cailliet *et al.* 2001). The important question that comes from this observation

has to do with the number of years past age at maturity over which these females continue to produce eggs and larvae. In addition, both fishing and natural mortality can cause the relative numbers of females in successively older age classes to decline, further reducing their net reproductive rates over these years.

Knowing the longevity and age-specific fecundity for rockfishes allows us to speculate about the potential impact of size-specific mortality from fishing. Without fishing mortality, bocaccio, canary and yelloweye rockfishes would start spawning from the ages of approximately 7, 8, and 19 years, to the end of their lifetime at ages approaching 37, 84, and 118 years, respectively (Berkeley 2006; modified by our recent results). Hence, the longer-lived, deeper-dwelling canary and yelloweye rockfishes would have up to 76 and 99 reproductive years, versus up to 30

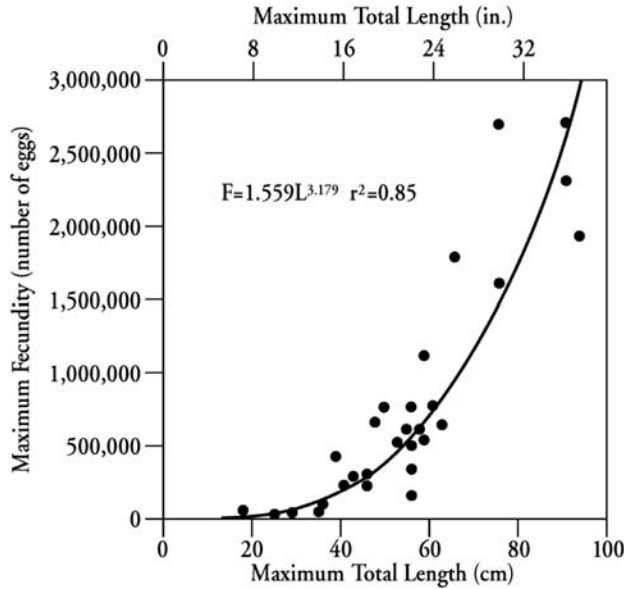


Fig. 5. A compilation of data used to establish a relationship between rockfish maximum length (L , cm) and fecundity (F) for 33 rockfish species from the eastern North Pacific (Data and figure from Figure 5.8, page 37, in Love *et al.* (2002) with permission to reprint). This relationship may be an indication that larger, and presumably older, fish are typically more fecund; typifying the concerns associated with size-specific management strategies.

years for the shallower, shorter-lived bocaccio rockfish. By taking this into consideration, it is relatively straight forward to predict that size-specific fishing mortality (i.e. heavily fishing the larger, older fish) would have a deleterious impact on lifetime egg (larval) production. This would be even more convincing if age-specific natural mortality, the growth rate and age at maturity were known.

Using this line of thinking, it is interesting to consider several other rockfishes that have greater life spans. The rougheye (*Sebastes aleutianus*) and shorttraker (*Sebastes borealis*) rockfishes have estimated longevity ranging up to 157 and 205 years, respectively (Munk 2001); however, these estimates are based on growth zone counts from otolith sections. Neither lead-radium or bomb radiocarbon dating has been applied and the development of other tech-

niques is necessary to accurately ascertain ages of this magnitude. Assuming these estimates are accurate, these exceedingly long-lived fishes would be more vulnerable to size-specific fishing mortality that targets deep-dwelling individuals that are larger and potentially much older.

Likewise, age validation is an important factor in managing other long-lived fishes that are heavily exploited. For example, Baker and Wilson (2001) and Milton *et al.* (1995) validated longevity in snappers (family Lutjanidae) occupying relatively deep water in the Gulf of Mexico and off Australia, respectively. Another prime example, with a bit of a twist, is the orange roughy (*Hoplostethus atlanticus*) with its contrasting reproductive strategy of bestowal (maximizing investment in a smaller number of offspring; Pankhurst and Conroy 1987). This factor, combined with its validated

centenarian longevity (Fenton *et al.* 1991; Smith *et al.* 1995; Andrews and Tracey 2007), make orange roughly a particular concern in a size-selective management strategy. Other deep-water fishes with potentially high longevity, like oreos (family Oreosomatidae: Stewart *et al.* 1995), need to be further considered in this regard. This kind of vulnerability would be especially true for those targeted preferentially for egg production, like anglerfishes (family Lophiidae, genus *Lophius*: Duarte *et al.* 2001; Landa *et al.* 2001).

6. Conclusions

It is a combination of age structure (keep the big ones), life-time egg production (greater productivity), and genetic diversity (potential enhanced survivorship) that beg for further consideration of age validation and abandonment of only size-specific management practices. Age validation for fishes that are recognized as long-lived (e.g., Burton *et al.* 1999 and Cailliet *et al.* 2001), such as chondrichthyans (sharks: Cailliet and Goldman 2004; Cailliet *et al.* 2006), chondrosteans (sturgeon & paddlefishes: Burton *et al.* 1999), and tarpons (Andrews *et al.* 2001), either have required or will need to use a combination of techniques, including bomb radiocarbon, lead-radium dating, and/or tag-recapture and marking techniques to validate their growth characteristics and longevity estimates.

For chondrichthyan fishes and sturgeons, which are cartilaginous and do not have calcified otoliths that are useful, lead-radium dating did not work due to what appeared to be a violation of the closed system assumption (Welden *et al.* 1987; Burton *et al.* 1999). Radionuclides may move around from the vertebral column to the rest of the body, thus making lead-radium dating not useful for these fishes.

For some elasmobranch species, however, bomb radiocarbon dating has been suc-

cessfully applied because the signal is conserved in cartilage. Apparently, the bomb radiocarbon signal is conserved for some species, but may be: 1) free to move around in the vertebral column; or 2) mixed with radiocarbon-depleted sources in other species. This technique has worked well for the porbeagle (*Lamna nasus*: Campana *et al.* 2002), shortfin mako (*Isurus oxyrinchus*: Campana *et al.* 2002; Ardizzone *et al.* 2006), spiny dogfish (*Squalus acanthias*: Campana *et al.* 2006), and tiger shark (*Galeocerdo cuvieri*: Kneebone *et al.* in press). It did not work as well for white shark (*Carcharodon carcharias*: Kerr *et al.* 2006).

In summary, both lead-radium and bomb radiocarbon dating provide solid, age-based evidence as tools in age validation of fishes. Lead-radium dating is useful for the groups of fishes with otoliths large enough, and with enough radioactivity, for core applications. Bomb radiocarbon dating has often corroborated age estimates, growth characteristics, and life spans of rockfishes, and deeper-dwelling species. Results so far indicate that these deep-water fishes can live a long time, and in a growing number of cases exceed a centenarian life span. As pointed out by Longhurst (2002, 2006), longevity is extremely important to insure effective, age-based management practices. In addition, the metabolism and growth of deep-water fishes appear to be lower and slower than near-shore fishes (Haedrich *et al.* 2001; Roberts 2002; Marriott *et al.* 2006; Drazen and Seibel 2007). Prevention of fishing down the larger and more productive age, not just size, classes is of paramount importance.

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Monitoring Prey Availability via Data Loggers Deployed on Seabirds: Advances and Present Limitations

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Seabirds constitute a key group of marine top-predators. While foraging seabirds prey mostly on schooling pelagic fish, crustaceans and squids. Because seabirds distribute over a wide spatial range they are sensitive to physical and biotic changes at several temporal scales. In the last 20 years bio-logging science has revolutionized our knowledge of how seabirds can act as monitors of prey stocks. One of the most interesting applications of data loggers on seabirds is determination of the distribution and availability of prey on which we have little knowledge such as mesopelagic fish, squid, and krill. There are now known to be several variables measurable by data loggers which estimate the number of prey caught by free-ranging seabirds. Such data loggers, in combined deployment on seabirds with time-depth or movement loggers (which record acceleration in one or more dimensions) provide data sets representing dependable indices of prey availability. While knowledge of seabird behaviour continues to improve, we still know little about the relationships between seabird behaviour and prey density/availability. Unravelling these relationships is a key step to calibrating the proxies of prey availability recorded by data loggers. Continuing to develop the use of instrumented seabirds as bio-indicators of marine resources is important in the quest to understand marine ecosystems and the conservation of top-predators.

KEYWORDS bio-indicator; data loggers; foraging ecology; monitoring; penguins; seabirds

1. Introduction

The concept of using seabirds as indicators of resource availability and distribution is not new. Fishermen in search of big fish interpret changes in the behaviour of seabirds like boobies and shearwaters, which are attracted to prey chased towards the water surface by species such as tuna (Batty 1989). Seabirds constitute a key group of marine consumers in several ecosystems because of the large amount of biomass they consume (de L. Brooke 2004). Seabirds forage at sea to sustain themselves, reconstitute their body reserves and to obtain food for their young. As marine predators they are dependent on the abundance of secondary and tertiary productivity but plays also a key role in the control of micronektonic species such as in "top-down" or "wasp-waist" ecosystems. The diet of seabirds is composed of combinations of small, schooling, pelagic fish, micro and macrozooplankton, and squids. Between species, diet diversity and foraging behaviours vary considerably. Many seabird species inhabiting high latitudes (auks, auklets) are mostly monophagous (Furness 1978; Cairns 1992; Montevecchi and Myers 1997). Several groups feed in the upper trophic levels and are top-predators (albatrosses, petrels). Some families are highly specialized to search at depth (penguins, cormorans, auks). Among such pursuit divers some are typically benthic foragers (cormorants) and some others feed primarily on pelagic prey (most penguins, diving petrels, auks).

Recently, the importance of seabirds as bio-monitors of the marine ecosystem has begun to be regarded in a new light. The role of seabirds to assess changes in ecosystems at different spatial and temporal scales was highlighted at the International Symposium entitled 'Seabirds as indicators of marine ecosystems' held in 2006 under the auspices of the Pacific Seabird Group (Piatt and Sydeman 2007). Seabirds can distribute

themselves far from their colonies over wide foraging ranges. As a consequence of their dependence on marine resources these predators are, potentially, highly sensitive to environmental change at various temporal scales. Because seabirds are forced to periodically return to their colonies on land they are among the components of marine food webs most accessible for research. Some seabird breeding populations can be easily monitored over periods of up to decades (e.g. Weimerskirch *et al.* 2003). Long-term studies have successfully related changes in demographic parameters to climatic variability that has had an effect on the marine environment (Aebischer *et al.* 1990; Guinet *et al.* 1998; Barbraud and Weimerskirch 2001; Croxall *et al.* 2002). Climate change has been reported to affect seabirds through changes in weather conditions and prey availability (e.g. sea ice and krill, Barbraud and Weimerskirch 2001; sea surface rising in the North Atlantic and decreases in sandeel stocks, Wanless 2006).

Seabird breeding success and population changes are strongly affected by fish stocks and fishery activities (Montevecchi and Myers 1997). Numerous models and empirical studies have shown that seabird and fishery harvests can be in competition (Furness 1978; Croll *et al.* 1998). Decreases in breeding success and population sizes in response to increased fishery activity have been demonstrated through seabird monitoring (e.g. Wanless 2006). Changes in reproductive performance and feeding rates of seabirds have been monitored over several decades to assess variation in food availability (Cairns 1992; Bost and Le Maho 1993; Monaghan 1996; Croxall *et al.* 1999). Some species of seabirds are however, able to buffer breeding success by increasing their foraging effort when prey density declines (Piatt *et al.* 2007).

Given that seabirds travel out to sea in order to feed and presumably strive to forage efficiently so as to maximise their

chances of reproductive success, their foraging behaviours could be effective indicators of short-term prey availability (Cairns 1987; Wilson 1992; Bost and Le Maho 1993). The marine distribution of seabirds is strongly related to increased productivity and prey abundance at physical processes such as frontal areas, shelf slopes and ice edges (Hunt *et al.* 1999; Ainley *et al.* 2005). Until the beginning of the nineties, knowledge of seabird activity and movement at sea was very limited. During the last twenty years the fast development of bio-logging technology (Naito 2004) has revolutionized our vision of seabirds as monitors of the marine environment. Behavioural (diving depth, travel speed, location), energetic (notably heart rate; Green *et al.* 2003; Grémillet *et al.* 2005), environmental (sea temperature, salinity) and biotic parameters (fluorescence, prey catching) can be continuously recorded by instrumented, free-ranging animals thanks to rapid progression towards high-resolution, multi-channel data loggers. These high-tech devices have large memory sizes (several tens of megabytes) and very fast acquisition rates (up to at least 32 Hz; Ropert-Coudert and Wilson 2005).

Over the last two decades there has also been growing evidence that foraging behaviours of instrumented predators can be used as bio-indicators of available resources (Wilson *et al.* 1994, 2002; Bost *et al.* 1997, 2007; Croll *et al.* 1998; Austin *et al.* 2006; Ropert-Coudert *et al.* 2006; Garthe *et al.* 2007; Grémillet *et al.* 2008). Several species of schooling fish that are of commercial interest are patchily distributed, highly mobile and hard to localize (e.g. Cushing 1988; Davoren *et al.* 2003; Piatt *et al.* 2007). Inter-year changes in their distribution, typical depth in the water column and recruitment are thus difficult to assess. Moreover, few data are available for some fish stocks that are still not commercially exploited. This is the case for some key species of food webs such as Antarctic krill (*Euphausia superba*;

Nicols *et al.* 2000) and pelagic fish (myctophids; Ichii *et al.* 2007). Most seabird prey are difficult to locate by traditional sampling techniques because of their patchy distribution, periodically inaccessibility (e.g. due to sea-ice) and thus the costs of adequate sampling.

The present paper discusses progressions in our understanding of marine resources gained through the instrumentation of seabirds. However, we do not to promote the use of seabird derived parameters to detect new unexploited stocks of fish; modern fisheries are highly efficient, employing advanced technologies to locate fish stocks. Instead, we endeavour to show how independent information derived from seabirds at sea can help to 1) better assess changes in prey availability and ecosystems by giving relevant informations of spatial aggregation or presence of preys in unsampled areas or throughout the year, and 2) promote protection of prey stocks and the main foraging areas of seabirds in the context of ecosystem conservation. The majority of the data used in the present paper have been obtained through extensive work conducted over the last 20 years on several top-consumers in the southern oceans such as penguins and albatrosses.

2. Foraging at Sea

Seabirds forage in a heterogeneous environment where the resources are patchily distributed (Ashmole 1971; Weimerskirch 1997). Bio-logging studies have demonstrated that many seabirds exhibit high predictability in their directional movements at macro- and meso-scales. Tracked seabirds from polar and temperate marine regions generally leave their colony quickly and head in a set direction (Weimerskirch 2007). The destination of their trips usually corresponds to particular meso-scale features such as fronts, eddies or shelf breaks (Fig. 1), which hold predictable prey. Flighted birds

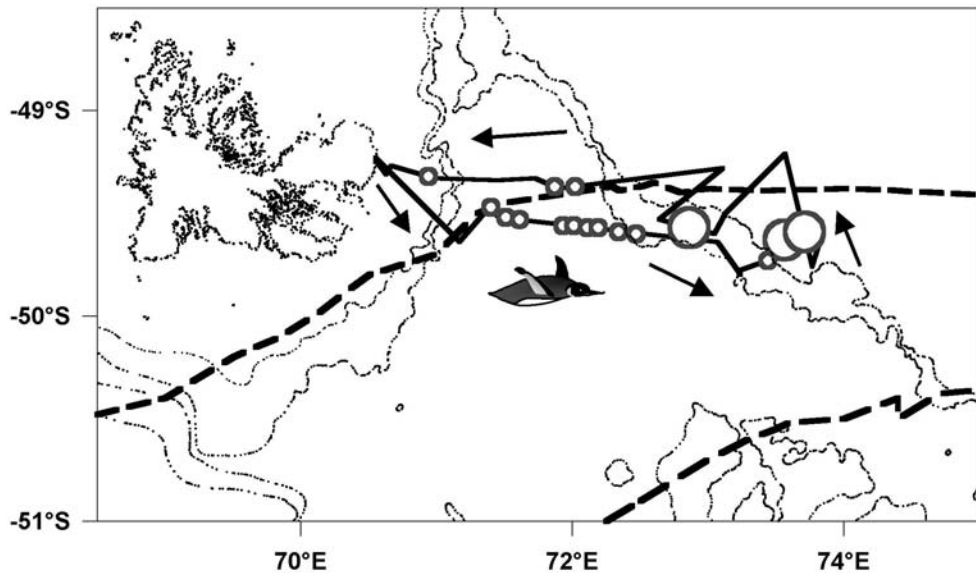


Fig. 1. Example of a foraging trip by a typical pelagic forager (king penguin), which was satellite tracked (Argos) at the polar frontal zone near Kerguelen Island, south Indian Ocean. The bird was instrumented with two loggers: a time-depth recorder and a stomach temperature recorder. The size of the circles corresponds to the amount of foraging activity estimated from the stomach temperature records. The dotted line corresponds to the limit of the polar front. Most of the foraging activity occurs at the limit of the maximal foraging range although significant activity is recorded over the whole trip including the return phase towards the colony.

searching for patchy prey can deviate from a straight line during travelling and decrease flight speed after catching prey. Such searching behaviour is called “area restricted searching” (ARS). Area-restricted searching is as a response to an increase in prey density, i.e. the discovery of a prey-rich area (Kareiva and Odell 1987; Fauchald and Tveraa 2003; Pinaud 2008).

In contrast to flighted seabirds, diving seabirds exploit the water mass in the three dimensions when searching for food. Highly specialized diving species such as penguins travel towards predictable physical features (Bost *et al.* 1997; Hull *et al.* 1997; Rodary *et al.* 2000; Lescroël and Bost 2005). Penguins are more constrained than flying species in terms of travelling speed and hence foraging distance. When foraging many penguin species rely on three main prey types accord-

ing to prey locality or availability: small crustaceans aggregated in swarms, small pelagic, schooling fish and pelagic squids (Croxall and Lishman 1987). Once penguins have left their colony they quickly find favourable areas by alternating shallow travelling dives with deeper prospecting and feeding dives (Wilson 1995). During the central phase of the foraging trip, travel speed decreases in relation to an increased frequency of deep dives and foraging effort (Bost *et al.* 1997; Cotté *et al.* 2007). Penguins are visual hunters (Wilson *et al.* 1992) and exhibit a diel pattern of diving with respect to light transmission at depth. Deepest dives are thus undertaken during daylight and only relatively shallow dives, if any, at night (Wilson *et al.* 1992; Bost *et al.* 2002). During the return phase, heading back to the colony, penguins increase their horizontal speed (Wilson 1995; Cotté *et al.* 2007).

3. Determining How Seabirds Feed

3.1. Feeding recorders

To date, three different biological variables recordable by data loggers have been determined as reliable proxies of the number of prey caught by free-ranging seabirds. The first of these variables recorded was stomach temperature, used to monitor feeding activity and success in large volant seabirds such as albatrosses (Wilson *et al.* 1992; Weimerskirch *et al.* 1994). Stomach temperature temporarily decreases after ingestion of a prey item (whose body temperature is below that of the predator). In species that ingest large prey (fish, squid), recording stomach temperature enables estimation of the mass of each prey. After the ingestion of such prey, a characteristic temperature drop occurs. It is followed by an approximate exponential rise as the prey is warmed to the bird normal body temperature. The integration of the curve from the timing of ingestion until the beginning of the asymptote allows calculating the mass of big prey (Wilson *et al.* 1992). The location of prey capture can provide new information on the distribution and patchiness of poorly understood species such as squids (Catry *et al.* 2004; Weimerskirch *et al.* 2005, 2007). The data logger is swallowed by the bird prior to a foraging trip and recovered by stomach lavage upon return (Wilson 1984). There is a risk that data are lost due to the bird regurgitating the logger at sea. However this can be circumvented by the addition of a back-mounted receiver that enables the logger to transmit recorded data to remote storage.

For diving predators such as penguins, accurate estimations of prey ingestion and prey size are more complicated. This is due to changes in abdominal temperature during the long periods of submersion associated with their foraging dives (Handrich *et al.* 1997), coupled with the relatively small size of prey caught. However, recording oesopha-

geal temperature (at a high temporal resolution) is more reliable for detecting small prey. In king penguins (*Aptenodytes patagonicus*), temperature drops of $\geq 0.06^{\circ}\text{C s}^{-1}$ in the oesophagus correspond to prey ingestion (Charrassin *et al.* 2001; Bost *et al.* 2007). Thus these temperature recordings have been used for quantifying penguin foraging success (Ancel *et al.* 1997; Charrassin *et al.* 2001; Bost *et al.* 2007). The data logger can either be attached to the back of the bird, with implanted leads linking the logger to the temperature probe in the oesophagus (Ropert-Coudert *et al.* 2000, 2001; Charrassin *et al.* 2001; Bost *et al.* 2007), or the logger can be swallowed by the bird before departure, with the leads suspended in the oesophagus by a thin thread glued to the beak. The third measurement suitable for detecting prey ingestion is beak-opening activity, which can be recorded using a Hall sensor (Fig. 2; Wilson *et al.* 2002a). On one mandible of the beak a Hall sensor is attached to record the magnetic field produced by a magnet attached to the other mandible. When the bird opens its beak (to catch a prey, drink, preen or vocalise), the strength of the magnetic field decreases. This can be calibrated with degree of beak opening. Penguins open their beaks underwater mostly to catch prey. Therefore it is potentially possible from Hall sensor data to know when a penguin has ingested a prey and furthermore, by calibrating beak opening angle with prey size, the size of the food ingested. The associated data logger has been implanted in larger penguins (e.g. *Aptenodytes*) or fitted to the back of smaller penguins (e.g. *Pygoscelis*, *Spheniscus*; Wilson *et al.* 2002a, b). The accuracy and reliability of the second and third proxies of prey ingestion, oesophageal temperature and beak opening angle, have been compared in captive penguins, for which both proxies were recorded simultaneously (Wilson *et al.* 2002a, b). Particularly when feeding rates are high, measuring periods of beak opening detects more ingestions than does measuring changes in oesophageal temperature

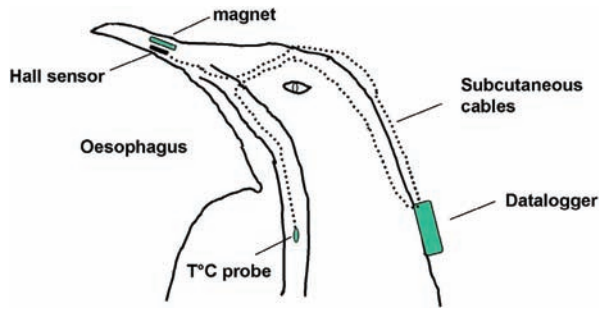


Fig. 2. View of a king penguin fitted with two kinds of feeding data loggers: a beak-opening recorder (Hall sensor) and an oesophagus temperature recorder. The leads connecting the Hall and temperature sensors to the data logger (attached to the back) were either implanted under the skin or attached to the feathers.

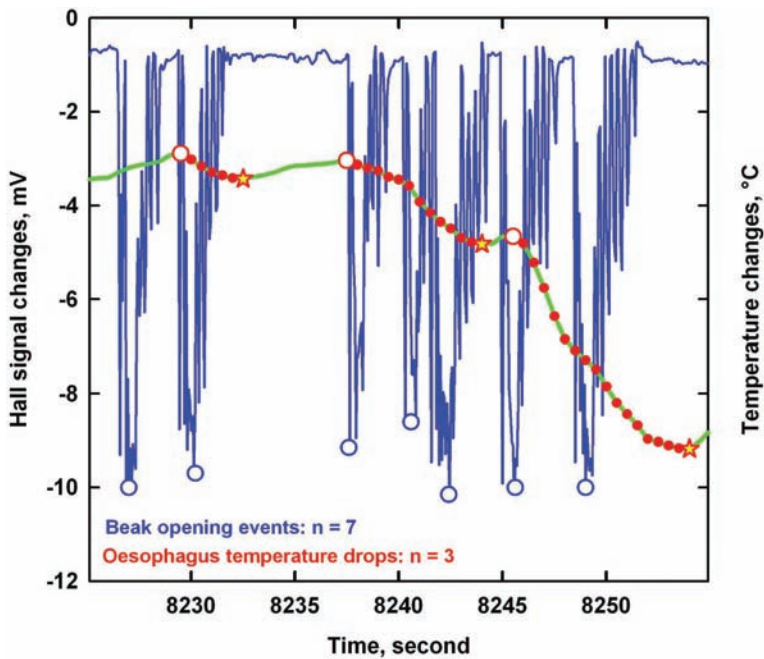


Fig. 3. Differences in response time of an oesophagus temperature sensor and a beak opening sensor (Hall sensor) on the same instrumented king penguin (this study). The blue circles correspond to the feeding events detected by the Hall sensor method. The red circles correspond to the feeding events detected by the oesophagus temperature method. The stars indicate the end of the ingestions events following Charrassin *et al.* (2001) criteria.

(Fig. 3). Recordings of beak-opening are reliable when applied to species feeding on small, aggregated prey such as pelagic fish

or euphausiids (Wilson *et al.* 2002a, b; Hanuise, Handrich and Bost, in prep.). Until now these two systems have mostly been

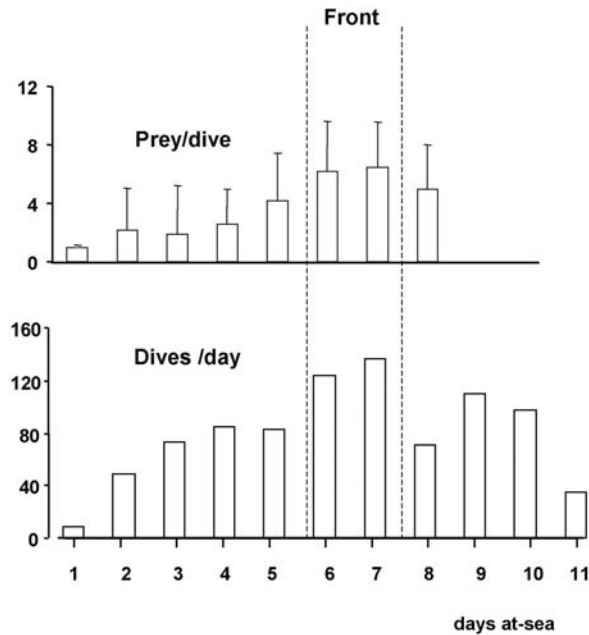


Fig. 4. Changes in feeding success per dive and diving frequency per day of a king penguin while travelling towards the polar front (Crozet Island, South Indian Ocean).

applied to penguins (Wilson *et al.* 2002a, b; Takahashi *et al.* 2004b; Bost *et al.* 2007). Deployed in conjunctions with an activity recorder (TDR Time-Depth-Recorder) such feeding recorders have allowed scientists to accurately monitor the change in penguins feeding activity. In turn these data have been used to assess the distribution and availability of mesopelagic fish (Fig. 4), whose distribution from conventional surveys are still very poorly known (Lancraft *et al.* 1989; Pakhomov *et al.* 1996).

4. Changes in Foraging Behaviour as Proxies of Feeding Success

Data loggers measuring proxies of prey ingestion are now used to assess 'catch per unit effort' (CPUE) in seabirds. However some ethical issues concerning instrumentation of the birds still exist and technical problems in data acquisition often occur. Birds need

to be immobilized or anaesthetized in order to be instrumented and the recovery of ingested loggers upon the return of the bird can be complicated. To recover an ingested logger a magnet embedded in silicone tubing is introduced via the oesophagus (Wilson and Kierspel 1998). In fact, methods of data logger recovery involve minimal stress so long as the logger and sensors remain intact and the fieldworker has experience in bird handling. Even with the logger attached to the back the feeding data recovered may often cover only part of the foraging trip if, for example, the leads break part way through deployment. This is because in diving birds, the mechanical stresses placed on the oesophagus temperature or beak opening sensors and leads are high. As a consequence the leads connecting the sensor to the logger are sometimes broken before the end of the foraging trip. An attractive alternative to the aforementioned proxies of prey ingestion is

the measurement of a behavioural variable that requires less invasive instrumentation. Data loggers that record feeding success are indeed mostly deployed on the same birds instrumented with loggers recording fine-scale movement in the 3 dimensions of the marine environment and hence foraging behaviour (e.g. GPS, accelerometers, TDR).

4.1. Volant seabirds

New insights into the foraging behaviours of wandering albatross (*Diomedea exulans*) were obtained through the combined deployment of GPS loggers and stomach temperature loggers (Weimerskirch *et al.* 2002, 2007). Multiple logger deployment on individual birds allowed an examination of whether albatrosses adapt their foraging behaviour after they have detected and hunted prey. Albatrosses are long-distance foragers that travel by gliding and soaring, dispersing over long distances to find prey, typically squid. Wandering albatross searching for such patchy prey should exhibit ARS behaviour after catching prey. Biologging studies have shown most albatross food consumption occurs during two modes of feeding (Weimerskirch *et al.* 2007): foraging during flight over a generally straight travel path over long distances (up to 3000 km per foraging trip during breeding; Weimerskirch 1997). Secondly prey catching occurs while sitting on the water. However, wandering albatrosses do not catch more prey in areas where travel sinuosity is increased. At a small spatial scale, albatrosses can increase their foraging effort in response to the capture of prey but this behaviour is temporally limited. The ARS behaviour is only exhibited by wandering albatrosses when they are present around specific oceanographic features such as shelf breaks (Weimerskirch *et al.* 2007). Extrapolating information about prey capture and hence feeding success from changes in travelling sinuosity is thus complicate in species such as wandering albatross that forage over very large areas.

4.2. Diving birds

Accurate records of diving depths in relation to time (dive profiles) can be obtained relatively straightforwardly using high frequency (1 s to 4 ms) and high resolution (up to 22 bit) TDRs. Special attention must be provided to instrument diving predators with data loggers. This concerns especially penguins which are highly streamlined diving birds (Bannasch *et al.* 1994). Externally attached loggers can have deleterious effects on their behaviour and increase the energetic cost of movements, decreasing foraging efficiency (Wilson *et al.* 1986). Continuous advances in the development of miniaturized TDRs and accelerometers provide however reliable behavioural data that further elucidate the diving behaviour of predators (Ropert-Coudert and Wilson 2005). Diving behaviour has been classified from the temporal scale of a 'wiggle' within a dive to the scale of a foraging bout (a discrete series of foraging dives; Halsey *et al.* 2007). At the bottom of a foraging dive (bottom phase) a diving bird will search for, and attempt to capture, prey before ascending to the surface. During ascent, swimming angle and speed are constant, at least until close to the surface. However, foraging dive profiles can include a number of complexities. For example, maximum depth can vary considerably, while the periods of descent and ascent can include phases of horizontal travel, which create plateaus in the dive profile (Halsey *et al.* 2007). Several proxies of rate of prey capture have, however, been developed from details of diving behaviour (Ropert-Coudert *et al.* 2006; Bost *et al.* 2007; Sato *et al.* 2007).

4.3. Parameters at depth

4.3.1. Bottom duration

Model predictions have estimated the optimal duration spent in the bottom phase of a foraging dive in terms of overall prey capture

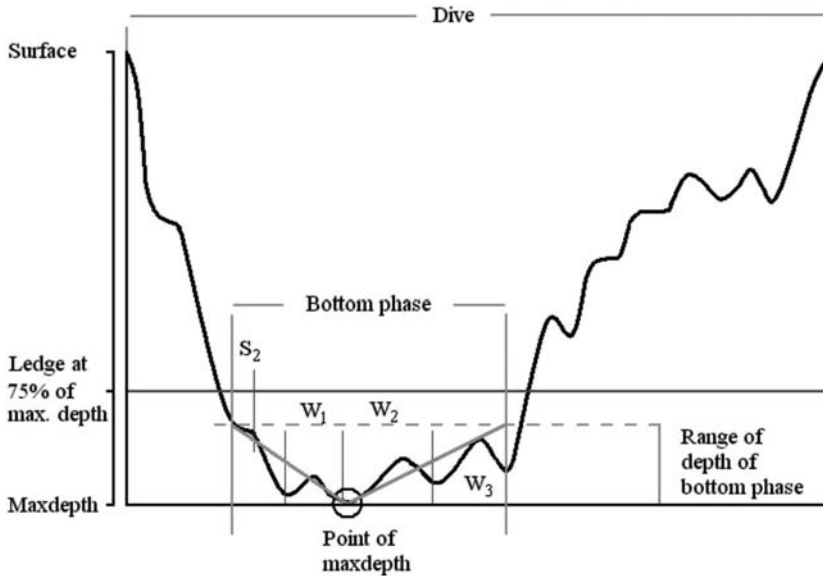


Fig. 5. Typical feeding dive of a king penguin. The different parameters of the dive are indicated as follows: S, step; W, wiggle. The ledge is a specific threshold corresponding to a percentage of maximum depth below which wiggles and steps are used to define the bottom phase of the dive (see Halsey *et al.* 2007).

rates (e.g. Houston and Carbon 1992; Mori *et al.* 2002). Diving predators should stay longer at depth when patch quality/density is higher. Preliminary results from the combined use of beak-opening records and oesophagus temperature records indicate that penguins stay longer at the bottom phase of dives when feeding success is high (Hanuise, Handrich and Bost, in prep.).

4.3.2. Number of wiggles in depth profiles

Wiggles correspond to short periods in the dive profile that are concave in shape (Halsey *et al.* 2007; Fig. 5). The exact definition of a wiggle, a period within a dive profile during which at three points the vertical speed passes below 0 m s^{-1} (Halsey *et al.* 2007), enables the exact number of wiggles within a dive to be counted, and for this process to be automated. Among penguins the number of ingestions recorded per dive is linearly related to the number of wiggles occurring

during the bottom phase and the ascent phase of the dives (king penguins and Adélie penguins, *Pygoscelis adeliae*; Bost *et al.* 2007; chinstrap penguins, *Pygoscelis Antarctica*; Takahashi *et al.* 2004b). Hence high resolution TDRs can be used to accurately assess the number of prey caught per dive, especially in divers feeding on small pelagic fish such as myctophids. King penguin wiggles correspond to prey ingestion events in the majority of dives by individuals (range 50–70%), determined from data sets including dive profiles and one or more proxies of prey ingestion (Bost *et al.* 2007; Hanuise, Handrich and Bost, in prep.).

4.3.3. Change in acceleration

Accelerometers can provide accurate information to monitor the fine-scale behaviour of divers at depth, potentially in three dimensions (Sato *et al.* 2002; Ropert-Coudert *et al.* 2006). A proxy of CPUE can be developed from the number of prey pursuits per

unit time submerged. Prey pursuits are indicated by clear changes in frequency and amplitude of wing or flipper beats during dives. The use of these data as proxies of prey (fish) capture assumes that an encounter with any potential prey will induce pursuit behaviour through an increased rate of flipper beating (Ropert-Coudert *et al.* 2006) and that the pursuit is successful. For instance in the little penguin (*Eudyptula minor*), which feeds on small schooling fish such as sprat, pilchard, garfish and anchovy in western Australia, 'pursuit phases' occur principally during the bottom periods of dives near the seabed. Plotting CPUE against time of day indicated a peak at midday with 90% limits between 05–06h and 18–19h. The higher rate of prey encounter during the middle of the day may be the consequence of greater light at depth, increasing the probability of prey detection by the predator (Ropert-Coudert *et al.* 2006).

Recently the deployment of accelerometers on cormorants, which dive for prey and continuously flap their wings during flight, have allowed finer-scale quantification of the number of prey caught. Furthermore, variation in the body mass of cormorants has been determined from accurate changes in wing stroke frequency after a series of dives (Sato *et al.* 2007).

5. Seabird-Derived Estimates of Prey Availability Compared to Survey Estimates

5.1. Comparison with conventional sampling

To validate the use of behaviours at sea as proxies of prey availability, independent data from extensive simultaneous surveys using relevant sampling methods are needed. Very few studies, however, have tackled the validity of estimates derived from seabird foraging and feeding data (Grémillet *et al.* 2004). This is because it remains difficult to combine effective monitoring of foraging

success with simultaneous and unbiased sampling of prey (Guinet *et al.* 2001; Bost *et al.* 2002). Recently Ichii *et al.* (2007) provided one of the first detailed reports on the distribution of Antarctic marine predators while simultaneously assessing the abundance of their prey (krill and myctophid fish) within their foraging areas. Brooding chinstrap penguins must return frequently to their chick to feed it and thus forage in shelf areas where high krill concentrations are found. Incubating chinstrap penguins are less time constrained and forage in more distant slope and oceanic areas. They have to spend more time foraging due to lower krill abundance. However, this is somewhat counter-balanced by the fact that the krill have a higher energy content (Ichii *et al.* 2007).

At Dumont D'Urville (Eastern Antarctica), 'real-time' sampling of euphausiids was carried out with net hauls in the same areas visited by tracked Adélie penguins (Jaeger, Koubbi and Bost, in prep.). When the penguins returned to their colonies, individuals were carefully stomach pumped to reconstitute their diet composition. In 2005, euphausiids comprised 80% of the penguin diet by number. Krill (*Euphausia superba*) was the modal prey, representing 69% of prey eaten in terms of reconstituted biomass. However, no krill were found in net hauls at the 'hot spot' of the tracked penguins. Overall data on prey sampling did not match the preferred foraging areas of the penguin (Fig. 6). The highest levels of krill biomass were not detected in the areas where the penguins concentrated their foraging effort. Such a lack of correspondence between penguin diet and net hauls has been reported previously (Bost *et al.* 1994; Hill *et al.* 1996) and may be a result of the inadequacy of some sampling methods, and the aggregative behaviour and mobility of prey swarms. Euphausiids are indeed difficult to sample with conventional methods using net hauls (Everson 2000; Siegel 2005). The difficult nature of sampling euphausiids highlights the

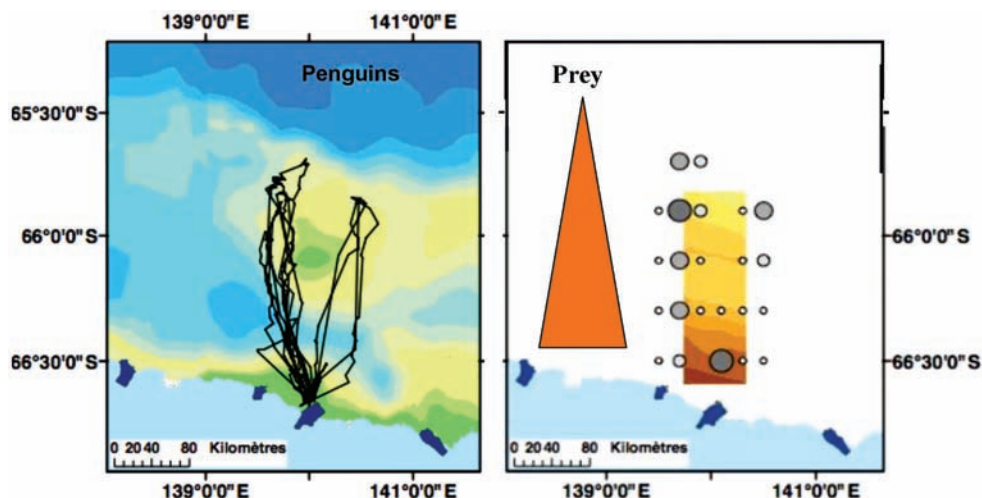


Fig. 6. **Left:** Foraging trips of 10 Adélie penguin satellite tracked at Dumont D'Urville, Antarctica (January 2005). Most of the birds performed extended trips toward the north (mean foraging range: 70 km). **Right:** Comparison of Adélie penguins foraging effort in terms of time spent by sector with the densities of adults euphausiids [range: from 0 (light yellow) to 40 individuals (dark brown). 100 m^{-3}]. The densities have been estimated from net haul data (Bongo net, diameter 0.61 m, mesh size: 500 μm).

need to use other sampling techniques such as multi-frequency echo-sounders (Everson 2000; Ichii *et al.* 2007) or wide-opening net hauls such as RMTs (Rectangular Mid-water Trawls), MOCNESS (Multiple Opening and Closing Nets, with an Environmental Sensing System), BIONESS (Bedford Institute of Oceanography Net and Environmental Sampling System) or KYMT nets (*Kaiyo Maru* Midwater Trawl) (Herman 1988; Green *et al.* 1998; Wiebe and Benfield 2003) coupled with powerful random sampling designs. Technical limitations in euphausiid sampling, however, do not always explain the mismatch between seabird foraging activity and prey abundance. This is especially so because conventional sampling does not estimate prey abundance in the same conditions as those experienced by predators (Ainley *et al.* 2005). Furthermore, prey densities may need to reach certain levels before seabirds aggregate (Schneider 1990; Piatt *et al.* 2007). Such aggregative response

of seabirds to their main prey can be also scale-dependant. Transects at sea have shown a clear response by seabirds to increased indices of prey abundance at the meso-scale (Hunt *et al.* 1999; Fauchald and Erikstad 2002; Ainley *et al.* 2005). However, at finer scales, seabird distributions have been directly related to prey biomass with only limited success (van Franecker *et al.* 2002; Fauchald and Erikstad 2002). A close relationship between the aggregative response of murre (*Uria* sp.), capelin abundance and patchiness has been found (Fauchald and Erikstad 2002). At the large scale, the overlap between capelin and murre increased with increasing capelin density. Such large-scale concordance between seabirds and capelins could indicate the profitability of this prey relative to other prey items. On the other hand, within the large-scale structures of capelin, the small-scale concordance observed between murre and capelin might reflect the search efficiency of murre

relative to the escape abilities of their main prey. A reduction of patchiness and abundance might reduce the search efficiency of seabirds foraging in a stochastic hierarchical patch system (Fauchald and Erickstadt 2002).

6. Discussion

Over the last twenty years, our knowledge of seabird foraging and feeding behaviour at sea has greatly advanced. We can now reconstruct movement and foraging success of an instrumented bird in the three dimensions of the ocean. Bio-logging studies have shown in detail how pelagic seabirds utilise specific oceanic features. Furthermore, seabird species have been found to adapt their foraging movements and effort over the course of a year (Charrassin *et al.* 2002). The number of prey ingested per unit of foraging time can now also be estimated for seabird predators.

Tracking instrumented seabird predators to get information on prey abundance offers several advantages. Firstly, bio-logging studies can provide a considerable amount of additive information on the distribution and availability of prey. Bio-logging studies can be routinely performed year round (although data collection during winter still remains more difficult). The cost of bio-logging studies is also moderate with respect to conventional surveys (Cairns 1992; Piatt *et al.* 2007). However, inter-individual variation (Weimerskirch *et al.* 2007) requires a number of birds to be instrumented to guarantee a representative sample of the foraging and feeding behaviour of a particular population. Further, attachment of relatively large instrument can have deleterious effect on seabird foraging energetics (Wilson *et al.* 1986) especially during extensive deployment periods or during periods of resource scarcity.

6.1. Applications to conservation

The use of instrumented seabirds as bio-indicators of marine resources is relevant when applying ecosystem management that fo-

cuses on conserving marine predators. Tagged seabirds can be used to determine a feeding 'hotspot' for a population during a particular year, which could be made off-limits to fisheries. Yearly monitoring of seabird foraging behaviour can also provide important information about changes in ecosystem functioning and structure (Hooker and Gerber 2004).

6.2. Can we estimate prey availability from the monitoring of instrumented predators at sea?

While understanding of the foraging patterns of seabird predators is rapidly improving, little work has been done on the validation of proxies derived from instrumented seabirds. We still know very little about the relationships between foraging behaviour at sea and the density/availability of prey (Ropert-Coudert and Wilson 2005; Enstipp *et al.* 2007). To our knowledge no study has however succeeded in quantitatively relating CPUE in seabirds of known origin and breeding status with valid, simultaneous measurements of prey density. The logistic constraints of surveys at sea are indeed considerable, especially in the Southern and Arctic ocean because of the remoteness of the areas and the difficult sea conditions (rough waters, wind, ice).

In captivity, recent experiments into the effects of prey-density and size on foraging behaviour have been performed using double-crested cormorants *Phalacrocorax auritus*, a benthic forager, in a large dive tank (Enstipp *et al.* 2007). This study has shown a linear relationship between the CPUE of a bird and prey density. Feeding success was highly dependent on fish density, with cormorants being less successful and diving longer when targeting schooling fish. However at low fish densities the birds had a disproportionately low CPUE (Enstipp *et al.* 2007). Future experimental studies on prey capture rates should increase our knowledge about seabird-prey interactions at a fine

scale, allowing refinement of predator-prey models.

Future studies should also address whether seabird foraging effort is related to prey abundance. As pointed out by Montevecchi (1993), the significance of prey availability is highly correlated to prey abundance (in term of biomass). Physical factors can induce increased prey availability at the meso-scale. Thus pursuit-diving marine birds such penguins and auks are dependent on the depth of the thermocline that favours much higher accessibility to their preferential prey (Charrassin and Bost 2001).

6.3. Next steps

An important endeavour is to scale up individual records of CPUE and location of prey capture from instrumented birds to estimations of the entire prey base of an ecosystem. This long-term objective requires the use of bio-logging technologies on a large number of individual seabirds in several colonies or populations to develop a standard, international monitoring program. Monitoring of a large number of birds will allow integration of the possible effects of breeding experience and bird quality on the monitoring data.

Filling in the gaps between records of CPUE by individual seabirds during the course of a foraging trip and simultaneous, independent prey estimations should be a priority as it is an essential step towards utilising seabird distributions at sea as accurate proxies of prey abundance. This requires the use of integrated multidisciplinary prey surveys at sea, conducted at several spatial scales. One possible way to collect data on density/distribution of prey that are difficult to sample would be the use of seabird-borne miniaturized digital cameras. Such technology has been one of the most exciting recent advances for obtaining new insights into the social behaviour of seabirds at sea (Takahashi *et al.* 2004a) and can be compared with underwater video profiler or

Visual Plankton Recorder. However, recording video footage at great depths, where light conditions are low, is still a challenge (Watanuki *et al.* 2008).

Quantification of the rate of energy acquisition from behavioural data is also needed (Butler 2000). Measurements of the energetic costs of foraging throughout the annual cycle, and relating this to changes in determined levels of prey density is particularly relevant. The use of heart rate, to estimate energy expenditure have already been recorded in seabirds for an entire year (Green *et al.* 2002; Grémillet *et al.* 2001). This is of particular significance since metabolic rate is likely to differ with level of activity, through the year at each phase of the breeding cycle and as food availability varies (Nagy *et al.* 2001; Grémillet *et al.* 2005). Accurate knowledge of adult field metabolic rate and chick energetic needs (Halsey, Handrich, Butler, Bost, submitted) will allow estimation of the energy gain and the foraging time needed per trip for a given individual whose energetic balance is in equilibrium (Cairns 1992). Finally, research is still required to accurately determine the relationships between seabird foraging parameters and temporal/spatial changes in prey abundance. The usefulness of seabirds as bio-indicators should be enhanced in forthcoming years, especially given the ever growing pressures on marine resources and ecosystems.

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Refocusing Stock Assessment in Support of Policy Evaluation

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Fisheries stock assessment has traditionally been focused on providing the basis for tactical fisheries management advice. However, there is an increasing demand from decision makers for feedback control management strategies evaluated using Management Strategy Evaluation, MSE. MSE can be used to identify which uncertainties are likely to lead to poor performance of current management strategies and to which uncertainties such strategies are robust, and hence to the extent to which current management strategies need to be modified (if at all) to perform satisfactorily in the face of key uncertainties. Given this demand, there is a need for a refocus of the priorities of stock assessment science. In particular, more attention needs to be placed on exploring alternative model structures, including those that take spatial and multispecies considerations into account, while there is also a need to develop tools to assign weights or probabilities to alternative model structures using Bayesian and meta-analytic techniques. Finally, care needs to be taken not to overuse model selection methods when selecting model structures to use as the basis for the evaluation of management strategies.

KEYWORDS management strategies, meta-analysis, model selection, Monte Carlo simulation, simulation, stock assessment, uncertainty

1. Introduction

The objectives for conducting fisheries stock assessments during the 20th and early 21st centuries have been primarily tactical, such as providing management advice to decision

makers regarding current stock size, in absolute terms, and relative to target, limit or threshold reference levels, as well as yields (current and long-term) according to harvest control rules (such as $F_{0.1}$). Other outputs from stock assessments have included information on trends in fishing mortality, and

fishing mortality relative to reference points, such as F_{MSY} .

Fisheries stock assessment involves a wide variety of techniques ranging from catch curve analysis to estimate exploitation rates under the assumption that the population and fishery are in equilibrium, to the application of spatially-, age- and size-structured population dynamics models (such as CASAL (Bull *et al.* 2005); GADGET (Begley 2003; Stefansson 2003); MULTIFAN-CL (Hampton and Fournier 2001); and Stock Synthesis 2 (Methot 2005, 2007)) which estimate trends in a variety of measures of stock size and stock status (and their uncertainty). ‘State-of-the-art’ stock assessment continues to evolve to towards more complicated models that can use a broader range of data types in an increasingly statistically rigorous manner.

There is an increasing focus in stock assessment on quantifying and representing uncertainty, and including uncertainty in management advice, and the broad scope of assessment methods reflects this to some extent. Methods for representing uncertainty range include providing confidence intervals, calculating the probability of achieving various management goals, and showing the sensitivity of key model outputs to changing some of the assumptions of the assessment model, its data inputs and the emphasis placed on fitting different data inputs. One particularly common method for representing uncertainty in stock assessments and hence the consequences of future management actions is the decision table (see, for example, Table 1) which highlights the implications of choosing one set of assumptions (or state of nature) as the basis for management advice when another set of assumptions is true.

Although the information in a decision table has the potential to provide decision makers with the ability to evaluate the consequences of basing management decisions on one or other sets of assumptions, man-

Table 1. A decision table for black rockfish (*Sebastes melanops*) off California and Oregon (Sampson 2007). The entries indicate the spawning biomass from 2009 under a variety of catch series. The three states of nature reflect assumptions regarding values for sex-specific natural mortality and the sizes of the historical catches. The high, medium and low catch series are based on applying the $F_{50\%}$ harvest strategy (Ralston 2002) to the high, medium, and low states of natures respectively.

Year	Catch	State of nature		
		Low	Medium	High
Low catches				
2009	909	2195	3284	5710
2010	831	2099	3168	5518
2011	782	1981	3015	5258
2012	765	1860	2855	4982
2013	772	1756	2714	4737
2014	789	1683	2614	4555
Medium catches				
2009	1454	2195	3284	5710
2010	1303	2007	3077	5428
2011	1203	1804	2844	5092
2012	1156	1612	2616	4753
2013	1146	1450	2422	4458
2014	1153	1329	2277	4237
High catches				
2009	2660	2195	3284	5710
2010	2333	1802	2876	5231
2011	2112	1416	2467	4726
2012	1994	1072	2096	4252
2013	1945	796	1791	3854
2014	1930	583	1557	3551

agement decisions are nevertheless usually based on the “central” (or most likely) set of assumptions. This is often because assessment authors are unable (or unwilling) to assign probabilities to states of nature. Moreover, although management is based on a feedback control system, i.e. decisions are made, data are collected, and assessments are updated, there is no consideration of the implications of feedback in decision tables. Rather, decision tables are commonly based on the assumption that pre-specified

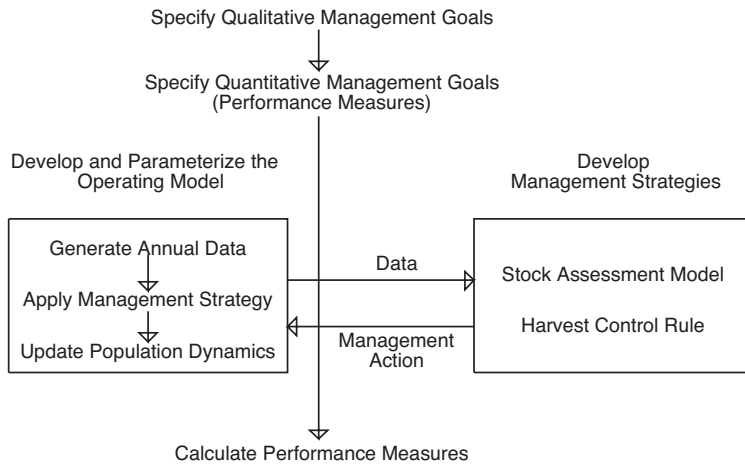


Fig. 1. Flowchart of the approach used to evaluate management strategies.

sequences of catches will be followed irrespective of the data that will be collected from the fishery in the future.

In cases in which management decisions are based on well-specified harvest control rules, it is possible to broaden the notion of stock assessment so that fuller account can be taken of uncertainty and the implications of feedback control. The Management Strategy Evaluation (MSE) approach is the use of simulation to evaluate the performance of combinations of stock assessment methods and harvest control rules (management strategies) in the face of uncertainty given (pre-agreed) objectives (Smith 1994; Smith *et al.* 1999; Kell *et al.* 2006). However, a focus on MSE rather than tactical management advice will require a change to the focus of stock assessment science. This paper first outlines MSE and then discusses how conventional stock assessment approaches will need to be modified so that they can be used to form the basis for MSE. The concepts outlined in this paper are illustrated using experiences from the International Whaling Commission, and the Pacific and North Pacific Fishery Management Councils as well as a simple illustrative situation (Appendix).

2. Management Strategy Evaluation

The MSE approach involves developing models that capture the entire management system, including the underlying fish population dynamics, the data collection scheme, the method of stock assessment used when providing management advice, and any harvest control rules. The MSE approach differs from earlier methods for evaluating management performance by explicitly including the impact of noise in the assessment data and by examining the use of incorrect assumptions when conducting stock assessments. MSE is considered to be state-of-the-art for evaluating and contrasting management strategies, and is fully consistent with the FAO precautionary approach to fisheries management (Punt 2006; Marasco *et al.* 2007). The steps followed when applying the MSE approach are (see also Fig. 1):

- 1) Identification of the management objectives and representation of these using a set of quantitative performance measures.
- 2) Development and parameterization of a set of alternative structural models (called operating models) of the system

under consideration; each of these operating models represents an alternative, yet nevertheless plausible, representation of reality.

- 3) Identification of the alternative management strategies which have the potential to satisfy the management objectives.
- 4) Simulation of the future use of each management strategy to manage the system (as represented by each operating model scenario) under feedback control. Each simulation trial usually involves about 100 replicates for a particular operating model specification. The following three steps occur for each year of the projection period.
 - Generation of the types of data available for assessment purposes.
 - Application of the method of stock assessment and the harvest control rule to determine a management measure for the next year (usually a Total Allowable Catch or Total Allowable Effort).
 - Determination of the implications of this management measure by setting the catch for the next year from the 'true' population represented in the operating model based on it.
- 5) Summary of the results of the simulations by means of the performance measures.

Conventionally, MSE, and hence the objectives and performance measures, has pertained to the direct effects of fisheries for target species (yield, stock status, and catch variability) (Butterworth *et al.* 1997; Punt and Smith 1999; Dichmont *et al.* 2006; Punt 2006), although, recently, it has also been proposed as the basis to evaluate whole-of-ecosystem management strategies and objectives (e.g. Sainsbury *et al.* 2000; Marasco *et al.* 2007; Fulton *et al.* 2007), including non-use objectives (Mapstone *et al.* in press). Equally importantly, MSE is considered a way to evaluate the implications of error and uncertainty when conducting stock assess-

ments and applying harvest control rules. Specifically, attempts are usually made when applying MSE to account for all major sources of uncertainty (*cf.* Francis and Shotton 1997), *viz.*

- **Process error:** variability in population dynamic processes such growth, recruitment and natural mortality due to unpredictable environmental factors.
- **Observation error:** variability in the data on which stock assessments (or management strategies more generally) are based.
- **Model error:** structural differences between the operating model and the models on which stock assessments are based.
- **Implementation error:** error that arises from an inability to adequately implement and enforce regulations.

Specification of the values for the parameters of the operating model ("conditioning" the operating model) is generally based on fitting it to existing data. However, this may lead to an overly restrictive set of operating model scenarios and it is often the case that operating model scenarios are also developed based on expert beliefs and other *a priori* information to reflect scenarios, while not the most likely, that could nevertheless occur in the future (Kell *et al.* 2006).

3. Implications of MSE for Stock Assessment Science

3.1. Multispecies and spatial models

Almost all of the population dynamics models on which fisheries stock assessments are based assume that the population being assessed constitutes a single homogenous population while only very limited account (if any) is taken of predation mortality when providing management advice. Also, it is well known that that conservation performance can be poor when multiple stocks are assessed and managed together, but the harvest is not proportional to stock size or the

intrinsic rate of growth (and hence productivity) differs among stocks. The poor performance of the IWC's Revised Management Procedure in the face of stock structure uncertainty (e.g. IWC 1992, 1993) was one reason for the development by the Scientific Committee of the International Whaling Commission (the IWC SC) of operating models that are specifically designed to examine the implications of stock-structure uncertainty, and the considerable focus in the IWC SC in recent years on developing processes for identifying the "full range" of "plausible" hypotheses, particularly those related to stock structure uncertainty (Punt and Donovan 2007).

The values for the parameters of the operating models developed by the IWC SC used when evaluating candidate management strategies are estimated, and their uncertainty quantified, by fitting them to data on absolute abundance by modeled region, proportions of different stocks in different areas based on analyses of (for example) genetic data, estimates of total mortality based on inferences from age-composition data, and movement rates based on tagging data (e.g. IWC 2004, 2007). Other studies of the performances of management strategies have considered the implications of spatial structure (e.g. Bentley *et al.* 2003; Punt *et al.* 2005; SC-CAMLR 2006; Hobday and Punt 2006), but the number of applications remains very low.

Given that the performance of management strategies can be sensitive to how spatial structure is treated, it is perhaps surprising that the number of spatially-structured stock assessment models which could form the basis for spatially-structured operating models is still relatively small. Examples, outside of the IWC of spatially-structured stock assessment models include those developed for hoki (*Macruronus novaezelandiae*) in New Zealand (e.g. Francis *et al.* 2003) and school shark (*Galeorhinus galeus*) in Australia (Punt *et al.* 2000). Assessment plat-

forms such as CASAL, GADGET, and SS2 all allow spatial-structure to be represented, but this feature has not been used extensively to date in actual applications.

Several assessment models have been developed that consider multispecies interactions (e.g. Magnússon 1995; Begley 2003; Jurado-Molina *et al.* 2005). However, their use for management advice has been limited, although they clearly have a role to play to when evaluating the robustness of management strategies (which usually ignore multispecies interactions). MSE analyses based on operating models which explicitly include biological multispecies interactions are rare (Schweder *et al.* 1998; Fulton *et al.* 2007 being notable exceptions). In contrast, an increasing number of evaluations of management strategies (e.g. De Oliveira *et al.* 1998; De Oliveira and Butterworth 2004; Punt *et al.* 2005; Dichmont *et al.* 2006; Fulton *et al.* 2007) have accounted for technical interactions.

Environmental variables are being included in an increasing number of stock assessments, for example as proxies for recruitment success (e.g. Maunder and Watters 2003; Schirripa 2007). In principle, if these environmental variables can be forecasted, based, for example, on downscaled IPCC predictions, the robustness of current management strategies to what is effectively non-stationarity in the parameters of the operating model can be evaluated.

Assessments that incorporate spatial structure, multispecies interactions, and environmental forcing will likely lead to less precise estimates of model parameters and estimates of quantities of management interest and so may not be appropriate as the basis for tactical fisheries management advice. However, considering such models routinely as part of assessments will allow two key questions to be addressed: (a) Are perceptions of stock status radically altered by including such factors, and (b) are the likely consequences of management actions in the

form of management strategies likely highly sensitive to these sources of model uncertainty?

3.2. The role of parameter estimation and weighting of models

The ideal management action or management strategy for a particular case will depend on the goals of management and the range of scenarios considered when evaluating alternative actions and strategies. However, if the aim of evaluating management strategies is to identify those that are (sufficiently) robust to uncertainty, each operating model scenario (and perhaps the objectives themselves) needed to be weighted. Table 2(a) contrasts the expected yield under five exploitation rates for four scenarios related to the steepness of the stock–recruitment relationship (see Appendix for technical details). The first four exploitation rates were determined by maximizing the expected yield for each of the four steepness values in turn, and the fifth exploitation rate by maximizing the expected yield over all the four values of steepness after giving each value for steepness equal weight. The optimal strategy differs among steepness values and there can be a large loss in yield if the exploitation rate used to determine management actions is based on the optimal exploitation rate for one value of steepness when another is true. This is quantified in Table 2(a) by the difference between expected value of perfect information about steepness (when each steepness value is equally weighted) and the expected yield for each of the five exploitation rates. The ability to avoid losses due to uncertainty can be reduced (and poor decisions avoided) if (appropriately selected) weights can be assigned to each set of assumptions about the dynamics of the system. This is, however, not always the case. For example, Table 2(b) shows that the expected yield (but not necessarily risk-related performance measures)

is insensitive to the extent of variation in recruitment.

Butterworth *et al.* (1996) proposed the following four-level scheme to assign “plausibility ranks” to the hypotheses underlying operating model scenarios:

- 1) How strong is the basis for the hypothesis in the data for the species or region under consideration;
- 2) How strong is the basis for the hypothesis in the data for a similar species or another region;
- 3) How strong is the basis for the hypothesis for any species; and
- 4) How strong or appropriate is the theoretical basis for the hypothesis?

Although this scheme is semi-quantitative, weights (if assigned at all) tend to be assigned to scenarios qualitatively in MSE using a ‘Delphi type’ approach. For example, the weighting scheme developed for use by the International Whaling Commission involves assigning operating model scenarios weights of ‘high’, ‘medium’, ‘low’ and ‘no agreement’ (with ‘no agreement’ being treated as ‘medium’) (IWC 2005). Nevertheless, stock assessment science has an important role to play in terms of the first two levels of this scheme. Specifically, given that appropriate care is taken to ensure that the likelihood function is valid for the problem at hand (many of the data sets used for stock assessment are grossly overdispersed given the probability distributions assumed for them), and operating model scenarios are only assigned low weight because the data suggest that other scenarios fit the data better (rather than the data having no ability to discriminate among different scenarios—see “Complex versus simple models” below), it should be possible to identify “unlikely” scenarios.

The development of Bayesian methods allows a focus on key (often structural) uncertainties by “integrating out” other sources of uncertainty (such as process error). In particular, basing projections on samples

Table 2. Relationship between exploitation rate, E , and expected yield for: (a) four scenarios related to stock-recruitment steepness, h , when $\sigma_R = 0.6$, and (b) four scenarios related to the extent of variation in recruitment, σ_R when $h = 0.7$. The five exploitation rates were chosen to maximize yield under each of four scenarios and when the results from each scenario are weighted equally. The shaded cells are those that were maximized to obtain the five exploitation rates.

(a) Stock-recruitment steepness (expected value of perfect information 70.9).

E	h				Expected
	0.9	0.7	0.5	0.3	
0.049	37.9	36.7	34.0	20.4	32.3
0.162	82.4	74.1	55.3	0.0	53.0
0.321	108.0	85.7	35.6	0.0	57.3
0.681	122.1	60.5	0.0	0.0	45.6
0.272	102.5	84.8	44.9	0.0	58.0

(b) Extent of recruitment variability (expected value of perfect information 86.8).

E	σ_R				Expected
	0.2	0.4	0.6	0.8	
0.364	90.9	88.7	85.2	80.0	86.2
0.346	90.8	88.8	85.5	80.7	86.4
0.321	90.4	88.6	85.7	81.4	86.5
0.294	89.5	88.0	85.4	81.7	86.1
0.328	90.5	88.7	85.7	81.3	86.5

from posterior distributions which represent parameter uncertainty reduces the number of scenarios which need to be examined, making to communication of results to decision makers substantially more straightforward and also reducing the number of scenarios that need to be weighted. Care should, however, be taken to avoid treating parameters which could have a substantial impact on the performance of alternative management strategies as estimable parameters that are automatically “integrated out” when constructing Bayesian posterior distributions. For example, the IWC SC based the analyses used to evaluate management strategies (Strike Limit Algorithms) for the Bering–Chukchi–Beaufort Seas bowhead whales

(*Balaena mysticetus*) and the eastern North Pacific gray whales (*Eschrichtius robustus*) on samples from Bayesian posteriors, but based operating model scenarios on specific choices for the parameter that determines productivity to allow the impact of this parameter on performance to be represented explicitly (even though the data appeared to be very informative about this parameter).

The use of Bayesian stock assessment methods requires that prior probability distributions be developed for all of the parameters of the model and these priors updated using data for the case in question. Moreover, given that prior probabilities can be assigned to models, Bayesian techniques can be used directly to compute the relative

weight that should be assigned to alternative models. Priors can be developed subjectively, by analyzing auxiliary information (e.g. Givens *et al.* (1995) for the Bering–Chukchi–Beaufort Seas bowhead whales) and using meta-analysis. Meta-analysis provides a formal basis for developing priors as well implementing step 3 of the approach to weighting developed by Butterworth *et al.* (1996). To date, meta-analyses based on data for multiple stocks have been conducted which could be used to assign priors to the parameters of the stock–recruitment relationship (Myers *et al.* 1995; Liermann and Hilborn 1997), the form of the relationship between catch-rate and abundance (Harley *et al.* 2001), and survey catchability (Harley and Myers 2001; Millar and Methot 2002). The further development of meta-analytic techniques and their application to fisheries data sets should provide a more rigorous (and replicable) basis for assigning weights to alternative models.

3.3. Complex versus simple models

One of the central tenants of contemporary fisheries stock assessment, and statistical modelling in general, is the desire to identify models that provide a parsimonious representation of the available data, using approaches such as likelihood ratio tests and the Akaike Information Criterion (Akaike 1974; Burnham and Anderson 2002). While simpler models may provide better short-term forecasts and, in fact, estimate biomass more accurately (Ludwig and Walters 1985; Punt 1993), ignoring models which include biological processes which are poorly supported by the data, but to which the performance of management strategies may be very sensitive may lead to the selection of a management strategy that is not sufficiently robust to uncertainty.

There are several areas where models that would not be supported by standard model selection approaches should not be ignored. For example, inclusion of depensation in

operating models. Most fisheries stock and recruitment data sets are incapable of selecting between alternative two-parameter stock–recruitment relationships such as Ricker and Beverton–Holt. Not surprisingly, therefore, only very few data sets provide statistically significant evidence for depensation (Myers *et al.* 1995) although, as noted by Liermann and Hilborn (1997), stock and recruitment data are generally uninformative about the presence of depensation rather than suggesting a lack of depensation. Ignoring depensation in the absence of information supporting such lack can, however, markedly under-estimate risk. For example, Fig. 2 contrasts two stock–recruitment relationships which differ in terms of whether there is depensation. The solid lines in Fig. 2 are based on the standard Beverton–Holt stock–recruitment relationship ($\gamma = 1$ in Eq. (A.1)), while the dashed lines are based on a stock–recruitment relationship that has the same equilibrium point and recruitment at 20% of the equilibrium unfished spawning stock biomass ($0.2B_0$) as the solid line, but for which the recruitment at $0.1B_0$ is 0.25 of that of the standard Beverton–Holt stock–recruitment relationship. The two stock–recruitment relationships are similar (arguably indistinguishable over a broad range of spawning stock biomass levels given typical stock and recruitment data sets), but have profound implications for both the level of catch that is sustainable and the fishing mortality associated with Maximum Sustainable Yield. Ignoring the possibility of depensation, given lack of (informative) data can consequently lead to unduly aggressive management strategies.

A perhaps more serious example of the impact of selecting perhaps overly parsimonious models when identifying operating models occurs in relation to stock structure uncertainty. The western North Pacific minke whales (*Balaenoptera acutorostrata*) provides an example of how stock-structure uncertainty can impact the selection of a

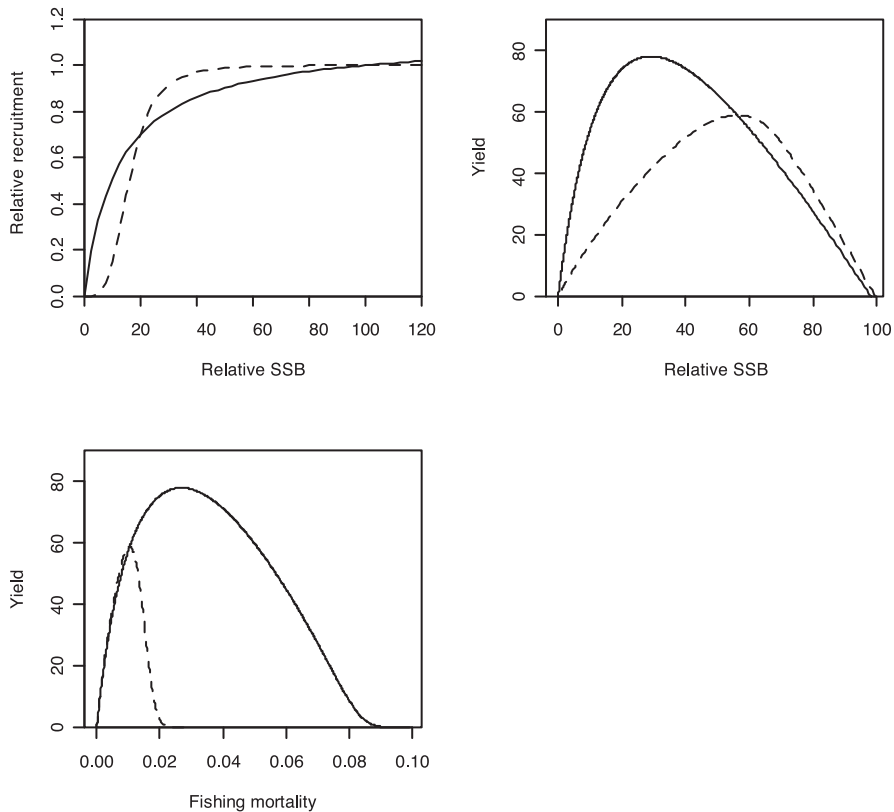


Fig. 2. Stock–recruitment relationships that include (dashed lines) and ignore (solid lines) depensation (upper left panel), and the implications of accounting for depensation for the (mean) surplus production function (upper right panel) and the relationship between yield and fishing mortality (lower left panel).

management strategy. Four broad stock-structure hypotheses were developed for this group of minke whales (IWC, 2004). These stock-structure hypotheses covered a range from two to four stocks in the region to be managed. The least complicated stock-structure hypotheses (two and three stocks) were justified primarily by the lack of evidence from statistical hypothesis tests applied to a range of genetic and non-genetic data. The most complicated hypothesis (four stocks) arose from the application of a clustering algorithm (Martien and Taylor 2001) to mtDNA data, noting that the power of most methods to detect stock structure can be very

poor (Martien and Taylor 2003). Perhaps not unexpectedly, the operating models based on four stocks posed more of a challenge to the candidate management strategies, and uncertainty about stock structure led, in part, to the IWC SC being unable to reach agreement on relative weights for different stock structure hypotheses.

4. Concluding Remarks

The discussion above might seem to suggest that improved management advice will require more sophisticated and complicated stock

assessments. In contrast, stock assessment science needs to consider two distinct types of assessments. Those which will form the basis for tactical management advice (e.g. setting of Total Allowable Catches and other management measures) by being part of management strategies, and those that will form the basis for the evaluation of candidate management strategies. In principle, the “stock assessment” component of a management strategy can be fairly simple (such as the largely empirical approaches on which management advice for sardine (*Sardinops sagax*) and anchovy (*Engraulis encrasicolus*) off south Africa are based; De Oliveira and Butterworth 2004) while great complexity should ideally only be found in the operating models used to evaluate management strategies.

The increased focus on an evaluation of feedback-control management strategies is likely to increase the demands on the stock assessment community owing to the move from trying to identify (and then justify) a single “best” model to identifying an appropriate range of plausible models, parameterizing the models, and assigning weights to them. However, the move to MSE has several advantages. Specifically, the evaluation of management strategies forces decision makers to be explicit regarding their management objectives and also helps to train them regarding the trade-offs among these management objectives (Walters 1994). In addition, attempting to explore a broad range of uncertainties has the advantage that it becomes possible to determine the relative importance of different factors in relation to achieving management goals. One almost general result of MSE analyses is that the presence of model uncertainty has a larger impact on the achieving management objectives than those sources of uncertainty considered routinely in stock assessments (observation and process error).

There are disadvantages associated with evaluating management strategies in addi-

tion to the increased technical demands on stock assessment scientists. Specifically, it is necessary to clearly define “risk”. Unfortunately, while decision makers are often willing to express an interest in “minimizing risk”, they are seldom willing to provide (let alone agree to) a definition for “risk” (and hence “acceptable risk”). Workshopping (e.g. Mapstone *et al.* in press) provides one way to explore different stakeholder objectives and attempt to find common ground among stakeholders who have perhaps markedly different objectives.

Data-poor situations pose major challenges for the conventional approach to fisheries stock assessment, and these problems are not alleviated when attention focuses on the performance of management strategies. For example, it is likely that the only management strategies that will perform adequately for all plausible scenarios are those which are very precautionary or which incorporate mechanisms (such as large MPAs) to protect the resource against the lack of information.

Schnute *et al.* (2007) identify the need for a global effort within the stock assessment community to develop software to implement general MSE frameworks. However, an equally important need at present is to identify whether there are “universal laws” which pertain to management strategies. Although they remain to be proved, such rules might include “management strategies based on empirical indicators are more likely than model-based management strategies to respond to major shifts in population abundance albeit at the cost of larger inter-annual variation in catches and stock sizes”. A global meta-analytic analysis based on MSEs for a variety of regions could be used to “test” such proposed “laws”. The availability of sets of such “laws” could be used for regions for which the resources needed to conduct MSEs are lacking.

Finally, Quinn (2003) speculates that the “Golden Age” of fisheries population

dynamics models may be over, noting that attacks on modelling and assessment paradigms based only on single-species considerations will continue because of the increasing number of well-publicized fishery collapses and the fact that increasingly it is becoming obvious to the broader scientific community that the single-species models conventionally used as the basis of management advice make assumptions (such as that natural mortality is time-invariant) that are not valid. While the push towards assessment and management paradigms which explicitly include a greater variety of biological processes (in particular multi-species interactions and climate impacts on recruitment, growth and movement) will continue, it seems unlikely that management advice will be based on such paradigms for many years (Marasco *et al.* 2007). Rather, the fact that “complexity is not necessary better” means

that changes to current management systems should continue to be made incrementally, using the MSE approach to confirm that proposed changes are both needed and beneficial. In fact, I predict that the MSE approach will show that in many cases well-designed management strategies based on single-species analyses are, in fact, fairly robust to the types of violations of the assumptions highlighted so frequently as long as management continues to be based on feedback control management systems and the decision makers follow the outputs from management strategies and enforce management regulations adequately.

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Appendix: A simple MSE analysis

The population dynamics are assumed to be governed by:

$$B_{t+1} = B_t e^{-M} + \frac{(5^y - 1)hR_0(B_{t-L}/B_0)^y}{(1-h) + (5^y h - 1)(B_{t-L}/B_0)^y} e^{\varepsilon_t - \sigma_R/2} - C_t; \quad \varepsilon_t \sim N(0; \sigma_R^2) \quad (\text{A.1})$$

where B_t is the biomass at the start of year t , M is the instantaneous rate of nature mortality (set to 0.2 yr^{-1} for the analyses of this paper), R_0 is the recruitment at unfished equilibrium ($=B_0(1 - e^{-M})$), B_0 is the average unfished biomass, C_t is the catch during year t :

$$C_t = EB_t \quad (\text{A.2})$$

E is the exploitation rate, L is the lag between spawning and recruitment to the exploitable (equals mature) biomass (set to 3 yr for the analyses of this paper), h is the “steepness” of the stock–recruitment relationship (the recruitment at $0.2B_0$ expressed as fraction of R_0), γ is the parameter that determines the shape of the stock–recruitment relationship (set to 1 for the Beverton–Holt form of the stock–recruitment relationship), and σ_R is the standard deviation of the random fluctuations in recruitment.

Hatchery Stocking for Restoring Wild Populations: A Genetic Evaluation of the Reproductive Success of Hatchery Fish vs. Wild Fish

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Potential impacts of hatchery programs on wild populations have long been discussed, and of particular interest is the reproductive success of hatchery-born fish in natural environments. Here I summarize our recent studies, in which DNA fingerprinting and genetic parentage analyses were used to estimate adult-to-adult reproductive fitness of steelhead trout (*Oncorhynchus mykiss*) in the Hood River, Oregon (USA). We found: (1) Hatchery fish left fewer adult offspring per parent than wild fish, but supplementation hatchery fish (from local, wild broodstock; H_{supp}) left larger numbers of offspring than traditional hatchery fish (from nonlocal, multi-generation hatchery broodstock; H_{trad}); (2) The reproductive fitness of H_{supp} declined unexpectedly fast (~40% per generation) when H_{supp} were reused as broodstock in a hatchery, suggesting that the negative effects of hatchery rearing are cumulative and heritable; (3) Effective population size was mainly restricted by variance in reproductive success among individuals, rather than by biased sex ratio and temporal fluctuation of population sizes; (4) H_{trad} showed particularly large variance in reproductive success, indicating another negative effect of traditional programs. Our case studies suggest that using local, wild broodstock reduces negative effects of hatchery rearing, but the repeated use of H_{supp} as broodstock should be minimized for efficient supplementation.

KEYWORDS hatchery stocking; supplementation; effective population size; reproductive success; parentage analysis

1. Introduction

Traditionally, hatchery programs have focused on a single mission: producing fish for harvest. The primary goal remains the same for the majority of the current hatchery programs. Fish culture technologies have been greatly improved in the last 100 years, and many of the current fisheries depend on hatchery fish (especially those for economically valuable species such as salmon, Leber *et al.* 2004; Williams 2006). Ecologically, however, there remain many uncertainties about the effects of hatchery fish on wild populations. For example, released or escaped hatchery fish might threaten wild populations by competing with wild fish for natural resources such as food, territory and mates (Jonsson and Jonsson 2006). Profound effects of hatchery stocking on wild populations and reproductive interactions between hatchery and wild fish have been predicted (Ryman and Laikre 1991; Waples and Do 1994; Lynch and O'Hely 2001; Ford 2002; Duchesne and Bernatchez 2002; Hutchings and Fraser 2008). Some studies suggest that traditional hatchery fish, which are typically non-local fish and maintained multiple generations in hatcheries, have low reproductive success in the wild, often only ~10% of reproductive success in wild fish (Berejikian and Ford 2004; Araki *et al.* 2008, for reviews). If the level of fitness decline in hatchery fish is common, the potential impacts of reproductive interactions between hatchery and wild fish may be large because humans release billions, if not trillions, of hatchery fish into the natural ecosystems annually (Heard 1995). Nevertheless, empirical evidence is not yet compelling enough to induce widespread changes in the design of hatchery operations for many fish species.

The risk of extinction and declines in wild populations of fish species have lead to an increasing number of hatcheries adopting a new set of goals: supplementing fish

for the restoration and conservation of wild populations (Cuenca *et al.* 1993; Waples and Drake 2004). Although there are many kinds of supplementation programs, a common idea behind them is to create hatchery fish from local broodstock (parents of hatchery fish), rear hatchery fish in well-protected captive environments, and stock them to 'supplement' wild populations. Main goals of the supplementation program are to have a large number of wild-born fish back to the population as a consequence of reproduction by hatchery fish in natural environments, and eventually to reestablish self-sustainable wild populations. Unlike traditional hatchery programs for harvest, therefore, having large numbers of hatchery-born fish in the populations itself is not a goal of the supplementation programs (although some hatchery programs have a hybrid goal, for conservation and harvest, at the same time).

An essential assumption of supplementation program is reasonably high reproductive success of hatchery-born fish in natural environments. However, it is largely untested whether supplementation hatchery stocks really reproduce better in the natural environments than do traditional hatchery stocks. In this review, I summarize our recent studies on this issue, in which we evaluated reproductive success of steelhead trout (*Oncorhynchus mykiss*) in the Hood River, Oregon (Araki *et al.* 2007a–d). Although these studies are based on an anadromous species in one system, they provide valuable information about genetic effects of hatchery rearing. Therefore I believe that the majority of conclusions are applicable to many other hatchery programs, including those for marine species.

2. The Study System

Steelhead trout is a sibling species of Pacific salmon (*Oncorhynchus* spp., Crespi and Fulton 2004), and an anadromous (sea-run) form of rainbow trout. Steelhead typically

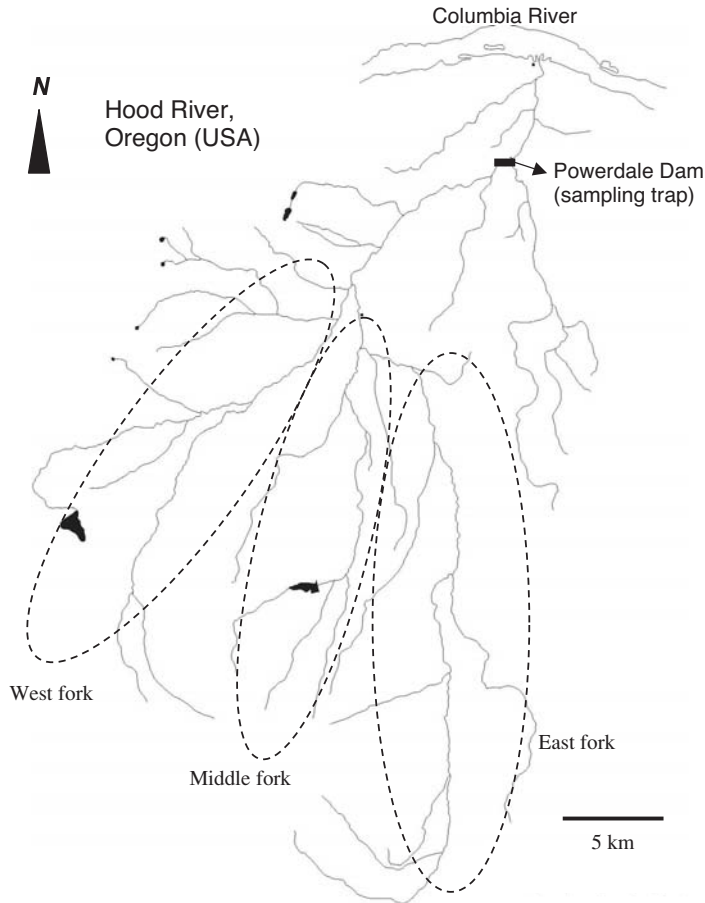


Fig. 1. Map of the Hood River, Oregon. The Hood River is a tributary of the Columbia River in Pacific Northwest, USA. The sampling trap, which is built in the Powerdale dam, is located at the mouth of the Hood River (4 river miles from the mouth). Dotted circles indicate three major branches of the Hood River and the locations of spawning grounds for steelhead trout (East fork for summer-run population and Middle and West forks for winter-run population). This map is kindly provided by Erik Olsen (ODFW).

return to the river for reproduction at age of 2–6 after 1–4 years of journey in the ocean. In contrast, rainbow trout remain in freshwater throughout their life cycle. We used DNA fingerprinting techniques and genetic parentage assignments (below) on the returning adult steelhead for three generations to evaluate adult-to-adult reproductive success of hatchery fish and the effective population size in the wild.

Two factors make the Hood River steelhead one of the best systems for this kind of genetic evaluations. First, genetic samples are available for almost all steelhead that returned and spawned in the river for last 16 years. The number of genetic samples used in our studies is more than 15,000. The whole population sampling was possible because the sampling trap is built in a dam on the mouth of the river (Fig. 1), and only fish

captured at the sampling trap and released above the dam can reach their spawning grounds in the river. The sampling has been operated by the Hood River conservation team in the Oregon Department of Fish and Wildlife (ODFW) since 1991 on a daily basis. The quality and quantity of biological and genetic data have been proven to be crucial for the fine-scale parentage and following analyses (Araki and Blouin 2005).

Second, Hood River was stocked with juvenile steelhead from traditional and supplementation hatchery programs during this period. Because both traditional hatchery fish (H_{trad}) and supplementation hatchery fish (H_{supp}) were allowed to pass the dam and spawn in the river, we could evaluate the effects of these hatchery programs in the same system. H_{trad} were created from non-local hatchery stocks and maintained in a hatchery for many generations (~10 generations), whereas H_{supp} were created from local wild stocks and maintained only one or two generations in a hatchery in the Hood River. In this article I use the term “wild fish” to refer to fish born and reared in natural environments (regardless of parentage), and the term “hatchery fish” to refer to fish that were born and raised in a hatchery through the juvenile stage before being released. In the Hood River programs, hatchery fish are released as yearling (Appendix for details).

3. DNA Fingerprinting and Parentage Assignments

DNA fingerprinting is a powerful tool for population studies. Polymorphic genetic markers, such as microsatellite (DNA repeat sequences in which the repeat number varies among individuals) are highly polymorphic and specific to individuals, and contain sufficient information to identify individuals (Jeffreys *et al.* 1985a, b). In addition, DNA is inherited by progeny from their parents. Therefore genetic markers provide pedigree information on the basis of Men-

delian inheritance. DNA fingerprinting was first applied to genetic identification of individuals humans (Jeffreys *et al.* 1985a, b), but the technique is now used for a variety species and for many purposes, including parentage analyses for reproductive success of individuals in wild populations (e.g., Avise 2004; Bishop *et al.* 2004). Genetic parentage analyses require temporal sample sets and many polymorphic markers, but once they are obtained, researchers can gain powerful insights regarding mating systems, population dynamics and natural selection in wild populations.

For Hood River steelhead, we surveyed 8 microsatellite markers after DNA extraction from sampled scales or fin clips (Araki *et al.* 2007a). These markers were highly polymorphic (average heterozygosity ~90%) and provided enough power for identifying parentages (Exclusion probability > 0.9996, Araki *et al.* 2007a). The 15000+ samples included fish from two steelhead populations (summer-run and winter-run), which are considered to be reproductively isolated from each other (Appendix). In the following studies, we treat them as two independent population samples. 96% of the samples were successfully examined for more than 6 microsatellite loci. Using the DNA fingerprinting and fine-scale parentage assignments, we reconstructed a two-generation pedigree for summer-run population and a three-generation pedigree for winter-run population (Araki *et al.* 2007a, b). The obtained pedigree information was used to estimate the relative reproductive success (*RRS*) of hatchery fish to wild fish and effective population size.

4. Reproductive Success of Hatchery Fish

RRS is defined as the ratio of average numbers of wild-born offspring from one type of parents (e.g., hatchery fish) to those from the other (e.g., wild fish) that returned in the

Table 1. Number of fish returned to the Hood River (updated from Araki *et al.* 2007a)

Run year of the parents	Winter-run population				Summer-run population			
	Parents		Offspring	RS	Parents		Offspring	RS
	Wild-born	Hatchery-born			Wild-born	Hatchery-born		
1991	716	292 (T)	273	0.27	—	—	—	—
1992	408	5	304	0.74	537	1677 (T)	87	0.04
1993	382	2	212	0.55	240	1108 (T)	128	0.09
1994	203	6	298	1.43	199	1652 (T)	199	0.11
1995	276	185 (S)	1237	2.68	132	518 (T)	212	0.33
1996	242	283 (S)	995	1.72	182	1310 (T)	615	0.41
1997	226	199 (S)	901	2.12	83	447 (T)	494	0.93
1998	299	220 (S)	628	1.21	134	4	271	1.96
1999	920	267 (S)	520	0.44	182	0	283	1.55
2000	1013	657 (S)	466	0.28	208	0	>191	—
2001	1025	684 (S)	>362	—	491	115 (S)	>144	—
2002	725	413 (S)	>390	—	641	482 (S)	>50	—
2003	625	535 (S)	>17	—	241	189 (S)—	—	—
2004	352	242 (S)	—	—	217	148 (S)—	—	—
2005	502	295 (S)	—	—	195	128 (S)—	—	—
2006	493	350 (S)	—	—	208	164 (S)—	—	—
Total	8407	4635	6603	—	3890	7942	2674	—

Parents: Number of wild-born fish or hatchery-born fish returned and passed above the dam for spawning in each run year (Appendix for definition). (T): Traditional hatchery fish; (S): Supplementation hatchery fish. Offspring: Number of fish born in the wild in that specific run year and returned in the subsequent run years as adults (typically after 2–6 years). The years of birth of the returned fish were estimated from year rings on scales by ODFW. RS: an absolute reproductive success (= number of offspring per total number of parents) among all the potential parents for each run population. Data as of August 24, 2007 from ODFW.

same year (Appendix). We used RRS to evaluate reproductive success of hatchery fish because absolute values of reproductive success fluctuated considerably among years (Table 1). Analyses on RRS in the Hood River steelhead revealed a rapid fitness decline of hatchery fish as they are released in the wild after the hatchery rearing for more than one generation (Araki *et al.* 2007a, b). One extreme example is H_{trad} . The broodstock of H_{trad} in the Hood River were reared in a hatchery for ~10 generations. On the basis of the pedigree information, we found that the overall RRS of H_{trad} to wild fish was only 8% for winter-run and 33% for summer-run (Fig. 2). Although non-local origins of H_{trad} might also affect the reproductive success due to the lack of local adaptation, these results indicate that domestication through the generations of hatchery

rearing has a large potential to reduce reproductive fitness of hatchery fish in the wild.

The first generation of H_{supp} (H_{supp} created from wild broodstock) reproduced better than H_{trad} in the wild, but generally worse than wild fish (Fig. 2). The difference in reproductive success between first generation H_{supp} and wild fish was not statistically significant in the first three run years (winter-run 1995–1997, Araki *et al.* 2007a), but it was significant when another three run years of data are included (Overall RRS of first generation H_{supp} to wild fish in winter-run 1995–2000 = 0.85, $P < 0.001$, Araki *et al.* 2007b). These results suggest that when H_{supp} are created from wild parents, hatchery fish can leave 85% of offspring as do wild fish (in contrast to only 8–33% RRS in H_{trad}). The difference in RRS between H_{supp} and H_{trad}

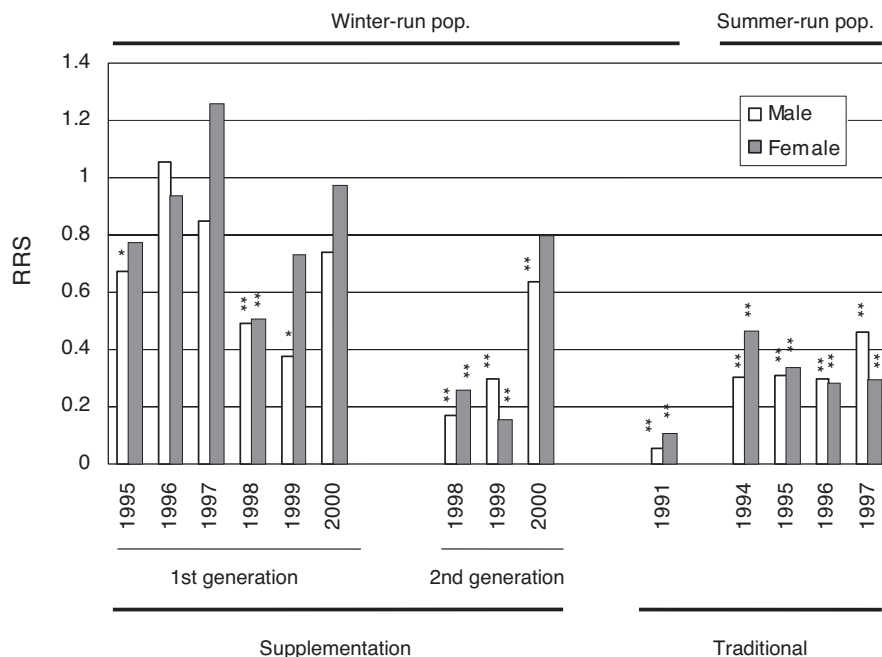


Fig. 2. Relative reproductive success (RRS) of hatchery fish to wild fish in the Hood River steelhead. Original data are shown in Araki *et al.* (2007a, b). $RRS = 1$ (average reproductive success of hatchery fish is equal to that of wild fish in the same run year) was used as a null hypothesis of the permutation test. RRS was estimated for fish from two types of hatchery programs operated during 1991–2000. In winter-run population, overall RRS of hatchery fish to wild fish was 0.848 for the first generation of supplementation hatchery fish, 0.379 for the second generation, and 0.079 for the traditional hatchery fish. In the summer-run population, overall RRS was 0.334 for the traditional hatchery fish ($P < 0.01$ in all four cases above). Reproductive success of supplementation hatchery fish in summer run population was not evaluated because enough number of offspring from the summer-run supplementation hatchery has not returned yet (Table 1). Angling of hatchery fish was allowed above the dam between run years 1992 and 1997 but ignored in this table because the correction for harvest had only minor effects on our conclusions (see Araki *et al.* 2007a). * $P < 0.05$, ** $P < 0.01$.

indicates that the supplementation hatchery practice indeed improves the effectiveness of hatchery program for the restoration of wild population, at least in a short term. Whether the 15% decrease in reproductive fitness compared to wild fish is acceptable or not and what that means to the long term (evolutionary) consequences of wild populations remain open questions.

In the second generation of the supplementation program (winter-run 1995–1999), the hatchery managers started using the returning first generation H_{supp} as broodstock

to create more hatchery fish. Although [hatchery $\times$ hatchery] broodstock cross was avoided, both [hatchery $\times$ wild] and [wild $\times$ wild] broodstock crosses were allowed in the hatchery (Fig. 3). By reconstructing parentages between second generation H_{supp} and their wild-born offspring, the reproductive success of the second generation H_{supp} in the wild was evaluated. RRS of the second generation H_{supp} from the [hatchery $\times$ wild] crosses to wild fish was significantly lower than 1.0 (Fig. 2, Overall $RRS = 0.38$, $P < 0.001$, Araki *et al.* 2007b).

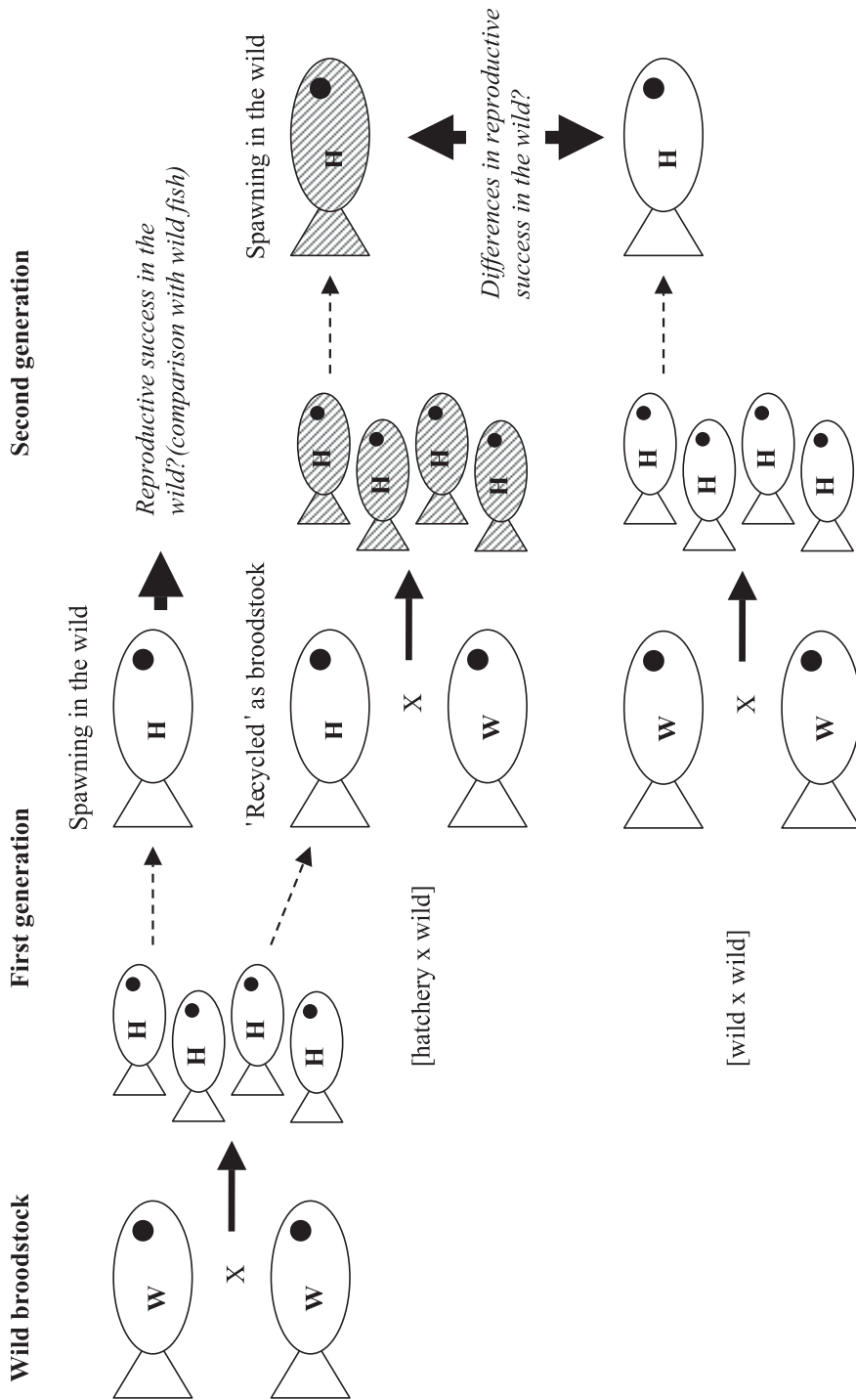


Fig. 3. Conceptual diagram for the evaluation of natural reproductive success in supplementation hatchery fish. Shaded fish represent hatchery fish from a hatchery parent and a wild parent, which are compared with hatchery fish from two wild parents.

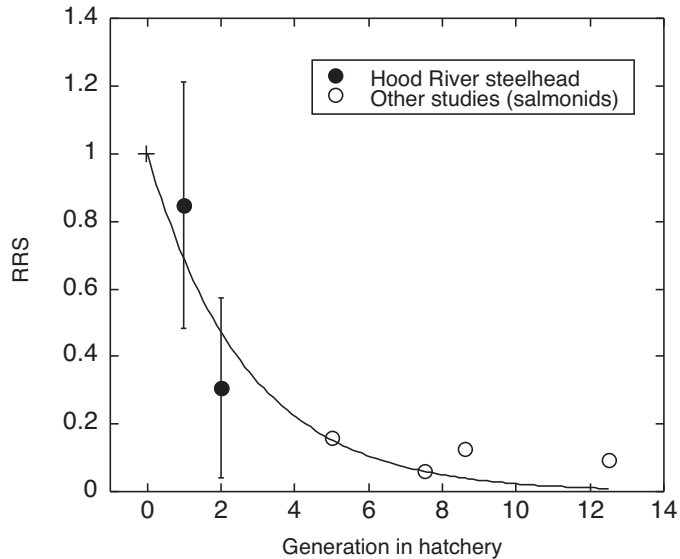


Fig. 4. Relative reproductive success of salmonid hatchery fish to wild fish plotted against generation time in captivity (cited from Araki *et al.* 2007b). Closed circles represent the estimates from the Hood River steelhead (weighted geometric means from Fig. 2). The open circles represent four other estimates from studies on local hatchery stocks of various salmonid species (See text for details). The exponential regressions suggest a 37.5% fitness decline per captive reared generation ($y = e^{-0.375x}$, correlation coefficient = 0.962).

We also performed a meta-analysis including data available for other salmonid hatchery stocks that have local origins. In this analysis, we combined the *RRS* estimates from the Hood River steelhead with those from four other studies, two on steelhead, one on Atlantic salmon, and one on brown trout (Salmon Recovery Science Review Panel 2004; Araki *et al.* 2007b and references therein). These data fit very well on an exponentially declining curve (Fig. 4), despite the fact that these data include *RRS* estimates using different species and methods. The clear decline in reproductive success as a function of the number of generation in hatchery suggests: The reproductive fitness of hatchery fish in the wild can decline very quickly when the broodstock have been reared for multiple generations in a hatchery. Reproductive fitness decline can be as high as 37.5% per generation in the hatch-

ery (Fig. 4); The cause of the fitness decline is cumulative through captive-reared generations, indicating that genetic changes in the hatchery rearing are largely responsible for the fitness decline. Our results are consistent with those from other studies, in which genetic degradation of fish in hatchery is suggested to have a large effect on reproductive traits of hatchery fish (Reisenbichler and McIntyre 1977; Reisenbichler and Rubin 1999; but also see Dahl *et al.* 2006; Ford *et al.* 2006). The genetic effects of hatchery rearing are further discussed in the next section.

5. Genetic Effects of Hatchery Rearing

One problem for determining effects of hatchery rearing is the confounding effects of genetic and non-genetic factors. Hatchery

environments differ radically from natural environments, thus the reproductive success of hatchery fish might be directly affected by environmental (non-genetic) factors. While the cumulative fitness effects of hatchery rearing indicate that the major cause of the decline is heritable (and hence genetically-based), we cannot completely exclude the effects of the different rearing environments in the comparisons between hatchery and wild fish. In addition, H_{trad} had non-local origins in the Hood River steelhead, and the difference in their genetic origins also confounded this comparison.

A major advantage of the second generation study on Hood River steelhead (Araki *et al.* 2007b) is that we could eliminate the environmental factors. As mentioned above, we had two types of hatchery fish in the second generation (hatchery fish from the [hatchery $\times$ wild] cross and those from the [wild $\times$ wild] cross, Fig. 3). Comparison of the reproductive success of the two types of hatchery fish, instead of the comparisons between hatchery and wild fish above, was used to directly evaluate the genetic effect of hatchery rearing for one additional generation. Both types of hatchery fish were reared in the same hatchery at the same time, and shared the same local origin. The only difference between these hatchery fish was the number of generations for which half of the genome was exposed to the hatchery environment. Our data show that the overall RRS of H_{supp} from [hatchery $\times$ wild] to H_{supp} from [wild $\times$ wild] was only 55% ($P = 0.009$, Araki *et al.* 2007b), suggesting that H_{supp} from a hatchery parent and a wild parent had lower reproductive success than H_{supp} from wild parents. This result also suggests that the extended hatchery rearing for only one generation can lower reproductive fitness of the next generation in the wild, and that the negative effect is most likely genetically-based.

6. Effective Population Size

In the above sections we focused on the average reproductive success of hatchery fish in the wild. The individual-based parentage analysis also provides the direct estimate of variance in reproductive success, which is another key factor to understanding how genetically effective the individuals are in producing the next generation (Crow and Morton 1955). In this section, I briefly summarize a study on effective population size (N_e) of the Hood River steelhead, on the basis of parentage assignments and the estimates of the variance in reproductive success (Araki *et al.* 2007c). Although I focus on the demographic estimates of N_e , it is noteworthy that empirical comparisons of N_e estimates using different methods in the Hood River steelhead also provided an important finding that a temporal estimation of N_e (a genetic method) contains a systematic bias when reproductively inferior groups (e.g., hatchery fish) are included in the estimation (Araki *et al.* 2007d).

N_e can be defined as the size of an ideal population that has the same rate of genetic change as the population in question. An ideal population is one in which mating is occurring at random, population size is constant and the sex ratio is 1:1 (Wright 1931; Crow and Kimura 1970). N_e is a fundamentally important parameter in evolutionary and conservation biology because effectiveness of natural selection and the level of genetic variation within a population depend on the effective population size (Crow and Kimura 1970; Frankham *et al.* 2002). N_e is generally smaller than census population size (N) because the conditions for the ideal population are seldom, if ever, met in real populations (e.g., Frankham *et al.* 2002; Turner *et al.* 2002).

In Hood River steelhead, we found that overall N_e of steelhead per generation in the river was not small ($N_e \sim 1000$ –1500 per

generation, Araki *et al.* 2007c). This result is consistent with the results in the previous sections because natural selection is effective only when N_e is large, and we expect large variance in reproductive success when natural selection is strong (Crow and Kimura 1970). However, the N_e/N ratio was only 0.17–0.40, with large variance in reproductive success among individuals being the primary cause of small N_e/N (Araki *et al.* 2007c). The small N_e/N indicates that the level of population genetic diversity could be low in this population even if there are large numbers of individuals in the population (e.g., Frankham *et al.* 2002). In our case, biased sex ratio (male:female = ~2:3) and temporal fluctuation in population size within generation had relatively minor effects on N_e/N . Interestingly, H_{trad} showed large variance in reproductive success among breeding parents, whereas H_{supp} showed no sign of increased variance when compared with wild fish (Araki *et al.* 2007c). It suggests that H_{trad} , but probably not H_{supp} , impose a genetic risk of reducing N_e on the wild population.

7. Genetic Compensation between Life-History Forms

Another interesting result in the effective population size study (Araki *et al.* 2007c) is a novel function of reproductive interaction between life-history polymorphisms. As mentioned earlier, *O. mykiss* has two major life-history forms, anadromous (steelhead) and resident (rainbow trout). They have distinct morphologies at the adult stage, but they interbreed in natural environments when both forms coexist sympatrically (Zimmerman and Reeves 2000). Although we could not obtain genetic samples from the resident population, the near-complete sampling from anadromous population enabled us to infer when and how much the resident fish contributed to the production of the anadromous population (Araki *et al.* 2007a, c). We dis-

covered that the reproductive interaction between life-history forms can work as a genetic buffer, by which the relatively large effective population size is stably maintained: According to the parentage analyses, inferred reproductive contribution of resident parents to anadromous offspring was larger when fewer anadromous parents had returned to spawn in the Hood River (Araki *et al.* 2007c). It means that resident fish provided a maximum support for the reproduction of anadromous fish when the anadromous population was at the risk of genetic bottleneck. In fact, theoretical prediction suggests that the effective population size could have decreased 40–60% if there was no contribution from resident population (unpublished data). If such a genetic buffering between life-history forms is common, a conservation plan must be designed carefully so that the wild populations do not lose the buffering function via losing life-history polymorphisms in the same system. In the case of steelhead, unfortunately conservation plans often focus strongly on saving anadromous population and rarely take its resident counterpart into account.

8. Discussion

The most striking result from the studies of Hood River steelhead is the rate of fitness declines of hatchery fish—perhaps 30–40% per captive-reared generation (Fig. 4). The rate of fitness decline implies that hatchery fish rapidly lose their reproductive capabilities in the wild, presumably as a consequence of adaptation to hatchery environments (i.e., domestication). Strong influence of domestication is not generally expected for supplementation hatchery programs because intentional artificial selection is usually avoided in this type of hatchery program. However, quantitative genetics theory suggests that rapid domestication is not impossible if domestication selection works on multiple traits at the same time, or very strong selection

Table 2. Hypothetical stocking effects on population size in the next generation.

$N_{[wild]}$	$N_{[stocked]}$	$\%_{[stocked]}$	$N_{[offspring]}$ (No. of generation in hatchery)					
			0	1	2	3	5	10
100	10	9.1	110	106	103	102	101	100
100	100	50.0	200	163	139	124	110	101
100	1000	90.9	1100	725	491	344	195	109
100	10000	99.0	10100	6350	4006	2541	1054	191

This table illustrates expected demographic consequences of different scales of hatchery stocking into a hypothetical wild population with $N = 100$ ($N_{[wild]}$). $N_{[stocked]}$: Number of hatchery-born fish stocked into the population. $\%_{[stocked]}$: a percentage of hatchery-born fish in the stocked population. $N_{[offspring]}$: Number of wild-born offspring in the next generation. $N_{[offspring]}$ is a product of N after stocking ($=N_{[wild]} + N_{[stocked]}$) and the average reproductive fitness of the parents (W). For simplicity, absolute reproductive fitness of wild fish = 1, 37.5% fitness decline of stocked fish per hatchery-reared generation (see text), and no influence of the carrying capacity are assumed to calculate W . This table shows, for example, when ten fish are stocked into the wild population with $N = 100$ (first line in the table), the expected number of wild-born fish in the next generation is 110 if there is no fitness decline, but is 106, 103, 102, 101 and 100 if hatchery stock is from the first, second, third, fifth, and tenth generations in a hatchery, respectively.

works on a single trait both in captivity and in the wild and the trait under selection is highly heritable between generations (Araki *et al.* 2008). Another possible mechanism is an accumulation of deleterious mutations due to relaxation of natural selection, which would be certainly occurring in well-protected hatchery environments. However, mutation accumulation is unlikely to explain such a rapid fitness decline *per se*, while it might have fitness consequences in a long span of hatchery stocking (Lynch and O'Hely 2001).

The rapid fitness decline also implies that the repeated use (i.e., recycling) of hatchery broodstock to produce the next generations can result in a large loss of reproductive fitness in hatchery fish. The most obvious consequence of the low fitness of hatchery fish could be a failure of population size recovery with hatchery stocking. A simple calculation demonstrates the effects of fitness decline on the population size in the next generation: Let's assume that a current population is well under the carrying capacity and that there is no density dependence in popula-

tion growth for simplicity. Then the number of wild-born offspring ($N_{[offspring]}$) after hatchery stocking can be calculated simply as $N_{[offspring]} = NW$, where N is the total number of parents in a population and W is the average (absolute) reproductive fitness of the parents. Table 2 shows some of the demographic consequences of hypothetical stocking of hatchery fish, which have low reproductive fitness due to up to 10 generations of hatchery rearing. In these examples 37.5% fitness decline per captive-reared generation was assumed (from Fig. 4). It is clear that supplementation hatchery programs lose the potential for increasing the size of wild population very quickly as they use more heavily recycled hatchery stock. For instance, under the scenario of 50% stocking (i.e., when stocking intends to double the population size), population size increases only 39%, 10%, and 1% in the next generation if hatchery stocks are maintained for two, five, and ten generations in captivity, respectively. These percentages would be even lower if mortality of the stocked fish is higher than that of wild fish in natural environments

before reproduction. Of course larger scales of hatchery stocking increase the population size more in a short term (in the case of no restriction due to carrying capacity), but such a heavy stocking might reduce fitness of the wild population in a long term, considering the fact that hatchery fish have genetic differences that reduce reproductive fitness in the wild.

At this point, it is unclear whether hatchery programs are generally helping or harming the wild populations. However, evidence from the Hood River research suggests that hatchery programs, even the supplementation programs, produce reproductively inferior fish than wild fish. It means hatchery fish are not genetically or reproductively equivalent to wild fish. Especially, the negative effects of H_{trad} (small average and large variance in reproductive success) suggest that the hybridization between H_{trad} and wild fish should be carefully avoided in order to protect the wild population. Our data show that the use of local, wild fish has an advantage over the traditional hatchery programs for recovering the wild population. However, the cumulative effect of the hatchery rearing on the fitness of H_{supp} also suggests that the recycling of hatchery broodstock imposes a high risk both genetically and demographically. Genetic changes of hatchery fish occur rapidly, and the reuse of hatchery fish should be considered as a final option (e.g., when wild populations are strongly depressed and a collection of new wild broodstock is impossible). Given the fitness concerns, alternative conservation plans such as habitat restoration or control of over-fishing might be more effective in restoring wild populations in some cases.

Understanding the actual mechanism of fitness decline and the trait(s) under selection pressure will hopefully guide us to solutions for mitigating the negative effects of hatchery rearing. Previous studies show that many traits can be associated with fitness declines, such as egg size, growth rate, and

feeding and mating behaviors (Johnsson and Abrahams 1991; Arendt 1997; Berejikian *et al.* 2000; Einum and Fleming 2000; Heath *et al.* 2003; Reisenbichler *et al.* 2004; Tymchuk *et al.* 2007). However, none of these has yet been proven to be responsible for the observed rate of fitness decline to date. Monitoring and evaluating the effects of hatchery programs on wild populations are important for improving the designs of the hatchery and stocking programs. It is also noteworthy that the most appropriate design might be different among programs because it often depends on the goal of the program, conditions of wild populations, and species of interest.

The reproductive success of *wild* fish that have hatchery-born ancestors is another important issue for self-sustainable wild populations. Given that the fitness decline of hatchery fish is genetically-based, it is possible that even wild fish suffer low reproductive success after generations of supplementation due to introgression of 'hatchery' genes. However, it is also possible that one or a few generations of exposure to the natural environments are enough to eliminate the maladapted genes from the wild population. In the latter scenario, surviving wild fish from hatchery ancestors might show no difference in reproductive success when compared with wild fish from wild parents. Again, the DNA fingerprinting and the genetic parentage analysis are powerful tools to evaluate these possibilities, and the evaluation study is currently ongoing on the Hood River steelhead (Araki *et al.* in prep.).

Results from recent studies show that we are in a new era, in which we can directly test the effects of hatchery stocking on wild populations using various molecular techniques. I believe that we can reach to general conclusions on the effectiveness of hatchery stocking and more sophisticated means of hatchery/wild stock management—hopefully before too many wild populations go extinct.

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Appendix: Terminology

The Hood River hatchery programs for steelhead trout:

Traditional hatchery programs for the Hood River steelhead had been operated since mid 1900s until supplementation programs took place in 1990s. The Hood River supplementation programs of winter-run and summer-run steelhead (definitions below) started in 1991 and 1997, respectively (Olsen 2003). In the supplementation programs, broodstock (parents of hatchery fish) are collected from the Hood River each year. They are crossed at the Parkdale Hatchery on the Middle Fork of the Hood River, and hatchery fish are reared to yearling smolts. Smolt is juvenile fish that changed phenotypes from freshwater form to ocean form through a developmental process known as smoltification (e.g., growing silver scales). The smolts are first acclimated in the Parkdale Hatchery before release or are released directly into the Hood River. All hatchery fish are adipose-fin clipped before release, and fish without adipose fin are identified as hatchery fish at the sampling dam trap when they return as adults. The returning supplementation hatchery fish have been allowed to pass the Powerdale Dam since 1995 for winter-run and 2001 for summer-run (Table 1).

Run populations: The Hood River supports two run populations of steelhead (summer-run and winter-run), named for the time of year the most adults return to freshwater. Both runs breed in the spring. However, sum-

mer-run returns to the river up to one year earlier than the spawning, whereas winter-run returns to the river shortly before the spawning. They distribute to different forks of the river (Fig. 1). Our parentage analysis confirmed that there is little or no hybridization between them (Araki *et al.* 2007c).

Run year: Run year X of the parents represents a parental group that returned to the river between the year X and $X + 1$ and spawned in the year $X + 1$. For example, fish in the winter-run 1991 returned between 1991 and 1992, and spawned in 1992.

RRS: Relative reproductive success. *RRS* can be defined as a ratio of the average numbers of offspring from two types of parents. For example, if hatchery fish left one wild-born offspring per parent whereas wild fish left two, the *RRS* of hatchery fish to wild fish is 50% (1:2). We used an unbiased estimate of *RRS*, in which systematic bias due to errors in the genetic parentage assignments is corrected (Araki and Blouin 2005). The bias in the *RRS* estimation is inevitable especially when genetic markers are highly polymorphic, there are null alleles, and a part of the parents are not sampled (Araki and Blouin 2005). Overall *RRS* is a geometric mean of *RRS* between two groups of parent (e.g., hatchery fish and wild fish) over run year and sex. Each point estimate was weighted by number of parents. Geometric mean was used to summarize *RRS* because each estimate was obtained as a ratio of the absolute reproductive success.

A Review of Bycatch and Discard Issue Toward Solution

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A total of 27 million tons and a revised value of 6.8 million tons of global discards have been estimated, however, there is still no reliable discard estimation globally. The reason is the fuddled definitions of the terms used in this field such as bycatch and discards and methodological mystification in their applications. Despite the concerns, factual problems of discards have been limitedly discussed scientifically. The survival possibility for discarded organisms is a major subject, where high survival rates are found for matured individuals and large species of finfishes and crustaceans but low, for juveniles and small finfish species. The subsequent energy flow after discards is also a large research topic. Externalisation of discards by seabirds on surface and scavenging on seabed do not support the hypothesis of enrichment of surface water by nutrients from discards. Approaches toward solutions to discards include exclusion of bycatches underwater by improvement in fishing technology such as selectivity enhancement and development of bycatch exclusion devices and promotion of landing of discards otherwise. A simple analysis on the commonness between bycatch and discards is encouraged in realistic approach toward exclusion of juveniles of useful species and promotion of landing of unutilised species.

KEYWORDS bycatch; discard; incidental catch; capture fishery; resource; management; mortality; survival

1. Introduction

Discarding bycatch began drawing attention as the most serious wastage in capture fisheries against rational utilisation of marine resources during the 1970's. Researches on

bycatch and discards of fisheries organisms has been actively conducted around the world after that FAO promoted conversion from discards to utilisation in 1982 (FAO 1982). It has become an international issue in fisheries management since the estimation of global discards by FAO Technical Paper

339 (Alverson *et al.* 1994) and adoption of FAO Code of Conduct for Responsible Fisheries in 1995, where bycatch and discards were dealt repeatedly. A variety of researches have been conducted, such as *in situ* surveys of discard practices, development of technology to reduce bycatch and discards, and studies on the consequences of discarding in the ecosystem and formulation of the scientific basis approaching through sensory and behaviour of aquatic organisms. The orientation toward these researches during the mid 1980's was conjectured to be a reflection of, at least in part, the world-wide trends toward conservation of aquatic environment and resources those days. It is also a character of this issue that efforts have been devoted to the estimation of the discard amount in par with the researches toward solutions, because FAO Technical Paper 339 reported an estimate of a large amount of discards in regions. An increasing number of case studies are still being conducted in the 2000's globally, including those in the third world countries.

The bycatch issue involves those of both fisheries organisms such as finfishes, crustaceans, etc. and non-fisheries animals which are not aimed in fishing, such as marine mammals, marine reptiles and seabirds. The latter is referred to as an incidental catch, although the term of bycatch implies both. The bycatch and discard issue tends to refer only to the former. Two groups of animals have different characters both in implications in the fisheries management and from the viewpoint of the required technology toward solutions, therefore, it is difficult to deal with in one context. This paper deals with mainly the bycatch and discards of fisheries organisms and reviews the historical progress in the issue.

2. Definition of Terms

Definitions of the terms used in this field are chaotic. Catch is defined as those hauled up once onto deck with fishing gear, then re-

tained in part, while the remainder is discarded. Retention and landing are almost synonymous in the case of industrial fisheries, however, self-consumption in retention seldom appears in landing statistics. The definition of discard as those non-retained in catch is almost consensus, however, there are still different opinions such that release in sport fishing or safe returning to the sea after friendly sorting onboard should be excluded. Discard also takes place on land, which is not included in the landing.

Despite the title of this paper, bycatch is hardly defined scientifically. The definition of bycatch is different among countries and researchers (Alverson *et al.* 1994), and common basis is hardly pertained. Alverson used a concept of a set of target catch and bycatch, however, target catch is subjective and it is not a realistic approach to identify a single target species and to define all the others as bycatch. According to this definition, there is a landed bycatch, which could be referred to as by-products. The concept is hardly applicable to multi-species fisheries which are prevailing over Asian region and tropical waters. An enormous effort has been, therefore, devoted by e.g. SEAFDEC to define the terms applicable in Asian fisheries (SEAFDEC 1998). This paper temporally defines bycatch as is the irrational catch rather than the catch of species, sizes and sex acceptable in resource conservation and management.

3. Estimation of Bycatch and Discard Amounts

The amount of discards was first estimated in the prawn trawl fishery (Slavin 1982). A total of 5 million tons of annual discards in the world prawn trawl fishery was estimated on the basis of the discard ratios against the catch of prawns, which are usually 5 to 10, and are multiplied by a total production of prawns (a discard ratio and its application will be explained in detail later). This method

Table 1. Global and regional discards estimated in 1994.

Region No.	Nominal regions	Discards (1000MT)	(%)
61	Northwest Pacific	9,132	33.8
27	Northeast Atlantic	3,671	13.6
71	Central-west Pacific	2,777	10.3
87	Southeast Pacific	2,602	9.6
31	Central-west Atlantic	1,601	5.9
51	West Indian	1,471	5.4
67	Northeast Pacific	925	3.4
41	Southwest Atlantic	803	3.0
57	East Indian	802	3.0
77	Central-east Pacific	767	2.8
	Others	2,461	9.1
Total		27,012	100

*Tabulated from Alverson (1994)

was applicable only because the anticipated prawns and unwanted finfishes so-called as bycatch are distinctively segregated in this sector. The estimation of discards in later researches was unconsciously affected by this method.

FAO Fisheries Technical Paper 339 estimated 27 million tons of global discards annually (Alverson *et al.* 1994). Though discard estimates by country were concealed, it was presented that 47% of the global discards occurred in Asian waters, in particular, 9.13 million tons in Region-61, or Northwest Pacific, which was unrealistically large (Table 1). Responding to the criticisms, FAO convened an expert consultation meeting (FAO 1996). It was revealed that Paper 339 had methodological weakness which did not take the landings as non-target catches into account and fell into over-estimation of discards (Matsuoka 1996), although it was not described in the conclusion of the meeting (FAO 1996).

A revised estimation of global discards of 6.8 million tons was publicised by FAO (Kelleher 2005). This report included unacceptably unnatural assessments, for example, that the discard amount estimated for Korea was the smallest among Asian coun-

Table 2. Estimation of global and national discards revised in 2005.

Countries (Asia)	Discards (1000MT)	(%)
Cambodia	0.0	0.0
China	74.3	1.1
Indonesia	270.4	4.0
Japan	918.4	13.5
Korea	1.0	0.0
Malaysia	10.4	0.2
Myanmar	27.4	0.4
North Korea	1.1	0.0
Philippines	7.5	0.1
Thailand	27.8	0.4
Viet Nam	17.8	0.3
Others	5463.1	80.1
Global Total	6819.2	100.0

*Tabulated from Kelleher (2005)

tries and less than that for North Korea, despite no scientific data available for this criticism (Table 2). There is still no reliable estimation of global discards even nowadays.

There are several reasons of the confusion in discard estimation. One is the fuddled definitions of bycatch and discards as explained above. The other is that less logical consideration was given to the estimation

methodology, perhaps, because it is too simple for scientific studies. In order for discard estimation, a discard ratio r in a certain fishing sector is found by field survey as Eq. (1) (Alverson *et al.* 1994; Matsuoka 1999) where d_k and c_k are discard and landing amounts at the k -th sampling in K times. The total amount of discards D in the concerned sector is estimated as Eq. (2), with the total amount of landing C in the sector, which is usually available in landing statistics. However, a variety of discard ratios are definable, because the landing of either summed species, target species, or an individual species is adoptable as a denominator. In application of the Eq. (2), a discard ratio must be multiplied only by the corresponding landing statistics, e.g. where an individual species is used as a denominator, landing statistics of the particular species must be used as C .

$$r = \frac{1}{K} \sum_{k=1}^K \frac{d_k}{c_k} \quad (1)$$

$$D = r \cdot C \quad (2)$$

Matsuoka (1999) proposed that a discard estimation method with the discard ratio against summed species in each sector is the best in order to avoid the confusion. Though Alverson (1994) also defined the difference between the discard ratio and the discard rate, these are still disorderly used. This is partly attributed to improper translation of the results of bycatch and discards surveys published in local languages.

Another method based on 'discards per unit effort' (DPUE) q is also possible as Eqs. (3) and (4) where E is the total unit of fishing effort in a concerned sector (Matsuoka 1999). It is advantageous to use DPUE together with CPUE for an assessment of a fishing sector, however, Eq. (4) is applicable only where the fishing effort statistics are available.

$$q = \frac{1}{K} \sum_{k=1}^K d_k \quad (3)$$

$$D = q \cdot E \quad (4)$$

4. True Problems of Bycatch and Discards

Despite the prevailing concerns to bycatch and discards with emotional warnings sometimes, there had been a few scientific discussions on what are the problems in reality. Bycatch was detested as a hazard in trawl operations in early days (High *et al.* 1969) and smells of catches in bottom fishing due to rotten discard fish and contamination on beaches have been reported, however, most concerns were raised from a viewpoint of protection of wild life and management of resources (Seidel 1975). Bycatch includes the endangered or protected species or juveniles of commercially important species usually, therefore, the criticism is appropriate. Discarding juveniles of highly commercial species due to landing size regulations undermines the principal purpose of fishing regulations. There are many suggestions which indicate that mortality by discards may provoke negative impacts to aquatic ecosystems and biodiversity, consequently, discards of even non-utilised species are unacceptable. A variety of conflictions have been reported where bycatch species are those aimed in other fishing sectors (Juhl and Drummond 1976; Watson and McVea 1977). In developing countries, when bycatch and discards by industrial fisheries occur to the species utilised in subsistent fishing by nearby villagers, a unique problem arises against a national development policy such as conservation of the basis for rural life (Sujastani 1984; Matsuoka and Kan 1991).

Regarding the above deliberations, there is an essential subject if the discarded organisms cannot survive, because all the

criticisms are based on a presumption of mortality of discards. This is the issue on which the largest research effort has been devoted recently. The first experiment in this field was conducted in the Gulf of Carpentaria. Survivability after exposure for 10 to 15 minutes in air was higher than 50% for only crustaceans and matured fish of large species, while, many small finfishes and juveniles almost all died (Wassenberg and Hill 1989). Many researches have shown that the mortality of discarded small fishes and those with gas bladders is high (Suuronen 2005), e.g. 100% for whiting (Berghahn *et al.* 1992), and over 85% for juvenile snapper after 15 minutes of air exposure (Sumpton and Jackson 2005), while, the survival rate is high for large fishes, e.g. 100% for lingcod discarded immediately after the codend opened and 50% after 30 min exposure on deck (Parker *et al.* 2003), 78% to 90% for catshark (Rodriguez-Cabello *et al.* 2005), and 98% for dogfish (Revill 2005). Survival of discarded crustaceans is high, e.g. estimated 80.4% or 96.1% for snow crabs (Warrenchuk and Shirley 2002), 100% for brown-shrimp under low temperatures after stay on deck for a short time (Gamito and Cabral 2003), and 78 and 80% for discarded juvenile shrimps *Crangon crangon* (Lancaster and Frid 2002). Survival may be adversely affected by delayed effects by damages including injury and stress due to exposure in air and surface water (Bergmann and Moore 2001a, b; Bergmann *et al.* 2001; Harris and Ulmestrand 2004). Survival depends on the species, sex and size of animals, maximum fishing depth, sorting time on deck and deck conditions such as temperature, wetness, etc.

There are counter disputes as bycatch and discards are not problems, e.g. (1) bycatch may reduce predators to juveniles of high-value species and contribute to human utilisation of resources, (2) discards of demersal species contribute to vertical circulation of nutrients and enhance the productivity of fishing grounds, and (3) flat mortality to all

the species is rather friendly to ecosystem. These disputes appear mainly in media and at meeting floors, however, seldom, in scientific papers.

The above criticism has encouraged researches on the consequences of discards recently. Brewer (Brewer *et al.* 1991) first analysed stomach contents of bycatch finfishes by prawn trawlers in the Gulf of Carpentaria and found that they eat small non-commercial shrimp species. Mortality of small finfishes reduces the predation pressure to shrimps other than commercial prawn species and this does not support the hypothesis (1) above.

The energy flow from discards to subsequent consumption by organisms is one of the largest research subjects recently. Externalisation of discarded fishes by e.g. seabirds are actively studied, where discarded benthic-mesopelagic prey fishes are consumed by seabirds as supporting their population (Blaber 1995; Bertellotti and Yorio 2000; Pedrocchi 2002). Hill and Wassenberg (2000) conducted a precise investigation on the probable fate of discards from prawn trawlers. Floating fish was scavenged by dolphins, sharks and seabirds on the surface and 14% of discards were estimated to be available to seabirds. Discards over 80% by weight sink, where the majority are scavenged on the seabed mainly by fishes, small sharks and possibly cirrolanid isopods. Catchpole *et al.* (2006) demonstrated that seabirds utilise the estimated 57% of the discarded material and populations of scavenging species in the fishing ground is supported by sank materials. Mid-water scavenging of discards is relatively unimportant (Erzini *et al.* 2003). All these suggest that discards could not enrich surface waters in nutrients and do not support the hypothesis (2).

Poiner and Harris (Poiner and Hallis 1986) compared bycatch finfish compositions between before starting the prawn trawl industry and 20 years later in the Gulf of Carpentaria and found that species such as

ponyfishes *Leiognathus* spp. distinctively declined. This result suggests that the impacts of bycatch and discards are different among species and denies the hypothesis (3). Regardless of this analysis, such an assumption that bycatch induces flat mortality over all the organisms in an ecosystem is far from a reality, because of the biased sampling by selectivity of fishing gear.

There is no scientific evidence to refuse another assumption that bycatch mortality may give a positive effect such as the density of valuable species or nutrient condition. A research which considered an input from discards on the basis of carbon and nitrogen (Cabral *et al.* 2002) may reflect such a viewpoint. There is a discussion that discarding is not a problem as long as landing is maximised. It is suggestive to point out that the bycatch and discard issue is a matter how to utilise a fishing ground and resources rationally and maximisation of allowable catch is the final goal. It must be carefully studied if there is a way of resource use to increase MSY under a condition where bycatch and discards occur.

5. Solutions to Bycatch and Discards

Occurrences of bycatch are attributed to weak selectivity of fishing gear and multi-species characters of fishing grounds (Seidel 1975; Watson and McVea 1977), while, discards are attributable to mainly less profitability. Possibility of discards is high where the price difference between wanted species and others is large (Seidel 1975). Where fishing grounds are in rural areas far from markets, even utilisable species may be discarded. Some discards occur due to catch or landing regulations for resource management. Discards due to genuine non-edibility must be peripheral. The insufficient space of fish holds on fishing vessels is mentioned as a reason (Slavin 1982), however, this is a synonymous of no consideration given to bycatch in vessel designing.

There have been two ways of approaches toward countermeasures against bycatch and discards as; (1) underwater exclusion of unwanted species by improvement in fishing technology, and (2) utilisation of bycatch. Utilisation includes those for other sectors such as aquaculture feed and materials for other industries in addition to human consumption (Allsopp 1982).

5.1. Improvement in fishing technology

For development in fishing technology toward reduction of unwanted catch, there are two directions; (1a) improvement of fishing gear design toward enhancement of size- and species-selectivity, and (1b) development of bycatch exclusion devices to be added to fishing gear.

The typical approach in the former direction is the conventional mesh-size regulation to exclude small individuals of fishes to be discarded otherwise. A variety of tests have been conducted to activate size-selectivity by opening meshes widely, e.g. using square-mesh panels. It is the latest achievement that discards can be reduced by around 40% through simple modification to trawl gear with square-mesh panels (Cooper and Hickey 1989; Robertson 1989; Revill *et al.* 2007). Starting researches on species-selectivity over the world in the 1990's must be reflection of the bycatch and discard issue. Even peripheral modifications such as changing vertical opening and foot-gear arrangement for trawl nets (Valdemarsen 2001) and changing underwater height and giving spaces around a foot rope for gillnets result in altering the species selectivity. There are still extensive possibilities to exclude unwanted species and sizes by selectivity for most fishing gear.

Development of bycatch reduction technology is advanced in trawl, which has the oldest history of researches since the 1960–70's (Seidel 1975; Watson and McVea 1977). A variety of devices referred to as TED

(Trawl Efficiency Device), BED (Bycatch Exclusion Device) and BRD (Bycatch Reduction Device) have been developed particularly for prawn trawling. This is attributable to that prawn trawling is conducted in multi-species fishing grounds, it distinctively targets prawns, while, it is difficult to exclude small bycatch finfishes with size-selectivity by mesh size regulation because of the similar sizes between prawns and bycatch finfishes (Watson and McVea 1977; Watson *et al.* 1986).

Most bycatch reduction devices for trawl nets are composed of both/either net-webbing/grids and open sections such as windows or large-meshes. The former function is an induced exclusion by mechanical size-selectivity and the latter, passive exclusion based on difference in behaviour amongst animals (Matsuoka and Kan 1991; Matsushita 2000). Most bycatch reduction devices combine both functions. The net webbing used by High *et al.* (High *et al.* 1969) and Seidel (Seidel 1975) and a metal grid used by Karlsen and Larsen (Karlsen 1989) were in the former category, while, the large-meshed sky-light designed by Rulifson *et al.* (Rulifson *et al.* 1992), the latter. The famous NMFS TED (Watson *et al.* 1986) had a metal grids referred to as a deflector and lateral windows. Soft BEDs such as Morison type (Kendall 1990; Clark *et al.* 1991; Andrew *et al.* 1993) have merits of easy handling.

Rulifin (Rulifin *et al.* 1992) set an achievement criteria in bycatch exclusion in prawn trawl, as bycatch reduction more than 50%, while, loss of wanted catch less than 5% in weight. This criteria is still hardly achieved constantly. Watson *et al.* (1986) reported that unwanted finfishes were excluded at approximately 50% with almost no loss of targeted prawns, however, it was a result in a fishing ground where the major bycatch finfish was grouper which is easy to exclude (Matsuoka and Kan 1991). Comparable results were not obtained with the device in other fishing grounds (Sujastani

1984). This is because a function of a bycatch exclusion device depends on fish sensory and behaviour, therefore, the required designs of exclusion devices differ according to bycatch species compositions, or from fishing ground to fishing ground. This is one of the difficulties in development of bycatch exclusion technology. The latest achievement is that the Nordmore grid could reduce finfish bycatches by 48–74%, while, loss of targeted lobster and shrimp, 4–15% (Fonseca 2005).

Another reason of relatively low exclusion ratios for exclusion devices is that the chances for organisms to encounter with their grids or meshes are limited. Where the probability to pass through a grid or mesh space when an individual animal of size L at once encounter is denoted as $f(L)$, the probability of remaining after encountering for n times is described as Eq. (5).

$$S(L) = (1 - f(L))^n \quad (5)$$

$S(L)$ is the selectivity. The $S(L)$ curve becomes steeper with increasing n (Fig 1). In other words, small individuals which are statically excludable tend to remain when repetition of encountering is limited. Designing to let animals repeat encountering to grids or meshes is needed because they are usually short longitudinally. Suuronen (2005) also suggests that bycatch exclusion devices must be set at an appropriate position in net because the fish individuals pressed on the codend have no more chance to meet them.

Regarding selective harvest and exclusion of bycatch, questions have been raised to the survivability for the organisms excluded underwater. Code of Conduct for Responsible Fisheries repeatedly urges improvement of survivability for excluded escapees. Suuronen (1996, 1997), however, proved low survivability for Baltic herring *Clupea harengus* excluded from a trawl codend and suggested that the codend mesh size management must be reconsidered.

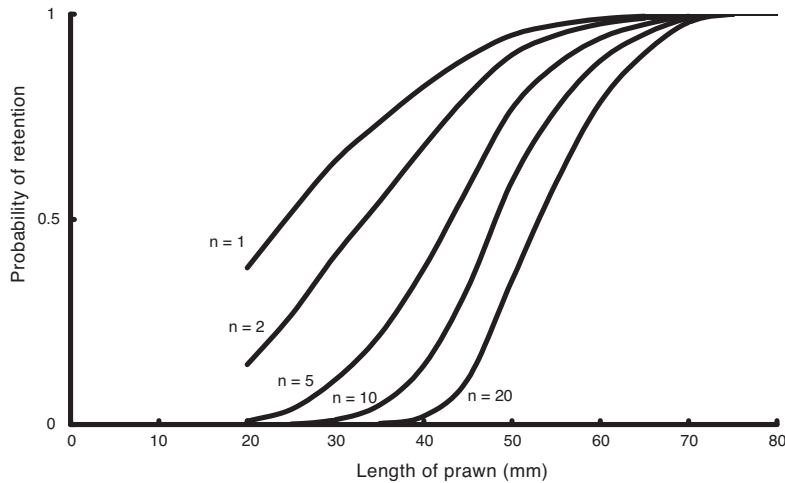


Fig. 1. Simulated selectivity at different numbers of encountering on net; An example of calculation of probabilities of retention of deepwater prawn, changing prawn size and number of encountering to a net webbing with fixed mesh size and hanging ratio.

Extensive works in this field have been conducted through an ICES research programme recently (Ingolfsson *et al.* 2007). Suuronen (2005) is the best summarisation of the researches on the survival issue.

There are trials of technical development towards friendly release of bycatch organisms, for example, release after selection in a shallow tank or under a shower, aiming at high survivability of released ones (Berghahn *et al.* 1992). There is, however, a criticism that the survival possibility for the released individuals are not very high, e.g. chances to return to the original environment may not be very high, in particular, in the case of deep water species, in addition to a question of mechanical damages after even friendly selection. High survivability by this approach is conjectured to be hardly guaranteed in many fishing sectors (Suuronen 2005). Segregation between friendly release and discarding is also questionable.

For further development of bycatch exclusion devices, the technology must give more consideration to merits for fishermen to compensate the possible loss of catches.

Watson (Watson *et al.* 1986) discussed on positive side-effects to be given by bycatch exclusion from a trawl net as; (1) reduction of resistance to a net and consequent fuel consumption, (2) reduction of on-deck work, and (3) improvement of quality of catches. Reduction of fishing gear materials is also a possible merit in such a case as a semi-demersal trammel-net with a space with no net material at the bottom part (Matsuoka 1999). In the case of static fishing gear, e.g. improved gillnets or trammel-nets, loss of wanted catch could be compensated by use of an increased number of fishing gear, as long as the bycatch exclusion ratio is higher than the wanted catch loss ratio. This implies, however, a declined catch efficiency is compensated by increased fishing effort and an essential consideration is needed from the viewpoint of rational development of capture fisheries.

5.2. Promotion of landing

Landing of bycatch has been assumed a fundamental solution to the bycatch and discard issue (FAO 1982). This is based on the idea

that there is no problem as long as catches are utilised. The officials and researchers from some Asian countries relied upon this logic and insisted that there is no discard problem in the region because of landing of small finfishes as trash-fish. It is truism that there are local utilisation practices in small-scale fisheries in many countries in Asia, such as catches are gradually sorted from those for human food to non-human consumptions depending on species, sizes and qualities through marketing and, consequently, catches are all utilised. There is, however, an essential problem that trash-fish usually includes juveniles of commercially important species. Careless promotion of landing may undermine the principle to avoid catching juveniles in the fisheries management. No discard is not necessarily a synonymous of rational utilisation.

Landing of bycatch is usually promoted with public investment. Promotion of landing of bycatch finfishes in prawn trawl is, for example, often conducted as official development assistance (ODA) projects. In an example of the author's observation of an ODA project, fishing companies became able to sell finfishes which were discarded before due to establishment of a marketing system to promote landing and newly born commercial value of those species. No additional investment was needed for fishing companies themselves and, consequently, the shift of the cost and income balance resulted in increased fishing effort and, then, the resource condition of wanted species declined.

5.3. Researches on sensory and behaviour

Active researches on the capture processes are conducted nowadays as the basis for development of selective fishing, and sensory and behaviour of aquatic organisms have been studied in fishing technology. Such works were oriented by, for example, Seidel (Seidel 1975) which indicated that difference

in the swimming ability between prawns and finfishes is the ruling factor in separation in a TED and Watson (Watson 1989) and Wardle (Wardle 1989) which emphasised the difference in behaviour against fishing gear for prawn trawl, in particular, Watson (1989) pointed out the difference between optomotor reaction by finfishes and reflex response by prawns in a trawl net. The author considers guidance by utilising visual responses, including the optomotor reaction, is the key concept in designing bycatch exclusion devices, i.e. discontinuity in the net structure and material colour, including windows and large-mesh sky-light, etc. of TED, is the stimulation to induce actions to result in exclusion of unwanted animals. Passive exclusion relying upon behavioural differences is functional even when the size difference is small between wanted and unwanted species. There are, however, species hardly excluded by this mechanism (Matsuoka and Kan 1991; Matsushita 2000), such as ponyfish *Leiognathus* spp. Utilisation of difference in swimming layer must be the most effective way in development of species-selective gillnets. In addition to the visual sensory, there are many researches on hearing, taste, chemical, tactile and lateral line sensory. Contribution from their results for development of bycatch reduction technology has been still limited, however, the researches in this orientation are important also toward the exclusion of incidental catches of sea turtles and sharks, beyond the mechanical selectivity of fishing gear.

6. Encouragement of Simple Assessment on Bycatch and Discards

In order to restructure the research basis for the bycatch and discard issue, the Division of Capture Fisheries, Japan Society of Fisheries Sciences convened an international meeting in 2005, where standardisation of bycatch and discard monitoring was proposed particularly from a viewpoint of Asian

Table 3. Example of analysis of commonness between retention and discards found in a set-net fishing in Japan.

Retained always			Discarded always		Common between ret. and dis.		
N of species	44		114		44		
5 major species and average weight per catch	Major species	(kg)	Major species	(kg)	Major species	(kg)	Ro
	<i>Acanthopagrus schlegeli</i>	6.7	<i>Strongylura anastomella</i>	9.4	<i>Sarda orientalis</i>	50.8	0.004
	<i>Thunnus albacares</i>	6.4	<i>Apogon notatus</i>	2.3	<i>Seriola dumerili</i>	21.9	0.002
	<i>Lethrinus haematopterus</i>	5.2	<i>Dasyatis akajei</i>	2.3	<i>Trachurus japonicus</i>	21.9	0.003
	<i>Istiophorus platypterus</i>	4.7	<i>Aetobatus narinari</i>	1.9	<i>Trichiurus lepturus</i>	8.7	0.794
	<i>Scomber japonicus</i>	3.9	<i>Myliobatis tobijei</i>	1.1	<i>Auxis thazard</i>	6.2	0.079
G. total		21.2		25.8		160.0	

*Table shows, e.g. that 44 species appeared in both retained and discarded catches throughout the sampling; a large amount of *Sarda orientalis* appeared in this category, however, the specific discard ratio (=specific discards/specific landing) is small; on the other hand *Trichiurus lepturus* is one of the major species in this category and the specific discard ratio is large; therefore, exclusion of *Trichiurus lepturus* must be the research target. The list of the species discarded always is useful to consider the possibilities of utilisations.

fisheries. A simple analyse on the commonness between landed and discarded species was encouraged for the purpose of assessment of bycatch and discards in individual fishing sectors. Table 3 is an example which was found in a survey of set-net fishing in Japan, where hairtail *Trichiurus lepturus* is found in the category of five major species common between landing and discards and its specific discard ratio is large. The species in this category are all commercial species and mainly their juveniles are discarded. It is, therefore, easily found that exclusion of juvenile hairtail must be the key point in the countermeasure research in this sector. This analysis anticipates that the combina-

tion of exclusion of small individuals of the species which appear in landing and discards and promotion of landing of the species which appear always in discards is the realistic approaches toward solutions.

As encouraged by FAO as early as in the 1980's, conversion of discards to landing is comparable to finding new resources for utilisation. The author wishes to urge that researches on bycatch and discards will open a new horizon in capture fisheries and, monitoring of bycatch and discard practices is important for responsible capture fisheries toward solutions to the bycatch and discard problems and it is the synonymous of finding of new food resources for human being.

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Environmental Impact of Aquaculture on Coastal Planktonic Ecosystems

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The main focus of this paper is the nutrient emission from cage aquaculture systems (CAS) in coastal waters and its potential environmental impact on pelagic ecosystems. The nutrient emission from CAS can be estimated based on mass balance using feed use, fish production, nutrients in feed and fish, and digestibility of nutrient components as input data. CAS release inorganic nutrients (NH_4 and PO_4), particulate organic nutrients, and dissolved organic nutrients. Pelagic ecosystems are primarily affected by the inorganic nutrients. A typical Norwegian salmon farm producing 1000 tonnes fish per year generated an amount of nutrient wastes comparable to a community of 7,500 to 10,000 people, with highest emission rates during summer. 3D hydrodynamic modelling is needed to estimate the dilution and transport of nutrients from CAS. Pelagic ecosystems have an inherent capacity of persistence, and smaller changes in nutrient input are mitigated through adaptive responses. There is an upper assimilation capacity above which pelagic ecosystems may lose integrity. The assimilation capacity of the pelagic ecosystem is mediated by two main mechanisms; the incorporation of nutrient in the organisms and a dilution process driven by hydrodynamics. There is a need for a systematic waste management from aquaculture in the future.

KEYWORDS cage aquaculture; nutrients; environmental impacts; eutrophication; pelagic ecosystem; hydrodynamics; nutrient assimilation; waste management

1. Introduction

There is now a growing attention and concern worldwide on societal aspects of the fast growing aquaculture industry. These concerns include for example animal welfare and particularly environmental impacts of marine aquaculture on coastal ecosystems (FAO 2006). Environmental concern and emerging new legislation, together with a growing competition for space in coastal waters, are becoming among the most important drivers for the development of marine aquaculture activities in western countries in the years to come. Two lines of development are apparent. Cage aquaculture systems (CAS) are gradually moved to more open sea locations, characterised by higher hydrodynamic energy, and land- and coastal based recycling aquaculture systems (RAS) are increasingly used for production of early stages and more local species. These trends of development are believed to continue (Olsen *et al.* 2008).

The social perception of the importance of an environmental problem is affected by the physical and biological characteristics of the coastal system as well as interactions with other important industries or human activities in the coastal zone. The major environmental concerns in Southern Europe are nutrient and organic waste emission from CAS in Mediterranean aquaculture producing mainly European sea bream and sea bass. Wastes from CAS are believed to cause ecosystem degradation and algal blooms. This is particularly regarded as a main threat for the blue water image of the stronger tourist industry, which is competing severely for space with aquaculture in coastal waters in Mediterranean countries (Katranidis *et al.* 2003). Escapes of fish from CAS and their interactions with fisheries are apparently not regarded as a major problem in Mediterranean countries, but these are the major environmental issues in many salmon producing

countries (Muir 2005). Escaped salmon may contribute to the spreading of disease and parasites to wild stocks and interact negatively with these stocks during their spawning in rivers. Associations and industries involved in river fisheries of salmon are important stakeholders opposing for example the Norwegian salmon industry (Porter 2003; McGinnity *et al.* 2003).

It is important to realise that aquaculture is the first industry to suffer from an inadequate coastal water quality and environmental damage. The industry is thus very dependent on minimising its own potential pollution, but also the pollution originating from other human activities like other industry or urban runoff. The concept of an "Ecosystem-based Approach to Aquaculture" (EAA) has recently been introduced by FAO representatives in cooperation with the scientific community (D. Soto, pers. comm.). According to the principles of this concept, the fish farms should be managed as a part of the marine ecosystem as any other source or sink of organic matter and nutrients.

The scientific literature on impacts on sediments and benthic ecosystems is very comprehensive, and there is a general scientific understanding on which we may base assessments of state and dynamics, management, and monitoring measures. The potential impacts of wastes from aquaculture on water column ecosystems is far less studied, presumably because it has been difficult to identify and quantify such impacts (Merceron *et al.* 2002; Soto and Norambuena 2004; Maldonado *et al.* 2005; Dalsgaard and Krause-Jensen 2006). There is apparently no scientific concept established for assessments of state and dynamics, management, and monitoring measures for open coastal waters. There is thus not sufficient knowledge as to how specific measured environmental variables can reflect potential harmful impacts on open water ecosystems. Thus, this paper will focus on potential impacts in pelagic waters of nutrient emission from

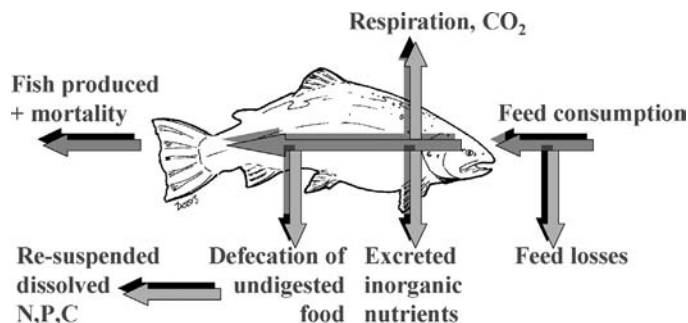


Fig. 1. Schematic allocation pathways of C, N, and P (energy and materials) in fish. Feed losses and mortality are relevant flows on the population level.

aquaculture and describe a preliminary general concept applicable for assessing such impacts.

2. Quantification of Nutrient Emission from CAS

The carbon (energy) mass balance for the flow of matter through a fish can be represented by the following simple mass balance equation:

$$I = A + F = G + R + F \quad (1)$$

where I is food consumed; A is assimilated food, or uptake in tissues; F is defecation; R is respiration, and G is growth and reproduction (all in terms of carbon or energy) (Fig. 1). The corresponding nutrient balance is expressed using the analogue equation:

$$I_{NP} = A_{NP} + F_{NP} = G_{NP} + E_{NP} + F_{NP} \quad (2)$$

where excretion of N and P (E_{NP}) replaces respiration. These general equations, together with knowledge on assimilation efficiencies of C, N, and P and the stoichiometric C:N:P composition of produced fish and feed are fundamental for estimating nutrient and carbon intake, metabolism, and losses from individuals of cultured fish.

The processes of respiration and excretion release inorganic carbon and excess inor-

ganic nutrients, respectively, from fish tissues (assimilated matter) to the water. Respiration is a loss of carbon dioxide (CO_2) reflecting the metabolic costs of growth and maintenance of the organisms. The excreted N and P species are mainly inorganic nutrients wastes, i.e. urine (urea-N, PO_4) and ammonia (NH_4). These losses of assimilated N and P are instrumental to maintain elemental homeostasis in fish tissues.

The assimilated food is the portion of the food that is digested by the fish and taken up in tissues, and the assimilation efficiency (AE) is defined as (similar for N and P):

$$AE = A/I \quad (3)$$

The undigested food (faeces) passes through the fish gut undigested or partially digested. This fraction constitutes mainly particulate organic substances, including particulate forms of N and P, but some part is rapidly released in molecular dissolved forms in the water. The assimilated food supports growth and weight increment, and the growth efficiency (GE) is generally defined as (similar for N and P):

$$GE = G/I \quad (4)$$

This term expresses the efficiency by which the food ingested is converted to new biomass, similar, although inverse, to the

food conversion ratio (FCR, feed consumed per fish produced) which is an operational term used in aquaculture.

The total wastes of carbon (TL_C) and nutrients (TL_{NP}) generated by cultured fish are expressed as:

$$TL_C = I - G = R + F \quad (5)$$

$$TL_{NP} = I_{NP} - G_{NP} = E_{NP} + F_{NP} \quad (6)$$

Respiration results in a release of inorganic CO_2 , the emission of organic carbon (L_{OC}) wastes is most easily estimated as:

$$L_{OC} = I - A = I(1 - AE) \quad (7)$$

Values for AE of carbon or energy can be obtained from literature and in some cases from feed companies. For the formation of dissolved components from faeces, there is no formal way to distinguish these dissolved organic component (DOC) from the particulate organic (POC) waste components, but the particulate fraction is the most important (see below).

The corresponding estimate of organic nutrient wastes (L_{ONP}) from fish is:

$$L_{ONP} = I_{NP} - A_{NP} = I_{NP}(1 - AE_{NP}) \quad (8)$$

I_{NP} can be estimated based on total feed intake times feed NP contents. The assimilation efficiency of N can be assumed to be equal to that of protein, widely reported in literature and by feed companies (Anderson *et al.* 1995). The assimilation efficiency of P is widely reported as well, but more uncertain because of the addition of indigestible P compounds in the feed such as phytate P (Hua and Bureau 2006). As for carbon, there is no formal way to distinguish between dissolved organic nutrients (DON, DOP) and particulate organic nutrients (PON, POP) waste components originating from faeces,

but the particulate nutrient fraction is the most important (see below).

The inorganic N and P release from the fish (L_{INP}) can be estimated as the difference between assimilation and production:

$$L_{INP} = A_{NP} - G_{NP} = (I_{NP} \times AE_{NP}) - G_{NP} \quad (9)$$

G_{NP} is N and P in produced fish, obtained as produced fish weight times N and P contents.

The waste production budget for an aquaculture facility differs from the fish budgets, as there is an additional waste component in the uneaten feed that may affect the environment. If mortality is significant, it should be included as well as a loss process of nutrients. Dead fish are usually collected for land deposition, and are as such not lost directly to the environment. To what extent a fish farm and the produced wastes affect the surrounding environment, depends on the size and type of the fish farm, the structure and functioning of the pelagic and benthic surrounding ecosystems, the overall management practice of the aquaculture facility, and perhaps most importantly on the hydrodynamics and physical conditions of the location.

The above method is most applicable for situations where the cultured organisms feed primarily on the added feed, and not on feed which are produced in the system (e.g., pond aquaculture systems). The method is robust and most accurate when there is adequate statistical input information on use of feed and fish production available.

If the statistical information on use of feed and fish production with time is more fragmented, the alternative to the mass balance model described above is a more general dynamic model describing fish metabolism, growth, and waste production (e.g., Fernandes *et al.* 2007). Such models are not that robust, but can be useful when input data are more fragmented.

Table 1. Characteristics and fate of nutrient components released from CAS.

Nutrient component	Acronym	Characteristics and fate
Particulate nutrients	PON and POP (particulate organic nitrogen and phosphorous)	• Whole feed pellets, small to very small particles originating from feed and fish faeces, other particles generated in fish farms (e.g., fouling). • Pellets and larger particles sink rapidly to the seafloor, are consumed by fish or other benthic organisms, or accumulate/decompose in sediments. • Small particles are suspended in the water column, consumed by filter feeders (mussels, zooplankton) and bacteria, within days. • Particles are not available for phytoplankton and macro algae.
Dissolved organic nutrients	DON and DOP (dissolved organic nitrogen and phosphorous)	Molecular nutrient components (organic), mostly complex chemical compounds, released from faeces particles and feed, and other biological activity. Stable components, available for phytoplankton on very long time scale. To some extent consumed by bacteria-microbial food web, can aggregate and sink (marine snow), relatively slow process.
Dissolved inorganic nutrients	DIN and DIP (dissolved inorganic nitrogen and phosphorous; NH_4 and PO_4)	Inorganic nutrients, i.e., ammonium and phosphate. Immediately taken up by phytoplankton, macro algae, and also by bacteria and used for growth, in the worst case they may cause algal blooms.

3. Characteristics and Fate of Nutrient Waste Components from CAS

Following the above considerations, CAS release nutrients as dissolved inorganic nutrients through excretion (NH_4 and PO_4), particulate organic nutrients (PON and POP) through defecation, and dissolved organic nutrients (DON and DOP) through resuspension from the particulate fractions (Table 1).

There will also be a direct loss of uneaten feed (feed N and feed P). These different waste components will affect different parts of the marine ecosystem; feed losses and the larger faeces particles will sink and affect sediments and benthic communities whereas dissolved inorganic nutrients, dissolved organic nutrients, and small faeces particles affect the pelagic communities and the state and quality of euphotic waters.

Inorganic nutrients released can affect phytoplankton in euphotic waters quite

Table 2. Estimated loading rates of organic C and specified nutrient components from a hypothetical salmon CAS producing 1000 tonne fish per year. The farm area is 51 000 m² (160 × 320 m²) the volume of the fish farm is 770 000 m³, the overall FCR of the farm (feed used per fish produced) is 1.16 kg dry feed used per kg fish produced (Gillibrandt *et al.* 2002), feed input is 1160 mt year⁻¹, direct feed loss is 5% (Mente *et al.* 2006), feed P content is 1 % (Islam *et al.* 2005), feed N content is 6% (Islam *et al.* 2005), and feed organic C content is 50% (Elser *et al.* 2000). Input data for feed use and fish produced are obtained from the Norwegian Association of Fish Farmers and feed characteristics from the feed company Biomar.

Pelagic loading rates	Tonnes farm ⁻¹ year ⁻¹	g m ⁻³ year ⁻¹	mg m ⁻³ day ⁻¹ (June–Sept)
OC-loading	20	26	100
NH ₄ -loading	28	36	140
PO ₄ -loading	2.1	2.7	11
Total N-loading	30	39	150
Total P-loading	3.0	3.9	15
DON + PON loading	17	22	86
DOP + POP loading	6.1	7.9	31

strongly in the upper mixed, illuminated layer of the water column where photosynthesis takes place. Organic dissolved nutrients are to a low extent available as nutrients for the phytoplankton. These nutrient components have long to very long residence times in marine waters (Bronk 2002; Karl and Björkman 2002). Particulate nutrients do not affect the phytoplankton in the mixed layer, but zooplankton is moderately affected all through the water column.

4. Nutrient Waste Emission from Typical Salmon Farm

The present section describes an annual mass balance for the main C, N, and P flows from a hypothetical salmon CAS producing 1000 metric tonnes wet weight of fish per year. Assumptions made in the calculations are shown in the legend of Table 2, which shows emission rates of the principal waste components from the fish farm. To put the numbers of Table 2 into a perspective, the total nutrient wastes generated of the fish farm corresponds to the emissions from a community of 7,500 to 10,000 people (2 g P per person per day, 13 g N per person per day, Norwegian standard). The N:P ratio of the

total and inorganic nutrient wastes to open waters was found to be 10 and 13, respectively, which is above the Redfield ratio (7.2, by weight). Contrary to this, not shown in Table 2, the N:P ratio of the particulate wastes affecting sediments and benthic ecosystems was 2.7 and therefore far lower than the Redfield ratio. The majority of the N wastes is released to open waters (68% of total) whereas the majority of the P is accumulated in sediments (63%).

For the defined salmon farm, there was a pronounced annual variation in fish production and waste emission over the year (mean pattern for all Norwegian salmon farms), among others because of variable fish biomass and low temperatures in the winter period (Fig. 2). The annual variations of the N- and P-allocation into biomass (growth) and different waste components released from the salmon farm were at their maximum in August, with rates less than half of this maximum during the winter months. As a general trend, a lower proportion of the P of the feed (feed-P) than that of the N in the feed (feed-N) was excreted as phosphate and ammonia, respectively. Contrary, the fraction of particulate P, mainly released through defecation, was throughout higher than that of particulate N.

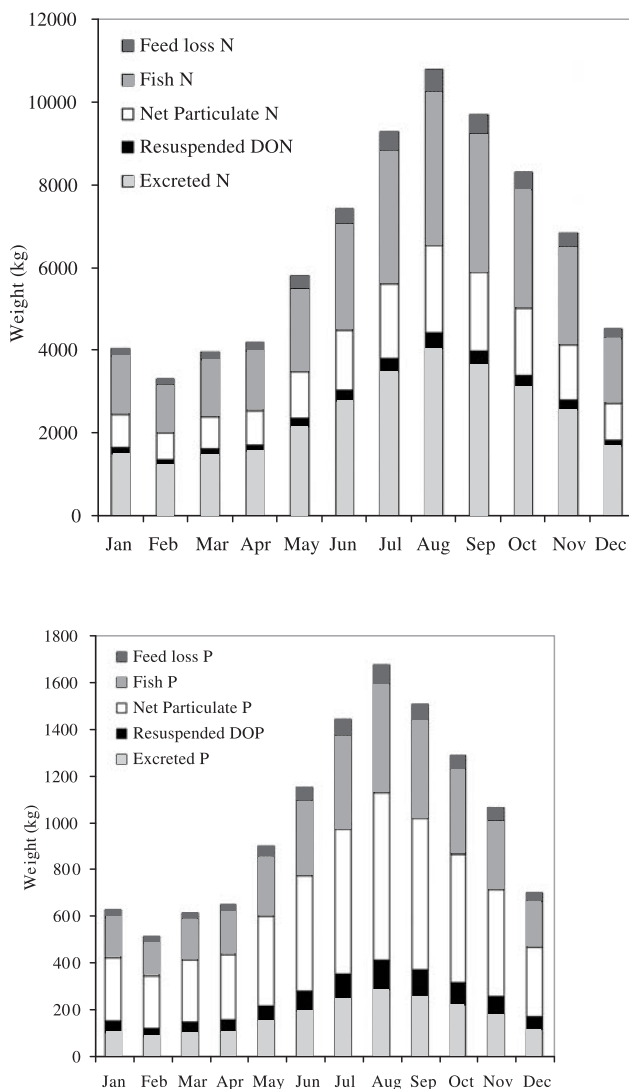


Fig. 2. Annual variation in nitrogen (upper panel) and phosphorus (lower panel) deposition in fish biomass and waste components for a hypothetical CAS producing 1000 tonnes salmon per year. The sum of the fractions equals the food nitrogen and phosphorous supplied.

The excretion of phosphate is highly dependent on the feed-P content, and the calculated P flows are relatively uncertain because of the problems in defining an accurate P content of the feed and its overall digestion efficiency (Sugiura *et al.* 2006). The values for N are probably more robust, because the protein content of the feed is less

variable and its digestibility is higher and better known (Anderson *et al.* 1995). It is finally noteworthy that the rate of feed losses and the emission rate of dissolved organic nutrients (re-suspended DON and DOP), which has a low availability for phytoplankton (Table 1), are relatively low as compared to other waste emission flows.

5. Nutrient Assimilation Capacity of Pelagic Ecosystems

There is no general scientific understanding of how wastes from CAS distribute and affect water column ecosystems, and there is a poor quantitative understanding of how these nutrients affect the structure and function of the pelagic ecosystem (Cloern 2001; Olsen *et al.* 2006). This lack of quantitative knowledge means that there is no scientific base for monitoring and managing environmental effects of CAS on open waters, meaning that management must comply with the principle of precautionary approach.

It can be hypothesised that the assimilation capacity of the water column ecosystem is mediated by two main mechanisms:

- Food web response: Nutrient uptake and assimilation by phytoplankton, with successive trophic transfers of energy and materials (e.g., nutrients) in the planktonic food web to the higher trophic levels.
- Hydrodynamics: Transport and dilution of nutrients and plankton organisms mediated by hydrodynamics at production sites and their surrounding, downstream water masses.

The dilution mechanism is independent on the organisms of the water column ecosystem; major physical forcing processes drive hydrodynamics. The assimilation capacity of the planktonic community is, on the other hand, strongly dependent on hydrodynamics, because dilution leads to a continuous reduction of nutrient concentrations and biomass, and therefore also to an increase in capacity of nutrient assimilation in the pelagic community. Both mechanisms are accordingly working in concert. Nutrient uptake and allocation in planktonic food webs and hydrodynamics are the fundamental processes determining the assimilation capacity of the water column of coastal and open waters.

5.1. Food web response

All ecosystems have an inherent capacity of persistence, and smaller environmental changes are mitigated through adaptive responses of the organisms. Major changes in ecosystem structure and function, whether it is reversible or irreversible, will only take place if the environmental signal, or the environmental interaction, is strong and sustained. For the planktonic ecosystem of the water column, we may deduce that nutrients are efficiently assimilated without any harm as long as the input rate remains below a critical upper level.

Enhanced inorganic nutrient supply to pelagic ecosystems results in a stepwise process where the first step is an increased nutrient uptake in phytoplankton (and bacteria) followed by an increased growth rate (i.e., primary production). If the zooplankton grazing rate of phytoplankton is not too high, the phytoplankton biomass may accumulate and increase, leading to higher food concentration for all groups of heterotrophs, and in turn to successive responses in their feeding activities and growth. It will most commonly take some 3–7 days before an increased nutrient supply rate results in an increased biomass, which is the last step of the chain reaction. This means that the effects normally are becoming realised far downstream of the nutrient source.

The typical responses in primary production and accumulated phytoplankton biomass in stagnant NE Atlantic coastal waters are illustrated in Fig. 3. There is a close to linear response below N loading rates of 11–18 mg N m⁻³ day⁻¹. Open dynamic systems will respond differently, advection and vertical mixing will reduce the response quite pronouncedly for biomass, dependent on the mixing rates. The response in primary production is less sensitive than biomass for physical mixing processes and grazing (Olsen *et al.* 2006).

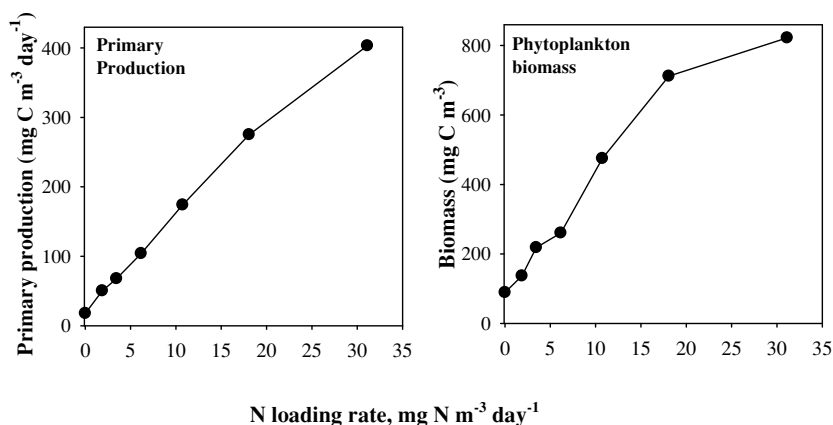


Fig. 3. Primary production (left) and phytoplankton biomass (right) as functions of N loading rate in stagnant NE Atlantic plankton ecosystems. (Norwegian data taken from Olsen *et al.* 2006).

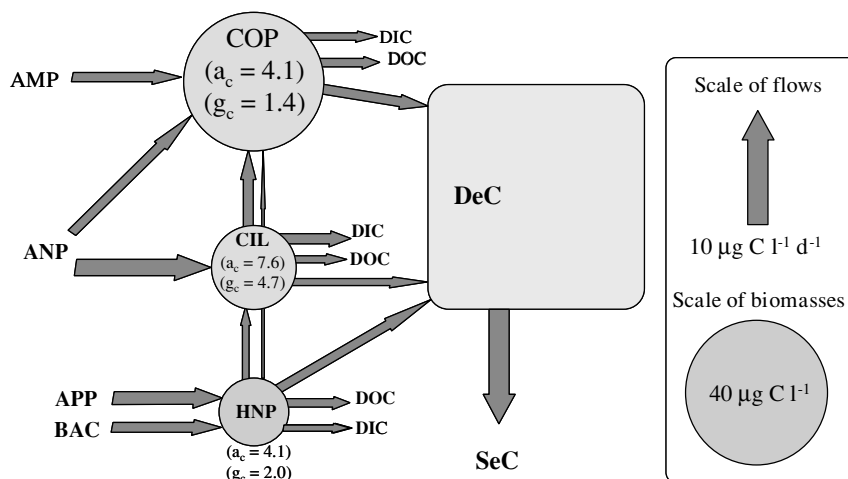
The responses and trophic interactions of the heterotrophic planktonic food web following enhanced nutrient input are complex, but the bottom line is that the food web acts like a buffer which, within certain limits, may mitigate negative ecological effects of enhanced nutrient input, for example any extensive blooms of phytoplankton. The main functional components of the planktonic food web will respond in a predictable way to increased nutrient supply, although not on species level (Fig. 4). The increased primary production represents increased food availability for the heterotrophic plankton groups (i.e., zooplankton and bacteria). The upper panel of Fig. 4 illustrates a representative flow network (structure and function) for the normal, undisturbed situation in the planktonic food web of NE Atlantic coastal water. The lower panel illustrates a situation with a nutrient supply 4–5 times above the natural level. The main take-home message of Fig. 4 is that enhanced nutrient supply affects carbon flows between the heterotrophic functional components more strongly than biomass of these functional groups. The phytoplankton biomass (not shown) is responding more strongly than the heterotrophic biomass (Olsen *et al.* 2006), as indi-

rectly indicated by the increased availability as food for zooplankton in the figure (input arrows). An apparent pattern of Fig. 4 is that structure and function of the microbial food web (bacteria, picocyanobacteria, small protozoans) is responding very little to nutrient addition. It is the larger groups of phytoplankton, metazoan, and protozoan (ciliates) grazers that primarily respond.

The most important message of Fig. 4 is the very pronounced increase in sedimentation rate that follows enhanced nutrient input. This flow of dead organic matter to deepwater and sediments represent an organic loading that becomes important for the oxygen requirements and concentration in aphotic waters and the sea floor communities. A non-linear, accelerating increase in sedimentation per primary production reflects the fact that the zooplankton grazers, at some point of nutrient input, are not any more able to consume, and efficiently remove, the enhanced primary production. A high sedimentation fraction of primary production is an indication that the planktonic ecosystem is no longer able to assimilate the enhanced nutrient input very efficiently.

Somewhere in between the loading rates of 3 and 20 mg N m⁻³ day⁻¹, we hypothesise

A: NE Atlantic coastal waters – Normal summer situation

(mean $L_N = 2.9 \pm 1.3 \mu\text{g N l}^{-1} \text{ d}^{-1}$; mean GPP = $57 \pm 18 \mu\text{g C l}^{-1} \text{ d}^{-1}$)

B: NE Atlantic coastal waters – High nutrient input

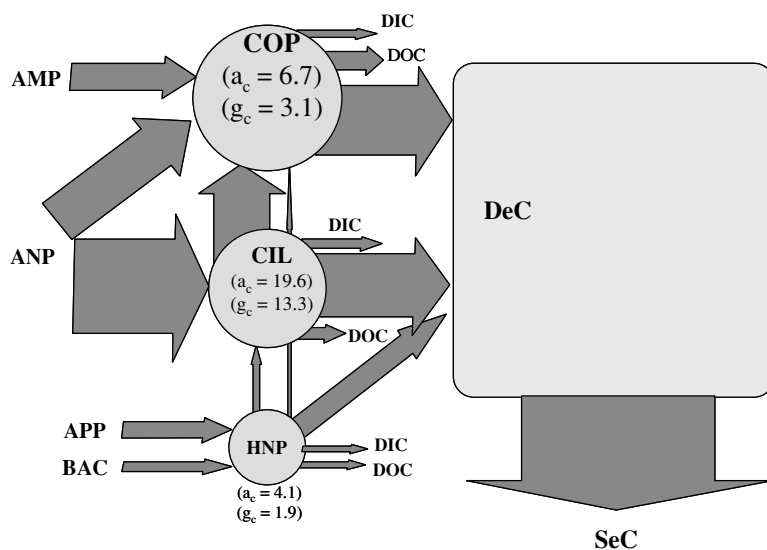
(mean $L_N = 19.5 \pm 5.9 \mu\text{g N l}^{-1} \text{ d}^{-1}$; mean GPP = $282 \pm 72 \mu\text{g C l}^{-1} \text{ d}^{-1}$)

Fig. 4. Schematic view of carbon flow networks during (A) a normal summer situation in NE Atlantic coastal waters and (B) conditions of high nutrient input. Arrows show flows, boxes show biomasses (and their allocation of energy). AMP: feeding of large sized phytoplankton (20–200 μm), ANP: feeding of medium sized phytoplankton (2–20 μm), APP: feeding of small sized phytoplankton (<2 μm); HNP: heterotrophic nanoplankton (<20 μm); CIL: ciliates, main constituent of microplankton; COP: copepods, main constituent of meso-zooplankton; DIC: CO_2 release (respiration); DOC: released of dissolved organic components; DeC: release of particulate organic components; SeC: sedimented carbon; a_c : assimilation rate; g_c : growth and reproduction rate. All concentrations are expressed in terms of mg C l^{-1} and rates as $\text{mg C l}^{-1} \text{ d}^{-1}$ (taken from Olsen *et al.* 2007).

a critical nutrient loading rate (CNLR) which cannot be exceeded without loss of ecosystem integrity. The food web organisms are capable to assimilate efficiently the nutrients input without major exports to sediments below this critical rate, but not above. There is so far no generally accepted method to determine a CNLR for open coastal waters experimentally or empirically, and there is no published value, as far as we understand. There is, however, evidence showing that the primary production and the zooplankton feeding rates in stagnant systems both level off for volumetric loading rates around $1 \text{ mmol N m}^{-3} \text{ day}^{-1}$ ($14 \text{ mg N m}^{-3} \text{ day}^{-1}$) in NE Atlantic coastal waters (see Fig. 3 and Olsen *et al.* 2006).

5.2. Hydrodynamics

A cage fish farm represents a point source of nutrients to the surrounding water. The hydrodynamic energy and the depth of the site will, to a great extent, determine the water volume that receives the daily nutrient emission of wastes. Due to coastal and tidal currents, coastal seawater is rarely or never stagnant. Usually the current velocity varies below 25 cm s^{-1} (Carroll *et al.* 2003). The currents will dilute the water in the cages and the nutrient waste flows and broaden the deposition area considerably.

The pattern of water currents in coastal waters is complex, and cannot easily be deduced, even after extensive field surveys. As a first approximation for estimating the receiving water volume of our defined salmon farm, we may assume that water are drained through the cage farm in a plug flow pattern with no further mixing downstream of the farm site (see legend of Table 2). If water enters the cage area directly from the length side, and there is no major resistance in the cages, Fig. 5A shows the number of water exchanges and the resulting total volume passing the cage farm as a function of the water current velocity. Already water currents of 10 cm sec^{-1} , which are relatively

slow, result in a high frequency of water renewal and a total exchanged volume of more than 50 million m^3 per day. This is for sure an underestimate of the real receiving volume, because the nutrients will become continuously exchanged with and diluted in neighbouring water masses downstream of the fish farm.

The volumetric loading rate (L_{VOL}) of inorganic nutrients is defined as the mass of nutrients released per volume of water per day. Quantification of the loading rate (mass of nutrients released per farm per unit of time, L_{time}) and the receiving water volume (V, m^3) allow estimation of L_{VOL} for inorganic N and P (Fig. 5B), which decreases rapidly as the water current velocity increases. The hypothesised critical loading rate (see above) to coastal waters is $14 \text{ mg N m}^{-3} \text{ day}^{-1}$ (10–20 $\text{mg N m}^{-3} \text{ day}^{-1}$, P in Redfield proportion). Another important reference is the natural supply rates of nitrogen to euphotic coastal waters. This value has been estimated to $4 \text{ mg N m}^{-3} \text{ day}^{-1}$ in a 5-year fertilisation experiment in a coastal lagoon off Central Norway (sedimentation measurements, seasonal mass balance).

With the critical and the natural loading rates in mind, Fig. 5B reveals that the volumetric nutrient loading rate of water passing through the fish farm will remain below the critical value as long as the water current velocity is $>2 \text{ cm sec}^{-1}$, which is indeed a very low velocity. The fish farm will contribute to a loading rate comparable to the nature when the water current velocity is around $5\text{--}6 \text{ cm sec}^{-1}$. These calculations assume that there is no further mixing of water downstream of the fish farm; they therefore represent worst-case situations.

The exercise illustrates how important hydrodynamics are to mitigate negative environmental effects of nutrients released from salmon fish farms and other point sources of nutrients. If the production conditions were stagnant, the nutrient concentration following one day of emission would correspond

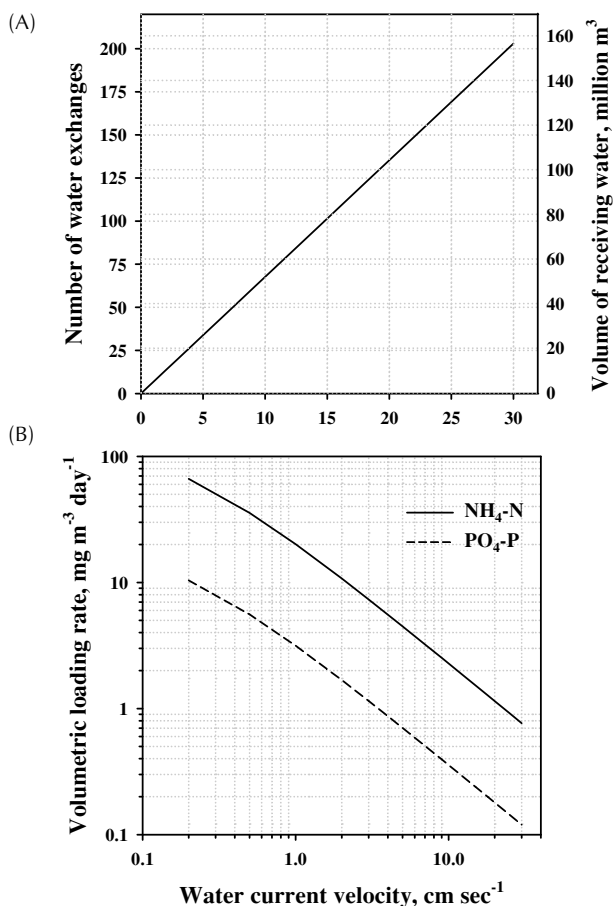


Fig. 5. Water exchange (A) and volumetric loading rates of N and P (B) as functions of the water current velocity (revised from Olsen *et al.* 2005).

approximately to a spring bloom event in Atlantic waters (typical DIN concentration of 140 mg N m⁻³). It is easy to understand that this situation would have become an immediate disaster for both the salmon and the producer.

This type of exercise clearly concludes that advanced 3D hydrodynamic modelling is needed to estimate the mean volumetric loading rates and to demonstrate the spreading pattern of the excess nutrients from the fish farm to the surrounding waters more consistently. This is particularly important for nutrient assessments undertaken on a re-

gional scale, with more than one fish farm draining to the same body of water.

5.3. Integrated scheme for food web and hydrodynamics

Food web and hydrodynamic contributions to the assimilation capacity of the water column ecosystem are schematically integrated in Fig. 6, forming a conceptual operational graphical tool for risk management of the water column ecosystem. In the “water current velocity–nitrogen loading rate”-space described as Area I, water dynamics are strong enough to maintain nutrient loading

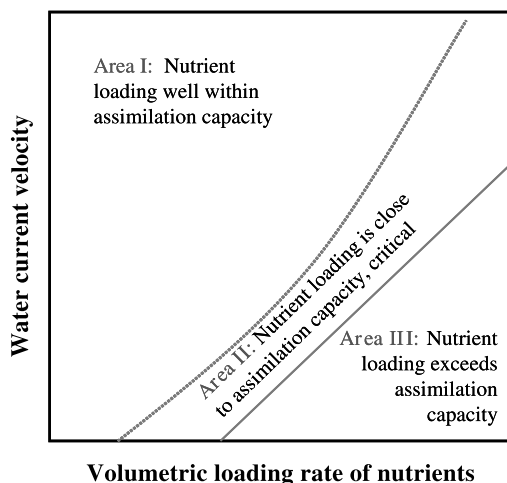


Fig. 6. Conceptual relationship describing the ability of the water column ecosystem to assimilate nutrient input as a function of the volumetric loading rate of inorganic nutrients and the water current velocity. Area I: Water dynamics are strong enough to maintain nutrient loading within the limits of the assimilation capacity of the water column ecosystems; Area II: The critical zone where loading rate is coming close to the critical nutrient loading that exceeds assimilation capacity. Situations represent increased risks and calls for special attention and a precautionary approach; Area III: Nutrient loading rate exceeds the limits of the assimilation capacity; the water column ecosystem can lose its integrity, which may cause harmful coastal eutrophication. The figure is preliminary, slopes and exact x-axis intersections of the indicated lines are unknown.

below the limits of the assimilation capacity of the water column ecosystems. In Area III, the nutrient loading rate exceeds the limits of the assimilation capacity of the pelagic ecosystem, the loading rate is then above the critical level (CNLR), and the water column ecosystem can lose its integrity, sedimentation rates are strongly enhanced, and harmful coastal eutrophication may occur. In the space described as Area II, the loading rate is coming close to the CNLR where nutrient loading exceeds the assimilation capacity. This situation represents increased risks and call for a precautionary approach. The solid curve expresses that the CNLR will increase with strong hydrodynamics.

The concept illustrated in Fig. 6 is preliminary. It will most likely intercept the L_{VOL} axis in $10\text{--}20 \text{ mg N m}^{-3} \text{ day}^{-1}$, but the slope of the indicated line is not known. Moreover, the axis legends are so far accidentally

chosen. Another proxy for hydrodynamic energy may be more suitable than the water current velocity. The volumetric loading rate can be translated into a standing stock of fish in fish farm. An ultimate R&D challenge is to examine different response variables for hydrodynamic energy and to quantify the borders of the areas in Fig. 6.

6. Monitoring and 3D Modelling of Nutrient Mixing

Monitoring techniques, which allow assessment of pelagic waters over a wider geographic scale, are paramount for detecting potential impacts from nutrient sources like CAS downstream of the farm. The most apparent options are regional scale satellite imaging and 3D hydrodynamic modelling. Satellite images can provide real situations for phytoplankton blooms in surface waters

Table 3. Hydrodynamic characteristics and mean excess nitrogen concentrations of the 3 virtual salmon farms studied. Values express the concentration of excess N in the water occupied by the fish cages (hot-spot, one model grid of $160 \times 320 \text{ m}^2$). The situations are representative for farms producing 1000 tonnes per year, which is well below today's production (up to 9,000 tonnes per year), but the results nevertheless demonstrates the options of the method. PON concentration in undisturbed coastal waters is set to 60 mg N m^{-3} , which is representative for the region. From Olsen *et al.* (2005), model data provided by D. Slagstad, SINTEF.

Fish farm number and location	Location conditions	Excess N in farm hot-spot, mg N m^{-3} (mmol N m^{-3})	% Excess N of natural PON in farm hot-spot (%)
1. Langøya, outer exposed area	Strongly exposed, water is efficiently mixed with open the ocean	0.6 (0.04)	<1
2. Langøysundet, a straight between islands	Tidal driven water exchange, efficiently mixed	6.4 (0.46)	11
3. Eidsfjorden, a relatively stagnant fjord bottom	Unidirectional, steady water currents	17.9 (1.3)	30

at any given time, but it cannot easily distinguish between the highly variable natural nutrient supply and the optional anthropogenic signal. 3D hydrodynamic models produce a virtual world, not a real one, but such models can cover the entire water column continuously with time over any geographic region. Moreover, modelling allows us to distinguish between natural and anthropogenic signals, and it can potentially predict phytoplankton production and effects on higher trophic levels. Models can accordingly be run with and without nutrient emission from fish farms included, and the difference, termed the “excess” nutrient concentration, can be estimated right away. Modelling are also well suited to assess the integrated effects of all CAS and other nutrient sources located in a region, and accordingly instrumental tools for ecosystem-based management of aquaculture. Classical measurements must be used to validate the major trends found by satellite images and modelling at specific locations.

A modelling study examining loading and spreading of inorganic N from three hypothetical fish farms in Norway can serve to

illustrate how a 3D simulation hydrodynamic modelling can be used to assess concentrations and distribution of released inorganic N and P from CAS (Olsen *et al.* 2005) (Table 3). The model predicts the concentration of excess N, i.e., the inorganic N released from the fish farm, whether it is dissolved or taken up by organisms after being released.

The mean excess N concentration at the farm hot-spot (see the legend of Table 3) in the outer exposed site at Langøya showed very low N concentrations, values that would not be measurable using analytical techniques (compare with the plug flow system, Fig. 5). Nitrogen was immediately dispersed, and neither enhanced primary production nor enhanced phyto-plankton biomass could be traced downstream of the farm in detectable amounts (not shown). Phosphorous will show an identical pattern of variation.

The excess N concentration inside the farm situated in Langøysundet, a strait between islands, was higher, corresponding to 11% of the natural PON concentration at the site (see legend of Table 3). Tides moved the water back and forth in the strait, and the surrounding water masses on both sides were

to some extent affected locally (<1 km). The hydrodynamic forces were still strong, and the nutrients were widely spread. The residence time of the water was too short to allow a significant enhanced primary production and phytoplankton biomass around the fish farm (data are not shown).

The third site Eidsfjorden, which is a relatively stagnant fjord bottom, was characterised by regular anti-clockwise water currents with a main pattern not much affected by the tidal cycle. The concentration in the farm oscillated, however, quite pronouncedly, meaning that water current velocity varied with tides. The fish farm affected the water masses downstream of the site, and the mean hot-spot concentration of excess N was around 30% of the natural PON concentration. Excess N, primary production, or biomass did not significantly affect the outer water masses of the fjord. Hydrodynamics were surprisingly strong downstream of the farm along the north coast of the fjord, and the resources were rapidly spread to large water masses. There is not much space for a bigger salmon farm on this site, which might be suitable for integrated aquaculture of mussels (Whitmarsh *et al.* 2006).

7. Concluding Remarks

As aquaculture continues to grow, there will be a need for a more comprehensive waste management, not only for CAS, but for other systems as well. The means available for reducing the potential environmental impact is highly diverse and include feeding management, site selection, alteration of site, and an active use of wastes from feeding aquaculture to produce organisms on other trophic levels, organisms that can take advantage of the wastes from CAS (Integrated multi-trophic aquaculture). The environmental impacts of sediments and benthic ecosystems are relatively well understood and managed, but there is a need to improve the general understanding of how pelagic ecosystems are impacted in order to establish a science-based management and monitoring practices for open waters. This is important for the societal perception of aquaculture, but also for the industry itself which require pure water for its activity.

Acknowledgments

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Meeting the Future Demand for Aquatic Food through Aquaculture: the Role of Aquatic Animal Health

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The increasing importance of aquaculture, at present, is undoubtedly recognized. The sector is expected to effectively contribute to food security, nutritional well-being, poverty reduction and economic development. However, biosecurity lapses and transboundary aquatic animal diseases pose significant constraints to aquaculture sustainability. This paper discusses how aquaculture development brings new challenges to aquatic animal health. These challenges include compliance to international treaties, practical implementation of regional guidelines and national strategies, increasing biosecurity and biosecurity awareness at all levels, empowering farmers to manage disease and other risks through better management practices, and conducting targeted research that will support biosecurity assessments. A short brief on emerging and re-emerging issues such as chemical usage, climate change and disease ecology, fish welfare and fish health, fish-vet cooperation and certification scheme for aquatic animal health service providers is included.

KEYWORDS aquatic animal health; aquaculture; biosecurity; transboundary aquatic animal diseases; risk analysis

1. Introduction

Aquaculture is now known as the emerging agriculture, the catalyst of the 'blue revolution', the answer to world's future fish sup-

ply, the fastest food producing sector, the future of fisheries.

The increasing importance of aquaculture is, at present, undoubtedly recognized. Aquaculture production has substantially grown accounting for nearly 50 percent of

the world's aquatic food supply. It is anticipated that aquaculture will further contribute 85 million tonnes of aquatic food by 2030, an increase of 37.5 million tonnes over the 2004 level (FAO 2007a), with minimum impact to the environment and maximum benefit to society. Thus, the sector is expected to effectively contribute to food security, nutritional well-being, poverty reduction and economic development.

Aquaculture's contribution to the food fish supply continues to increase. Given that production from capture fisheries will likely stagnate, it can be foreseen that a greater share of future food fish supply will come from aquaculture. Figure 1 illustrates the past and projected contribution from capture fisheries and aquaculture. The green line shows that in 2005, aquaculture supplied nearly half (45.5%) of all food fish for human consumption; the yellow line shows that by 2015, aquaculture will be required to supply half of the food fish supply; the white line shows that aquaculture will be required to supply 85 million tonnes by 2030. In both scenarios, the assumption is that capture fisheries production and the proportion of the catch destined for fish meal and fish oil (non-food uses) remain equal to the average 2000–2005 figures (FAO 2007a).

The 2006 State of the World Aquaculture (FAO 2007a) identified five major trends, namely: (i) intensification of aquaculture production driven by the availability of sites and ability to exploit non-agricultural land; (ii) diversification of species use, e.g., high value marine species; (iii) increasing attention on better management of the sector; (iv) increasing influence of markets, trades and consumers—greater attention to food quality and safety, moving towards adding and processing of products for export; and (v) enhancing regulation and improving governance, with emphasis on self-regulation by farmer association.

The levels of projection shown in Fig. 1 and the major trends identified will pose a big challenge to the aquaculture sector.

2. International Trade

Alongside with the expansion, intensification and diversification of aquaculture is the enhancement of trade in aquaculture species, products and services. The global trade in live aquatic animals (fish, crustaceans, molluscs, other invertebrates, aquatic plants, amphibians, etc.) and their products is carried out for a variety of reasons such as: ornamental/aquarium trade, aquaculture development,

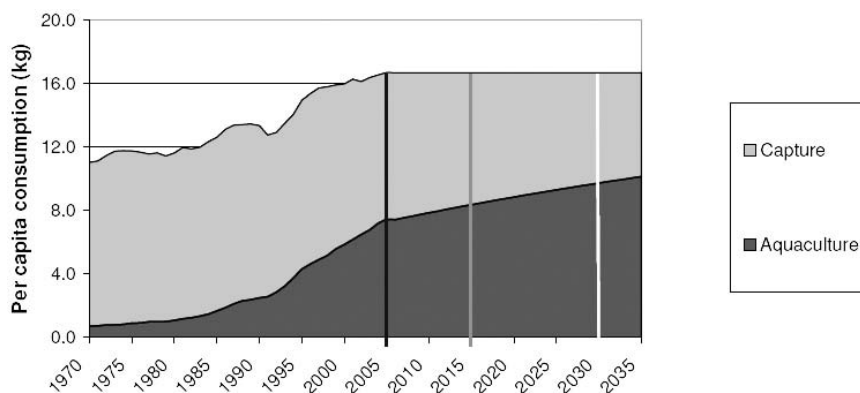


Fig. 1. Past and projected contribution of aquaculture to per capita food fish supply.

food (live food market), baitfish, capture fisheries development/enhancement, biological control, angling/sport development, others (e.g. animal feed, medicine and health products, etc.) (Arthur 2004). Trade of aquaculture commodities, whether live animals or products, is an important income-generating activity for primary producers which contributes significantly to their economic well-being. It is thus expected that trade will continue, legally or otherwise, and new market opportunities will be seen. Since all movements of live aquatic animals involve an element of pathogen risk, it can be expected that unregulated and irresponsible trading will facilitate new mechanism by which pathogens and diseases may be spread to new areas together with host movement. Aquatic animal health will therefore continue to be a challenging issue to aquaculture development and sustainability.

3. Biosecurity Lapses and TAADs— Significant Constraints to Aquaculture Production

Transboundary aquatic animal diseases or TAADs, similar to transboundary animal diseases (TADs) in the livestock sector, are diseases that are highly contagious, have the potential for very rapid spread irrespective of national borders and cause serious socio-economic consequences (Baldock 2002; Bondad-Reantaso 2004). The introduction and spread of many of these pathogens across many aquaculture regions of the world is one of impacts of trade globalization. Other factors responsible for the current disease problems faced by the aquaculture sector include intensification of aquaculture through translocation of broodstock, post-larvae, fry and fingerlings; development and expansion of the ornamental fish trade; enhancement of marine and coastal areas through stocking with aquatic animals raised in hatcheries; the misunderstanding and misuse of specific pathogen free (SPF) stocks; unanticipated

interactions between cultured and wild fish populations; slow awareness on emerging diseases; climate change. In addition to the above, inadequate or poorly implemented biosecurity measures ranks high in the factors which have led to significant losses due to aquatic animal diseases on a global scale (Bondad-Reantaso *et al.* 2005).

Infectious diseases are constraining the development and sustainability of the aquaculture sector through direct production losses and increased operating costs and indirectly, through restrictions on trade and impacts on biodiversity. Even though systematic assessments of the impacts of transboundary aquatic animal diseases are lacking, some data are available in the grey literature. Bondad-Reantaso *et al.* (2005) reviewed these impacts which have been expressed in terms of monetary estimates, percentage decrease in production, export losses, unemployment, closure of aquaculture operations, lost of consumer confidence, etc. Economic impacts have also been estimated in terms of cost of investment in disease research and other health management programmes as well as economic investments (e.g. development of national strategies, setting up of research institutes, operating costs for a reference laboratory, funding research and disease control programmes and investments in development programme). Available data reveal high figures for both economic impacts and investments. It could range from national level estimates as low as US\$ 17.5M (white spot disease (WSD) of shrimp in India in 1994) to as high as US\$ 650M (for yellowhead virus and WSD in Thailand in 1994) to a global estimate of US\$ 3019 (Israngkura and Sae-Hae 2002).

4. Strategies for Reducing the Risks of Aquatic Animal Diseases

There are a number of strategies and approaches all aimed at minimizing the risks

of aquatic animal disease incursion. They are briefly described below.

4.1. Compliance with international codes, regional guidelines through national strategies and other implementation mechanisms

Table 1 lists a number of instruments (international treaties/codes, regional guidelines, national strategies) concerned with aquatic animal health. The general objective of these guidelines is to assist countries in reducing the risk of introduction and spread of serious TAADs via the international and domestic movement of live aquatic animals. These instruments are in a state of constant change requiring revisions and modifications which are necessitated by factors such as rapid development in the aquaculture sector, increasing knowledge on diseases and better understanding of the dynamics and epidemiology of aquatic diseases, improved diagnostic and detection methods, emergence of unknown diseases, changing trade patterns which reflect the changing political, social, industrial and economic environments of trading countries, etc. Practical field application of such guidelines will be required.

Improved compliance to these international, regional and national instruments will encourage adoption of appropriate practices, assist in minimizing the risk of disease transfer and other adverse effects on wild and cultured stocks, promote responsible movement of live aquatic animals and healthy aquatic production.

4.2. Increasing biosecurity and bio-security awareness at all levels

General biosecurity issues had recently gained an increased profile on a global basis. A range of factors are behind it. International trade in animals, plants and their products have diversified and increased in volume. As previously mentioned, this is now accepted as a key contributor to the spread

of pathogens from one region to another. Many examples of this scenario exist in aquaculture. Changing agricultural practices result in new hazards to health that are able to easily cross border. Changes in human ecology and behaviour have also contributed to the increased incidence and spread of hazards of public, animal and plant health importance. Alongside with trade liberalization, the global community is increasingly demanding improved biosecurity in order to ensure public health, protect the environment and conserve biodiversity. In the long term, effective biosecurity can be expected to improve human health, support agricultural development, improve food safety, maintain biodiversity, protect the environment, increase trade, improve genetics, and enable greater access to market.

In aquaculture, increasing effective biosecurity at all levels can be done, for example, through the following:

1) Meaningful health certification and integrating quarantine as part of national biosecurity framework

Previous efforts in implementing quarantine have not been successful in preventing the spread the entry and spread of exotic TAADs into national territory. Quarantine was seen as a separate activity and as a procedure that should be applied to all imports of live aquatic animals. Quarantine measures should now be fully integrated into national strategies on aquatic animal health within the overall biosecurity programmes as part of the risk analysis process. Health certification is a prerequisite for international movement of live aquatic animals and an important and integral part of the quarantine process. Standardization of science-based identification of all risk pathways and high-risk organisms, and implementation of pre-border, border and post-border measures to prevent pests and diseases from entering a country will be necessary (Subasinghe and Bondad-Reantaso 2006).

Table 1. Examples of different instruments at different levels concerned with aquatic animal health issues.

	Reference
International codes/treaties/guidelines	
OIE's Aquatic Animal Health Code	OIE 2007
Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES)	ICES 1995
Code of Conduct for Responsible Fisheries (CCRF) of the Food and Agriculture Organization of the United Nations (FAO)	FAO 1995
Sanitary and Phyto-sanitary (SPS) Agreement of the World Trade Organization	WTO 1994; Chilaud 1996
FAO Technical Guidelines for Responsible Fisheries. No. 5, Suppl. 2. Health management for responsible movement of live aquatic animals.	FAO 2007b
Regional guidelines	
Codes of Practice and Manual of Procedures for Consideration of Introductions and Transfers of Marine and Freshwater Organisms of the European Inland Fisheries Advisory Commission (EIFAC)	Turner 1988
FAO/NACA Asia regional technical guidelines for the responsible movement of live aquatic animals	FAO/NACA 2000
National strategies	
AQUAPLAN: Australia's National Strategic Plan for Aquatic Animal Health	http://www.daff.gov.au/animal-plant-health/aquatic/aquaplan
NAAHP: Canada's National Aquatic Animal Health Programme	Olivier 2004
USA's National Aquatic Animal Health Plan	Amos 2004
Thailand's Strategic Plan for Aquatic Animal Health	Kanchanakhan and Chinabut 2004

2) *Surveillance programme and diagnostic services to detect and identify the emergence and spread of diseases and rapid response*

Two decades ago, the term 'surveillance' is totally unheard of in the field of aquatic animal health. Surveillance programmes are now becoming important tools to detect the emergence of diseases. The focus should be on early detection followed by a rapid response to disease epizootics to prevent its

spread and establishment and if possible to quickly eliminate the disease. With experience gained in establishing surveillance programmes and realizing the benefits of such program, we now see a recent trend where countries are now quick to implement targetted surveillance for emerging diseases.

3) *Risk analysis: timely assessment of the threat from new or expanding species*

Owing to the current global climate of free trade, there is now a growing demand

for public health and environmental protection. Risk analysis has become an important tool to achieve the goals of protecting human, animal and plant health and biodiversity. Governments and the private sector must often make decisions based on incomplete knowledge and a high degree of uncertainty. Such decisions may have far-reaching social, environmental and economic consequences. Risk analysis is a structured process that provides a flexible framework within which the risks of adverse consequences resulting from a course of action can be evaluated in a systematic, science-based manner. The risk analysis approach permits a defensible decision to be reached on whether the risk posed by a particular action or hazard is acceptable or not, and provides the means to evaluate possible ways to reduce the risk from an unacceptable level to one that is acceptable. Aside from using risk analysis procedures as basis for assessing requests to import live aquatic animals, more efforts should be directed towards prioritizing risks and looking at ways to reduce them effectively by drawing on the entire arsenal of risk management measures that are available.

4) *National frameworks to regulate, manage and control biosecurity*

Responsible and good health management will be key to healthy and sustainable aquaculture production. The development and implementation of national strategies on aquatic animal health within the broader national aquaculture development plans and biosecurity frameworks must be continuously pursued. The model for a national strat-

egy is comprehensive enough and provides a good entry point for capacity building for many countries, at whatever level of national economic development they may currently be. The focus should be centered on prevention, responsible and better health management practices and ensuring and maintaining healthy aquatic production. A clear strategy is required; policy decisions should be based on best available science. Involvement of partners (stakeholders) at all levels will be necessary. Essential components of a biosecurity and national strategy framework are shown in Box 1 below:

In many countries, efforts are being made to improve biosecurity and reduce risks of aquatic diseases. However, the required trained expertise, human capacity, institutional capacity and arrangement, and adequate allocation of resources is significantly lacking. This shortfall needs to be clearly addressed and rectified to make any meaningful improvements in national aquatic biosecurity. In order for the national framework to be effective, it needs an implementation plan, the authority and necessary resources required for its implementation. Therefore, strong national commitment from concerned authority is necessary.

4.3. Empowering farmers to manage disease and other risks through implementation of better management practices

Farmers, small-scale farmers in particular, are key players in aquaculture production. Pond/farm health management is crucial in

Box 1. Essential components of a biosecurity and national strategy framework (FAO 2007b).

Policy, legislation and enforcement	Zoning
Risk analysis	Emergency preparedness
Pathogen list	Research
Information system	Institutional structure
Health certification and quarantine	Human resource development
Surveillance, monitoring and reporting	Regional and international cooperation

order to deal with day-to-day situations in farms in preventing and controlling serious disease. Inequities in aquatic animal medicine and/or husbandry education as well as inaccurate information dissemination are core issues. Empowering farmers with information and tools such as better aquaculture management practices or BMPs (e.g., good farm hygiene such as hand washing between tanks, separation of nets/tanks/stocks, maintaining good water quality, proper handling of fish; good record keeping, for example, gross and environmental observations and stoking records including movement records of fish in and out of facility) or simple and practical biosecurity measures at farm level (e.g. proper quarantine of incoming fish; removal of dead fish from ponds; drying and liming of ponds; regular and correct disinfection procedures; exclusion of wild fish) are all essential in understanding the disease situation at the farm level. Education of farmers and hobbyists on good health management is the key to disease prevention and early warning/reporting is the key to minimize disease spread (Bondad-Reantaso and Subasinghe 2005).

FAO's global guidelines on health management for responsible movement of live aquatic animals (FAO 2007b) have included guidance for health management at the farm and farm-cluster level. Box 2 lists a number of essential elements on health management which need to be addressed at the farm level.

Situation-specific BMPs developed with farmers and tailored to their farming systems and the investment capacity of individual farmers is an effective way to ensure farm level biosecurity. The cluster management

approach is an effective way of addressing biosecurity concerns at the farm level since it involves direct and active participation of a group of small-scale farmers, whom involvement individually will otherwise not be effective. Cluster management provides an opportunity for addressing a common biosecurity issue and finding a common solution which at the individual level may either not be possible or extremely difficult.

4.4. Scientific research and advice

Generating scientific information to support biosecurity assessments will be indispensable. Bondad-Reantaso *et al.* (2005) made an exhaustive list of research areas that will support aquaculture biosecurity. These include research on pathways of pathogen spread, methods for inactivation of infectivity, and "barrier" vaccination strategies. In addition, epidemiological research need to be carried out on biological factors (identification of at-risk populations, hazards, pathways, pattern of spread, incubation period, nature of the pathogen), risk factors, interventions and methodologies (e.g., surveillance techniques, disease outbreak modelling, use of geographic information systems). Other essential research areas identified include pathogen studies, information on trade and most importantly, biological pathways for the introduction (release assessment), establishment (exposure assessment) and spread (consequence assessment) of a pathogen, host susceptibility, modes of transmission, infectivity, virulence and stability, intermediate hosts and vectors, and effects of processing, storage and transport. Research on bioremediation and other related aquatic

Box 2. Elements on health management which need to be addressed at the farm level.

Compliance with national legislation	Cluster management
Better management practices	Surveillance and reporting of disease outbreaks
On-farm disease prevention	Emergency preparedness
Certification	Information sharing and farmer education

husbandry management tools will assist in implementing better management practices. For newly emerging diseases as well as some diseases in poorly studied aquatic animal species, basic studies on their pathology and methods for rapid and accurate diagnosis (including standardization, validation and inter-calibration) are essential to facilitate accurate risk assessment and biosecurity management. Increased surveillance of wild fish to detect significant disease problems at an early stage will also be required.

5. Emerging Issues

5.1. Responsible use of chemotherapeutants in aquaculture

As in other food producing sector, the use of chemicals in aquaculture is essential for a successful production (Subasinghe 2004). They are used in various ways (e.g. pond and tank construction, soil and water management, enhancement of natural aquatic productivity, feed formulation, growth promotion, health management, etc.) and there are many benefits in the use of chemicals in aquaculture. However, their use also presents a number of potential risks (e.g. risk to the environment, risks in human health and risks to production systems, etc.). Particularly important is for farmers to be informed of the benefits and the risks of chemical usage in aquaculture so that chemicals can be used in a responsible and prudent way.

5.2. Climate change and disease ecology

Very few studies have direct links on the impact of climate change on aquatic animal diseases. In general terms, changes in rainfall and weather patterns triggered by global warming may affect the distribution of disease vectors and thus change the distribution of diseases that they transmit. In order to understand the correlation of climate change and disease ecology, it will be nec-

essary to direct some of current research efforts in doing retrospective analysis/case studies that can illustrate the potential effects of climate change on disease dynamics and any link between climate and disease.

5.3. Fish welfare and fish health

There is now a growing interest in fish welfare. One of the so-called “five freedom” is freedom from pain, injury or disease—by prevention or rapid diagnosis and treatment (Damsgard *et al.* 2006). In practical fish farming, fish reared under poor welfare conditions are more prone to disease. Studies on fish welfare and its integration with fish behaviour, fish physiology and fish health are now used as tools in better understanding fish welfare in farmed fish. As well, guidelines for health and welfare monitoring of fish used in research will also be forthcoming. The issue of fish welfare and health will become more prominent in the future and the aquatic animal health community should be prepared to tackle this.

5.4. Improving and strengthening fisheries-veterinarian dialogue and cooperation

This is an area where joint efforts are required to achieve tangible outputs. As fisheries and veterinary authorities are the focal points dealing with aquatic animal health issues, a concerted effort is required to enhance the dialogue and to improve capacity of appropriate authority/ies particularly in, for example, in complying to the OIE standards, FAO guidelines and other related international treaties and agreements. At the corporate level, there is limited experience in sharing responsibilities. Animal health has been and still is the mandate of the veterinary administration. For example, the use of “competent authority” vs “veterinary authority” in the OIE Code is quite recent. At the corporate level, aquaculture is a new area of animal production for veterinarians. For

example, “aquatic veterinary surgeons” do not have the same image as that of “veterinary surgeons” for dogs or other farm animals. The study and practice of aquatic animal disease and health management has been with biologists using the science approach and not the medical approach. The veterinarian legacy is needed by the aquaculture sector. In order to move forward, the current challenge for veterinarians will be to position themselves on aquatic animals as they have done for farm animals two hundred years ago. A restructuring of the veterinary curricula to include aquatic species will be of central importance. The challenge for fishery biologists will be to embrace the veterinary knowledge. Mutual respect for each profession, more dialogue and real cooperation will be desirable.

5.5. Certification of aquatic animal health service providers

A professional standards mechanism that promotes and authorizes a certification protocol for aquatic animal health providers will be useful in improving issues related to disease diagnosis, aquatic animal medicine, and information dissemination. Certified aquatic animal health service providers can offer farmers an opportunity to identify and choose qualified individuals; they will also provide credibility to importers and consumers regarding product quality; such standards will also lead to production of valid health certificates and ensure the competence of those who are involved in providing and interpreting information on the health status of aquatic animals. As aquaculture continues to expand as the fastest food producing sector, we can expect an increasing demand in certification schemes for aquatic animal health service providers, particularly in countries or regions where disease diagnostic services are provided by non-veterinarians.

6. Conclusions

Aquaculture development will continuously bring new challenges to aquatic animal health. A variety of strategies, approaches and options have been proposed, experimented and implemented. This is happening within the context of increasing social scrutiny. International and regional guidelines continue to be improved and added. National strategies enable ad hoc compliance to these standards with due respect to national priorities, objectives and means. Increased cooperation between fisheries and veterinary authorities provides timely support to national plans. Certification is certainly one critical issue, currently, because of its multiple and significant implications. Empowering farmers to manage disease and other risks is seen as the key to success under reducing public funds. Global aquaculture development therefore calls for innovative response in health management.

Diseases do matter. It requires major response by the government. Diseases can disrupt the sector, international trade and food supply but the impacts go beyond these as it has social and financial consequences as well, and in some cases, may have human health issues. The credibility of the government/country is at stake and failure to control will be a major risk. No amount of effort can eliminate the risk of danger from diseases. Aside from the technical challenge, the other challenges in dealing with disease control are resource management, public relations, communication, information management and endurance challenge. Dealing with disease emergencies require the following: (a) speed of response, decision-making and action; (b) systems of management, of information and of communication; and (c) good science. The overall objective must be to minimise the risk of disease entering a country; maintaining alertness or vigilance will be essential to achieve this.

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Spermatogonial Transplantation in Fish: Production of Trout Offspring from Salmon Parents

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Many salmonid species have recently become at risk of extinction. For fish species where eggs cannot be cryopreserved, establishment of techniques to preserve genetic resources other than egg and embryo cryopreservation is imperative. In the present study, spermatogonia from male trout were transplanted into the peritoneal cavity of newly hatched sterile triploid salmon. Transplanted trout spermatogonia colonized the gonads of sterile salmon recipients. In male recipients, transplanted spermatogonia underwent spermatogenesis. Further, in female recipients, transplanted spermatogonia underwent oogenesis. At 2 years after transplantation, sterile salmon recipients only produced donor-derived trout sperm and eggs. Fertilization with the sperm and eggs obtained from the triploid salmon recipients, only the donor-derived trout offspring was successfully produced. Combined with cryopreservation of spermatogonia, the present technique would make it possible to preserve fish genetic resources and to revive extinct species when necessary.

KEYWORDS spermatogonia; germ cell transplantation; vasa; surrogate broodstock

1. Introduction

In recent years, researchers have reported that if environmental destruction and commercial fishing continue at the current pace, the entire fishery resource will be depleted in about 40 years (Worm *et al.* 2006). Over

the last decade, numbers have declined for many salmonid species, with some species becoming extinct while others have become endangered (U.S. Fish & Wildlife Service 2008). Particularly in North America, many salmonids, such as certain stocks of Chinook salmon (*Oncorhynchus tshawytscha*), Gila trout (*O. gilae*), and bull trout (*Salvelinus*

confluentus) are endangered. Many salmonid species have been placed on the endangered species list (U.S. Fish & Wildlife Service 2008) and urgent measures appear necessary. Although the first choice for saving endangered species is to preserve and improve the environment, some species and local populations are likely to disappear before habitats can be restored. Urgent backup technologies, therefore, need to be developed while preserving and improving the environment (Myers *et al.* 2003). At present, one backup technique is to raise endangered species in captivity, but this involves risks such as facility accidents, outbreaks of infectious disease, and the inability of farmed fish to adapt to the original natural environments. Cryopreservation of gametes and early embryos is a backup technique that does not carry such risks. In principle, semi-permanent preservation is possible, and this technique for genetic resource preservation is less expensive and less risky than raising fish in captivity. However, cryopreservation of fish eggs has not been accomplished due to the large size, low permeability of chorion, and their high fat content (Chao and Liao 2001). As an alternative method of genetic resource preservation for fish, we have been developing surrogate broodstock technologies. Surrogate broodstock technologies refer to techniques where immature germ cells from a target fish species are transplanted into a related species that is easy to raise and mature, so that the related species can produce sperm and eggs of the target species (Yoshizaki *et al.* 2002, 2003; Okutsu *et al.* 2006a). Immature germ cells, such as primordial germ cells (PGCs) and spermatogonia, are sufficiently small to be easily cryopreserved (Kobayashi *et al.* 2007). By cryopreserving the germ cells of an endangered species, even if that species goes extinct, the species can be revived by transplanting thawed PGCs or spermatogonia into a related species. Recently, we have succeeded in obtaining functional rainbow trout (*O. mykiss*) sperm from masu salmon (*O.*

masou) by transplanting trout PGCs into the peritoneal cavity of newly hatched salmon and allowing the recipient salmon to mature (Takeuchi *et al.* 2004). However, the following issues must be resolved for surrogate broodstock technology using PGCs. First, fish PGCs can only be collected within a period of a few weeks before and after hatching, and since PGCs are not great in quantity, gathering sufficient numbers for transplantation is difficult. Second, when transplanting PGCs, most spermatozoa produced by recipients are of recipient origin, and few donor-derived spermatozoa are produced. Third, production of donor-derived functional eggs in xenogeneic recipients has not been possible by the method with trout PGCs. Okutsu *et al.* reported that a great quantity of spermatogonia could be obtained from the testes of male fish in all developmental stages and that spermatogonia could differentiate into both eggs and sperm after transplantation into newly hatched embryos (Okutsu *et al.* 2006b). The present study thus aimed to produce trout-derived sperm and eggs by transplanting spermatogonia into salmon recipients. Further, by using sterile triploid salmon as recipients, production of all donor-derived offspring was attempted.

2. Salmon Recipients Produce Donor-Derived Trout Sperm and Eggs Following Interspecies Transplantation of Spermatogonia

To verify whether the fish spermatogonial transplantation technology (Okutsu *et al.* 2006b) is valid for interspecies application, trout spermatogonia were microinjected into the peritoneal cavities of 90 salmon recipients by the method previously established in our laboratory (Takeuchi *et al.* 2003; Okutsu *et al.* 2006b). In this experiment, *pvasa-Gfp* (Yoshizaki *et al.* 2000; Takeuchi *et al.* 2002) hemizygous (*pvasa-Gfp*/–), dominant orange-colored mutant (Boonanuntanasarn *et al.* 2004) heterozygous (OR/wt), adult male rainbow trout and wild-type masu salmon

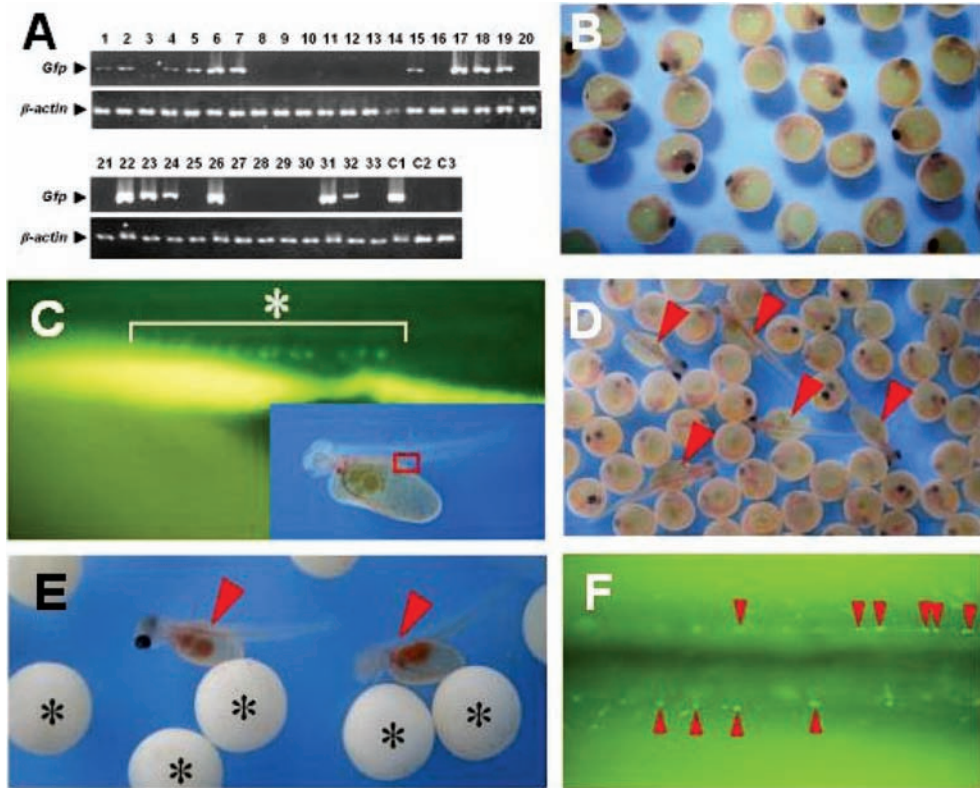


Fig. 1. Germline transmission of donor-derived haplotype to F1 progeny of diploid salmon recipients. **A)** PCR analysis of recipient milt samples with *Gfp*-specific primers. Lanes were labeled as follows: C1, positive control (milt of pvasa-*Gfp* trout); C2, negative control (milt of wild-type trout); C3, negative control (milt of wild-type salmon); 1–33, milt samples from diploid salmon male recipients. Positive results for the *Gfp* gene were seen in 16 milt samples. **B)** Hybrid obtained from wild-type male salmon and wild-type female trout at 34 dpf. No hybrids started hatching. **C)** Lateral view of orange-colored offspring that hatched before 34 dpf (inset), and a magnified image of the frame indicated in the inset showing *Gfp*-expressing PGCs (asterisk). The donor-derived haplotype was confirmed by *Gfp* expression in PGCs. **D)** F1 offspring obtained from a diploid salmon male recipient and wild-type female trout at 34 dpf. Most offspring had not hatched at this stage, although some had (arrowheads). **E)** F1 offspring obtained from diploid salmon female recipient and wild-type male trout at 34 dpf. Most offspring died before 34 dpf (asterisks), whereas some with normal morphology had hatched (arrowheads). **F)** Ventral view of the peritoneal cavity of F1 orange-colored offspring in E. The donor-derived haplotype was confirmed by *Gfp* expression in PGCs (arrowheads).

were used as donors and recipients, respectively. The masu salmon is a Pacific salmon found only in East Asia, whereas the rainbow trout is native to North America. These two species have been phylogenetically separated for at least 8 million years (McKay *et al.* 1996). Furthermore, only lethal hybrids

can be produced from these two species. At 2 years after transplantation, 33 mature male and 38 mature female recipients were successfully obtained. Of these, 16 males were identified to produce donor-derived spermatozoa following PCR analysis with *Gfp*-specific primers (Fig. 1A). Progeny tests

Table 1. Appearance rate of donor-derived rainbow trout among F1 generation of diploid masu salmon male recipients

Male recipient	Recipient-derived hybrids (n)	Donor-derived rainbow trout (n)	Total F1 offspring (n)	Donor-derived/Total (%)
5	1097	2	1099	0.2
6	970	34	1004	3.4
6 –2nd	406	12	418	2.9
18	960	26	986	2.6
18 –2nd	198	116	314	36.9
19	4	42	46	91.3
20	766	4	770	0.5
24	720	176	896	19.6
27	600	26	626	4.2
27 –2nd	757	116	873	13.3
32	412	140	552	25.4
33	769	8	777	1.0

were performed using 16 *Gfp*-positive male salmon with wild-type female trout. If male salmon produced donor-derived trout sperm, the next generation would consist of hybrids from recipient-derived salmon sperm and trout eggs and pure trout from donor-derived trout sperm and trout eggs. When raised at a water temperature of 10°C, almost all trout eggs hatched before 34 days post-fertilization (dpf), but no trout egg/salmon sperm hybrid eggs hatched before 34 dpf (Fig. 1B). As a result, in the following generation, nine recipients (9/33, 27.3%) produced offspring that hatched prior to 34 dpf and retained orange-colored body and *Gfp*-labeled PGCs (Figs. 1C, D), clearly identifying these offspring as donor-derived trout. Mean germline transmission rate for the donor-derived haplotype was 18.9% (range, 0.2–91.3%) (Table 1). When mating female salmon recipients and male wild-type trout in a progeny test, one of 38 female recipients produced two normal hatchlings (hatched within 34 dpf) among 324 hatchlings (0.6%) (Fig. 1E). One hatchling displayed orange-colored body and *Gfp*-labeled PGCs (Figs. 1E, F). In addition, salmon egg/trout sperm hybrids did not develop normally and could not hatch (Fig. 1E; Table 4). These normal hatchlings

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3. Sterile Triploid Salmon Male Recipients Produce Only Trout-Offspring

Spermatogonia were collected from donor trout displaying the above-mentioned characteristics (*pvasa-Gfp*^{+/–}; OR/wt) and transplanted into 100 newly hatched triploid salmon recipients. Just prior to the spawning season at 2 years after transplantation, testes were extracted from some male recipients and analyzed histologically. The results showed that while the testes of triploid salmon in the control group (no transplanta-

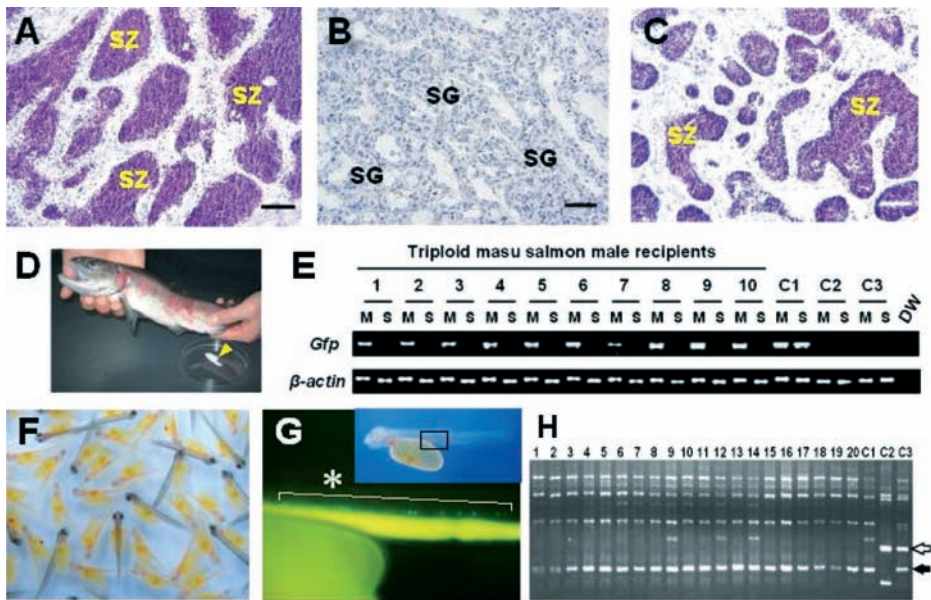


Fig. 2. Germline transmission of donor-derived haplotype to F1 progeny of sterile triploid salmon male recipients. **A–C)** HE-stained section of testis. Wild-type diploid salmon (**A**), intact triploid salmon (**B**) and triploid salmon recipient that received spermatogonial transplantation (**C**). Normal spermatogenesis (spermatozoa, **SZ**) was seen in the testes of triploid recipients in addition to wild-type diploid salmon, whereas intact triploid salmon testis retained only undifferentiated spermatogonia (indicated as **SG**). Bar scales: 50 μ m. **D)** The milt was successfully obtained (arrow-head) from a triploid salmon recipient. **E)** PCR analysis of milt (**M**) and soma (**S**) of triploid recipients with *Gfp*-specific primers. Lanes were labeled as follows: C1, *pvasa-Gfp* trout; C2, wild-type trout; C3, wild-type salmon; 1–10, triploid salmon male recipients. *Gfp* genes were detected in all milt of recipients not in soma. **F)** F1 offspring obtained from triploid salmon male recipient at 34 dpf. Orange-colored and wild-type hatchlings were obtained in similar proportions. **G)** Lateral view of orange-colored offspring that hatched before 34 dpf (inset), and a magnified image of the frame indicated in the inset. *Gfp* was expressed in PGCs (asterisk). **H)** RAPD analysis of F1 offspring. Lanes are labeled as follows: C1, wild-type trout; C2, wild-type salmon; C3, hybrid between trout and salmon; 1–20, F1 offspring of triploid salmon recipients. All offspring retained trout-specific bands (black arrow), not salmon-specific bands (white arrow). Although 20 out of 50 are shown here, the remaining samples showed identical banding patterns as lanes 1 to 20.

tion) were immature and filled with mostly spermatogonia (Fig. 2B), testes of recipient salmon contained spermatozoa just like normal salmon (Figs. 2A, C). At 2 years after transplantation, 10 of the 29 male triploid salmon recipients (34%) exhibited normal secondary sexual features and produced milt containing a large quantity of spermatozoa (Fig. 2D). Conversely, none of the triploid salmon without transplantation produced sperm.

PCR detected the *Gfp* gene specific to donor trout in the genomic DNA of sperm collected from all 10 recipients (Fig. 2E). Next, milts collected from these 10 recipients were used to fertilize wild-type trout eggs. F1 fertilized eggs all hatched before 34 dpf (Fig. 2F). In the F1 generation, the ratio of orange to wild-type colors was approximately 1:1 (302:304) (Fig. 2F; Table 2). In addition, an average of 49.3% possessed *Gfp*-labeled PGCs (Fig. 2G; Table 2). At this

Table 2. Appearance rate of F1 offspring with donor-derived haplotype among F1 generation of triploid salmon male recipients

Triploid masu salmon male recipient	Age of recipients (years)	Wild-type	Albino	Albino/Total (%)	Gfp+	Gfp-	Gfp+/Total (%)
#1	2	282	238	45.8	39	40	49.4
#2	2	388	423	52.2	21	15	58.3
#3	2	345	366	51.5	NE*	NE*	NE*
#4	2	321	342	51.6	41	40	50.6
#5	2	350	321	47.8	NE*	NE*	NE*
#6	2	275	301	52.3	41	51	44.6
#7	2	263	265	50.2	48	60	44.4
#8	2	284	255	47.3	42	34	55.3
#9	2	258	257	49.9	47	52	47.5
#10	2	251	272	52.0	44	56	44.0
Wild type**	2	452	0	0.0	0	61	0.0
#1	3	107	108	50.2	NE*	NE*	NE*
#3	3	113	84	42.6	NE*	NE*	NE*
#4	3	75	89	54.3	NE*	NE*	NE*
Wild type**	2	191	0	0.0	NE*	NE*	NE*

*NE: not examined; **Wild type: wild-type rainbow trout

time, development rate in the F1 generation was comparable to that in control wild-type trout (Table 3). Five F1 fish were collected from each of the 10 recipients, and these 50 fish were subjected to random amplified polymorphic DNA (RAPD) analysis for species determination. All 50 fish displayed the same DNA fingerprinting pattern as trout (Fig. 2H). These findings proved that the generation following male triploid salmon recipients produced only donor-derived trout (Okutsu *et al.* 2007). Furthermore, to measure the contamination rate of salmon cells in milt produced by triploid salmon recipients, semi-quantitative PCR analysis was performed using a masu salmon growth hormone gene-specific primer (Nagano *et al.* 1994). Contamination rates of salmon genomic DNA were low, ranging from 0.0–1.3%. While low levels of contamination by salmon cells were confirmed, the generation after male triploid salmon recipients exhibited development rates comparable to those of normal trout (Table 3), and all 50 F1 fish subjected to RAPD exhibited the same fin-

gerprinting pattern as normal trout, suggesting that salmon genomic DNA originated from somatic cells including blood cells in milt. These findings clarified that the 10 triploid salmon recipients only produced donor-derived trout sperm. It has been known that male triploid salmonids occasionally produce aneuploid sperm (Carrasco *et al.* 1998), but the resulting next generation cannot survive. Furthermore, DNA content was measured in 67 F1 fish, and the DNA content for these F1 fish was identical to that for normal diploid trout, suggesting that triploid salmon recipients only produced donor-derived trout sperm (Fig. 3). In addition, triploid salmon recipients only produced large quantities of donor-derived sperm at 2 and 3 years old to spawn only donor-derived trout (Tables 2 and 3). These findings suggest that trout spermatogonia functioned as spermatogonial stem cells in the testes of triploid xenogeneic recipients. The cause of sterility in triploid male salmonids has not been clarified, but the present study is the first to indicate that the

Table 3. Development of F1 trout derived from triploid masu salmon male recipients at 2 and 3 years old

Triploid masu salmon male recipient	Age of recipients (years)	Total eggs (n)	Fertilized (%)	Eyed (%)	Hatched (%)	Swim up (%)
#1	2	697	73.3	74.6	73.5	73.2
#2	2	817	100.0	99.3	99.3	98.5
#3	2	715	100.0	99.4	99.2	98.9
#4	2	668	100.0	99.3	99.3	98.7
#5	2	676	100.0	99.3	89.3	88.8
#6	2	578	100.0	99.7	99.7	98.6
#7	2	532	100.0	99.2	98.5	97.4
#8	2	550	100.0	98.0	90.7	90.2
#9	2	523	100.0	98.5	98.5	98.1
#10	2	533	100.0	98.1	94.4	94.4
Wild type*	2	831	100.0	99.0	97.8	96.5
#1	3	238	100.0	99.6	98.7	96.6
#3	3	210	100.0	93.8	91.0	88.6
#4	3	168	100.0	97.6	97.6	96.4
Wild type*	2	201	100.0	95.0	93.5	89.6

*Wild type: wild-type rainbow trout

cause of sterility in male triploid salmonids is the germ cells themselves, and the gonadal microenvironment of male triploid salmonids is capable of maintaining the “stemness” of spermatogonial stem cells and has the proper endocrine system for spermatogenesis.

4. Successful Production of Only Trout Offspring from Sterile Salmon Parents

Some female 17-month-old triploid salmon recipients were dissected to examine the ovaries, and in four of eight recipients, the ovary contained donor-specific green-fluorescent oocytes (Fig. 4A). At this age, these oocytes had proceeded to vitellogenesis just like oocytes produced by normal diploid salmon (Figs. 4A, B). At the same age, ovaries of triploid salmon that had not received spermatogonia were underdeveloped, containing no vitellogenic oocytes (Fig. 4C). When recipients reached 2 years, five of the 50 female triploid salmon recipients ovulated from 38 to 213 eggs (Table 4). In order to

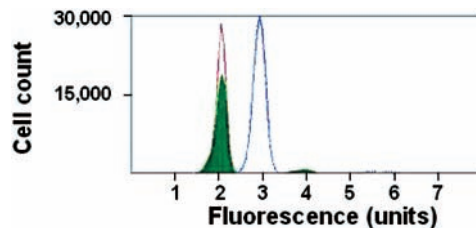


Fig. 3. Ploidy analysis of F1 offspring obtained from triploid salmon male recipient. Red line indicates wild-type diploid trout. Blue line indicates triploid salmon. Green indicates F1 offspring. DNA amount (ploidy) for F1 offspring was identical to that for wild-type diploid trout.

develop the present surrogate broodstock technology as a practical technique, successful production of donor-derived offspring from xenogeneic recipients is essential. Therefore, these eggs were fertilized using milt harvested from male triploid salmon recipients that had been shown to produce only trout sperm, in an attempt to produce donor-derived next-generation trout from

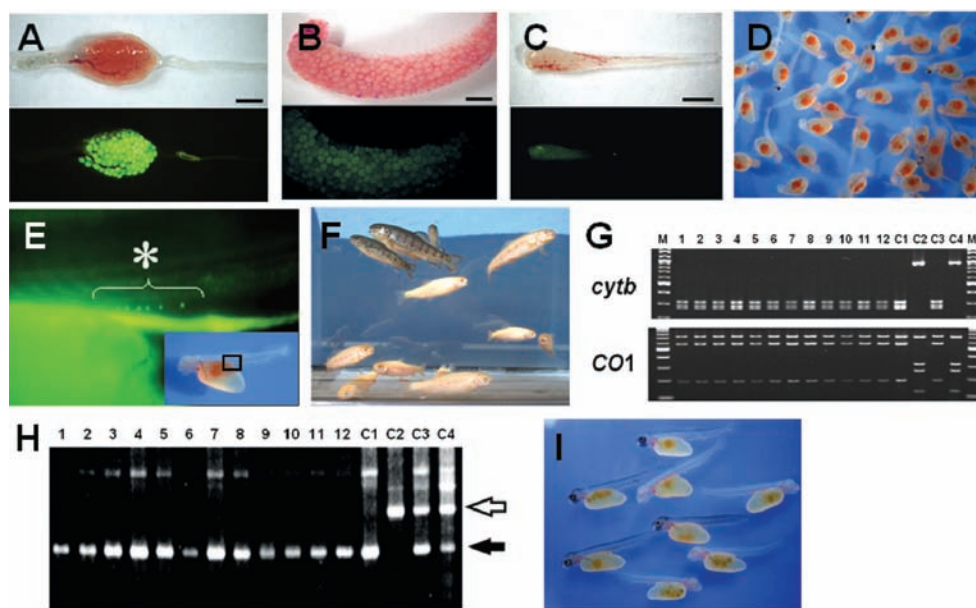


Fig. 4. Development of donor derived-oocytes and F1 offspring generated from surrogate parents. **A–C)** Oocyte colony derived from donor trout-spermatogonia in the ovary of triploid salmon recipient 17 months after transplantation (A), and ovaries of diploid salmon (B) and intact triploid salmon (C) at the same age as the recipient. Lower panels, fluorescent view. Bar scales: 5 mm. **D)** F1 offspring obtained from surrogate salmon parents at 34 dpf. **E)** Lateral view of orange-colored offspring (inset), with a highly magnified image of a frame in inset. *Gfp* was expressed in PGCs (asterisk). **F)** Juveniles generated from surrogate salmon parents at 6 months old. **G)** RFLP analysis of mitochondrial genes, *cytb* and *CO1*. Lanes were labeled as follows: M, MW marker; 1–12, PCR products obtained from template DNA of F1 offspring produced by surrogate salmon parents; C1, wild-type trout; C2, wild-type salmon; C3, hybrid between trout female and salmon male; C4, hybrid between salmon female and trout male. All F1 offspring showed digestion patterns identical to trout. **H)** RAPD of nuclear genome of F1 offspring produced by surrogate salmon parents. Lanes are labeled in the same way as in G. All offspring retained a trout-specific band (black arrow), not salmon-specific band (white arrow). **I)** F2 offspring obtained from F1 male.

xenogeneic male and female recipients. Although development rate of the next generation varied markedly from one female brood-stock to the next, hatching and swim-up rates reached 89.5% and 57.9%, respectively, in some recipients (Table 4). All F1 hatchlings obtained from these triploid salmon parents hatched before 34 dpf, as in normal trout, and the ratios of orange-colored trout to wild-type trout and of *pvasa-Gfp*(+) and *pvasa-Gfp*(–) were both about 3:1 in the F1 generation (Figs. 4D, E). These findings show that the inheritance of OR/wt and *pvasa-Gfp*/– was Mendelian, and in other

words, the F1 generation was generated from donor-derived sperm and eggs. Swim-up juveniles also developed normally (Fig. 4F). Next, restriction fragment length polymorphism (RFLP) analysis was performed to ascertain the mitochondrial origin of the F1 generation obtained from triploid salmon parents, clarifying that all 18 F1 fish carried trout mitochondria (Fig. 4G). These findings clarify that female triploid salmon recipients that received trout spermatogonia only produced normal functional donor-derived trout eggs. In addition, RAPD analysis of nuclear DNA showed that the DNA fingerprinting

Table 4. Development of F1 generation derived from xenogeneic surrogate parents

Total masu salmon female recipient	Eggs (n)	Eyed eggs (n, %)	Hatched embryos (n, %)	Swim-up juveniles (n, %)
#1	178	0 (0.0)	0 (0.0)	0 (0.0)
#2	38	36 (94.7)	34 (89.5)	22 (57.9)
#3	154	4 (2.6)	3 (1.9)	3 (1.9)
#4	312	14 (4.5)	7 (2.2)	2 (0.6)
#5	310	155 (50.0)	155 (50.0)	65 (21.0)

Table 5. Development of F2 offspring produced using milt of F1 males of xenogeneic surrogate salmon parents

F1 male derived from surrogate parent	Egg numbers used for insemination	Fertilized (%)	Eyed (%)	Hatched (%)
F1-1	262	97.2	91.2	91.2
F1-2	212	100.0	92.0	91.0
F1-3	225	97.2	83.6	82.2
F1-4	242	97.2	97.5	96.7
F1-5	209	100.0	99.0	98.1
Trout ♀ × Trout ♂	287	100.0	98.6	96.5
Trout ♀ × Salmon ♂	888	100.0	96.6	96.3*
Salmon ♀ × Trout ♂	489	100.0	0.0	0.0

*These hybrids all died by 30 days after hatching

pattern of the F1 generation was the same as that of trout (Fig. 4H). Furthermore, the F1 generation was fertile to produce normal F2-generation trout (Fig. 4I; Table 5). We thus succeeded in establishing a surrogate brood-stock technique for salmonids where spermatogonia are transplanted into sterile triploid xenogeneic recipients to produce a next generation consisting of all donor-derived fish (Okutsu *et al.* 2007). Spermatogonia collected from one donor can be transplanted to >100 recipients. In the present study, spermatogonia were transplanted into 100 recipients. Indeed #1–4 female recipients and all male recipients were prepared using spermatogonia collected from one donor trout. Therefore, juveniles (in total 27) obtained from #1–4 female recipients were produced from a single donor. In other words, these

F1 fish were self-fertilized fish produced from one donor trout. This suggests that even when only one male remains, more fish containing both male and female can be produced using the present technique. Furthermore, after cryopreserving fish spermatogonia, a species can be efficiently revived by transplanting cryopreserved spermatogonia into sterile recipients using the present technique. Even if a species has gone extinct, the species can be revived using cryopreserved spermatogonia if the natural habitat is first restored, individuals of the species are produced using the present technique, and those individuals are released to its natural habitat.

In the present study, great individual differences were seen in the development rate of eggs obtained from female triploid salmon

recipients, and rates were low in some recipients (Table 4). Female triploid salmon produce small ovaries (Fig. 4A), and individual differences were attributed to low hormone secretion from the ovary and subsequent abnormal feedback regulations of the hypothalamus and pituitary. In the future, the efficiency of the present technique will be improved by clarifying the secretion profiles of reproductive hormones in female triploid salmon recipients and administering the necessary exogenous hormones.

We propose application of the present results to marine fish, to markedly improve the efficiency of seed production for tuna to counter the rapidly decreasing numbers seen in recent years. Bluefin tuna takes 3–5 years to mature, and since adult bluefin tuna weigh about 100–600 kg, farming requires great

facilities, manpower and costs. Mackerel mature in 1–2 years and the small weight of about 500 g allows the fish to be raised in a small tank. If mackerel could be used as a surrogate for bluefin tuna, large quantities of seeds could be produced in small land-based fish tanks over a short period of time. In this manner, the present technique could be used to not only conserve endangered species, but also drastically improve the supply efficiency of marine products that have been in great demand worldwide in recent years.

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Biotechnology of Marine Invertebrates —Recent Advances in Shrimp and Shellfish

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Marine invertebrates possess a variety of interesting functions and some of them have been well studied from the standpoint of biotechnology. Shrimp and shellfish are useful animals among marine invertebrates. In this section, we describe the recent advances in biological defense system of shrimp and the biomineralization system of shellfish as examples of which molecular mechanisms have been well elucidated.

First, we focus on shrimp antimicrobial peptide (AMPs) which are considered to play a major role in the innate immunity against invading microbes. A total of 767 ESTs encoding putative AMPs were identified in the *Penaeus monodon* EST database (<http://pmonodon.biotec.or.th>), which were comprised of homologues of the three AMP families; penaeidins, crustins and antilipopolysaccharide factors. Sequence analysis revealed that each AMP family had sequence diversity and constituted multiple isoforms or subgroups. The relevance of this sequence variability to the potential antimicrobial function was investigated. These shrimp AMPs with high selectivity to severe pathogens are potential candidates as an alternative to antibiotics in aquaculture.

As a second example, we present the recent advances in the molecular mechanism of shell formation. A variety of animals and plants have the ability to make hard biological structure called biominerals made of calcium, silicon and other minerals. Molluscan shell is a typical biomineral composed of CaCO₃, but a small amount of organic matrix proteins included in the shell direct the crystal growth that is specific for the shell species. Ca²⁺ adsorbed through specific transporters and HCO₃²⁻ synthesized from CO₂ are mixed under well regulated conditions, thereby forming shell.

KEYWORDS antilipopolysaccharides; antimicrobial peptides; biomineral; calcification; crustins; penaeidins; shell

1. Shrimp Antimicrobial Peptides: Sequence Diversity and Functional Characteristics of Different Isoforms

1.1. Introduction

Organisms lacking an adaptive immunity rely on antimicrobial peptides (AMPs) as the first line of defense against invading pathogens (Hancock and Diamond 2000; Jenssen *et al.* 2006). AMPs are typically small cationic molecules (15 to 100 amino acids) which are considered to play an important role in the innate immune system. They are widely distributed in the whole living kingdom ranging from bacteria to plants, invertebrate and vertebrate species, including mammals. They display a broad spectrum of activity against bacteria, fungi, viruses, parasites and even tumor cells. Although they differ in amino acid sequences, most AMPs adopt an amphipathic secondary structure that is believed to be essential for their antimicrobial action.

Numerous AMPs are found in invertebrates, particularly in insects, where they play a major role in protection from invading pathogens (Bulet and Stocklin 2005). In crustaceans, AMPs were first described in the hemocytes of the shore crab *Carcinus maenas* (Schnapp *et al.* 1996) as a 6.5 kDa proline-rich cationic protein displaying activity against both gram-positive and gram-negative bacteria. Not until 1997 was the first shrimp AMP family, named penaeidin, discovered in the Pacific white shrimp, *Litopenaeus vannamei*, by means of reverse-phase chromatography (Destoumieux *et al.* 1997). Subsequently, penaeidin sequences have been found in several penaeid shrimps by genomic approaches (Cuthbertson *et al.* 2002; Kang *et al.* 2004; Supungul *et al.* 2004). The other two shrimp AMP families, the crustins and antilipopolysaccharide factors (ALFs), have been identified from haemocyte cDNA libraries of several shrimp

species. The crustins, which are homologues of the shore crab 11.5 kDa antibacterial protein, were identified by expressed sequenced tag (EST) analysis from *L. vannamei* and *L. setiferus* (Bartlett *et al.* 2002; Vargas-Albores *et al.* 2004). Since then, crustins and crustin-like peptides have been identified from a variety of other species (Rattanachai *et al.* 2004; Hauton *et al.* 2006; Brockton *et al.* 2007; Jiravanichpaisal *et al.* 2007; Zhang *et al.* 2007; Supungul *et al.* 2008). Antilipopolysaccharide factors (ALFs), first reported in horseshoe crabs (Morita *et al.* 1985; Aketagawa *et al.* 1986; Muta *et al.* 1987), have also been identified in shrimps (Supungul *et al.* 2004; Liu *et al.* 2005; Somboonwiwat *et al.* 2005). Moreover, an anionic antimicrobial peptide, astacidin, which is derived from the limited proteolysis of hemocyanin, has been reported in *L. vannamei* (Destoumieux-Garzón *et al.* 2001). In this paper, we present a recent finding on the sequence diversity of cationic AMPs identified from the black tiger shrimp *Penaeus monodon* database (<http://pmonodon.biotec.or.th>), their antimicrobial properties and potential application for disease control in aquaculture.

1.2. AMPs identified from the *Penaeus monodon* EST Database

As of December, 2007, the *Penaeus monodon* EST database (<http://pmonodon.biotec.or.th>) contained a total of 40,001 EST sequences. Approximately 30,000 additional ESTs were obtained after the first establishment of the database according to the report by Tassanakajon *et al.* (2006). Clustering and sequence assembly identified 10,536 unique sequences represented by 3,227 contigs and 7,309 singletons. Each unique sequence was subjected to similarity searches against the GenBank database using BLASTx and BLASTn with an e-value cut off of $<10^{-4}$. Of 10,536 unigenes, 5,648 (53.6%) showed a significant match whereas 4,888 (46.4%) did not match to any sequences in the database.

Among the known (annotated) genes, a total of 767 clones were identified as putative antimicrobial proteins. They were homologues of the three AMP families; penaeidins (284 clones), crustins (275 clones) and antilipopolsaccharide factors (208 clones). Sequence analysis revealed that each family of AMPs displays sequence diversity and constitutes multiple isoforms or subgroups (Table 1). The sequence and functional diversity of the different isoforms of *P. monodon* AMPs are described below.

1.3. Penaeidins

The shrimp AMPs in the penaeidin family contain a unique structure comprised of an N-terminal proline-rich domain and a C-terminal cysteine-rich domain with six conserved cysteine residues engaged in three disulfide bonds. Penaeidins 1, 2 and 3 (PEN1, -2 and -3) were first isolated from haemocytes of the white shrimp *L. vannamei* (Destoumieux *et al.* 1997). Subsequent phylogenetic analysis indicated that *Litvan*PEN1 is a variant of *Litvan*PEN2 (Cuthbertson *et al.* 2002). To date, penaeidin sequences have been identified from different penaeid shrimps by genomic approaches and can be classified into four different subgroups (PEN2, -3, -4 and -5) based on their primary sequence diversity. The conserved key residues that appear to be a signature for PEN-2, -3 and -4 have been established in PenBase, <http://www.penbase.immunology.com> (Gueguen *et al.* 2006) while the distinct sequence characteristics of PEN-5, recently identified in *Fenneropenaeus chinensis*, have also been described by Kang *et al.* (2007).

From the *Penaeus monodon* EST database, 284 ESTs representing penaeidins were identified and subjected to sequence analysis. Based on the sequence lengths and the conserved signature residues, they were classified into two different subgroups: *Penmon*PEN3 and -5 (Fig. 1). The major penaeidin sequences (260 clones) found were classified as PEN3 whereas PEN5 sequences

Table 1. The abundance of putative antimicrobial peptides in the *Penaeus monodon* EST database (<http://pmonodon.biotec.or.th>).

Antimicrobial peptide	Frequency
Antilipopolsaccharide factors	208
ALF <i>Pm</i> 1	1
ALF <i>Pm</i> 2	2
ALF <i>Pm</i> 3	184
ALF <i>Pm</i> 4	1
ALF <i>Pm</i> 5	2
ALF <i>Pm</i> 6	16
ALF <i>Pm</i> 7	2
ALF <i>Pm</i> 8	1
Crustins	275
crustin <i>Pm</i> 1	55
crustin <i>Pm</i> 2*	2
crustin <i>Pm</i> 3	1
crustin <i>Pm</i> 4	158
crustin <i>Pm</i> 5	1
crustin <i>Pm</i> 6	2
crustin <i>Pm</i> 7 (crus-like <i>Pm</i>)	56
Penaeidins	284
<i>Penmon</i> Pen3	260
<i>Penmon</i> Pen5	24

*not full length

(24 clones) were found much less frequently. *Penmon*PEN3 and -5 sequences encode predicted peptides of 74 and 79 amino acid residues, respectively, including a signal peptide of 19 amino acids. All *Penmon*PEN3 predicted peptide sequences possess the following signature: Gln¹, Gly⁵, Arg¹³, Gly¹⁸, Ser³⁵, His³⁷, Gln⁴³ and Ala⁴⁶, similar to the conserved key residues of PEN3 described by Gueguen *et al.* (2006). However, *Penmon*PEN5 sequences do not contain all the reported conserved key residues (Gln¹, Ser⁵, Arg¹³, Ser¹⁸, Arg³⁷, Gln⁴³ and Ala⁴⁶) proposed by Kang *et al.* (2007), but show

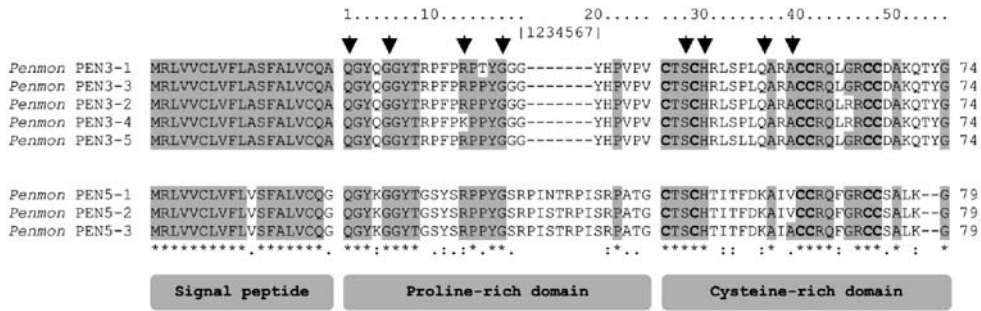


Fig. 1. Predicted amino acid sequence comparison of PEN3, -5 and their variants from *Penaeus monodon*. The full sequences of the penaeidins were aligned using ClustalW and gaps were introduced to optimize the alignment. Cysteines are in boldface, identical residues and conservative replacements are shaded. The asterisk (*) indicates amino acid identity and (.) and (:) indicate amino acid similarity. The arrows indicate conserved residues of penaeidin subgroups.

variations at Gly⁵ and His³⁷ which are identical to the residues in PEN3, and at Ser¹⁸ which is a specific residue in the subgroup 5 and was found in all *Penmon*PEN5 sequences. Variants of *Penmon*PEN3 (*Penmon*PEN3-1 to *Penmon*PEN3-5) and *Penmon*PEN5 (*Penmon*PEN5-1 to *Penmon*PEN5-3) show polymorphisms at four and two amino acid residues, respectively (Fig. 1). It should also be noted that the penaeidin-5 cDNA from *P. monodon* reported previously by Hu *et al.* (2006) is identical to *Penmon*PEN3-3 reported here. According to the conserved key residues for PEN3 proposed in PenBase by Gueguen *et al.* (2006) and those proposed for PEN5 by Kang *et al.* (2007), this cDNA sequence should be classified as a PEN3 isoform and not PEN5. Analysis of penaeidin sequences through PenBase, <http://www.penbase.immunaqua.com>, would allow a systematic nomenclature and classification of the penaeidin family and prevent confusion with the increasing number of independently derived penaeidin sequences from a variety of shrimp species.

In *L. vannamei* and *L. setiferus*, genes from each of the three penaeidin subgroups (PEN2, -3, and -4) have been found and shown to be expressed in a single individual

(Cuthbertson *et al.* 2008), whilst in *P. monodon* only two subgroups (PEN3 and -5) were found with the expression level of the later being much less abundant. This probably indicates that *Penmon*PEN5 is not constitutively expressed but rather may be regulated in response to certain physiological stimuli. Further expression studies are needed to test this notion.

In general, penaeidins possess antibacterial activity predominantly directed against gram-positive bacteria and antifungal activity against filamentous fungi. PEN2 and -3 from *L. vannamei* both exhibit similar antimicrobial properties although PEN3 is more effective at low concentrations (Destoumieux *et al.* 1999). In *L. setiferus*, the antimicrobial properties of PEN3 and -4 were compared and it was found that *Litset*PEN3 has a broader range of microbial targets whilst *Litset*PEN4 is generally more effective against fungi (Cuthbertson *et al.* 2004). *Fenchi*PEN 5 displayed activities against gram-positive and gram-negative bacteria and fungi (Kang *et al.* 2007). Since antimicrobial properties of the four different subgroups of penaeidins have been well characterized by several research groups, we do not further characterize *Penmon*PEN3 and -5. The structure and function of penaeidins

have been subjected to extensive review elsewhere (Bachère *et al.* 2000; Cuthbertson *et al.* 2008).

1.4. Crustins

Crustins are homologues of 'carcinin', an 11.5 kDa antibacterial protein from the shore crab *Carcinus maenas*. The cDNA of crustins have been reported from a variety of crustaceans including *L. vannamei* (Bartlett *et al.* 2002; Vargas-Albores *et al.* 2004), *L. setiferus* (Bartlett *et al.* 2002), *P. monodon* (Supungul *et al.* 2004), *Marsupenaeus japonicus* (Rattanachai *et al.* 2004), *Fenneropenaeus chinensis* (Zhang *et al.* 2007), *Panulirus argus* (Stoss *et al.* 2004), *Homarus gammarus* (Hauton *et al.* 2006), *C. maenas* (Brockton *et al.* 2007) and *Pacifastacus leniusculus* (Jiravanichpaisal *et al.* 2007). Those crustins that have been described to date have diverse amino acid sequences but with a relatively conserved C-terminus of 12 cysteine residues including a single whey acidic protein (WAP) domain. The WAP domain generally consists of 50 amino acid residues with eight cysteine residues at defined positions. They form four intracellular disulfide bonds creating a tightly packed structure (Grütter *et al.* 1988). The WAP domain-containing proteins are widespread throughout vertebrates and invertebrates and have diverse biological functions including antibacterial activity and protease inhibitory activity (Sallénave 2000; Hagiwara *et al.* 2003).

From the *Penaeus monodon* EST database, 275 ESTs representing crustins were identified. Clustering analysis revealed the presence of five isoforms including the four isoforms reported previously (CrusPm1–4) (Supungul *et al.* 2004). The amino acid sequence alignment of the full length sequences of all isoforms except crustinPm2 suggested that isoform 3 is a subset of isoform 4. Each isoform was found at a different frequency (Table 1), and sequence variation was also observed within each

isoform suggesting both differential expression and potential functional polymorphisms. The crustin isoforms are very different in length and primary sequence and they are the most diverged representatives of the AMP families in the species. Alignment of the crustin sequences revealed very diverse amino-terminal signal peptide and glycine-rich regions whilst the carboxyl-terminal region, where the WAP domain resided, is more conserved (Fig. 2). CrustinPm4, one of the major isoforms, contained a very long glycine-rich repeat and this isoform is 92% identical at the amino acid sequence level to the *P. monodon* crustin reported by Chen *et al.* (2004). All isoforms contain 12 conserved cysteine residues in the C-terminus that participate in the formation of six disulfide bonds. The phylogenetic analysis of WAP domains from various invertebrate WAP domain-containing proteins showed a high diversity and could be divided into three distinct groups (Fig. 3). The first group composed of crustacean single WAP proteins. The second group contained crab carcinins, lobster and crayfish crustins. The last group contained all the shrimp crustins including all isoforms of *P. monodon* crustins. However, CrustinPm7, later named the crustin-likePm, is quite distinct from the other isoforms and formed a subgroup with a crustin-like protein Fc1 from *F. chinensis* (Zhang *et al.* 2007). The difference of the WAP domains may indicate different biological activity of the proteins.

Expression analysis in various shrimp tissues revealed different patterns of expression of different crustin isoforms. For example, crustinPm1 was expressed in all tissues examined including haemocytes, lymphoid organs, gill, intestine, eyestalk, hepatopancreas, epipodite and antennal gland whereas the crustinPm5 transcript was observed only in epipodite and eyestalks (data not shown). The difference in tissue distribution of the crustin transcripts probably indicates diverse functions of crustins as immune effectors,

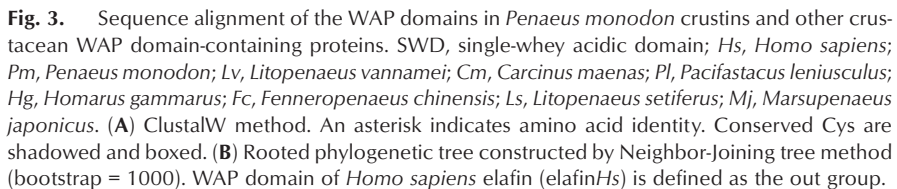
crustinPm1	MKGLGVILFC-VLAMASA-----	17
crustinPm6	MKGVSVAIICCVLAASA-----	18
crustinPm3, 4	MKGVKAVILCSLLATALA-GKFRGSGTYGGGSGYGGGSGYGGGLGGGVSGGGLGGGVNNGG	59
crustinPm5	MRVAGYLVVAVASVAVTDG-----	19
crus-likePm	MLKFVVLSSVAVAVVHA-----	17
	*	
crustinPm1	-----	17
crustinPm6	-----	18
crustinPm3, 4	GLGGGVNNGGGLGGGLGGGVNNGGLGGGGAYGGGLGGGLGGGVNNGGLGGGLGGGVQGGG	119
crustinPm5	-----QY	21
crus-likePm	-----	17
crustinPm1	-----QSWHGGRPG---GFPG---GGRP---GGFPGGG---RPGGRP---GGFP---	51
crustinPm6	---NERQGSNRFNNGGFGGGVNSGFGGGFGGGFPAGGFGQGGG---FPGGFGGIGGFPFPG	71
crustinPm3, 4	LGGGLGGGVHGGGLGGGLGGGVHGGGLGGGLGGGLS-GGLGGGLG---RPGGGLGRPGGGL	176
crustinPm5	IGFGVPGQGLVDSLNLISGG---GFPG---GHFPGQGGHFPQGGHFPQGGNFPQGQ---	74
crus-likePm	-----QDKGNADTRFLGG---LGVPG---GGVPGVGGGFLPGV---PGHGGVVPGGGG	61
	* * * * *	
crustinPm1	-SVTAPPAS CRRWCETPENAFYCC ---SRYEPEAPVGT KILDCPKVRDTCPPVRFLAVE	107
crustinPm6	AGISQSS CRYYWCRNPENQVYCC ---TDLEPEGPVGT KPLDCPIVRPTCP-VSVRG-L	126
crustinPm3	SPGSRGPST CRYWCTTPEGKQYCC ---DKNEPEIPVGT KPLDCPQVRPTCP--RFQG--	229
crustinPm5	-GNYPGQGS CKYWCSPENQYCCD RGNNQGGNYP-GSKPGFCPAVRDVCPPTRFGVG-	
crus-likePm	-LLPGGQFE CN-YCRT-RYGYVCC ----- KPGRCPIRDTCPGLRKGV-	102
	* . : * . . ** * * * : * . *	
crustinPm1	QPVPCSSDYKCGG-LDKCCFDRCLGQHVCKPPSFY--EFFA	145
crustinPm6	RPITCSNDYKCGG-VDKCCYDRCLQEHVCKPPS-----FFG	161
crustinPm3, 4	PPVTCSHDFKACAG-LDKCCFDRCLGEHVCKPPSFYGRNVKG	269
crustinPm5	RPIQCAHDGQCYASNDKCCFDRCLGEHVCKPATYNGR---	169
crus-likePm	---ICRQDTCDFG-SDKCCFDTCLNDTVCKPIV---AGSQG	136
	* * * . * * * * : * * * : * * *	

Fig. 2. Multiple alignment of deduced amino acid sequences of *Penaeus monodon* crustins. The asterisk (*) indicates amino acid identity and (.) and (:) indicate amino acid similarity. The signal peptides, glycine rich regions, and WAP domains are indicated by white, gray, and black boxes, respectively. The bolds letters correspond to the cysteine rich regions.

although multifunctional roles in development or differentiation cannot be excluded.

To further investigate the biological function of *P. monodon* crustins, three isoforms (crustinPm1, -5 and crus-likePm) were selected for the production of recombinant proteins in the *E. coli* system. The recombinant proteins expressed mainly in insoluble inclusion bodies were solubilized, purified and characterized for their antibacterial activity against several strains of gram-positive and gram-negative bacteria. Recombinant crus-likePm showed the broadest spectrum of activity against various strains of bacteria including all five tested gram-positive bacteria (*Staphylococcus*

aureus, *S. haemolyticus*, *Aerococcus viridans*, *Bacillus megaterium* and *Micrococcus luteus*) with MIC values ranging from 0.312 to 10 μ M, and five of the six gram-negative bacteria (Table 2). However, potency against gram negative bacteria ranged from strong (*Escherichia coli* 363 and *Vibrio harveyi* with MIC values ranging from 2.5 to 5 μ M), intermediate (*Klebsiella pneumoniae*) to weak or no activity (*E. cloacae* and *S. thyphimurium*). In contrast, the other two isoforms were far less active (Table 2). Recombinant crustinPm1 was active against gram-positive bacteria with the highest activity against *S. aureus* (MIC of 3.13 to 6.25 μ M) and no detectable activity against



values ranging from 1.56 to 50 μM . Further studies on the mechanism of inhibition of crustin $Pm1$ (Supungul *et al.* 2008) and crustin-like Pm (Amparyup 2008) against bacteria has revealed crustins to have bactericidal effects. Besides the antimicrobial

Table 2. Antimicrobial activities of crus-like*Pm*, crustin*Pm*1 and crustin*Pm*5 peptides against gram-positive and gram-negative bacteria in a liquid growth inhibition assay

Microorganism	MIC value (mM)		
	crustin <i>Pm</i> 1	crus-like <i>Pm</i>	crustin <i>Pm</i> 5
Gram (+) bacteria			
<i>Aerococcus viridans</i>	50–100	0.312–0.625	>100
<i>Staphylococcus aureus</i>	3.13–6.25	5–10	0.78–1.56
<i>Staphylococcus haemolyticus</i>	50–100	2.5–5	12.5–25
<i>Micrococcus luteus</i>	25–50	2.5–5	25–50
<i>Bacillus megaterium</i>	6.25–12.50	1.25–2.5	>100
Gram (–) bacteria			
<i>Enterobacter cloacae</i>	>100	>100	>100
<i>Klebsiella pneumoniae</i>	>100	10–20	>100
<i>Salmonella thyphimurium</i>	>100	>100	>100
<i>Escherichia coli</i> 363	50–100	2.5–5	>100
<i>Erwinia carotovora</i>	>100	ND	>100
<i>Vibrio harveyi</i> 639	>100	2.5–5	>100

MIC are expressed as the interval a–b, where a is the highest concentration tested at which microorganisms are growing, and b the lowest concentration that caused 100% growth inhibition. ND = not determined

activity, the WAP domain is also recognized as a signature motif for a serine protease inhibitor. However, the recombinant crustin*Pm*1, -5 and crus-like*Pm* do not possess a detectable protease inhibitory activity (data not shown).

Most crustins reported so far are active against only gram-positive bacteria, excepting the crus-like*Pm* which also kills some gram-negative bacteria. Although it is quite clear that crustins are antimicrobial proteins, some isoforms which showed low killing activity may be engaged in other biological functions, or else require different physiological conditions or partners to be active. Variations in the response of crustin expression to various pathogens have been observed in several shrimps (Supungul *et al.* 2004; Vargas-Albores *et al.* 2004; Amparyup *et al.* 2008) as well as in *H. gammarus* (Hauton *et al.* 2006) and *P. leniusculus* (Jiravanichpaisal *et al.* 2007). Moreover, we found that the expression of crustin*Pm*5,

which is a novel crustin recently isolated from a gill-epipodite cDNA library of *P. monodon* was significantly up-regulated under heat stress when the animals were maintained at 35°C, compared to those at 30°C (unpublished data). Inconsistent patterns of correlation between expression of crustin isoforms in response to microorganisms and the response of some crustin isoforms to other physiological stress may indicate potential other functions for these proteins outside of immunity. Further investigation is required to elucidate the true functions of various crustins from crustacean species.

1.5. Antilipopolysaccharide factors

Antilipopolysaccharide factor (ALF) is a small basic protein originally isolated from hemocytes of the horseshoe crab, *L. polyphemus*, (LALF) and *Tachypleus tridentatus* (TALF) (Tanaka *et al.* 1982; Aketagawa *et al.* 1986). LALF binds to and

ALF Pm6	MRVSVFS-MILVVVVAASFAPQCQA SGWEALVPAIANKLTSLWESGEFELLGHYCSFNVT	59
ALF Pm7	MRVSVLT-MALTVALVALPSQCSA AGWGAFMPSIATRLTGLWETGELELLGHYCTYSVK	59
ALF Pm3	MRVSVLVSLVLVSLVALFAPQCQA OGWEAVAAAVASKIVGLWRNEKTELLGHECKFTVK	60
ALF Pm2	MR--VLVSFLMALSLIALMP-RCQG QGVQDLLPALVEKIALGLWHSDEVEFLGHSCRYSR	57
ALF Pm8	MTSSTARGMFSLVCFLLIASA SARPLGDLGSLVETFEVNAIKTTEITILDNYCLLSRS	60
	* . : : . . . : : . . : : * .	
ALF Pm6	PKFKRWQLYFRGRMWC PGWTTIRGQAETRS-RSGVVGRTTQDFVRKAFRAGIITESEAQA	118
ALF Pm7	PTFQQWQLYFIGSMWC PGWTPIRGVAETRS-RSGVVGKMTQDFVRKALRADLLSKEEAET	118
ALF Pm3	PYLKRFQVYYKGRMWC PGWTAIRGEASTRS-QSGVAGKTAKDFVRKAFQKGLISQEQANQ	119
ALF Pm2	PSFYRWELYFNGRMWC PGWAPFTGRSRTSR-PSGAIEHATRDFVQKALQSNLITEEDARI	116
ALF Pm8	PYLKKFEVHYRAELKCP GWTTIVGKGRDHTNPTNSELAIAIKDFIGQALKKGLVTDEEAAQ	120
	* : : : : : . : * * * : : * . : : . : * : : * : : : : : *	
ALF Pm6	WLNN	122
ALF Pm7	WLSH	122
ALF Pm3	WLSS	123
ALF Pm2	WLEH	120
ALF Pm8	YL--	122
	: *	

Fig. 4. Comparison of the deduced amino acid sequences of ALF cDNAs. The asterisk (*) indicates amino acid identity and (.) and (:) indicate amino acid similarity. The bold letters represent the putative signal sequences. The gray box indicates the putative LPS-binding site.

neutralizes LPS and exhibits an anti-bacterial effect on the growth of the gram-negative bacteria *Salmonella minnesota* but not on the gram-positive *S. aureus* (Morita *et al.* 1985). Since, the discovery of horseshoe crab ALFs, various lipopolysaccharide-binding proteins and derivatives have attracted great interest as candidate therapeutic agents for the management of septic shocks (Vallespi *et al.* 2000).

In penaeid shrimps, the cDNAs of ALF were identified and characterized in *Penaeus monodon* (Supungul *et al.* 2002, 2004), *L. setiferus* (Gross *et al.* 2001), *F. chinensis* (Liu *et al.* 2005) and *M. japonicus* (Nagoshi *et al.* 2006). Five different ALF sequences (ALFPm1–ALFPm5) previously reported in *P. monodon* could be divided into two groups: the ALFPm1 and 2 as group A and the ALFPm3, 4 and 5 as group B. Each group has a unique LPS binding site (CRYSQLRPSFYRWELYFNGRMWC for group A and CKFTVKPYLKRFQVYYKGRMWC for group B). Recently, we showed that the two groups of *P. monodon* ALFs are encoded by different genomic loci (Tharntada *et al.*

2008) and that ALFPm2 and 3 were the major or authentic ALFs in the haemocytes whereas the other isoforms may result from aberrant RNA splicing. In addition, sequence variation of the major isoform, ALFPm3, has also been reported (Somboonwivat *et al.* 2006).

From the *Penaeus monodon* database, 208 ESTs representing candidate ALF sequences were identified. Clustering analysis indicated the presence of five isoforms, and three of them (ALFPm6, -7 and -8) are novel ALFPm isoforms rather than aberrant RNA splices. Sequence alignment showed that all ALF sequences contained a signal peptide at the N-terminus and a predicted LPS binding site with clustered positive charges between two conserved cysteine residues (Fig. 4). Interestingly, each isoform contained quite a distinct putative LPS-binding site which suggested that they each might have a different ability to bind the microbial cell wall components or to different LPS isoforms.

ALFPm2 and -3 isoforms were expressed as recombinant proteins in the yeast *Pichia pastoris* for further characterization of their

Table 3. Antimicrobial activities of ALFPm3, LivanPEN-3 and crus-likePm against gram-positive and gram-negative bacteria in a liquid growth inhibition assay

Microorganism	MIC [μ M]		
	ALFPm3	LivanPEN3 (Destoumieux <i>et al.</i> 1999)	crus-likePm
Gram-positive bacteria			
<i>Aerococcus viridans</i>	1.56–3.12	0.3–0.6	0.312–0.625
<i>Bacillus megaterium</i>	0.19–0.39	2.5–5	1.25–2.5
<i>Micrococcus luteus</i>	1.56–3.12	1.25–2.5	2.5–5
<i>Staphylococcus aureus</i>	50–100	>40	5–10
<i>Staphylococcus haemolyticus</i>	ND	ND	2.5–5
Gram-negative bacteria			
<i>Enterobacter cloacae</i>	3.12–6.25	>40	>100
<i>Erwinia carotovora</i>	1.56–3.12	>40	ND
<i>Escherichia coli</i> 363	0.095–0.19	5–10	2.5–5
<i>Klebsiella pneumoniae</i>	3.12–6.25	>40	10–20
<i>Salmonella thyphimurium</i>	6.25–12.5	>40	>100
<i>Vibrio alginolyticus</i>	0.39–0.78	>40	ND
<i>Vibrio harveyi</i>	0.78–1.56	>40	2.5–5
<i>Vibrio penaeicida</i>	25–50	>40	ND
Fungi			
<i>Botrytis cinerea</i>	3.12–6.25	5–10	ND
<i>Fusarium oxysporum</i>	1.56–3.12	5–10	ND

MIC are expressed as the interval a–b, where a = the highest concentration tested at which microorganisms are growing, and b = the lowest concentration that caused 100% growth inhibition. ND = not determined

antimicrobial properties. Overexpression of rALFPm3 but not rALFPm2 was attained. The purified rALFPm3 and the crude protein of rALFPm2 were then used for further characterization. The rALFPm3 has a broad activity spectrum with anti-fungal properties against both tested filamentous fungi, and anti-bacterial activities against both gram-positive (4/4 tested species) and gram-negative (8/8 tested species) bacteria, with high potency against the natural shrimp pathogens, *V. harveyi* (MIC of 0.78 to 1.56 μ M) and *V. alginolyticus* (MIC of 0.39 to 0.78 μ M) (Table 3). The bactericidal activity against *E. coli* 363 and *B. megaterium* was also evidenced (Somboonwivat *et al.* 2005). The synthetic peptide corresponding to a part of the LPS binding site of ALFPm3 was shown

to only exhibit activity against gram-positive bacteria suggesting the involvement of the full molecule for activity against gram-negative bacteria. The crude protein of rALFPm2 was also active against the gram-positive bacteria, *B. megaterium*, and the gram-negative bacteria, *E. coli* 363 (data not shown). However, it would be interesting to compare the antimicrobial properties between the two isoforms once sufficient rALFPm2 is available. ALF cDNAs have been identified in other shrimps but their antimicrobial properties remain to be characterized. Nevertheless, amongst the three families of shrimp AMPs, the ALF displayed the broadest spectrum of antimicrobial activities including against natural shrimp pathogens. The other two shrimp AMP

families, crus-like *Pm* and *LivanPEN-3* revealed lower activities (*LivanPEN-3*) or a reduced target spectrum (crus-like *Pm*) under these conditions compared to *ALFPm3* (Table 3). Moreover, it has recently been shown by RNA interference (RNAi) in both whole animals and in cell cultures that the crayfish ALF can interfere with the replication of white spot syndrome virus (Liu *et al.* 2006). The exciting potential antiviral property of *rALFPm3* is now under investigation, but clearly signals the need for a more extensive screening of other host proteins including ALF proteins for anti-viral activity against the suite of shrimp viral pathogens.

1.6. The potential use of antimicrobial peptides for disease control in aquaculture

Aquatic animals including shrimps live in an environment enriched with pathogenic organisms and, therefore, antibiotics are commonly used in aquaculture to prevent or treat disease outbreaks. Most farmers use antibiotics prophylactically, some on a daily basis. In addition to the elevated costs, the continuous use of antibiotics has resulted in residues of varying concentrations of antibiotics in the water and mud, and subsequently, the development or selection for antibiotic resistance in bacteria in the environment. Moreover, there could be an adverse effect on species biodiversity which ultimately may affect the cultured shrimps, humans and environment.

Antimicrobial peptides provide a good therapeutic alternative for the treatment of diseases in aquaculture. They are natural molecules with a broad spectrum of activity against a wide range of microorganisms, are easy to produce, and are less prone to induce resistance. Some antimicrobial peptides are already in clinical and commercial use (Reddy *et al.* 2004) but their use for disease control in aquaculture has yet to be demonstrated. However, it is quite promising that the shrimp AMPs reported here could be potential candidates as an alternative to an-

tibiotics in shrimp farming. As shown here, *rALFPm3* possess broad spectrum of antimicrobial activity and effectively kills the shrimp pathogen, *V. harveyi*, at a MIC of 0.78 to 1.56 μM whereas the commonly used antibiotic in aquaculture, oxytetracycline kills the bacteria at a MIC of 72.1 to 90.3 μM (Vaseeharan *et al.* 2005). More recently, the shrimp AMPs *LivanPen3* and *ALFPm3* were tested for their antiviral activity against herpes simplex virus type 1, human adenovirus respiratory strain, and rotavirus SA11 (Carriel-Gomes *et al.* 2007). Both shrimp AMPs exhibited significant activity against HSV-1 although they were active at near cytotoxic concentrations. The activity of shrimp AMPs against shrimp viruses has not yet been clearly demonstrated due to the lack of shrimp cell lines. Nevertheless, their potential use in shrimp farming to overcome severe disease outbreaks is quite promising. Besides their antimicrobial function, AMPs are also known to act as mediators of inflammation influencing diverse processes such as cell proliferation, wound healing, cytokine release and immune induction (Zaiou 2007). The application of AMPs as a food additive to enhance shrimp immunity is another strategy to combat microbial infections. Evidently, the genes encoding these AMPs represent good candidates for the genetic improvement of shrimps for resistance to severe pathogens.

2. Biom mineralization of Marine Organisms

2.1. Biom mineralization

A variety of animals including bacteria, fungus, plants and animals have the ability to synthesize mineral crystals called biominerals. Calcium phosphate in vertebrate bone and teeth, calcium carbonate in mollusk shell and some sea algae, silica in sponges and diatoms, calcium sulfate in jellyfish, small magnetic particles in chitons and magnetic bacteria are well-known examples of biominerals.

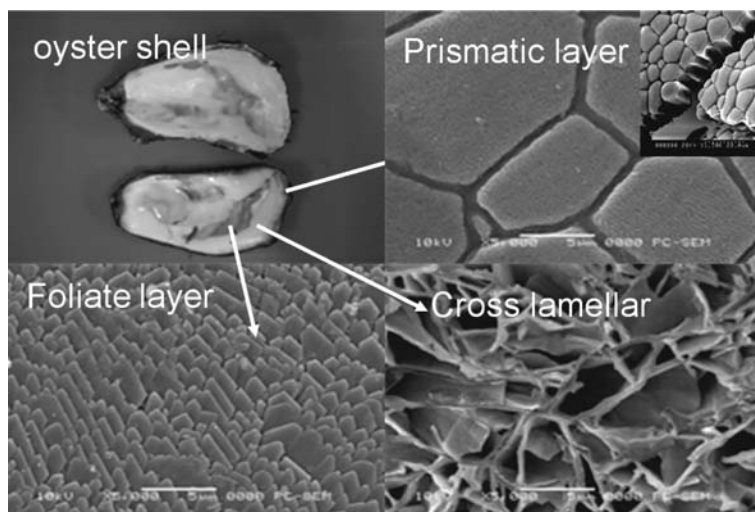


Fig. 5. Structure of oyster shell. Oyster shell is composed of prismatic layer, cross lamellar and foliate layer from outside to inside that are all made of CaCO_3 . Small amount of organic matrix components possibly play important roles to produce the structure of crystal structures specific for each layer.

These biominerals function to maintain the framework of the body, besides regulation of the mineral balance in the body to function as a reservoir, recognition of the direction by detecting the earth magnet (biological magnets in birds), sense of hearing and body balance (statolith of fish and jellyfish), teeth of vertebrates and chitons.

A variety of biominerals have been utilized in human life: Coins, dishes and music apparatus, jewelries such as pearl, cameo and corals, industrial materials such as chalk, cement and marble, functional food additives such as chitin. Biominerals of fish statolith and fossil are biomarkers to evaluate the age of fish and era of the fossil.

In the 17th century, biominerals especially having economical value were studied by microscopic observation to judge the truth or fake. In the late 20th century, electron microscopy has been used for the observation, followed by the biochemical analysis of the components of the shell.

Synthesis of the shell proceeds under physiological condition, moderate tempera-

ture and pH, suggesting that there are so many merits in this system to be learned in the utilization for the implant of the bone and teeth in the medical field, production of light and tough materials in the industrial field.

2.2. Structure of shell

In this section, the author would like to explain the structure of the shell by taking oyster (*Crassostrea gigas*) as the example. Molluscan body consists of periostracum and ostracum. The structure of oyster shell is shown in Fig. 5. The periostracum that is not calcified and organic matrix covers the whole shell body. In the case of oyster, most of the periostracum is lost except for the edge of the shell.

The ostracum is calcified and separated into three parts, prismatic layer, cross lamellar and foliate layer from outside to inside. The prismatic layer is composed of column structure made of calcite. The cross lamellar, also called chalk, demonstrates the rough matrix structure formed with thin plate-like



Fig. 6. Naturally obtained calcite (left) and oyster shell (right). Both have same crystal structure, but the apparent features are clearly distinctive.

crystal. Foliate layer is composed with distinct plate-like crystals accumulating each other.

Naturally, CaCO_3 forms calcite or aragonite crystals or otherwise amorphous structure. In the case of shell, most CaCO_3 crystal is calcite (Fig. 6), but the inner side of pearl oyster is aragonite, which produces rarely a pearl. Pearl is composed of thousands of CaCO_3 layer having a $0.4\ \mu\text{m}$ thickness. When light shines into pearl layers, beautiful pink interfering color occurs by the mechanism of multiple layer interference. Pigments among CaCO_3 layers sometimes causes specific colors such as gold and black. The color of the bottom layer derived from organic materials sometimes causes a beautiful blue color.

After hatching, oyster larvae float 2–3 weeks and settle on the bottom of the sea with their left side shell down. Since most oysters live on the mud of estuaries under natural condition, they suffer from the change in mud condition according to the tidal change. They have selected two living strategy. One is that they settle on the other adult oyster of the prior generation to maintain the gill opened to the seawater. Another strategy is to lower the shell weight not to be buried in the mud. Chalk structure of the shell is helpful for them to lower the body weight. Due to their living strategy, the

morphology of oyster is quite different from other bivalves.

2.3. Function of organic substances for biomineralization

Although CaCO_3 is a main constituent of the shell, morphology of the shell is highly species-specific (Fig. 7). In addition, the structure of the shell is different even in the same species as mentioned above. A possible assumption so far proposed is an implication of organic matrix compounds contained in the shell. Although the amount of the organic matrix component is very small (0.1–10%), these components possibly lead the shell structure specific for the shell species and portion specific of the shell even in the one species. The organic matrix components are roughly classified into soluble and insoluble fractions into water or EDTA solution. Generally, water or EDTA soluble fraction contains aspartate rich proteins and insoluble fraction contains glycine, serine and alanine rich proteins (Crenshaw 1996, Sudo 1997).

Calcification in the shell proceeds according to the following reaction.



Ca^{2+} is adsorbed in the body through specific transporters expressed in the membrane of the epithelial cells of mantle, gill and

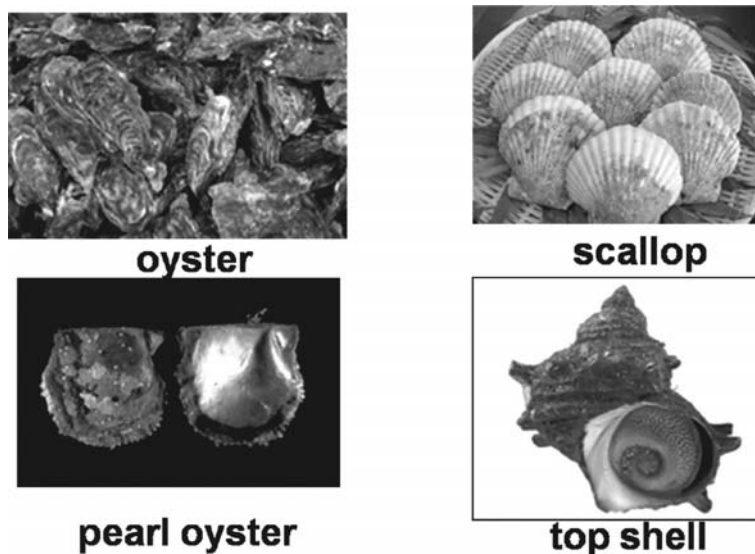


Fig. 7. Species specific structure of mollusk shells. All of shells are composed of CaCO_3 crystals, but the apparent features are clearly different.

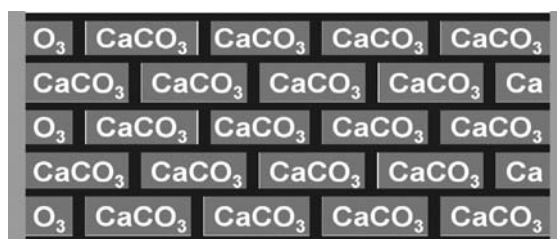


Fig. 8. Putative functions of organic matrix proteins. These proteins assume to play the function to combine the CaCO_3 crystals and lead the form of these units by suppressing the growth of the crystal formation.

intestine. HCO_3^{2-} is partly adsorbed from the surrounding water, but most of them are synthesized from CO_2 produced through TCA cycle.

Ca^{2+} and HCO_3^{2-} are mixed and concentrated in the specific space around the specific area surrounded by the shell and the epithelia of the mantle and crystallized there. The more the core materials exist, crystallization starts under the lower concentration of both compounds. Some kind of organic materials are possibly involved in the initia-

tion of crystallization. After crystallization begins, sheets composed of other organic materials surround the crystals and lead the form of crystals to the expected form.

Some matrix protein fractionated in the soluble fraction exhibits an enzyme activity, Nacrein isolated from pearl oyster (*Pinctada fucata*) in 1996 showed carbonic anhydrase activity that catalyzed the formation of CO_3^{2-} from HCO_3^{2-} (Miyamoto 1996).

Figure 8 demonstrates the putative function of organic matrix proteins. Direct

evidences to show the function of organic matrix proteins are not available at present, but these proteins assume to play the function to combine the CaCO_3 crystals and lead the form of these units by suppressing the development of the crystal formation.

2.4. Common proteins involved in biomineralization among animals

Among studies so far reported, evolutionary relationship of shell matrix proteins are few. Dermatopontin gene has been detected in mammals, arthropods and sponge, and Nacrein and N66 found in pearl oyster has been also found among various animals (Sarashina 2006).

Perlucin, perlustrin and perlwapin reported from abalone demonstrates homology with known proteins. Perlucin is a kind of C-type lectin having carbohydrate binding domain and shares a variety of function involved in biomineral formation of mammal, bird, fish and sea urchin (Mann *et al.* 2000). Perlustrin shows a homology with N-terminal domain of insulin-like growth factor binding protein (Weiss *et al.* 2001). Perlwapin exhibits a homology with mammalian whey acidic protein (Treccani 2006).

As described above, there have been only limited reports suggesting the evolutionary relationship of shell matrix proteins. Thus, most proteins involved in the synthesis of biominerals have evolved independently. Acidic amino acids such as aspartate and glutamate implicating in the chelating Ca^{2+} may play an important function in the biomineralization and the proteins having these amino acids apparently showed homology among different animals.

2.5. Transportation of Ca^{2+} for biomineralization

Metal ions are essential for a variety of biological systems in animals. Metal ions are transported into cells by membrane associ-

ated proteins including divalent metal transporters (DMTs), which have been identified from various species (Andrews *et al.* 1999). DMT1 (also called DCT1 or Nramp2) was reported as a homologous protein of Nramp which was implicated in natural resistance to infection by intracellular parasites in mouse (Vidal *et al.* 1993; Gruenheid *et al.* 1995; Govoni and Gros 1998). DMT1 was identified in rat as DCT1 through expression cloning to search for an mRNA that promoted Fe^{2+} uptake activity (Gunshin *et al.* 1997). Mutations in the DMT1 gene caused a defect in intestinal iron absorption and red cell iron utilization in rat and mouse, suggesting that DMT1 plays an important role in iron absorption by intestinal cells (Fleming *et al.* 1997, 1998; Su *et al.* 1998). The presence of an iron regulatory element (IRE) in mammalian DMTs suggests that DMT mRNA is implicated in post-transcriptional regulation by intracellular Fe^{2+} concentration (Lee *et al.* 1998; Andrews 1999). Accordingly, iron deficiency in rat induced a remarkable accumulation in the mRNA level of DMT1 due to the function of IRE (Gunshin *et al.* 2001).

We cloned a DMT gene from scallop *Mizuhopecten yessoensis* as a transporter possibly involved in the extraordinary accumulation of cadmium (Toyohara *et al.* 2005). Scallop DMT (scDMT) mRNA was strongly expressed in the gill and intestine. As a result of the functional analysis using electrophysiological method using *Xenopus laevis* oocytes, scDMT transported Fe^{2+} and Cd^{2+} , the same as mammalian DMT. Interestingly, scDMT differs from mammalian DMT in possessing an ability to transport Ca^{2+} .

It must be stressed that scDMT most effectively transports Ca^{2+} at 10 mM (Takagi *et al.* 2007). Considering that the Ca^{2+} concentration of sea water is approximately 10 mM, this result indicates that scallop possibly absorbs Ca^{2+} directly from sea water by scDMT. For further characterization of

scDMT, it is necessary to examine the Ca^{2+} transport activity in a wide range of Ca^{2+} concentration.

Ca^{2+} transport activity of DMT, specifically detected for the mollusks, suggests the implication of mollusks DMT in the shell formation process. Recently, it was reported that the crystal of CaCO_3 is formed in the hemocytes, not in the mantle, and released CaCO_3 crystals are used for the shell synthesis (Mount *et al.* 2004). Together with the fact that the higher level of mRNA expression of scDMT was detected in the gill and intestine, Ca^{2+} in sea water is transported by scDMT localized in the epithelium of these tissues and presumably transferred into the hemocytes to make CaCO_3 crystals.

2.6. Conclusions

In this paper, we described the recent advance in shrimp antimicrobial peptide and

shell formation as examples of the studies of marine invertebrates. Marine invertebrates include a variety of animals and some of them are very important for human life. Shrimp and shell are the most useful animals among marine invertebrates, but there remain so many animals to be studied. We hope the studies of marine invertebrates will serve the development of biotechnology possibly promising the prosperous human life in the future.

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Molecular Biotechnology of Development and Growth in Fish Muscle

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The myotomal muscles are the main edible part of teleost fish. This short review summarises current information on myogenic genes of particular biotechnological interest from the perspective of growth regulation and food chemistry. The cellular and molecular mechanisms underlying myogenesis at different stages of the life-cycle are described. The importance of temperature as a factor regulating growth and tissue phenotype is discussed with reference to developmental plasticity and temperature acclimation responses. The applications of muscle biotechnology to fisheries and aquaculture are highlighted together with areas for future research.

KEYWORDS myogenesis; growth regulation; gene paralogues; developmental plasticity; temperature acclimation; myosin

1. Introduction

The central goal of finfish aquaculture is the production of myotomal muscle. It is therefore important to understand the interactions of fish nutrition and environment on muscle development and growth at the molecular and cellular level. Although it is likely that

the functions of genes regulating muscle development and growth are generally conserved in fish relative to mammals, some important differences have been documented. Embryonic myogenesis in teleosts has several unique features relative to amniote relatives. For example, cells are specified to the muscle lineage much earlier in teleosts, during mid-gastrulation (at 70–75%

epiboly; Weinberg *et al.* 1996), which probably reflects the early requirement to generate body movement imposed by external fertilization. These first muscle cells, the adaxial cells, then migrate from the medial to lateral somite to develop into a layer of slow muscle fibres anatomically separated from the fast-twitch muscle, a trait maintained throughout adult stages. In addition, teleost somites develop an external cell layer that has some conserved molecular and cellular characteristics of the amniote dermomyotome (Hollway *et al.* 2007), which supplies myogenic precursors for postembryonic growth (Gros *et al.* 2005).

The main cultured fish are teleosts, which form more than 95% of the ray finned fishes (the Actinopterygii) (Nelson 2006). The most important aquaculture species are found in some of the most successful teleost superorders including the Acanthopterygii (Perciformes, e.g. tilapia sp., sea bass [*Dicentrarchus labrax*], halibut [*Hippoglossus hippoglossus*]), Ostariophysi (cyprinids, e.g. the common carp [*Cyprinus carpio*]), Protacanthopterygii (salmonids, e.g. rainbow trout [*Oncorhynchus mykiss*] and Atlantic salmon [*Salmo salar*]), and Paracanthopterygii (gadoids, e.g. Atlantic cod [*Gadus morhua*]). The recent sequencing of the green spotted pufferfish (*Tetraodon nigroviridis*) genome, which is most compact vertebrate genome known (~365 Mb; Brenner *et al.* 1993) provided compelling evidence for the long suspected whole genome duplication (WGD) event at the base of the Actinopterygian lineage some 320–350 Mya (Van de Peer 2004). For example, ~1000 pairs of duplicated genes in *T. nigroviridis* and the tiger pufferfish (*Takifugu rubripes*) genome tended to have a single duplicated copy on a single distinct chromosome (Jaillon *et al.* 2004). Additionally, a striking pattern of double conserved synteny was observed in the *T. nigroviridis* genome, where two chromosomal segments were conserved relative to mammals (Jaillon

et al. 2004). It has been estimated that around 15% of the duplicated genes from this basal WGD have been retained in extant species (Jaillon *et al.* 2004). Several teleost families, including the salmonids, have undergone a second WGD with an estimated ~50% of duplicated paralogues retained (Allendorf and Thorgaard 1984). The evolutionary history of each target species is therefore an important consideration in the goal of distinguishing conserved and divergent features of muscle growth regulation in teleosts, requiring rigorous phylogenetic analysis and careful characterisation of gene paralogues (e.g. Kerr *et al.* 2005; Macqueen and Johnston 2008a).

Significant progress has been made in understanding the main events occurring in embryonic myogenesis in a model species, the zebrafish *Danio rerio* L. (e.g. Devoto *et al.* 1996; Barresi *et al.* 2000; Hammond *et al.* 2007; Hollway *et al.* 2007; Stellabotte *et al.* 2007). The advantages of working with zebrafish in terms of its draft genome sequence, small size, short generation time and embryonic transparency, make it the organism of choice for investigating muscle development in teleosts. Further, the availability of multiple mutant lines with aberrant development including muscle growth, as well as the now routine use of morpholino antisense RNA to knockdown genes of interest, have made zebrafish the most important teleost as a comparative model for studying conserved molecular and cellular features of myogenesis across vertebrates.

2. Myogenic Genes of Biotechnological Interest

The short review in this section is focused on a sub-set of the genetic pathways regulating muscle growth that are of particular interest from an aquaculture biotechnology perspective.

2.1. The MyoD gene family

Myogenic regulatory factors (MRFs) are a family of four basic helix-loop-helix transcription factors that are highly conserved between mammals and fish and are required for muscle lineage determination (MyoD, Myf5, Mrf4 [also known as Myf6]) and for the initiation and stabilization of muscle differentiation (Myogenin, Mrf4) (Rudnicki *et al.* 1993; Hasty *et al.* 1993; Kassam-Dushosoy *et al.* 2004). These “master” transcription factors play a central role in regulating muscle development and growth (Tapscott, 2005). Each protein contains two conserved motifs, the basic region and the helix-loop-helix (HLH) domain. The HLH domain enables MRFs to form dimers with bHLH containing E-proteins allowing binding via the basic domains to a conserved CIS-acting site (with the sequence CANNTG) found in the regulatory regions of most muscle-specific genes. Further a motif towards the C-terminal of MRFs, the amphipathic Helix-3, which is most strongly conserved in MyoD and Myf5, partially conserved in Mrf4 and poorly conserved in Myogenin, underlies the different abilities of the family members to act as muscle determination factors (Bergstrom and Tapscott, 2001). Although the MRFs show significant redundancy, each gene has evolved a unique expression pattern and specialised role in myogenesis. *myod* and *myf5* are expressed prior to segmentation in adaxial cells in several teleost species (Weinberg *et al.* 1996; Temple *et al.* 2001; Tan and Du 2002; Cole *et al.* 2004). Morpholino “knock-down” of either gene in zebrafish had no effect on slow muscle formation, whereas double “knockdowns” lacked a viable myogenic program (Hammond *et al.* 2007). This result is reminiscent of mice mutants lacking functional genes for both Myf5 and MyoD, where the skeletal muscle lineage was entirely absent (Rudnicki *et al.* 1993) and indicates a conserved function in myogenic specification. However, unlike the situation

in mammals, where Mrf4 can compensate for MyoD or Myf5 as a muscle specification factor (Kassar-Dushosoy *et al.* 2004), in zebrafish lacking Myf5 and MyoD expression, *mrf4* transcripts and muscle differentiation are absent (Hinitz *et al.* 2007).

The teleost *myod* gene is conserved as duplicated copies in more than one lineage. For example, two salmonid MyoD paralogues were characterised in rainbow trout (*Oncorhynchus mykiss*) and named TmyoD and TmyoD2 (Rescan and Gauvry 1996). Further, several species of the Acanthopterygii have two paralogues, including the Gilthead seabream (Tan and Du 2002), Atlantic halibut (Galloway *et al.* 2006) and the tiger pufferfish (Macqueen and Johnston 2006). The Acanthopterygian MyoD paralogues, were, originally denoted as MyoD1 and MyoD2 (Tan and Du 2002). Conversely, the zebrafish genome contains a single copy of MyoD. More recently a third MyoD sequence was characterised that is commonly conserved in salmonids (Macqueen and Johnston 2006). Phylogenetic reconstruction by maximum likelihood clustered teleost MyoD proteins into two clades, the first containing the single MyoD protein of the Ostariophysi (i.e. zebrafish) as well as MyoD1 of the Acanthopterygii and all three salmonid MyoD sequences, whereas the second clade contained solely the MyoD2 protein of the Acanthopterygii (Macqueen and Johnston 2006). An investigation of the genomic neighbourhood encompassing *myod* across the vertebrates revealed that genes on the single *myod*-containing chromosome of tetrapods were retained in both zebrafish and Acanthopterygians in a compelling pattern of double conserved synteny (Macqueen and Johnston 2008a). Phylogenetic reconstruction of these *myod*-neighbouring genes using Bayesian and maximum likelihood methods supported a common origin for teleost paralogues following the split of the Actinopterygii and Sarcopterygii. These results strongly suggested that a *myod*-containing

chromosome was duplicated during the teleost WGD, but that the one of the duplicated copies was subsequently lost in the Ostariophysi (including zebrafish) and Protacanthopterygii (including salmonids) lineages (Macqueen and Johnston 2008a). We have proposed a consensus nomenclature in which the ancestral gene, as conserved in diploid tetrapods, should be named *myod* and teleost paralogues should be first identified by their orthology to the *myod1* or *myod2* genes that arose during the WGD and then more recently derived copies, such as those retained in salmonids, should be named within this framework as *myod1(a/b/etc)* or *myod2(a/b/etc)* (Macqueen and Johnston 2008a). Thus the single *myod* gene in zebrafish should be designated *myod1* and the salmonid genes, as *myod1a*, *1b* and *1c*.

In situ hybridisation was used to describe the expression pattern of all four MyoD family members and their known paralogues in the developing myotomal muscle of the tetraploid teleost, Atlantic salmon. Concurrent expression domains of slow muscle myosin light chain-1 (*smlc1*) and *pax7* (required for the maintenance of myogenic progenitor cells in mammals, Seale *et al.* 2000) were also recorded to place the information within the context of known muscle differentiation events. Adaxial myoblasts expressed *myod1a* prior to and during somitogenesis followed by *myod1c* (20 somite stage) and *mrf4* (25–30 somite stage), before they migrated laterally across the myotome, to form a single layer of slow muscle fibres (Macqueen *et al.* 2007). *myf5* was detected prior to somitogenesis in the presomitic mesoderm, but not in the adaxial cells in contrast to other teleosts studied (Macqueen *et al.* 2007). The expression of *myf5*, *myod1b* and myogenin was not confined to regions expressing *smlc1*, and transcripts were present in overlapping domains consistent with a role in fast muscle myogenesis (Macqueen *et al.* 2007). From the end of segmentation, each MRF was expressed to a greater or lesser extent in

zones of new fibre production, the progenitors of which probably originated in the external cell layer, which expressed *pax7* (Macqueen *et al.* 2007). Interestingly, the combined expression fields of *myod1a*, *myod1b* and *myod1c* in salmon recapitulated the expression pattern of the single *myod1* paralogue in zebrafish (Fig. 1A). It is plausible that where multiple teleost *myod* paralogues are found, such as in salmonids, that each gene has become subfunctionalised according to the model of Force *et al.* (Force *et al.* 1999), with each paralogue regulated by a sub-set of the *cis*-acting elements found in the promoter region(s) of the ancestral *myod* gene (Rescan 2001; Macqueen and Johnston 2006). In line with this model, it is also possible that a second, as yet uncharacterised salmonid *myf5* paralogue has inherited the *cis*-acting regulatory elements governing the adaxial cell expression domain conserved in other teleosts (Fig. 1B). Since hundreds of skeletal muscle genes are regulated downstream of MRFs in mammals (Blais *et al.* 2005; Bean *et al.* 2005), the presence of multiple paralogues may provide additional levels of control and complexity of expression patterns providing some selective advantage leading to their retention in the genome.

2.2. Myostatin

Myostatin (*Mstn*, also known as GDF-8) is a member of the transforming growth factor- β gene family (TGF- β), which was discovered to be a potent negative regulator of muscle growth in mammals (McPherron *et al.* 1997). The skeletal muscles of *mstn* null mice contain a larger number of fibres of greater diameter than wild-type controls, increasing muscle mass by up to 3-fold (McPherron *et al.* 1997). Naturally occurring *mstn* mutations are known to contribute to heavily muscled phenotypes observed in cattle, sheep, dogs and even humans (reviewed in Lee 2007a). *Mstn*-null mice also show a significant reduction in fat storage

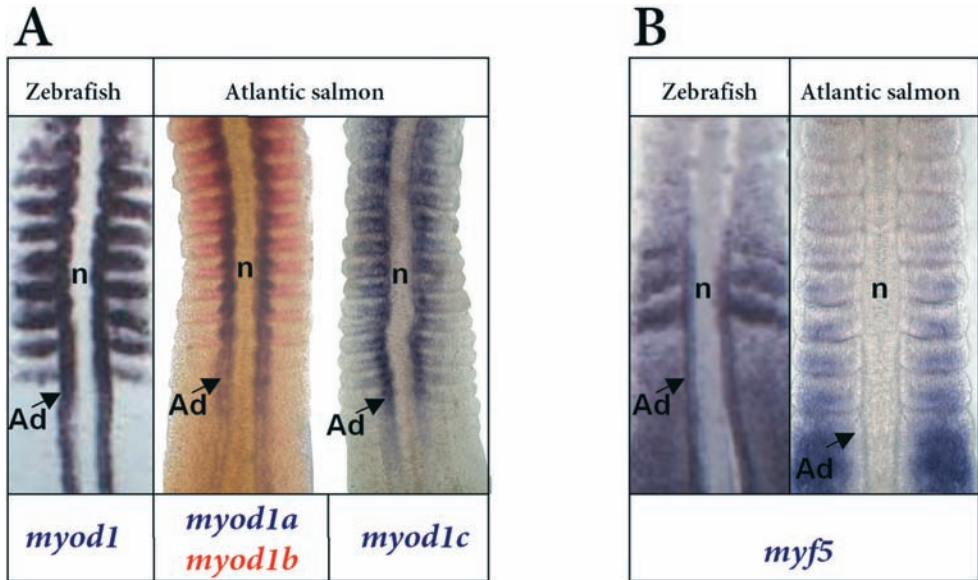


Fig. 1. (A) Embryonic expression of *myod1* paralogs in teleosts. Example images showing *in situ* hybridisation of either whole mount Atlantic salmon (30 somite stage (ss), right of box A) or zebrafish (10 ss, left of box A) embryos to specific complementary RNA probes to *myod1* (zebrafish) and *myod1a*, *myod1b* and *myod1c* (salmon). In salmon, the three *myod1* co-orthologues (*myod1a*, *myod1b*, *myod1c*) have distinct but overlapping expression domains, that together recapitulated the zebrafish *myod1* expression domain. This likely reflects the portioning of distinct *cis*-acting regulatory motifs conserved in the single zebrafish *myod1* gene, between the regulatory regions of the salmonid co-orthologues, following the duplication-degeneration-complementation (DDC) model (Force *et al.* 1999). (B) In zebrafish (10 ss, right of box B) as well as Acanthopterygian teleosts where the expression domain has been recorded, *myf5* transcripts accumulate strongly in the adaxial cells of the presomitic mesoderm and newly formed somites. In Atlantic salmon (30ss, right of box B) this *myf5* adaxial cell expression domain was absent at an equivalent stage of development, although the remaining expression pattern was comparable to zebrafish. We suggested that a second, as yet uncharacterised *myf5* paralogue is conserved in the salmonid lineage that fulfils this expression domain under the DDC hypothesis. Zebrafish panels were adapted from Hamade *et al.* (2006) with permission from Elsevier.

(McPherron and Lee 2002). These features of Mstn make it a most interesting biotechnological candidate for potential therapeutic applications in human muscle diseases (e.g. Nakatani *et al.* 2008), but also as a potential target to improve muscle growth by breeding or genetic engineering programs in agriculture (e.g. Pirottin *et al.* 2005) or aquaculture.

In mammalian cell culture, Mstn downregulates several MRFs (Langley *et al.* 2002) and prevents the transition of

myoblasts from G1 to the S phase of the cell cycle (Thomas *et al.* 2000). Mstn is thought to inhibit myoblast proliferation and differentiation through a tumour growth factor-B pathway involving the activin receptor ActRIIB and the phosphorylation of Smads 2 and 3 (reviewed in Joulia-Ekaza and Cabello 2007). Transgenic mice expressing high levels of a dominant negative form of ActRIIB exhibited dramatic increases in muscle mass comparable to those seen in Mstn knockouts (Lee and McPherron 2001). Smad4 potentiates

Mstn signalling whereas Smad7 and Smurf1 are inhibitors (Joulia-Ekaza and Cabello 2007). Myogenin and the p21 cyclin-dependent kinase inhibitor are probably the major physiological targets of Mstn (Joulia *et al.* 2003). Promoter analysis of *mstn* in mammals reveals MyoD binding sites, glucocorticoid response elements and several FoxO boxes (Allen and Unterman 2007; Joulia-Ekaza and Cabello 2007). FoxO1 is of particular interest as an activator of *mstn* expression since it upregulated ubiquitin ligase expression, leading to protein degradation during muscle wasting (Allen and Unterman 2007).

The structure of the *mstn* gene has been elucidated in numerous teleost species because of its potential biotechnological interest (e.g. zebrafish, Xu *et al.* 2003; Atlantic salmon, Østbye *et al.* 2001; rainbow trout, Rescan *et al.* 2001 and Gilthead seabream, Maccatrozzo *et al.* 2001). The teleost gene has two introns and three exons that encode a 374–377 amino acid protein, which includes a signal peptide, conserved cysteine residues and a RPXXR proteolytic signal domain. Cleavage at the conserved RXXR region gives rise to the N-terminal latency associated peptide and a ~26 kD active processed peptide which is highly conserved across species. *Mstn* is conserved as two genes in most teleosts (*mstn1* and *mstn2*), as a result of the basal WGD (Maccatrozzo *et al.* 2001; Kerr *et al.* 2005). In salmonid teleosts, all the paralogues from the lineage-specific genome tetraploidization have been retained, as there are two paralogues per gene, namely *mstn1alb* and *mstn2alb* (*mstn2b* is a pseudogene) (Kerr *et al.* 2005; Garikipati *et al.* 2007). The ActRIIB receptor has also been cloned and characterised from Atlantic salmon and was found to be expressed in multiple tissues (Østbye *et al.* 2007).

There is somewhat contradictory evidence for *mstn* being a major negative regulator of muscle growth in teleosts as shown

in mammals. In adult mammals the expression of *mstn* is almost exclusively restricted to muscle, whereas in teleosts expression occurs in a diverse range of tissues, including brain and ovaries (e.g. Østbye *et al.* 2001; Roberts and Goetz 2001; Garikipati *et al.* 2006). Such findings are consistent with a wider physiological role for *mstn* genes in teleosts. An interesting recent finding was that both *mstn1* and *mstn2* transcripts were upregulated 600-fold in the spleen of zebrafish exposed to overcrowding stress (Helterline *et al.* 2007). While most authors have generally been unable to detect embryonic *mstn* mRNA using *in situ* hybridisation (e.g. Xu *et al.* 2003; Kerr *et al.* 2005), Amali *et al.* (2004) reported ubiquitous expression of *mstn* in zebrafish embryos by this approach. Further, they reported that injecting morpholino mRNA for *mstn* into one to four cell zebrafish embryos caused later upregulation of MRFs and major changes in somite morphology consistent with a role in negative regulation of growth and myogenesis (Amali *et al.* 2004). However, the validity of Amali and co-workers findings has been questioned by other authors (Kerr *et al.* 2005; Helterline *et al.* 2007). Microinjection of double-stranded DNA (dsRNA) corresponding to the biologically active C-terminal myostatin domain of tilapia into zebrafish embryos was reported to increase body mass relative to controls as well as to augment muscle hypertrophy and hyperplasia (Acosta *et al.* 2005). In contrast, overexpression of the *mstn* propeptide (a negative regulator of the active peptide) in zebrafish produced only a relatively minor increase in muscle fibre number (~12%) and no change in fibre diameter or MRF expression (Xu *et al.* 2003). Furthermore, forced exercise in rainbow trout produced a marked hypertrophy of fast muscle fibres, but only a 6–7% decrease in myostatin active peptide relative to tank-rested controls (Martin and Johnston 2005). Taken together, these results do not provide strong evidence for a conserved

role for teleost *mstn* as a potent negative regulator of myogenesis, but certainly indicate a need for further functional studies.

2.3. Follistatin

Follistatin (*Fst*) is a secreted glycoprotein that is expressed in many mammalian tissues where it was first identified as a potent inhibitor of follicle stimulating hormone (Phillips and de Krestor 1998). *Fst* is an inhibitor of *Mstn* and Bone Morphogenic Proteins (BMPs) and a known regulator of amniote myogenesis (Amthor *et al.* 2002, 2004). Null *fst* mice showed retarded growth and reduced muscle mass (Matzuk *et al.* 1995) whereas mice over-expressing *Fst* showed significantly increased muscle fibre hyperplasia and hypertrophy relative to wild-type controls (Lee and McPherron 2001). In chick embryos, *fst* is expressed in myogenic progenitor cells of the dermomyotome and it antagonises the inhibitory effect of *Mstn* on transcription factors including *myoD* and *pax3* (Amthor *et al.* 1996, 2004). *Fst* protein was also shown to bind BMPs at a level that stopped BMP-induced apoptosis and promoted excessive muscle growth, with upregulation of *pax3* (Amthor *et al.* 2002). Furthermore, transgenic mice that lack a viable *Mstn* gene, but overexpress *Fst* display an even more exaggerated muscle-growth phenotype than *mstn* mutants (Lee 2007b).

In teleost fish, research with *fst* is limited from a myogenic perspective, despite its potential to manipulate muscle mass. We recently demonstrated by phylogenetic reconstruction (maximum likelihood) and comparative analyses of genomic synteny, that *fst* was duplicated during the teleost WGD and retained as two paralogues (*fst1* and *fst2*), in the Ostariophysi superorder (in zebrafish and in catfishes) but not in the Acanthopterygii or Protacanthopterygii (Macqueen and Johnston 2008b), which have a single gene (*fst1*). In adult salmon, zebrafish and seabream, *fst1* was shown to be expressed

in multiple tissues, including fast and slow muscles (Macqueen and Johnston 2008b; Funkenstein *et al.* 2008), whereas zebrafish *fst2* showed a more restricted pattern and was virtually absent from fast muscles (Macqueen and Johnston 2008b). A potential conserved role for teleost *fst* in embryonic myogenesis was demonstrated, as *fst1* transcripts were expressed concurrently to *pax7* in presumptive myogenic precursors of the anterior somite, external cell layer and pectoral fin buds (Macqueen and Johnston 2008b).

2.4. The insulin-like growth factor system

In mammals, insulin-like growth factor-I (IGF-I) and IGF-II represent the main endocrine and autocrine regulators of skeletal muscle growth. IGF-I is synthesised in liver and other tissues including skeletal muscle. The liver expression of the *IGF-I* gene is controlled by the pituitary/growth hormone axis. Circulating levels of IGF-1 are regulated by six binding proteins (IGFBPs) in mammals and there are several receptor subtypes (Denley *et al.* 2005). IGF-I is synthesized as a pre-pro-peptide that is processed by proteolytic cleavage to produce the mature IGF-I and E-peptide, and both peptides are co-secreted. Multiple isoforms of pro-IGF-I have been identified from fish to human with differences only in their C-terminus E-peptides. IGF-I Ea and IGF-IEb splice variants have been described in rodents (Yang *et al.* 1996). IGF-IEa corresponds to the liver form of IGF-I whereas IGF-IEb, also referred to as mechano-growth factor (MGF), is produced locally in the muscle in response to mechanical stimuli (Goldspink 1999). IGF-IEb differs from IGF-IEa by a 52-bp insert in exon 5, and also with respect to its promoter region (Yang *et al.* 1996; Goldspink 1999). In humans, there is evidence that following mechanical stress the IGF-pre-propeptide is initially spliced towards MGF (which is designated IGF-IEc,

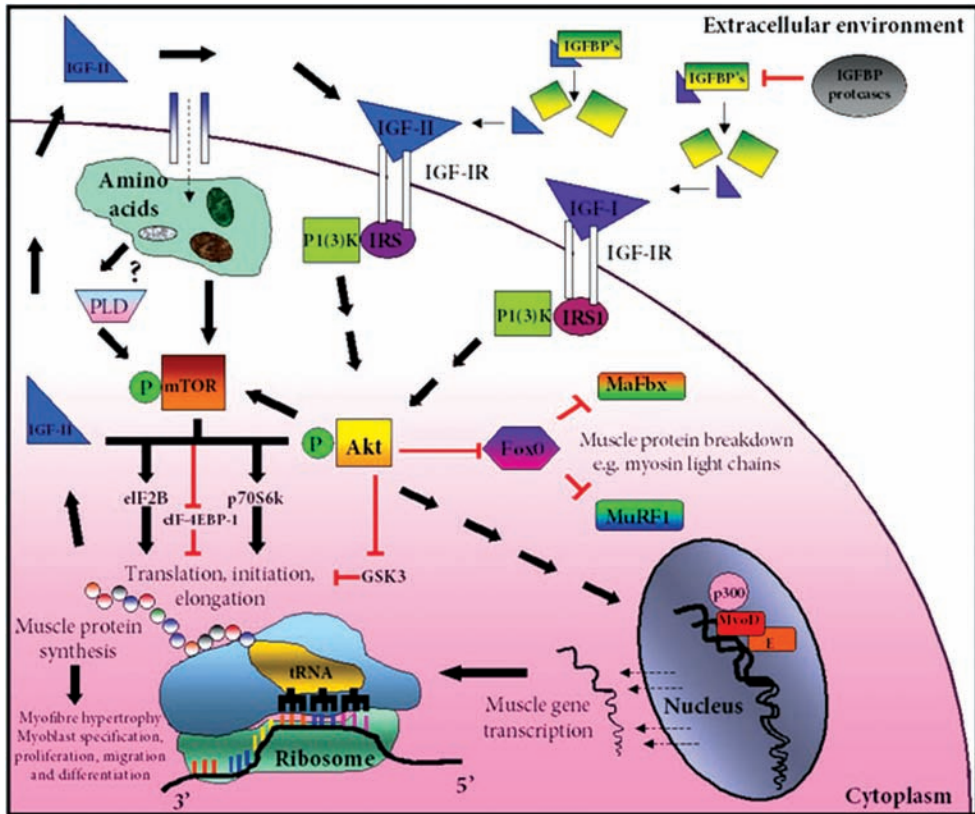


Fig. 2. A diagrammatic representation of the the IGF axis regulating muscle growth and atrophy through the mTOR/Akt/P70S6K signalling cascade (see text for abbreviations and details).

but is still equivalent to rodent MGF/IGF-IEb), resulting in the activation and proliferation of muscle progenitor cells (Ates *et al.* 2007) and the facilitation of myoblast migration (Mills *et al.* 2007). Subsequently, the IGF-pre-propeptide is spliced towards the IGF-I Ea splice variant, which is the main source of mature IGF-I required for myogenic differentiation and MGF is downregulated (Ates *et al.* 2007). Although IGF-IEa and MGF may have different roles in muscle growth (Yang and Goldspink 2002), much remains to be discovered about their regulation and function.

The signalling pathways that are activated by IGF-I and IGF-II in mammals have been studied using a combination of *in vivo*

and cell culture models of muscle hypertrophy in combination with pharmacological and gene “knock-out” experiments (reviewed in Glass 2005) and are summarized in Fig. 2. Briefly, the binding of IGF-I to a membrane receptor triggers the activation of several intracellular kinases, including phosphatidylinositol-3-kinase (PI3K). PI3K phosphorylates the membrane phospholipids phosphatidylinositol-4,5-bis-phosphate to phosphatidylinositol-3,4,5-trisphosphate, creating a lipid binding site on the cell membrane for a serine/threonine kinase called Akt (or PKB-protein kinase B). The subsequent translocation of Akt to the membrane facilitates its phosphorylation and activation by PDK-1. Downstream targets of Akt include

the mammalian target of rapamycin (mTOR), which activates (p70S6K, e1EF2) or represses (GSK3, glycogen synthase kinase 3, ef1-4EBP1 [aka PHAS-1]) key proteins regulating translation and protein synthesis (Fig. 2). Significantly, the IGF-1/PI3K/Akt signalling pathway also functions as a switch between protein synthesis and degradation, suppressing catabolic pathways by inhibiting the FoxO family of transcription factors preventing induction of the muscle-specific ubiquitin ligases MAFbx and MuRF1 (Fig. 2). The autocrine IGF-II transcription required for skeletal myocyte differentiation is regulated by mTOR and the availability of branched chain amino acids such as isoleucine (Erbay *et al.* 2003) (Fig. 2). Phospholipase D (PLD) has recently been demonstrated as an upstream regulator of mTOR and may provide a molecular link between amino acid availability and mTOR activity (Yoon and Chen 2008) (Fig. 2). Thus the mTOR-IGF axis provides a direct molecular link between nutritional levels and protein synthesis leading to muscle fibre growth.

Fish are ectotherms and in nature temperate species are often subject to marked seasonal fluctuations in food supply resulting in annual cycles of growth. It is therefore likely that many aspects of the IGF-system regulating growth in fish differ from those described for mammals. Nevertheless the main features of the growth hormone/IGF system appear to be highly conserved between fish and mammals. Numerous studies have characterised the coding sequences of teleost orthologues of IGF-I, IGF-II, IGF-receptors and IGFBPs (reviewed in Wood *et al.* 2005). Several teleost genes in the IGF system have paralogues that probably arose from genome duplication events. Four different isoforms of pro-IGF-I containing identical mature IGF-I but different lengths of E-peptides have been identified in rainbow trout (Shamblott and Chen 1993). However, the majority of teleost studies of IGF-I and

IGF-II expression have not distinguished between different splice variants or simultaneously investigated IGF-receptor and IGF-binding expression which may be important in the regulation of the bioavailability and effective concentration of the hormones. Furthermore, it has not yet been established whether any of the teleost IGF-I splice variants responds to mechanical stimuli as in mammals. The relative importance of IGF-I and IGF-II expression may also be species dependent (Chauvigné *et al.* 2003; Peterson *et al.* 2004). Fasted fish reduce metabolic rate and can survive for long periods on energy stores whilst displaying negative growth. Re-feeding following fasting results in a rapid growth spurt often called compensatory growth. In fasted rainbow trout, refeeding resulted in an 8- and 15-fold increase in IGF-I mRNA after 4 and 12 d respectively, but only a small and much slower increase in IGF-II expression (Chauvigné *et al.* 2003). Consistent with this finding, plasma *IGF-I* increased four days after refeeding in fasted trout, while plasma *IGF-II* concentration increased after one day (Gabillard *et al.* 2006). Further, IGF receptor Ia expression declined following refeeding whilst IGF receptor Ib transcript levels were unchanged (Chauvigné *et al.* 2003). Transcripts for *IGFBP4*, *IGFBP2*, *IGFBP5* and IGFBP-related protein 1 were also reduced in fasted trout, before being upregulated upon refeeding (Gabillard *et al.* 2006).

2.5. Calpain/calpastatin

The calpain-calpastatin system has been extensively studied in mammals because of its importance in regulating a wide range of physiological processes including protein turnover and growth (Goll *et al.* 2003), cell cycle progression, as well as myoblast differentiation, migration and fusion (Dedieu *et al.* 2004; Barnoy *et al.* 1996). There is also considerable biotechnological interest in these proteins because of their effects on meat quality. Proteolysis by these proteases

post mortem influences muscle texture with an inverse relationship between calpastatin levels and meat tenderization (Duckett *et al.* 2000). Calpains are calcium-dependent cysteine proteinases that are present in all cells. The ubiquitous μ - and m-calpains catalyse the limited proteolysis of cytoskeletal and membrane proteins and are regulated by Ca^{2+} concentration and the specific protein inhibitor calpastatin (Goll *et al.* 2003). μ -calpain (calpain 1) is active at μM calcium concentration and m-calpain (calpain 2) is active at mM calcium concentrations. Each calpain has a common 30 kDa regulatory subunit and a unique 80 kDa catalytic subunit. In typical calpains, the catalytic subunit has four domains: Domain I (autolytic activation), Domain II (cysteine catalytic site), Domain III (switch domain) and Domain IV (calmodulin-like calcium binding domain). Molecular cloning has also identified a large number (15 to date in mammals) of Calpains, some of which are predominantly tissue specific, including Calpain 3 (p94), that is expressed principally in skeletal muscle in mammals (Jones *et al.* 1999), and others that are expressed more ubiquitously (Goll *et al.* 2003). Calpain 3 mRNA transcripts are ten times more abundant in muscle than those of Calpains 1 and 2 and its genetic disruption underlies limb girdle muscular dystrophy type 2A (Goll *et al.* 2003). The purified protein is unstable on isolation and *in vivo* it is thought to be stabilised by interaction with titin, a giant 3.7 Mda cytoskeletal protein that spans the muscle half sarcomere from M to Z line (Duguez *et al.* 2006). It has also been suggested that calpain 3 plays a central role in sarcomere remodelling (Duguez *et al.* 2006).

Calpastatin is a specific inhibitor of calpains. In mammals, calpastatin has four homologous C-terminal inhibitory domains (I–IV) downstream of a non-inhibitory leader domain (L) and an N-terminal XL sequence (Goll *et al.* 2003). Several isoforms of calpastatin have been reported that are ex-

pressed in a muscle fibre type-specific fashion. The different isoforms arise from alternative splicing, different start sites of translation/transcription and different states of phosphorylation (reviewed in Sentandreu *et al.* 2002). Studies with transgenic mice that over-express human calpastatin have provided evidence that calpains regulate muscle glucose metabolism and muscle mass (Otani *et al.* 2004). Calpastatin transgenic mice showed decreased levels of the myocyte enhancer factors, MEF2A and MEF2D, and a significant hypertrophy of muscle mass (Otani *et al.* 2004). In another study, it was shown that overexpression of calpastatin prevented myoblast fusion and strongly downregulated myogenin expression (Barnoy *et al.* 2005).

The calpain/calpastatin system is poorly studied in fish compared with mammals. Calpain 1 and 2 have been partially purified from a number of aquaculture species. Recently, full-length cDNAs have been obtained for calpain 1 and 2 from the rainbow trout that show around 65% identity with mouse orthologues (Salem *et al.* 2005a). Starvation for 35 d in the rainbow trout resulted in the up-regulation of mRNA transcripts for calpain 1 (2.2-fold), calpain 2 (6.0-fold) and calpastatin (1.6-fold) (Salem *et al.* 2005a). These results indicate that season of harvest and pre-slaughter starvation period are likely to affect the calpain/calpastatin system and hence flesh texture and storage characteristics. In rainbow trout, two CAST isoforms have been reported, a long (CAST-L) and a short (CAST-S) form (Salem *et al.* 2005b). Fish CASTs have fewer repetitive inhibitory domains than found in mammals (one or two versus four).

Whereas tenderisation is a positive attribute in red meat, in fish softness represents a loss of quality and hence economic value. Verrex-Bagnis *et al.* (2002) used Western blotting to show that calpain 2 released α -actinin and desmin from myofibrils following *in vitro* degradation of

myofibrils. Calpain was shown to degrade troponin T and α -actinin in sea bass (Delbarre-Ladrat *et al.* 2004). The carboxyterminal region of dystrophin, a cytoskeletal actin binding protein, is highly sensitive to degradation by calpain 2 (Bonnal *et al.* 2001). Several studies have shown that during the pre-rigor period, cytoskeletal proteins are affected by the first proteolytic events. These cleavages disrupt connections between myofibrils and the extracellular matrix, induce segmentation of myofibrillar cores, and modify the rheological properties of the tissue (Bonnal *et al.* 2001). Dystrophin release has proved to be a very useful marker of the early events of proteolysis during fish storage.

3. Embryonic Myogenesis

The somites are transient embryonic structures common to all vertebrates that form from mesodermal tissue in a rostral to caudal wave. Somites give rise to the myotomes (the future skeletal muscle) and the sclerotome (the future skeleton), which is much reduced in teleosts compared to amniotes, a likely adaptation to the additional buoyancy provided by water relative to a terrestrial environment. Prior to segmentation two distinct cell types can already be distinguished morphologically and by their gene expression patterns. In the epithelial zebrafish somite, a four by five layer of cuboidal cells (the adaxial cells) reside adjacent to the notochord which differentiate into a single stack of twenty elongated cells that migrate radially through the somite to form a superficial layer of slow muscle fibres (Devoto *et al.* 1996). This migration of adaxial cells is driven by differential cell adhesion through the actions of Cadherin transmembrane proteins (Cortés *et al.* 2003). Undifferentiated adaxial cells express *myod1* and *myf5* (Weinberg *et al.* 1996; Coutelle *et al.* 2001) and their specification is regulated by Hedgehog (Hh) morphogens in a dose-dependent

manner (Blagden *et al.* 1997; reviewed by Ingham and Kim, 2005). Another sub-population of adaxial derived cells, termed the muscle pioneers, remain in a medial position in the somite and express engrailed genes (Weinberg *et al.* 1996; Devoto *et al.* 1996). The cells of the lateral pre-somitic mesoderm of zebrafish were shown to differentiate into the embryonic fast muscle fibres (Devoto *et al.* 1996) and two main sub-populations have since been characterised. A population in the posterior-lateral epithelial somite requires Fibroblast growth factor 8 (*fgf8*) signalling for *myod1* expression and terminal differentiation (Groves *et al.* 2005). The other population arises medially within the somite and like the slow muscle pioneers, expresses engrailed genes, in response to Hh signalling (Wolff *et al.* 2003). Interestingly, in *fgf8* mutants, a residual medial fast fibre population was identified that was not dependent on Hh signalling and presumably is regulated through some other midline signal (Groves *et al.* 2005). Hh and Fgf8 signalling suppress Pax3/7 and promote the expression of *myf5* and *myod1* in specific muscle progenitor cell populations (Feng *et al.* 2006, Hammond *et al.* 2007). A combination of vital dye staining and lineage-tracking techniques in zebrafish were used to show that somite sub-domains are generated through a whole-somite rotation of 90° from the starting position, which begins during mid-somitogenesis and is complete by the end of the segmentation period (Hollway *et al.* 2007). Somite-rotation has been shown to require Sdf cytokine signalling (Hollway *et al.* 2007). The anterior component of the somite forms the external cell layer of Pax3/7 expressing cells (Hollway *et al.* 2007; Stellabotte *et al.* 2007). A sub-set of these cells migrate from the external cell layer to form fast muscle fibres in the late embryo and larval stages, whilst others remain in the external cell layer and are thought to be a source of myogenic progenitor cells for later stages of post-embryonic growth (Hollway

et al. 2007; Stellabotte *et al.* 2007). The external cell layer also provides progenitors used in the growth of the dermis and pectoral and dorsal fin muscles suggesting it has a functional role equivalent to the amniote dermomyotome (Hollway *et al.* 2007).

4. Postembryonic Myogenesis

In the late embryo and early larval stages, primary embryonic slow and fast muscle fibres are supplemented by fibres from discrete germinal zones, a process which has been termed stratified hyperplasia (SH) (Rowlerson and Veggetti 2001). Three anatomically distinct, but overlapping waves of SH have been observed in zebrafish, resulting in a continuous increase in the number of slow fibres per myotomal cross-section from hatching until the maximum adult length (~40 cm standard length, snout to caudal peduncle) (Lee and Johnston, unpublished results). Growth of the myotome also involves an increase in the length and diameter of fibres, a process that requires the absorption of myogenic progenitor cells to provide additional nuclei (reviewed in Johnston, 2006). Using mutant lines of zebrafish deficient in midline Hedgehog signalling it was shown that the first wave of SH does not require a scaffold of embryonic slow muscle fibres and proceeds independently of sonic Hh (Barresi *et al.* 2001). The origin of the myogenic precursors that fuel later phases of SH remains to be established, but could also be the external cell layer which persists in adult stages (Hollway *et al.* 2007; Stellabotte, *et al.* 2007).

Expansion in the number of fast muscle fibres with growth also proceeds via an initial phase of stratified hyperplasia (reviewed in Rowlerson and Veggetti 2001). The intensity and duration of this growth phase varies considerably between species, but usually involves the addition of new fast fibres at the dorsal and ventral surfaces of the myotome. Fate mapping studies have shown that

a Pax7 expressing sub-set of the external cell population migrate through the embryonic slow muscle and give rise to fast muscle fibres in larvae (Stellabotte *et al.* 2007; Hollway *et al.* 2007). Within lineage-related clusters it was also possible to detect the original undifferentiated cell at the site of muscle fibre generation, indicating a stem cell self-renewal system (Stellabotte *et al.* 2007; Hollway *et al.* 2007) as proposed for mammalian satellite cells (Schultz 1996). SH is the only mechanism of fast fibre expansion reported in the guppy (*Poecilia reticulata*) (Veggetti *et al.* 1993) and in two out of the eight families of Antarctic notothenioid fishes (Harpagiferidae and Channichthyidae) (Johnston *et al.* 2003a). The adaptive radiation of the Notothenioidei has been associated with a general reduction in fibre number and an increase in fibre diameter from the basal to more derived genera, and this includes species that reach a large body size (~85 cm) (Johnston *et al.* 2003a).

The second and main mechanism for increasing fast fibre number in all other species examined, including zebrafish, is mosaic hyperplasia (MH). MH involves myogenic progenitor cells distributed throughout the myotome and results in successive waves of myotube production producing a characteristic mosaic pattern of fibre diameters. Myotube production in fast muscle only continues until the fish has attained ~40–45% of its maximum body length, with subsequent growth restricted to an expansion in the diameter and length of muscle fibres, and nuclear accretion (Weatherley *et al.* 1988; Johnston *et al.* 2003b). However, in response to injury and as a component of the repair response, new myotube production can be initiated even after the end of fibre recruitment (Rowlerson *et al.* 1997). In those species that show sexual dimorphism in body size, such as the Argentinean hake (*Merluccius hubbsi*) (Calvo 1989) and the Atlantic halibut (*Hippoglossus hippoglossus*),

the duration of fibre recruitment differs between male and female fish (Hagen *et al.* 2006), although the underlying mechanism is unknown. A genome-wide screen in the Tiger pufferfish (*Takifugu rubripes*) revealed a sub-set of genes that are specifically upregulated concomitant with the end of fibre recruitment in fast muscle, although whether any of these genes has a role in establishing the final fibre number (FFN) remains to be established (Fernandes *et al.* 2005). The origin of myogenic precursor cells required for MH has also not been determined, although the external cell layer must be a strong candidate. In this regard it is interesting to note that in zebrafish the external cell layer only contains at most a few dozen Pax7 positive cells per somite whereas in adult fish ~3,500 fast muscle fibres are eventually produced per myotomal cross-section. Furthermore fast muscle fibres with the maximum diameter (80 μm) contain ~1,700 nuclei per cm length (Lee and Johnston 2008). If the external cell layer is indeed the sole source of cells for juvenile and adult growth then this implies that a very small number of self-replicating stem cells gives rise to an enormous number of myogenic progenitors via some lineage restricting mechanism. The number of resident myogenic progenitors can be increased in fasted fish by feeding (Brodeur *et al.* 2003) and can also be manipulated by photoperiod regime (Johnston *et al.* 2003b). For example, in seawater stages of farmed Atlantic salmon that were transferred from short winter days to continuous light there was a transient 70% increase in the number of myogenic progenitors (c-met positive mononuclear cells) relative to controls (Johnston *et al.* 2003b). Continuous light treatment for 6 months over the winter/early spring was associated with longer term benefits in growth performance, as well as a higher final fibre number and greater levels of nuclear accretion in muscle fibres compared to fish subject to natural photoperiod (Johnston *et al.* 2003b).

5. Developmental Plasticity and Adult Growth

It has long been known that exposing fish eggs to different conditions of temperature and/or salinity fish influences a range of meristic characteristics such as vertebral number in the adult stage (Hempel and Blaxter 1961). Later it was discovered that egg incubation temperature affected the number and size distribution of myotomal muscle fibres in Atlantic salmon alevins at hatching (Stickland *et al.* 1988). Since this first paper there have been numerous further studies on the developmental plasticity of muscle growth at different temperatures and/or oxygen levels in a wide range of species (reviewed in Johnston 2006). Most of these studies have been restricted to the larval or early juvenile stages and have typically involved just two embryonic treatments. Recently, we demonstrated that in Atlantic salmon, altering temperature treatments from fertilisation until the embryonic eye became completely pigmented (the “eyed stage”) was sufficient to affect growth trajectory over the entire lifecycle (Macqueen *et al.* 2008). Furthermore, embryonic temperature set the final number of fast muscle fibres per myotomal cross-section (FFN) and altered fibre size distribution and the myonuclear content of individual muscle fibres in adult fish (Johnston *et al.* 2003c; Macqueen *et al.* 2008) (Fig. 3). For the Salmobreed (Norway, A/S) strain, FFN showed a distinct optimum for the 5°C treatment, and was reduced at higher or lower embryonic temperatures (Fig. 3). Experiments showing embryonic environment can affect adult growth can only be explained by direct temperature effects on embryonic tissues, such as the external cell layer. The period to eye pigmentation encompasses the window in which the external cell layer is formed (Hollway *et al.* 2007; Macqueen *et al.* 2008). Undifferentiated *pax7* expressing

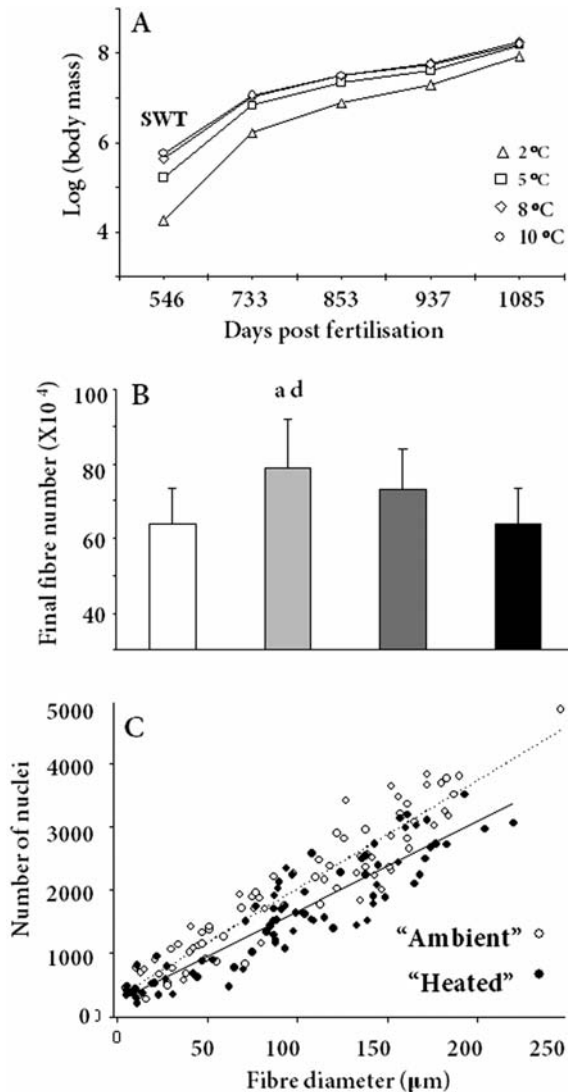


Fig. 3. Embryonic temperature modulates growth and the final muscle fibre number in adult Atlantic salmon (*Salmo salar* L.). (A) Shows the different seawater growth trajectories of Atlantic salmon reared for a short embryonic developmental window at either 2, 5, 8, or 10°C and then provided equal growth opportunity. While 10 and 8°C treatments were significantly heavier at seawater transfer (SWT), strong compensatory growth was observed at lower temperatures, although 2°C fish did not attain the body sizes reached by 5, 8 and 10°C treatments. (B) Shows the norm of reaction response of the final number of fast myotomal muscle fibres in adult Atlantic salmon from the same experiment as A. The final number of fibres showed an optimum at 5°C and was reduced at either higher or lower temperatures by a maximum of 17%. a d indicates a significant difference ($p < 0.001$) compared to 2 and 10°C respectively. (A, B) From Macqueen *et al.* (2008). (C) Shows the nuclear content of fast myotomal muscle fibres of a range of diameters in adult Atlantic salmon reared from fertilisation to smoltification at either ambient temperature or in water heated by 1–3°C and then provided equal growth opportunity. The muscle fibres of fish from the ambient treatment had significantly more nuclei at equivalent fibre diameters ($p < 0.001$). From Johnston *et al.* (2003c).

MPCs remain in the external cell layer of adult zebrafish and are a possible source of myogenic progenitors for MH (Hollway *et al.* 2007). In zebrafish there is an inverse relationship between Pax7 and MRFs in terms of protein expression. Ablation of *myod1* and *myf5* by morpholino antisense RNA in zebrafish increased the number of Pax7 expressing cells (Hammond *et al.* 2007). In Atlantic salmon, we have shown heterochronies in MRF expression (*myf5* and *mrf4*) with respect to developmental stage for different temperature treatments to the “eyed stage” (Macqueen *et al.* 2007), a finding paralleled at the mRNA and protein level in rainbow trout (Xie *et al.* 2001). A plausible working hypothesis is that temperature alters the timing and intensity of expression fields for MRF transcripts with downstream consequences for the number of Pax7 expressing anterior cells. Since the external cell layer MPCs are self-renewing (Hollway *et al.* 2007; Stellabotte *et al.* 2007), an increase in the number derived from the anterior somite could have long-term consequences for muscle fibre recruitment and nuclear accretion. Whatever the mechanism, the finding that a brief period during embryogenesis can “set” the adult final fibre number without significantly compromising somatic growth is of practical significance to the fish farming industry since muscle fibre density has been shown to be an important flesh quality trait affecting texture (Johnston *et al.* 2000). Given the pervasive effects of embryonic environment on adult growth it is also entirely possible that developmental plasticity has long-term effects on other tissues controlling feeding, foraging and growth e.g. the appetite centre in the hypothalamus and/or various endocrine/neuroendocrine tissues.

6. Temperature Acclimation Responses

Temperate fish living in habitats with stable seasonal temperature cues can modify their

swimming performance following several weeks acclimation to a new temperature regime (Fry and Hart 1948). The mechanisms underlying the plasticity of locomotory performance are complex and vary between species, but are typically reversible in juvenile and adult stages (Johnston and Temple, 2002). Proximal mechanisms include changes in the relative amounts of cellular organelles (Johnston and Maitland 1980) and altered expression of hundreds of genes (Gracey *et al.* 2004). In the goldfish *Carassius auratus* (Johnston *et al.* 1975) and common carp *C. carpio* (Heap *et al.* 1985) changes in muscle myofibrillar ATPase activity constitute a major element of the acclimation response. The common carp has become a model species for the study of temperature acclimation in fish (Watabe 2002). Studies with fully activated skinned fibres found that maximum tension and shortening speed increased at low temperatures in both fast and slow muscles following a period of cold acclimation (Johnston *et al.* 1985). Changes in the expression of myosin heavy chain (MYH) isoforms play a key role in the plasticity of myofibrillar ATPase activity and contractile properties with temperature acclimation (Hwang *et al.* 1990; Watabe *et al.* 1992; Guo *et al.* 1994). Three distinct MYH cDNAs have been cloned from the fast muscle of the common carp acclimated to either 10°C or 30°C (Imai *et al.* 1997; Hirayama and Watabe 1997). The relative proportions of each isoform varied with acclimation temperature (Imai *et al.* 1997) (Fig. 4); the 10°C-type MYH (MYHF10) and the 30°C-type MYH (MYHF30) cDNAs were the predominant transcripts in 10°C- and 30°C-acclimated fish, respectively. A third cDNA (intermediate-type) was expressed over a relatively broad temperature range (MYHFint) (Imai *et al.* 1997).

Myosin heavy chain expression in fast muscle also varies with acclimation temperature in the medaka *Oryzias latipes* (Liang *et al.* 2007). The complete medaka MYH

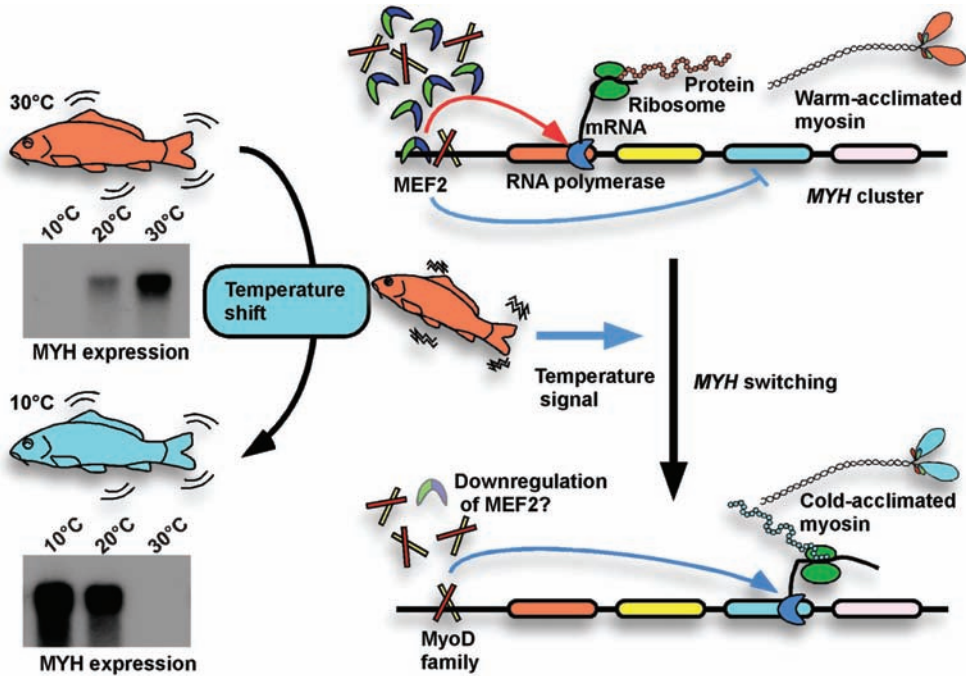


Fig. 4. The expression of fast muscle myosin heavy chain genes with temperature acclimation in the common carp (*Cyprinus carpio* L.) are regulated by members of the MyoD and MEF2 gene families.

(*mMYH*) locus of 219 kbp revealed a cluster of 11 tandemly arrayed *mMYHs*, in which eight genes are transcribed and three are pseudogenes. Expression analysis revealed that two genes were highly expressed in medaka acclimated to either 10 or 30°C whereas there was comparatively low expression level of three other genes exclusively in 30°C-acclimated fish. The ~6 kb 5'-flanking region of the predominantly expressed *MYHs* at 10°C (*mMyH10*) and 30°C (*mMyH30*) contained various *cis*-elements that were putative binding sites for transcription factors such as MyoD, myocyte enhancer factor 2 (MEF2) and nuclear factor of activated T cells (NAFTc). The truncation of the MEF2 binding site located at -966 to -957 in the 5'-flanking region of *mMYH10* resulted in the activation of gene expression at 10°C; a result confirmed using a mutation construct (Liang *et al.* 2008). An E box site

for MyoD family members was located at -613 to -607 in *mMYH10*, and was found to be responsible for transcriptional activity. In contrast, the MEF2 binding site located at -960 to -951 of *mMYH30* was involved in the activation of this gene at 30°C. Thus, the MEF2 binding site is crucial for the temperature-dependent expression of *mMYHs* (Fig. 4).

7. Applications and Perspectives for Future Research

An integrated understanding of the genetic pathways regulating muscle development and growth is important for the identification of particular genes that have large phenotypic effects on production and quality traits in aquaculture species. Natural population variation in these key genes will provide the basis for future programs involving

marker-assisted selection to produce superior strains with advantageous characteristics. Such studies may also identify gene and protein biomarkers that could have utility in new types of growth trial, enabling the cost effective development of novel diets and the identification of nutritional components with strong biological effects. Research on the “cross-talk” between regulatory systems controlling growth of the muscle, skeleton and immune system are likely to be of particular importance in understanding abnormal development leading to the relatively high incidence of body deformities observed in some aquaculture species. Although such studies are in their infancy, progress is expected to be rapid, particularly given the adoption of new high throughput sequencing technologies leading to the completion of more fish genomes and transcriptomes to the draft level. This chapter has not considered the role of microRNAs in muscle development and growth, as there are currently few relevant teleost studies. MicroRNAs are con-

served small regulatory RNAs that regulate the stability and translation of hundreds of gene targets. The role of microRNAs in fish growth will be an important topic for future research. The finding that embryonic environment has profound effects on adult muscle growth through mechanisms of developmental plasticity is of direct relevance for the production of juvenile fish for on growing. Future research should also focus on maternal or cross-generational effects of broodstock environment on larval and juvenile muscle growth that may act via maternally transmitted mRNA transcripts in the yolk.

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Molecular Innate Immunity in Teleost Fish: Review and Future Perspectives

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Innate immune response is a primitive form of defense mechanism existent in plants and animals. It is a complex system which, in vertebrates, is composed of cellular and humoral responses. The vertebrate teleost fish, which diverged from the tetrapod lineage about 450 million years ago, has an innate immune component that shows considerable conservation with higher vertebrates particularly in mammals highlighted by the presence of orthologous pattern recognition receptors (PRRs) and stimulated cytokines. However, there is also increasing evidence of teleost fish components and functions that are not observed in mammals suggesting complexity and diversity in teleost fish innate immune function. Knowledge about the network and function of these innate immune-related molecules in model and/or economically important fish is therefore important and new molecular biological tools such as large scale gene expression profiling including DNA microarrays and protein-based techniques will help elucidate such information. This review focuses on the molecular innate immune mechanisms in teleost fish in reference to the known mammalian system. It also includes future perspectives on how this basic knowledge coupled with existing molecular biological techniques can contribute to the control of fish disease and thereby improve aquaculture.

KEYWORDS molecular immunity; teleost fish; pathogen recognition receptors; inflammatory cytokines

1. Introduction

Innate immunity is the first line of defense against infection and is regarded as the pri-

meval and hence the universal form of host defense (Janeway and Medzhitov 2002). It exists in animals (vertebrates and invertebrates) and plants, and recent data suggests that it is a product of convergent rather than

divergent evolution (Ausubel 2005). Common features of the system across species include conserved receptors for microbe-recognition, antimicrobial peptides and mitogen-associated protein kinase signaling cascades.

Innate immunity is generally subdivided into two parts, the cellular and humoral defense responses. Cellular responses include the physical barrier such as mucus and epithelial tissues lining the skin, gills and stomach, which keeps infectious microorganisms from entering the body, and specialized cells (like monocytes/macrophages, granulocytes and nonspecific cytotoxic cells) capable of killing and digesting pathogens if the latter breaches the physical barriers. These cells are recruited in the infection site primarily by inflammatory cytokines. Humoral responses, on the other hand, employ a variety of proteins and glycoproteins capable of destroying or inhibiting growth of infectious microorganisms, which include among others anti-bacterial peptides, proteases, complement, transferrins and the antiviral myxovirus resistance-1 protein (Mx1).

In mammals, innate immunity is triggered by the recognition of conserved microbial products by receptors generally called pathogen recognition receptors or pattern recognition receptors (PRRs) (Fig. 1). These molecules can distinguish 'infectious nonself' from the 'noninfectious' self. PRRs, which include both extracellular and cytosolic recognition, mediate several pathways that give rise to the production of inflammatory cytokines and interferons (Lee and Kim 2007). In teleost fish, such mechanisms are believed to be conserved mainly because of the presence of PRR and cytokine orthologs (Plouffe *et al.* 2005; Stein *et al.* 2007). Furthermore, microarray analysis has revealed transcriptional modulation of various cloned fish PRRs and cytokines following bacterial agents (Kurobe *et al.* 2005; Gerwick *et al.* 2007; Peatman *et al.* 2007;

Peatman *et al.* 2008). On the other hand, increasing evidence also shows that there are molecules and mechanisms that are specific to teleost fish (Plouffe *et al.* 2005; Stein *et al.* 2007).

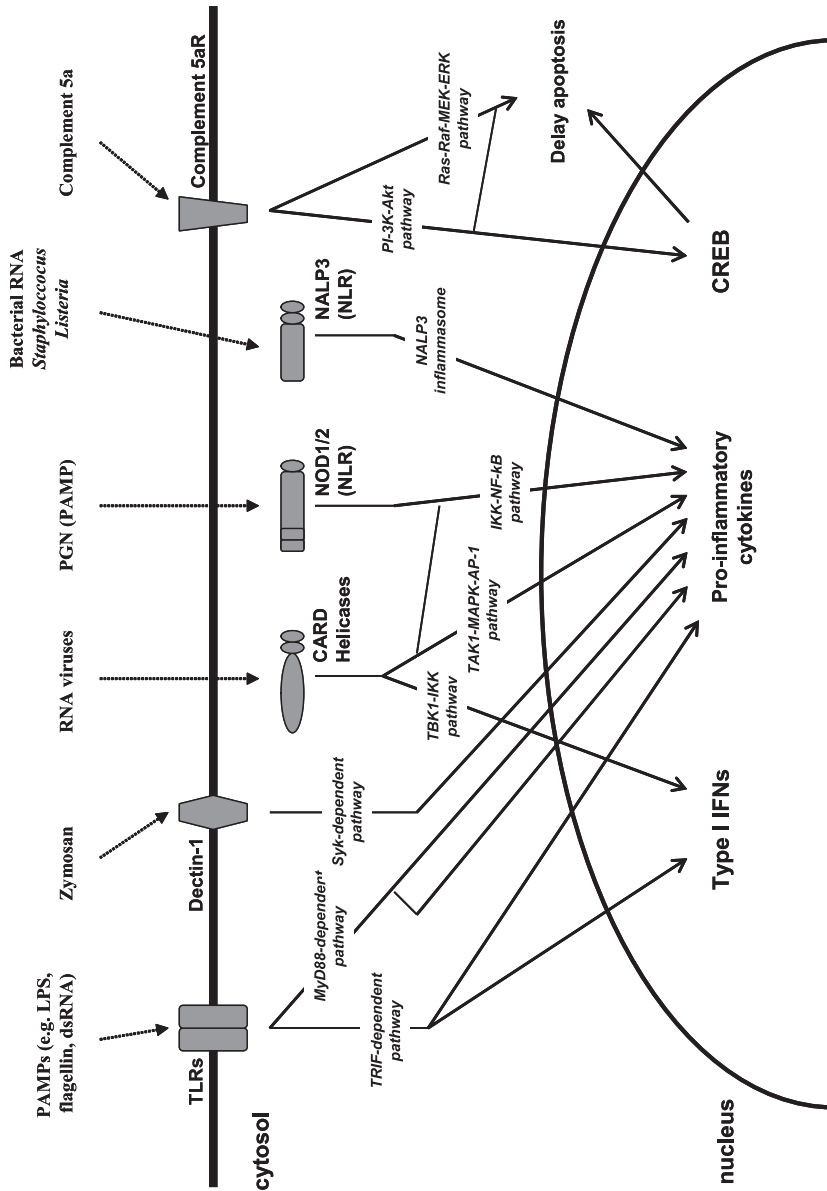
In this paper, we review the innate immune system network in fish gathered from available information and studies including pathogen recognition and the ensuing cytokine cascade output as well as innate immune-related cellular functions. We draw comparisons of these with the known molecules and mechanisms in mammals, in the absence/limited functional information in teleost fish, as well as highlight recent and growing innate immune functions that are teleost fish-specific. We also present some future perspectives on the basic study of fish immunology and the techniques that would help produce this knowledge, as well as the possible applications that could be generated for the improvement of aquacultural productivity.

2. Mammalian Innate Immune Responses

2.1. Pathogen recognition

In mammals, innate immune response is initiated through the recognition of pathogens by the PRRs. PRR families that have been identified thus far include the extracellular Toll-like Receptors (TLRs), C-type lectins and complement receptors CRs, and the cytosolic nucleotide-binding domain and LRR proteins (NLRs) (Table 1). It is known that these PRRs are able to trigger various innate immune responses including complement pathway, apoptosis, leukocyte activation and migration, and cytokine production (Lee and Kim 2007).

TLRs, now one of the most studied biological molecules discovered because of the Toll receptor in *Drosophila*, are considered to be vital to microbial pathogen recognition. Its extracellular leucine-rich repeat (LRR)



Source: modified from Lee and Kim, 2007

Fig. 1. Molecular mechanism of innate immunity in mammals: from recognition to signaling to inflammatory cascade. Orthologous molecules/mechanisms known in teleost fish are shown (discontinuous ring).

Table 1. Selected mammalian and teleostean pattern-recognition receptors

Family	Function/Structure	Taxon	Member	Source
TLRs	Pathogen associated molecular patterns (PAMPs) recognition on the cellular membrane. Extracellular leucin-rich repeats (LRRs) and intracellular Toll/IL-1 receptor (TIR) domain.	Mammals	TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, TLR13	Lee and Kim 2007; Roach <i>et al.</i> 2005 Jault C, <i>et al.</i> , 2004
		Teleost Fish	TLR1*, TLR2, TLR3, TLR4*(Z), TLR5, soluble TLR5, TLR7*, TLR8*, TLR9, TLR14 (P), TLR21*, TLR22, TLR23 (P)	
Lectins	Involved in the carbohydrates recognition. Secretion form and membrane form are exist, and generally function as multimeric structure.	Mammals	Mannose binding lectin (MBL), Dectin-1 #, Intelectin, Pentraxins	Lee and Kim 2007; Russell and Lumsden
		Teleost Fish	MBL, Intelectin, Pentraxins	
Complement receptors (CRs)	Involved in the leukocytes activation and migration.	Mammals	CR3 and CR4 (integrins), C5aR	Lee and Kim 2007; Boshra H, <i>et al.</i> 2004
		Teleost Fish	CD18 (β chain for the CR3 and CR4), C5aR	Qian Y <i>et al.</i> 1999
NLRs	Involved in the PAMPs recognition in the cytoplasm. The receptors posses the central nucleotide oligomerization domain (NOD), caspase activation and recruitment domain (CARD) and LRRs.	Mammals	NOD1, NOD2, NALPs, IPAF, NAIP5	Lee and Kim 2007; Stein <i>et al.</i> 2007
		Teleost Fish	NOD1, NOD2	
CARD helicases	Implicated in the cytoplasmic detection of viruses. The molecules possess the RNA helicase activity in its C-terminal portion and CARD.	Mammals	Retinoic acid-inducible gene 1 (RIG-I), melanoma-differentiation-associated gene 5 (MDA5)	Lee and Kim 2007
		Teleost Fish	Unknown	

*Isotypes were reported.
(Z) Reported from only the zebrafish.
(P) Reported from only the pufferfish.
Only dectin-1 is a lectin that be able to mediate the intracellular signaling directly after the ligand recognition.

domains are responsible for specifically recognizing molecular signatures of various pathogens while the cytoplasmic Toll/IL-1R (TIR) domain directs signals that induces pro-inflammatory cytokine, Type I interferon and chemokines production (Kawai and Akira 2005). To date, 10 members of TLRs have been identified in human, and 13 in mouse (Krishnan *et al.* 2007).

On the other hand, C-type lectins, of which the mannose-binding lectin (MBL) the best characterized, recognize enveloped virus, bacteria and fungi structure via cell to cell interactions following Ca^{2+} -dependent manner (Epstein *et al.* 1996; Vasta *et al.* 1999). Through the binding of extracellular MBL to the pathogen then to MBP-associated serine proteases (MASPs), the lectin complement pathway is induced and produces pro-inflammatory cytokines (Gupta and Surolia 2007). Recently, another C-type lectin, *dectin1*, was found to recognize fungus and β -glucan (Robinson *et al.* 2006). Upon binding of *dectin1* to pathogen, the immunoreceptor tyrosine-based activation motif (ITAM) also induces immune responses (Ariizumi *et al.* 2000; Brown 2006). Another lectin, the mammalian *intelectin* (*IntL*), is a Ca^{2+} -dependent enteric molecule that has specific affinities to D-pentoses and D-galactofuranosyl residues as well as recognizes *Nocardia rubra* arabinogalactan (Tsuji *et al.* 2001). Pentraxins recognize a wide range of pathogenic substances and altered self molecules and acute phase proteins (Mantovani *et al.* 2007). Examples of which are the C-reactive protein (CRP), serum amyloid P component (SAP) and Pentraxin 3 (PTX3) (Mantovani *et al.* 2007).

The complement receptors such as CR3 and CR4 are able to mediate phagocytosis of opsonized pathogens and/or apoptotic cells. Further, C5aR is known as immunoregulatory signal generator. C5a is a potent inflammatory agent that has diverse functions including chemotaxis, phagocytosis enhancement, reduction of neutrophil

apoptosis, and IL-12 production through C5aR. The PI-3K-Akt and ERK signaling pathways are known C5aR cascade that lead to the expression of the anti-apoptotic protein Bcl2 so far (Lee and Kim 2007).

The NLR family, are recently discovered PRRs involved in the cytoplasmic recognition of whole bacteria and their products such as flagellin and peptidoglycan (Kaparakis *et al.* 2007). It is composed of 2 major sub-families, the NODs and the NACHT-LRR-PYD-containing proteins, or NALPs. NODs and NALPs induce the production of pro-inflammatory cytokines and even antimicrobial peptides upon pathogen recognition via the NLR signaling pathway and the "inflammasome" pathway, respectively (Kaparakis *et al.* 2007). The other cytoplasmic PPRs of retinoic acid-inducible gene I (*RIG-I*) and melanoma-differentiation-associated gene 5 (*MDA5*) possesses RNA helicase activity to mediate the antiviral responses (Kaparakis *et al.* 2007).

2.2. Cytokine cascade

Cytokines, specifically pro-inflammatory cytokines and Type I interferons, are known to be induced following the activation by signal pathways as result of the stimulation of PRRs (Table 2). Cytokines have pleiotropic (multiple), overlapping and sometimes contradictory functions such that their classification is oftentimes difficult. These molecules have been grouped based on general names (e.g. lymphokines, monokines, chemokines, interleukins, interferons, tumor necrosis factors, colony stimulating factors etc); on functions (e.g. pro- and anti-inflammatory or innate and adaptive immunity-related); on structure (e.g. short and long chain cytokines); and on receptors used (immunoglobulin superfamily, hematopoietic growth factor (type 1-family), interferon family (type 2-family), tumor necrosis factor (type 3-family) and chemokine receptors (7 transmembrane helix

family). Cytokines here were grouped based on available recent reviews for each of the stated group; Cytokine Class 1, Cytokine Class 2, Chemokines, TNF Super-family and IL1 Family (Table 1).

Class 1 cytokines are often involved in the proliferation, recruitment to inflammation/infection sites, survival and maturation of cells (Huising *et al.* 2006). Because of their pleiotropic nature, they can also be involved in other physiological processes like reproduction, food intake and metabolism. They share similar 4α -helical- 2β sheet 3D structure and use the class 1 cytokine receptors. Although they share little primary sequence identity, these cytokines are considered to have expanded from a single ancestor (Bazan 1990).

Class II cytokines, in contrast to Class I, generally acts on minimizing damage to host after noninfectious and infectious insult, and include the interferons which mediates antiviral responses and IL10 known to be anti-inflammatory in nature (Krause and Pestka 2005). Their structure is likewise different with Class 1 cytokines in that their β sheets have been replaced by α -helices (Langer *et al.* 2004).

Chemotactic cytokines or chemokines, are mostly involved in leukocyte migration and are known as "second-order" cytokines, being induced by pro-inflammatory "first-order" cytokines like IL-1s, TNFs, IFNs. These are a family of small proteins that regulate cell migration under both inflammatory conditions or homeostasis (Peatman and Liu 2007). Chemokines are subdivided based on the arrangement of the 1st 2 of the 4 cysteine residues; CC, CXC, C and CX3C (Bacon *et al.* 2003).

TNF ligand superfamily (TNSF) cytokines, which includes the tumor necrosis factor- α (TNF- α), lymphotoxin- α and lymphotixin- β , are structurally related proteins, either soluble or membrane-bound, that are produced during lymphoid organ development and inflammation. (Gruss 1996)

These molecules are involved mainly in cellular regulation such as tumor cell killing during immune responses and inflammatory reactions.

Interleukin 1 family is one of the critical early pro-inflammatory cytokines that down- or up-regulate other cytokines during infection or inflammation (Huising *et al.* 2004). These molecules are produced by monocytes, activated macrophages, neutrophils, endothelial cells, fibroblasts, Langerhans cells of the skin and other types of cells. IL18, which is a recently discovered member, was observed to stimulate (IFN)- γ production, T-helper1 (Th1) cell differentiation and enhances cell cytotoxicity of natural killer cells (Akira 2000).

3. Teleost Innate Immune Responses

3.1. Pathogen recognition

Many components of the innate immune signaling are conserved between teleost fish and mammals, with very clear orthologous relationships. Examples of the latter are the presence of teleost fish PRRs such as TLRs, Lectins, complement receptors, NLRs (Table 1).

Teleost fish have been shown to functionally react to a number of TLR agonists and that this recognition induces cytokine expression similar to those observed in mammals (Purcell *et al.* 2006). In particular, TLR signaling molecules have been established to be evolutionary conserved in teleost fish including myeloid differentiation factor 88 (MYD88), TIR domain-containing adapter protein (TIRAP), TIR-domain containing adaptor including INF- β (TRIF), TRIF-related adaptor protein (TAM), sterile α and HEAT-Armadillo motifs (SARM), interferon regulatory factor 3 (IRF3) and interferon regulatory factor 7 (IRF7) (Purcell *et al.* 2006). As such, it is now believed that the basic program of gene regulation by TLR-signaling pathway is conserved across

vertebrates. A total of 11 types of teleost fish TLRs (TLR 1, 2, 3, 5, 7, 8, 9, 14, 21, 22 and 23) have been including isotypes for TLR 1, 4, 8 and 21 (Jault *et al.* 2004; Roach *et al.* 2005). Their orthology suggests that they also possess the same type of pathogen recognition mechanism as with their mammalian counterparts.

The complement system of teleost fish, which is composed of soluble plasma proteins involved in immune responses, has been reviewed recently (Boshra *et al.* 2006). It has been shown to be involved in opsonization, phagocytosis and inflammation. Fish complement components include the C1q/MBL family, C1r/C1s/MASP family, C2/Bf and factor D molecules, C3/C4/C5 family, membrane attack complex (MAC) assembly, complement regulatory proteins and complement receptors.

Lectin is well studied in various teleost fish, although the detail of correlation to the fish immunity is unclear (Russell and Lumsden 2005). Teleost fish MBL is able to recognize mannose, N-acetyl-D-glucosamine (GlcNAc) and glucose (Nakao *et al.* 2006). This has also been demonstrated with lamprey MBL suggesting that lectin complement pathway exist as early as in agnathans (Takahashi *et al.* 2006). IntL homologs have likewise been identified in teleost fish through gene expression profiling (Gerwick *et al.* 2007; Peatman *et al.* 2007; Peatman *et al.* 2008).

3.2. Cytokine cascade

Similar to PRRs, there are also clear orthologous cytokines in teleost fish, particularly of the Class I, Class II, chemokines, TNF superfamily and IL1 families (Table 2). Coupled with the PRR orthologues, this information suggests that the innate immune system in teleost fish also exhibits the same general mechanism of recognition and cascade seen in mammals. Recent reviews also discussed the similarities of innate immune

mechanisms between teleost fish and mammals where the presence of fish orthologues for antimicrobial peptides, pro-inflammatory cytokines, e.g. TNF- α , IL-1 β , IL-18, interferons, chemo-kines and IL-8, and complement have been established (Plouffe *et al.* 2005).

Gene or protein orthology and conservation allows speculation for similarity of function but functional genomics and protein assays are desirable to ascertain function. With the whole genome sequences in fish, recent publications have started to report functionalities of immune-related genes and their proteins. An example of gene structure and function conservation between fish and humans is the action of erythropoietin (EPO) and erythropoietin receptor (EPOR) in red blood cell formation and hematopoiesis and under hypoxic conditions (Paffett-Lugassy *et al.* 2007). Another is the macrophage colony-stimulating factor (CSF-1), which like in mammals, was observed to induce proliferation of monocytes to macrophages (Hanington *et al.* 2007).

4. Only in Teleost Fish

The Whole Genome Duplication (WGD) that happened early in the life of ray-finned fishes is now increasingly believed to have happened about 350 to 450 million year ago and is the main reason for the explosion of the fish species diversity at >23,500 spp. (Volff 2005). The gene duplication that happened resulted in the creation of numerous novel or semi-novel genes and functions in fish, known as "more genes in fish than mammals" concept (Ohno 1970). It is therefore not surprising that there are already 5 genomic databases sequenced thus far for this taxon; the zebrafish, medaka, stickleback, tiger pufferfish and the green spotted pufferfish (www.ensembl.org). Large scale teleost fish genome analysis has revealed numerous gene duplications that are thought to originate from the WGD (Stein 2007). This

Table 2. Selected mammalian and teleostean cytokines

Family	Function/Structure	Taxon	Selected members	Source
Cytokine Class 1	Involved in expansion and differentiation of cells. Have a 4- α helix bundle structure	Mammals	IL6, GCSF, IL11, IL12p35, IL23p19, IL27p28, ILF, OSM, CNTF, CLC, CT-1, CT-2 NP, Leptin, Epo, PRL, GH	Bazan 1990; Huisling <i>et al.</i> 2006; Santos <i>et al.</i> 2006; Hwang <i>et al.</i> 2007
		Teleost Fish	IL6-a and -b, IL11-a and -b*, GCSF-a and -b*, Leptin, Epo, PRL, GH, M17* and M17 Homologue (MSH)*	
Cytokine Class 2	Involved in minimizing damage to host after insult. Contain more than 4- α helices.	Mammals	Type I IFN (IFN α 2, IFN α 4, IFN β , IFN ω 1, IFN γ 1), Type II IFN, IFN- γ , IL10, IL28-like, IL19, IL20, IL22, IL24, IL26	Krause and Pestka 2005; Robertson 2006
		Teleost Fish	Type I IFN (IFN α -1, IFN α -2), IFN- γ , IL10, IL20, IL24	
		Mammals	CXC (IL8, PF4, PBP, NAP-2), CC (MIP-1 α , MIP-1 β , MCP-1, MCP-2, MCP-3, RANTES); C (Lymphotactin)	Bacon <i>et al.</i> 2003; Peatman and Liu 2007; Kim <i>et al.</i> 2007
TNF Super Family	Involved in inflammation and lymphoid organ development. Compact trimers as membrane bound or soluble proteins	Teleost Fish	CXC (CXC8-like, CXC-10, -12, -13, -14) CC (CCL19/21/25, CCL20, CCL27/28, CCL17/22, MIP, MCP)	
		Mammals	TNF- α , lymphotactin- α (TNF- β) and lymphotactin- β	Gruss 1996; Kono <i>et al.</i> 2006
		Teleost Fish	TNF- α 1, TNF- α 2, TNF-N, lymphotactin- β 1 and - β 1	
Interleukin 1 Family	Involved in pro-inflammatory responses. Fold rich in β -strands	Mammals	IL1- α , IL1- β , IL18	Huisling <i>et al.</i> 2004
		Teleost Fish	IL1- α , IL1- β , IL18	

*Only found in fish.

was clear with genes involved in signal transduction downstream of the transmembrane receptors but not with molecules involved in interactions with pathogen components. Genetic diversity translates to protein diversity and as such it is therefore very possible that in teleost fish, there will be a lot of unique and differing functionalities amidst the background of conserved functions. In fact, many of these fish-specific features are now starting to be unraveled.

4.1. Pathogen recognition

Teleost fish TLR14, 21, 22 and 23 have not been found from mammals so far. TLR 14 is reported from lamprey, teleosts and amphibians, TLR 21 in teleosts, amphibians and birds, TLR 22 in teleosts and amphibians, TLR 23 in Fugu (Roach *et al.* 2005). The soluble form TLR5 (sTLR5) is also unique in teleost fish (Tsujita *et al.* 2004). Its production is facilitated by the NF- κ B activated by membrane TLR5 (mTLR5) upon recognition of Gram-negative bacteria flagellin (Tsujita *et al.* 2004). Conversely, there are no reports yet of mammalian TLR6 orthologues in fish.

Fish complement/anaphylatoxin PUEF-8, which is composed of C3a, C4a and C5a, greatly enhance particle uptake in teleost fish leukocytes, increased the number of phagocytic cells by 3- to 4-fold and acted as strong chemoattractant to peripheral blood and kidney leukocytes. These observations were not observed in mammalian anaphylatoxins and are therefore novel functions only found in fish (Boshra *et al.* 2006).

NLRs have been identified in teleost fish including a conserved NACHT-domain protein which is unique in fish and species-specific subgroup of NLRs with a novel N-terminal domain were found from zebrafish and pufferfish (Stein *et al.* 2007). Taken together, the divergence of the fish PRRs may indicate the species-specific pathogen recognition pathways and immune responses.

4.2. Cytokine cascade

The differing functional attributes between teleost fish and mammals is now also being observed not only at the genetic but at the protein level as well. Th2 cytokines (IL4, IL5, IL13) as well as the neighbor IL3 and granulocyte-macrophage colony stimulating factor (GM-CSF) are apparently missing in teleost fish (Huising *et al.* 2006). Recently, there have been reports of members of the IL6-cytokine subfamily that appear to only be found in teleosts. A novel class-1 helical cytokine M17 (named after the original clone number of the carp leukocyte cDNA library), cloned in 2003, was shown to be induced by alginate by (Fujiki *et al.* 2003) and later shown to be induced by nitric oxide and can stimulate proliferation of monocytes to macrophages (Hanington and Belosevic 2007). Its apparent paralogue named M17 homologue (MSH) have been cloned recently and was shown to be induced by bacterial agents, lipopolysaccharide, peptidoglycan and the dsDNA, viral mimic polyI:C (Hwang *et al.* 2007). Another recently published fish-specific report showed that goldfish possesses a soluble macrophage colony stimulating factor receptor (CSF1R). In terms of cytokine which can inhibit CSF1-induced proliferation of monocytes into macrophages and monocyte-like cells (Hanington *et al.* 2007).

In terms of cytokine receptors, there have also been reports of such genes that do not possess clear orthology to known receptors and appears to be more of ancestral in structure. The Leukemia Inhibitory Factor Receptor (LIFR)-like molecule identified in goldfish was suggested to be the ancestral molecule for mammalian LIFR and Oncostatin M Receptor (OSMR) (Hanington and Belosevic 2005) although it still not clear whether the LIFR-like genes predicted in the chromosome 12 of the green spotted pufferfish is orthologous to the goldfish

LIFR-like genes or to teleost OSMR as well (Jaillon *et al.* 2004). Another class 1-cytokine receptor in fish which appears to be ancestral in structure is the Japanese flounder glycoprotein 130 homologue or JfGPH discovered very recently (Santos *et al.* 2007). This receptor, which contains the classic WSXWS motif, the cytokine binding domains, and the Jak/STAT signaling boxes 1 to 3 in its cytoplasmic region, does not possess a detectable Ig-like N-terminal domain. It appears to be an ancestral gene for class 1 cytokine receptors that is ubiquitously expressed in tissues and down-regulated during viral-induced pathways.

Novel immune-type receptor (NITRs) is another group of molecules belonging to the immunoglobulin superfamily that have no known homolog in mammals. These are expressed mainly in spleen, kidney and intestine and were suggested to play important roles in the regulation of cellular immune responses (Hawke *et al.* 2001). Recently, we have identified an NITR in Japanese flounder that is localized in both the T and B cells pointing to its specific importance in adaptive immunity (Piyaviriyakul *et al.* 2007).

4.3. Cellular mechanisms

Fish rely heavily on innate immune responses to deal with pathogens. With this is the use of cells to combat pathogens. Novel types of cells that have been found in teleost fish but not mammals include non-specific cytotoxic cells (NCCs) (Plouffe *et al.* 2005).

Because of cellular and morphological differences, blood cells between mammals and teleost fish, has always been suggested to be functionally different (Rowley *et al.* 1988). Recently, direct studies have identified immune-related cellular functions in fish that defies present paradigms in mammals. For example, unlike mammalian B cells, rainbow trout B cell populations were shown to have potent phagocytic capability i.e. following uptake of particles, these cells could

induce “downstream” degradation pathways leading to formation of phagolysosome and intracellular killing of microbes (Li *et al.* 2006). Zebrafish primitive macrophage lineage was shown to give rise to a number of neutrophilic granulocytes in contrast to mammalian primitive macrophage which is considered to give rise to no other cell types. Only a small fraction of these zebrafish larval neutrophils phagocytose microbes as compared to the macrophages and are more attracted to stressed and malformed tissues suggesting that these cells are involved in a wider role than biodefense (Guyader *et al.* 2007). The same functional adaptation has been seen in seabream professional phagocytic granulocytes, where these cells, other than immune surveillance, have a role in the reorganization of the testis during post-spawning (Chaves-Pozo *et al.* 2005).

5. Future Perspective

5.1. Basic knowledge in fish innate immunity

There are undoubtedly numerous innate immune mechanisms in teleosts that resemble or are conserved with mammalian processes. This is not unexpected since it is regarded that innate immunity is the universal and ancient form of host defense against infection (Janeway and Medzhitov 2002). Present studies on orthologous teleost fish genes, albeit many are only up to expression studies, points to general similarity in function to higher vertebrates. Going forward, however, it is the fish-specific molecules and processes that would usher a new era in basic teleost fish immunological studies. The apparent duplication of many of the teleost fish genes adds to the complexity of fish immunology and could potentially reveal new mechanisms unheard of in the study of immunology. The availability of 5 fish genomic databases (for zebrafish, fugu, green spotted pufferfish, stickleback and medaka)

will certainly fast track the study of these fish-specific phenomenon. This will allow for a better understanding of fish immunity as well as how this relate to the evolution of innate immunity in vertebrates.

5.2. Technologies for fish innate immunity

The advent of various molecular biological techniques have paved the way in increasing our knowledge of fish innate immunity and these same technologies will still be very useful in uncovering this new information. For example, we have successfully used the Expressed Sequence Tag (EST) platform to identify orthologous, paralogous and novel genes in Japanese flounder (Aoki and Hirono, 2005). We likewise have successfully employed cDNA microarray platform to evaluate efficacy of vaccination using different vaccine agents like formalin-killed cells, DNA and recombinant protein vaccines (Byon *et al.* 2006; Byon *et al.* 2005) as well as comparing between vaccine agents like viral G and N protein DNA vaccines (Yasuike *et al.* 2007). Technologies that are important for the future include PCR-based techniques including Quantitive Real-Time PCR, immunological-bases assays, recombinant protein analysis and microarray studies. In mammals, these assays have been very effective in elucidating innate immunity and is expected that it will do the same for fish.

5.3. Application to aquaculture

The detailed analysis of the innate immune-related molecules fish including their function and network will certainly generate new technologies that can be applied to improve aquaculture.

5.3.1. Biomarkers

The production of healthy quality fish is important to consumers in terms of food safety and nutrient content, and to aquacul-

ture in terms of profitability. Biomarkers could be utilized to monitor such quality. In innate immune responses, several important molecules are involved and the up- or down-regulation of these molecules provides some measure of the biological state of the fish, whether it is in normal condition or stressed due to infection. For instance, C-reactive protein is an indicator for the internal inflammatory responses in mammals. From our studies of the innate immune responses of Japanese flounder at the molecular level, we have discovered a number of potential biomarker genes such as antimicrobial agents, acute stress response factors, and various cytokines during the infectious pathogen invasion (Aoki and Hirono 2005). The antiviral Mx1 gene is an interesting example of a biomarker to measure vaccine efficiency. We observed that it was only expressed by rhabdovirus glycoprotein gene vaccinated but not in rhabdovirus nucleocapsid gene vaccinated fish (Yasuike *et al.* 2007).

5.3.2. Vaccine/adjuvant

Our immunostimulation studies with the aid of extensive gene expression profiling like ESTs, cDNA microarray and Serial Analysis of Gene Expression (SAGE) generated information on various pro-inflammatory cytokines that are potential for vaccine/adjuvant development. For example, we have previously shown that Japanese flounder IL-1 β can induce various cytokines, cell surface antigens, signal transduction genes, suggesting that IL-1 β is a potential vaccine adjuvant (Emmadi *et al.* 2005).

5.3.3. Transgenic

Fish transgenesis to produce transgenic fish with commercially important traits such as growth enhancement and disease resistance is still being pursued and many molecules involved in innate immunity are target molecules for such. We have, for example, successfully shown that the TNF promoter can

be a tissue-specific, inducible promoter that can be utilized to express foreign genes in Japanese flounder (Yazawa *et al.* 2005a–c). The transgenic technique itself could be used to augment or complement the fish innate immune response. For instance, the recombinant Japanese flounder lysozyme has been shown to be infective against Japanese flounder pathogens like *Edwardsiella tarda* (Hikima *et al.* 2001). However, when chicken

lysozyme was expressed in transgenic zebrafish, the fish acquired resistance against the bacteria (Yazawa *et al.* 2006).

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Molecular Interaction between Fish Pathogens and Host Aquatic Animals

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We have studied the host-pathogen interactions between Atlantic salmon (*Salmo salar* L.) and *Aeromonas salmonicida*. Sequencing the genome of the bacterium allowed us to investigate virulence factors and other gene products with potential as vaccines. Using knock-out mutants of *A. salmonicida*, we identified key virulence factors. Proteomics studies of bacterial cells grown in a variety of media as well as in an *in vivo* implant system revealed differential protein production and have shed new light on bacterial proteins such as superoxide dismutase, pili and flagellar proteins, type three secretion systems, and their roles in *A. salmonicida* pathogenicity. We constructed a whole genome DNA microarray to use in comparative genomic hybridizations (M-CGH) and bacterial gene expression studies. Carbohydrate analysis has shown the variation in LPS between strains and reveals the importance of LPS in virulence. Salmon were challenged with *A. salmonicida* and tissues were taken to construct suppressive subtractive hybridization libraries to investigate differential host gene expression. We constructed an Atlantic salmon cDNA microarray to investigate the host response to *A. salmonicida*. Real-Time qPCR and NMR-based metabolomics have revealed important information about host responses to infection and to chronic stress. By linking genome sequencing, functional genomics, proteomics, carbohydrate analysis, metabolomics, and whole animal assays, we took integrated and innovative approach to pathogenesis research.

KEYWORDS host-pathogen interactions; genomics; proteomics; furunculosis; DNA microarrays; knock-out mutants

1. Introduction

The development of genomics, proteomics and metabolomics, have enabled the transition from sequential studies of single genes, proteins or metabolites to the simultaneous study of many components and their interactions at a variety of biological levels (from pathways to cells, tissues and whole organisms). The development of these fields has been supported by the concurrent development of new technologies and methods such as quantitative PCR or Real Time PCR, RNA interference (RNAi) assays, and *in situ* hybridization. These fields and their supporting technologies and methods are now widely used in the study of disease of higher vertebrates as well as in the development of new therapeutants and vaccines. The field of aquatic animal health has only recently begun to benefit from their application.

It is well known that farmed aquatic animals are susceptible to a wide variety of infectious diseases. Direct and indirect costs of preventing and controlling infectious diseases in aquaculture are estimated to exceed 10% of total production costs. This represents an appreciable loss of profits to the aquaculture industry. Coupled with this is an increasing global concern about the use of therapeutants (antibiotics and chemical treatments) in the production of food animals and issues related to animal welfare. Development of more effective vaccines for aquaculture is necessary to reduce losses due to disease, to enable further reductions in the use of therapeutants, and to improve animal welfare.

Vaccines against infectious diseases in fish have been effective and available for years. For example, multi-valent vaccines based on whole bacterins in formalin- or oil-based preparations have provided protection to salmon against *Aeromonas salmonicida*, *Listonella anguillarum*. Vaccines are also available against viral pathogens. However,

it is notable that several problems are associated with commercially available vaccines for fish. Oil-based and formalin-based vaccines have been associated with adhesions and other pathologies including weight loss (Midtlyng and Lillehaug 1998), secondary infections (Bruno and Brown 1999), injury and inflammation (Mutoloki *et al.* 2004). Therefore, research into vaccines and vaccine delivery systems is ongoing.

Similarly, effective husbandry tools and practices have been used since the inception of aquaculture. Fallowing farm sites, separation of year classes, culling of infected broodstock, and reduction of anthropogenic stressors are all examples of successful husbandry techniques. However, these are largely based on trial and error and on empirical observations.

In order to devise effective aquatic animal health management tools in a timely and cost-effective fashion, it is essential that we identify meaningful biological targets, by knowing which antigens, host immune factors, and stress response genes to target. In order to do that, it is necessary to increase our understanding of the interactions between host aquatic animals and pathogens. Genomics, proteomics, and other biotechnological approaches help us to do that.

Aeromonas salmonicida is a non-motile, Gram-negative bacterium that is the etiological agent of furunculosis. Furunculosis has been recognized as a widespread important disease in wild and farmed fish since the 1890s (Emmerich and Weibel 1894). Due to its early recognition as an important pathogen it is arguably the best studied bacterial pathogen of fish. Several excellent reviews of earlier research on *A. salmonicida* and furunculosis are available (Bernoth 1997; Wiklund and Dalsgaard 1998; Hiney and Olivier 1999).

In this chapter we will demonstrate how a multi-disciplinary approach, combining genomics, proteomics and metabolomics with more traditional methods was used

within the National Research Council Genomics and Health Initiative Program "Genomics Approaches to Aquatic Animal Health Management," to understand the biology of the bacterial pathogen *Aeromonas salmonicida* and its interactions with one of its hosts, the Atlantic salmon (*Salmo salar* Linnaeus). We will also show how this approach was used to identify candidate protein and carbohydrate antigens for use in vaccines, in conjunction with whole animal assays.

2. Development of an *in-vivo* Growth System for *Aeromonas salmonicida*

It is well known that the conditions under which the bacterium are grown influence their gene expression and ultimately their phenotype (Dalsgaard *et al.* 1998). This was originally demonstrated for *A. salmonicida* grown within chambers in the host by Garduño *et al.* (1993). These authors demonstrated that cells grown under *in vivo* growth conditions had increased resistance to bacteriolysis, phagocytosis and oxidative killing. We developed a method for the *in vivo* growth of large volumes of *A. salmonicida* (Dacanay *et al.* 2003). Briefly this method involves the use of growth chambers made of autoclaved 12 to 14-kDa molecular-mass-cutoff dialysis tubes that are filled with *A. salmonicida* suspensions and surgically implanted into the abdominal cavity of Atlantic salmon for a period of 24 hours, then harvested. Bacteria grown within these implants have been used in studies of gene expression, for proteomics, carbohydrate analysis and for studying the host response to infection as described below.

3. Development of Genomics Resources and Tools for *Aeromonas salmonicida* subsp. *salmonicida* (A449)

The acquisition of full genomic sequences for pathogens has facilitated rapid advances

in infectious disease research. Genomic sequencing has facilitated the development of new methods of pathogen detection, our understanding of genetic relationships between species and investigations into the nature of host-pathogen interactions. Through the use of bioinformatics methods, which allow for genomic comparisons between species, putative virulence factors, as well as genes that encode proteins that may be suitable for use in vaccines can be identified. Genomic sequencing also facilitates the use of other technologies such as the development of knock-out mutants, and other genomics (e.g., microarray), and proteomic studies, all of which serve to increase our understanding of host-pathogen interactions and aid in the rational design of effective vaccines.

At the initiation of our research program there was little genomic information available for *A. salmonicida* or closely related species. In order to understand the infection process and possible interactions between *A. salmonicida* and its hosts, as well as to aid in the rational design of new vaccines the whole genome of wild-type strain A449 was sequenced and assembled (GenBank Accession #s NC_009348-009350). The A449 genome consists of a circular chromosome and five plasmids that encode more than 4700 genes (Boyd *et al.* 2003; Reith *et al.* unpublished). During this program a full genomic sequence became available for *Aeromonas hydrophila* (Seshadri *et al.* 2006). Comparison of the *Aeromonas salmonicida* genome with genomes of related organisms such as *A. hydrophila* enabled the identification of large numbers of genes encoding potential virulence factors (Boyd *et al.* 2003; Nash *et al.* 2006; Reith *et al.* unpublished). Virulence genes were targeted for future study and for use as vaccine candidates. Table 1 summarizes some of the results of the projects described above.

Genome arrays which contain large numbers of genes or whole genomes have

Table 1. Summary of *Aeromonas salmonicida* genes identified via genomic sequencing and knock-out mutants. We also elucidated the role of the expressed proteins in virulence

System	Gene(s)	Role	In vivo		Ex vivo	Reference
			Injection	Immersion		
Type I pili	<i>fimABCD</i>	Type I pilus subunit, tip and usher proteins	No change in virulence			
Reduced covert infection?	No change in virulence					
	Reduced adherence	Unpublished				
Type IV pili	<i>tapA</i>	Type IV pilus subunit	No change in virulence	Reduced virulence	No change	Boyd <i>et al.</i> 2008
Type III Secretion (TTSS)	<i>flp1</i>	Type IV pilus subunit	No change in virulence	No change in virulence		
	<i>ascC</i>	TTSS secretome protein	Avirulent	Avirulent	Altered macrophage cytokine profiles	Dacanay <i>et al.</i> 2006
	<i>aexT</i>	Secreted effector	Reduced virulence	No change in virulence	—	
	<i>aopH</i>	Secreted effector	Reduced virulence	No change in virulence	—	
	<i>aopO</i>	Secreted effector	No change in virulence	No change in virulence	—	
Iron sequestration	<i>fstB</i>	Anguibactin-like siderophore receptor	Avirulent	Avirulent	—	Unpublished
	<i>fstC</i>	Amonabactin-like siderophore receptor	No change in virulence	No change in virulence	—	
	<i>hupA</i>	Haemoprotein receptor		Reduced virulence	—	
Oxidative stress	<i>sodA</i>	Superoxide anion detoxification	No change in virulence	Reduced virulence	Increased enzyme levels in virulent strains	Dacanay <i>et al.</i> 2003 (unpublished)
	<i>sodB</i>	Superoxide anion detoxification	No change in virulence	Reduced virulence	Increased enzyme levels in virulent strains	Dacanay <i>et al.</i> 2003 (unpublished)
	<i>katA</i>	Hydrogen peroxide detoxification	No change in virulence	—	—	Unpublished

been used to study genomic variability among strains of a variety of bacterial pathogens through a process known as microarray-based comparative genomic hybridization (M-CGH) (Ong *et al.* 2004; Taboada *et al.* 2004). From the genomic sequence Nash *et al.* (2006) developed a DNA microarray of 2024 genes for *A. salmonicida* subsp. *salmonicida* and used M-CGH to compare a variety of *A. salmonicida* subspecies, strains and isolates obtained from different hosts and geographical locations. Using this technique these authors were able to show variable carriage of the virulence-associated genes and generally increased variation in gene content across sub-species and species boundaries. Variable genes included those encoding outer membrane proteins, pili, and flagella. Their data showed little correlation between geographic region and degree of variation for all isolates tested. The greatest variation was observed among genes associated with plasmids and transposons. However, many known virulence genes were common to all strains tested, including superoxide dismutase, and those associated with iron-restriction and sequestration.

Wang *et al.* (2007) examined the LPS structures of the same strains and isolates that were used by Nash *et al.* (2006) in their M-CGH studies. The carbohydrate analysis showed three distinct structural types. The separation of the isolates into the three LPS types corresponded to the clustering of the gene variation seen in the M-CGH studies (Nash *et al.* 2006). Wang *et al.* (2007) speculated that an LPS-based classification system of *A. salmonicida* sub-species consisting of two serologically distinct types: type A and non-type A, could be developed.

Identification of common proteins, carbohydrates, and other antigens is an important first step in identifying genes important in virulence, as well as vaccine candidates that are conserved across all strains known to cause disease. In addition the importance of some of these genes as virulence factors

was confirmed with knock-out mutants as described below.

4. Transcriptional, Proteomics and Biochemical Responses of *A. salmonicida* Grown under Selected Conditions: Understanding Virulence Mechanisms of *A. salmonicida* Using Mutants and Live Challenges

In order to investigate effects of host responses and other growth conditions on *A. salmonicida* we conducted studies of gene expression and protein expression, as well as carbohydrate analysis. These studies examined how the phenotype of *A. salmonicida* changed in response to the host factors (*in vivo* growth condition) by comparing *in vivo*-grown bacteria to bacteria grown under variable *in vitro* conditions such as high temperatures, and within iron-restricted media. Such data can aid understanding the host-pathogen interaction as well as in the selection and development of antigens for use in vaccines. These data also highlight that interpretation of data from studies of expression single genes or gene sets, proteins or carbohydrates must be taken within the context of the strain, subspecies, or isolate used.

For example, the *A. salmonicida* genomic DNA microarray was used to examine changes in transcription levels and proteomics was used to examine changes in the translation levels for *A. salmonicida* in response to iron-restricted *in vitro* and *in vivo* growth conditions (Brown *et al.* unpublished). Based upon this work it was possible to identify genes that are differentially expressed when the organism is under these two conditions. Not surprisingly many of the genes that were identified as differentially expressed were involved in the utilization of iron or heme. However, there were several genes which were upregulated only *in vivo*, including genes homologous to an ABC-type galactoside transporter and components of the lateral flagellar system. Comparison of

the expression data to proteomic data obtained from the same samples showed that for many of the genes and proteins there was a strong positive correlation between gene expression level and protein expression, thus confirming the conclusions on the roles of the proteins and genes in virulence and pathogenicity. These results demonstrate how microarray analysis of *A. salmonicida* under varied growth conditions can be used to determine how different host factors influence gene expression, as well as emphasize the importance that interpretation of data from studies of expression single genes or gene sets, proteins or carbohydrates must be taken within the context of the growth conditions.

Within our research program two dimensional gel electrophoresis (2DE)-based proteomics methods were used to identify outer membrane proteins (OMP) of *A. salmonicida*, as well as to describe changes in the OMP profile resulting from *in vitro* culture in low iron media and *in vivo* culture (Ebanks *et al.* 2004, 2005). Ebanks *et al.* (2005) were able to identify 76 proteins from a carbonate-enriched *A. salmonicida* membrane preparation which corresponded to approximately 60% of all of the protein spots that could be visualized by 2DE (pI 4–7). Within these were a number of proteins such as endolase, which due to their lack of a classical export sorting signals were not predicted to be OMPs, i.e., based on motif sequence. Ebanks *et al.* (2005) determined that they were in fact, OMPs, based on protein analysis. Being able to identify these proteins within the OMPs of *A. salmonicida* demonstrates the value of combining genomic and proteomic approaches. In another study in our research program, outer membrane protein profiles of *A. salmonicida* were compared between *in vitro*-grown bacteria and bacteria grown in low iron media and *in vivo* (Ebanks *et al.* 2004). With respect to the OMPs, growth under both iron-restricted conditions and *in vivo* caused a marked up-

regulation of two iron regulation systems (ferric siderophore and heme acquisition systems). Based on their results these authors felt that with respect to the OMPs that growth in low iron media was a good model system for *in vivo* growth. Microarray analysis of mRNA expressed in bacteria grown under the same conditions confirmed the results obtained in the proteomics experiments described above (Brown *et al.* unpublished). As these proteins were also seen to be expressed in avirulent strains of *A. salmonicida* under these conditions it is suggested that they may be necessary but not sufficient for bacterial virulence (Ebanks *et al.* 2004). Proteomics and RT-qPCR were also used to study the type III secretion system of *A. salmonicida* grown under selected conditions (Ebanks *et al.* 2006). In that study a variety of strains of *A. salmonicida* were grown *in vitro* at 16, 17 and 28°C, as well as *in vivo*. These authors demonstrated the increased expression, as well as assembly of, a functionally competent type III secretion system in cells grown at 28°C and in the presence of 0.19 to 0.39 M NaCl. Cells that were grown *in vivo*, in low iron media, at low pH or at high cell densities did not show increased expression of the type III secretion system.

Proteins are not the only virulence factors tested within the program. Wang *et al.* (2004) compared the structures of the capsular polysaccharide and lipopolysaccharide O-antigen of *A. salmonicida* grown on TBS and for 72 hours *in vivo*. This study demonstrated the presence of capsular polysaccharide and novel LPS O-chain polysaccharide in cells grown *in vivo* when compared to the *in vitro* grown bacterial inocula. These authors suggested that the use of *in vivo*-cultured cells for isolation and structural analysis of *A. salmonicida* polysaccharides leads to the development of more meaningful biological data.

The conclusions of Ebanks *et al.* (2004), based on proteomics data, were supported by the knock-out mutant results of Dacanay

et al. (2006). Those authors examined the contribution of the type III secretion system (TTSS) to virulence of *A. salmonicida*. The TTSS consists of an injection system (membrane proteins and a needle like structure) and a number of effector proteins that are transmitted into host cells, leading to modulation of components of both the innate and adaptive immune response that are advantages to the bacterium. In their study deletion mutant strains in the genes of the outer bacterial transmembrane pore (Δ ascC) and three effector genes (Δ aexT, Δ aopH, Δ aopO) were generated and the virulence of these strains tested by challenge of Atlantic salmon. These authors demonstrated that the TTSS was essential for virulence of *A. salmonicida* as the Δ ascC mutant strain was avirulent when challenged by both immersion and intraperitoneal (i.p.) injection. None of the three effector mutants showed significantly decrease virulence when compared to wild type in i.p. challenges. Interestingly, significantly lower morbidity of two of the mutant strains (Δ aexT and Δ aopH) when compared to wild type in immersion trials suggested a possible role of these effectors in host colonization. Furthermore fish that were i.p. challenged with the Δ ascC mutant were not protected upon re-challenge with the wild type strain. The authors concluded that the type III secretion system is absolutely essential for virulence of the bacterium, and that removal of individual effectors has little effect on virulence but has a significant effect on colonization.

As described above for the type III secretion systems, using information provided by the genomic sequence a number of mutant strains of *A. salmonicida* have been constructed and tested for virulence by live animal challenge (Table 1). For example, knock-out mutants for genes (*sodA*, *sodB* and *kata*) involved in protection against oxidative stress have been created and used in challenge trials with Atlantic salmon (J. M. Boyd and A. Dacanay, unpublished data).

When challenged by injection there was no significant change in virulence when compared to wild type, however the *sodA* and *sodB* mutants when used to challenge fish by immersion had reduced virulence (Table 1).

More recently, Boyd *et al.* (2008) examined the contribution of Type IV pili to the virulence of *A. salmonicida*. Pili are structures that allow bacteria to attach to surfaces such as host tissues and for this reason are important virulence factors for many pathogenic bacteria. In this work the authors constructed three mutant strains that were deficient in: a gene involved in encoding Tap pili (*tapA*), a gene involved in encoding FLP pili (*flpA*) and a double (*flpA-tapA*) mutant. Immersion challenge with the *tapA* and *flpA-tapA* mutants resulted in reduced cumulative mortality when compared to wild type. However, when challenged i.p. there was no significant difference between these mutants and the wild type. In contrast the Flp pili appear to contribute little to virulence as immersion and i.p. challenge with the *flpA* mutant resulted in mortalities that were not significantly different when compared to the wild-type (Boyd *et al.* 2008). The authors concluded that pili were essential factors of the initial adhesion and colonization processes.

Results within our research program showed that when determining the role of putative virulence factors of pathogens it is equally important to test various methods of presentation of the bacterial antigens, as seen by the studies cited above.

Knock-out mutants were also used to investigate pathways involved in host responses. For example, Fast *et al.* (2008, and unpublished data) using enriched cultures of head kidney macrophages exposed to the all of the type III secretion mutants, showed the same pattern of expression of Interleukin (IL)-8 and Tumour Necrosis Factor (TNF) when compared to cells exposed to wild type (parent) strain of *A. salmonicida*. With respect to expression of IL-10 and IL-12 the Δ ascC mutant strain (avirulent when

challenged by both immersion and i.p. injection) did not stimulate their expression. Exposure to the mutant Aop3 Δ (lacking 3 of the type III secretory system effectors) only stimulated IL-12 expression. Taken together these data suggest that the type III secretion system is not only essential for the virulence of the bacterium, but components of the system are also important for stimulation of host immune responses. IL-12 is an important cytokine released by infected macrophages, which stimulate T-helper type I cells driving towards cell-mediated immunity. As AscC Δ does not stimulate this cytokine, it may explain why infection with this knock-out does not provide protection from future A449 infection as immunological memory has not been triggered by the initial exposure. Furthermore, AscC Δ does not survive/replicate within macrophages as well as A449 (80% reduced).

Production of IL-10 by the wild type strain may assist in prolonging infection and down regulation of pro-inflammatory production and secretion, as IL-10 is inhibitory towards T_H-1 responses and generally towards inflammation, in mammals. Protection by Aop3 Δ therefore, would be expected due to stimulation of IL-12, but possibly in the absence of significant virulence (no IL-10), as seen in the wild type strain.

5. Transcriptional Responses of Atlantic Salmon to Infection with *A. salmonicida* and Chronic Stress

At the beginning of this research program there were relatively few genomic sequences available for Atlantic salmon. In order to characterize genes involved in the immune response of Atlantic salmon to infection with *A. salmonicida* reciprocal suppression subtractive hybridization (SSH) six cDNA libraries were constructed for liver, head kidney and spleen tissues following i.p. challenge (Tsoi *et al.* 2004). Control (uninfected, injected i.p. with saline only) cDNA was

subtracted against infected tissue, as well as the reciprocal subtractions, i.e., infected tissue was subtracted against that of the control fish. Tissues were kept separate, i.e., distinct libraries were each created for liver, head kidney, and spleen. This resulted in the identification of 1778 expressed sequence tags (ESTs) which were used to create a custom cDNA microarray that has been used to describe the transcriptional responses of Atlantic salmon following cohabitation challenge and Atlantic salmon macrophages exposed *in vitro* to *in vivo* and *in vitro* cultured *A. salmonicida* (Douglas *et al.* 2003; Ewart *et al.* 2005, 2007). Ewart *et al.* (2005) identified differentially expressed genes in head kidney, spleen and liver samples obtained from fish after 7 and 13 days of co-habitation challenge. Using this microarray and RT-qPCR validation they were able to identify in addition to known immune-related genes some unusual genes that were highly up-regulated. These genes were homologous to pufferfish plasma high-affinity saxitoxin-binding protein and snake peptide neurotoxin (also known as differentially regulated trout protein). They were also able to identify a large number of unknown genes that were also differentially regulated. This same microarray was used to examine differences in the transcriptional profile of primary isolates of Atlantic salmon macrophages infected with *in vivo* and *in vitro* cultured *A. salmonicida* (Ewart *et al.* 2007).

As mentioned previously there are significant and biologically meaningful differences in the phenotype of *A. salmonicida* culture under these two conditions (Ebanks *et al.* 2004; Dacanay *et al.* 2006; Brown *et al.* unpublished). Examination of gene transcription patterns at 0.5, 1 and 2 hours post-infection revealed commonalities, as well as significant differences in gene expression patterns between macrophages exposed to *in vivo* and *in vitro* grown cells. Differences in expression of genes such as JunB and TNF- α that were evident 30 minutes after

infection led these authors to suggest that these differences may be due to differential production by the bacteria of diffusible products (Ewart *et al.* 2007). As in the earlier study a number of unknown genes were also identified to be differentially regulated making them interesting candidates for further study. Overall the use of microarrays to examine the transcriptional response of *A. salmonicida* has led to the development of more questions than answers.

Fast *et al.* (unpublished data) isolated macrophages from Atlantic salmon and incubated them with the wild-type parent strain and with two type III secretion system knockout mutants, Aop3Δ (deficient in three of the type III secretion system effector genes) and AscCΔ (deficient in the gene encoding the outer-membrane pore of the type III secretion apparatus). The authors also incubated Salmon Head Kidney (SHK) cell culture with the same wild-type and mutant strains. Gene expression within salmon cells was measured using Real-Time qPCR. Their results showed *A. salmonicida* infection stimulates inflammatory gene expression and that expression of IL-8 and TNF are not affected by mutations within the type III secretion system. However, they did observe that the outer-pore membrane secretion apparatus seems to be essential for expression of IL-12 and IL-10, and the three effector genes are also essential for IL-10.

While investigating the mechanisms of host-pathogen interactions it is extremely important to determine the effect of the physiological state of the host on those interactions. The fish species involved in these investigations are important to aquaculture, and are subjected to anthropogenic and natural stressors, such as handling, grading, vaccinations, and fluctuations in temperature and water chemistry. These and other factors, contribute to the physiological state of the animal, and can directly or indirectly affect their ability to respond to pathogens.

Fast *et al.* (2008) showed that repeated

handling stress resulted in increased constitutive expression of IL- β in head kidney macrophages from Atlantic salmon, followed by decreased stimulation of leukocytes by extracellular antigens (LPS) and decreased leukocyte survival. This is important information and highlights the need for further studies. Aquaculture fish species can be subjected to acute and chronic stressors and it is essential to understand the effect of those stressors on the animals' ability to mount protective immune responses against pathogens. If the efficacy of vaccines is compromised because of the hosts' immune state, this will add to the cost of production to the farmer and will also lead to increased losses due to infection. If this can be avoided by avoiding stressors at the time of vaccination (for example), this would prove to be a low-cost approach to aquatic animal health management.

6. Metabolomics Responses of Atlantic Salmon to Infection with *A. salmonicida* and Vaccination

Metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind"—specifically, the study of their small-molecule metabolite profiles (Davis 2005). The metabolome represents the collection of all metabolites in a biological organism, which are the end products of its gene expression. Solanky *et al.* (2005) used NMR-based metabolomics to show that metabolites within the plasma of Atlantic could clearly separate into distinct groups: those fish that had been vaccinated with killed *Aeromonas salmonicida* bacterin, those that had been challenged, and those that had survived the challenge. Dacanay *et al.* (2006) showed that this technique can be used to investigate host response to specific virulence factors in pathogens, using plasma from fish challenged with selected knock-out mutants, and with the wild-type parent strain. Using

NMR-based metabolomics, the authors compared the plasma from vaccinated salmon immersion-challenged with the wild-type parent strain with plasma from fish challenged with the avirulent mutant strain AscCA. Their results showed that the plasma metabolite profiles correlated with the protective immune response, i.e., where a protective immune response was observed in whole animal challenge, the plasma profiles clustered separately. When there was no significant protection, the plasma profiles were indistinguishable (Dacanay *et al.* 2006). This study clearly demonstrates that metabolomics technologies have potential to provide non-invasive assays to monitor host immune responses.

7. Summary and Future Directions

In using these and other techniques, we have been able to identify virulence proteins and carbohydrates from *Aeromonas salmonicida*, and have been able to develop more effective vaccine candidates against the pathogen. Moreover, in examining host immune responses at the molecular level, we have developed antigen delivery strategies that target the immunomodulatory mechanisms of the host.

Our investigations into the molecular mechanisms of salmonid stress-related genes and disease susceptibility will allow us to develop more effective tools for husbandry. These and other studies will also aid in targeting specific disease-resistance markers for family selection and comprehensive breeding programs.

However, a word of caution is warranted. The tools and technologies as described above are very powerful, yet remain only tools. Genome sequencing, cDNA microarrays, EST libraries, etc., generate huge data sets, usually with hundreds, if not thousands of unknown genes and proteins. Currently, gene function is often tentatively assigned on the basis of BLAST annotation

and homology to known genes (e.g., GO annotation). If useful tools and knowledge is to be derived from the use of these genomics technologies, then it must be validated by complementary studies of the biological function of the genes and proteins. Knock-out mutants, transformative cell lines, and recombinant protein technologies are all useful in this sense. Ultimately, however, whole animal trials validate any assumptions made via the genomics and proteomics approaches described above. We have taken this systems approach in our research program.

Also required is an understanding of the physiological responses of the host, within the context of nutrition, stress, environmental effects. Equally important is knowledge of the biology of the pathogen: its genetic variability, its gene expression within the context of its environment.

As can be seen in describing the early and then later work of our team and others, these genomics and proteomics technologies can also be used to push earlier studies much further. Thus the techniques will allow us to achieve a greater understanding of the mechanisms of systems and processes that had been previously described at an higher organismal level.

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Progress on Processing and Utilization of Aquatic Products in China

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The development of fisheries in China has made constant progress and the output of fisheries products reached 52.5×10^6 tons in 2006. Changes in aquatic products processing and utilization have gradually developed along with the development of modern science and technology, improvement of distribution and marketing systems, and globalization of the world's economy. Attention to the quality and safety of aquatic products has been paid at all levels, and maintaining freshness and constructing processing systems have been enhanced gradually in China.

The general trend of aquatic product processing and utilization in China has been to select and breed suitable species as raw material for food processing, to process and create new products on an industrial scale, to develop safety/healthy/functional/convenience/recreational foods for the domestic market, to apply novel technologies from research to develop high quality/standard/suitable products for the international market, etc. In order to satisfy consumers' requirements and meet the needs for industrial development, we had better realize integral management among farming, processing and selling of aquatic products, specialize the processing species, make production based on raw materials, standardize the quality control system and scientific management on production, advance the processing technology, with large-scale, close network, utilizing more information, and marketing guidance, etc.

KEYWORDS aquatic products; processing; utilization; quality and safety

1. Current Situation and Review of Fisheries in China

Fishery production in China keeps increasing steadily. In 2006, the yield of fishery products reached to 52.5×10^6 tons, and a total output value of 820×10^9 RMB, while

the net income of a fisherman reached to 6,100 RMB. The total quantity of import and export was 6.337×10^6 tons, valued at 13.66×10^9 USD in 2006. Of which, the quantity of exports was 3.015×10^6 tons, and it amounted to 9.36×10^9 USD, increasing by 17.4 and 18.7% compared with the previous year, respectively, the import quantity

was 3.322×10^6 tons, and the amount of imports was 4.3×10^9 USD, decreasing by 9.2 and 4.4% from the previous year, respectively. Trade surplus was 5.06×10^9 USD, increasing by 1.63×10^9 USD compared with the previous year. The amount of exports of fishery products accounts for 30.2% of the total exports of agricultural products, and continues in first place of the large amount of agricultural products' export. From the information presented at the Fishery Specialized Meeting of the National Agriculture Workshop, according to the central government's requirement of constructing a resource-saving and environment-friendly society, we implement negative growth in marine fishing output, and vigorously explore changes in the methods of cultivation, so the quality of aquaculture and fishery development has been improved to some extent. The practice proved that only by implementing scientific developments, insisting on changing the main direction of the growth of fisheries, and unceasingly impelling the fishermen to transform from a quantity to an ecological quality style of fishing, can implement the continuous and healthy development of fisheries.

2. Current Situation of Aquatic Product Processing and Utilization in China

The processing and utilization of aquatic products are the extension of commercial fishing and aquaculture, and it is a bridge which links aquaculture and the fishery market of high value-added products. As the living standard of the Chinese people has significantly improved, and the consumption level enhances unceasingly with changes in the dietary preferences, there is a high demand for quality foods, especially high quality aquatic products. The demands for aquatic products present new characteristics of diversification, convenience, nutrition, safety, and individuality.

The demanded quantity of fishery products for the domestic and international market rises unceasingly and the export of high quality fishery products, increases to some extent compared to before. Besides live/fresh fishery products, as well as frozen, and/or dried, salted and/or smoked products are still the main processed fishery products in the domestic market, and the quantity has clearly increased. Because of the relatively lower processing cost of these three kinds of products, and with the improvement of transportation conditions and the establishment of the food cold-chain, these kinds of products will still dominate the consumer market for a long time in the future. The main products are entire frozen whole fish, frozen fish fillet, fresh fish fillet, salted and/or smoked products, canned foods, surimi products, roasted fish fillet, and so on. For the international export trade of aquatic products, besides the processed materials from buyers and feeding material in China, the processed goods for export include roast eel, roast laver, squid filament, tilapia fillet, channel catfish fillet, shrimp, naked body of squid, octopus, crayfish, canned mud fish food, crab meat, etc. These products are mainly exported to Japan, the USA, Korea and the EU, and the product-exporting market is unitary in which the roast eel is mainly supplied to Japan, tilapia fillet, channel catfish fillet and crayfish are mainly supplied to the USA, and roast laver is mainly supplied to the USA and Korea. In addition, the processing of value-added fishery products has also been enhanced to a certain degree, but in China about a 30% processing rate of fishery products was still the average level in the world, while the processing rate of aquatic products has reached 50–70% in some developed countries.

Fishery competent departments have paid more attention to the processing and utilization industry of aquatic products, and the regional distribution is clear in China. Important bases for processing aquatic

products have already been established in coastal provinces and cities, such as in Liaoning Province (Dalian), Shandong Province (Qingdao, Yantai, Weihai), Jiangsu Province (Yancheng, Nantong), Zhejiang Province (Zhoushan, Ningbo, Wenzhou), Fujian Province (Xiamen, Fuzhou, Dongshan), Guangdong Province (Zhanjiang, Shantou, Chaozhou), Guangxi Province (Beihai), and so on. But interior provinces, such as Hubei, Anhui, Jiangxi, have also gradually established their status as important areas for processing and utilization of freshwater fishes. In 2006, the overall situation of fishery product processing enterprises improved, and there had been a batch of highly profitable enterprises, such as the Dalian Zhangzidao Fishery Limited Company, whose deep processing project of marine products was awarded the honor of "The Demonstration Project of National Agricultural Product Deep-processing Special Project". The Dalian Zhangzidao Fishery Limited Company is the only deep-processing enterprise of marine products, among the 38 demonstration project enterprises announced at this congress. Through the exploratory development and expansion in the past few years, this company has led in the deep-processing of the marine products in the interior of our country. It has achieved international standards which provided the impetus for advancing seafood industrialization development and the industrial promotion in China.

Advances in processing technology and research into fishery product processing have also made marked progress, for example, in developing the natural food additive of phycoerythrin derived from red algae, production of alginate products derived from seaweeds, extraction of natural compounds from marine life and fishery processing offal, especially bioactive components, functional foods and medicines using aquatic products as raw material. The technology to sustain the freshness of aquatic products has

grown rapidly, especially in the food cold-chain. The preserving and processing methods of freshwater fishes have been improved unceasingly, in particular the level of sustaining the freshness of marine fish has achieved or approached international level. The aquatic product processing and the finished products present the tendency of comprehensiveness, high value, and multi-varieties, lengthening the industrial chain and enhancing the comprehensive benefit of fishery production. With the development of biochemistry, fermentation technology and applied technologies, the utilization level of low-valued fishery products and processing offal will be further enhanced.

The Ministry of Science and Technology, Ministry of Agriculture and all levels of local authorities in China have attached more importance and technology investment to the processing and utilization technologies of aquatic products. For example, the 11th Five-year Significant Project supported by the Ministry of Science and Technology has focused on the "Critical technology research and industrialization development of food processing—The development of new products made from large amounts of low-value fishery products and the industrialization demonstration of ultra-low temperature quickly frozen equipment", and "Critical technologies of food safety—Synthesis demonstration of the safety production of aquatic products". The ocean technology area in the "863" Plan directly targetting marine animals and plant resources has objectives to develop highly effective processing of marine products and new types of high value-added products, and conduct research into the quality and safety control of seafood, and develop new seafood (except functional foods) and high value-added products, for example, refer to the Year 2006 Topics "Deep-processing Technologies of the Marine Products". The implementation of these projects is expected to further promote the processing and utilization technical levels of fishery products

in China, to help some of the processing mainstay enterprises and play the role of demonstration and guidance, and to instill new vigor into the fast development of the fishery product processing industry.

As aquatic products are special food resources, they have the inherent characteristics of perishability, seasonality, regionality, and concentricity. At the same time, there are large differences from terrestrial food resources, and the facility condition of fishery product processing is generally simple and crude, the application of equipment is backward, efficiency is low, energy consumption is high, the technical craft is weak, types of products are few, the strength of the research and development of new products is not enough, the product package is simple and crude, and they are still at the stage of keeping-freshness and preservation of the traditional raw materials. So the simple processing and utilization of fishery products and small-scale manufacturing of the fishery foods results in failing to form a good management system of industrialized production. For example, edible freshwater fish that accounts for a large portion of China's fishery product are consumed mainly in live/fresh state, and this condition has obviously not been changed yet. Insufficient application of new technologies, new techniques and new equipment hinder the improvement of aquatic product processing. Faced with the ever-varying situation in consumption level and product structure, the freshness retention and preserving technologies of fishery products in distribution also requires thorough research and innovation. The history of the aquatic product processing industry was impressive and the system was self-formed, while its basic research started later, and applied and high-tech research were weaker. The mutual permeation among disciplines is still insufficient, lacking independent technological innovation. The transformation efficiency of achievement is low, lacking the technical pedestal and reserve

adapted for supporting the fast development of the aquatic product processing industry.

In general, the situation of aquatic product processing and utilization in China still has a large disparity when compared to the developed countries, and also has a large disparity when compared to domestic agricultural products processing industry, for example, cereals, oils, livestock, poultry and milk. However, the development potential of processing and utilization of aquatic products is certainly huge.

3. Developmental Tendency of Aquatic Product Processing and Utilization

Aquatic products are an important source of animal protein in human food, which plays a vital role in promoting human health. Along with the enhancement of people's living standards, aquatic products have received more and more favorable attention due to their high levels of protein and many kinds of highly unsaturated fatty acids. According to the "World Fishery Forecast for 2020" published by the American International Food Policy Research Society and World Fishery Center, the quantity of aquatic product consumption in the world will reach 127.8 million tons (mainly that of fish and shrimp). The demand for aquatic products in developed countries is still increasing. If bird and animal flu epidemics erupt in some nations, the quantity of consumption of aquatic product will increase; the cultivation of specialty and superior aquatic products has increased on a large scale in China. Therefore the issues of food quality and safety require a high level of attention. Because the export of aquatic products is sufficient, and the quality is unceasingly enhanced, Chinese aquatic products receive more and more favor due to their high quality and reasonable prices. Meanwhile, the average consumption quantity per capita of aquatic products is not so high, while the national

aquatic product ultimate output has reached 51.0165 million tons; annual aquatic product consumption per capita is 39.02 kilograms, in which aquatic product consumption per urban resident had reached approximately 16 kilograms (while it is 92 kilograms in Japan). But in rural areas, the consumption amounts to only one-third of that of the cities. Aquatic product average consumption per capita in China will reach to 44 kilograms at the end of "the 11th Five-Year Plan", and the average consumption per person will reach to 12 kilograms. Obviously, the broad countryside consumer market of aquatic products has not yet been truly established and developed. Along with the stable development of the economy in China, the rapid expansion of the tourism leisure industry, there has been rapid development of the export-oriented fishery and net loads of aquatic products entering supermarkets. Consequently, potential need for high quality, nutritional and safe aquatic products in the domestic market is greater. The gap in the international aquatic product consumer market will mainly rely on the supplementation of aquacultured products in the future because marine bio-resources are declining in the world. Fisheries in China have some advantages such as large-scale cultivation and production, advanced technology, rich labor force resources and strong processing ability. The growing demand in both domestic and international markets for aquatic products will contribute to China's competitive advantage and provide a broad opportunity for China's fishery development as well as it becoming a world fishery powerful nation.

According to the request from the central government for comprehensive construction of a well-off society and realization of the fishery modernization, during the period of the "11th Five-Year Plan", the fishery development must diligently complete four big basic tasks: guarantee secure supplies of aquatic products, a steady increase in income of fishermen (farmers), promote the sustain-

able development of fishery, and promote the harmonious development of society in rural areas supporting fishing. In comparison with the traditional fishery, the modern fishery follows the idea of resource conservation, environment amity and sustainable development, taking in modern science, technology and facility equipment in support, utilizing the advanced production modes and management means, forming an integrated industrial system of agriculture, industry and trade, as well as producing, processing and selling, and thus realizing a fishery industry that shapes the economy, ecology and social efficiency.

The development of an aquatic product processing industry will not only have an impact on fishery production but also promote the entire fishery economy. So it can impel transformation from a traditional to modern fishery, with extensive management to intensive farming, sole production to multiplex production; relieve bottlenecks caused by delays in the processing industry, and encourage economic growth. Aquatic product processing in one place will not only lead to the formation of multi-links among industry groups, realizing industrialized and serialized production, and making the regional economy prosperous; but also arrange re-employment for fisherman, maintain social stability as well as drive coordinated development among the inter-linked industries.

The developing guidelines of aquatic product processing will focus on technical innovation in refined and deep processing, raising comprehensive utilization levels and benefits. Based on the demands of international and domestic markets, it is necessary to adjust the product structure, and advance the establishment of a processing system for freshwater fish, shellfish, pelagic fish and algae, thus developing positively aquatic products which are nutritiously high, low in fat, non-polluting and environmentally friendly. We need to cultivate one batch of leading enterprises of aquatic product

processing which has large-scale management, a strong ability of management, good economic efficiency and with an independent brand name. We should construct and develop the logistics for aquatic products, strengthen the construction and management of aquatic product market, develop refined and deep processing of aquatic products, develop and utilize frozen surimi in particular, develop top grade, compound products and aquatic biological healthy products, which are all value-added. We should pay special attention to the comprehensive utilization of freshwater fish and pelagic fish, construction of bases, and matching cold-chain facilities for purifying and processing shellfish. The promotion of processing and exportation of aquatic products should be implemented. We can unceasingly enhance the exportation proportion of refined and deep processing products and can expand the international market further by developing well-known branded goods, supporting key enterprises, expanding the export trade of processed cultured products, steadily developing processing with buyer's materials steadily, and simultaneously persisting in the exportation of traditional primary products and refined and deep processing products.

We should promote the quality and safety level of aquatic products, because sea foods are different from other industrial consumables. When we are faced with a food technology barrier, we can't only depend on government negotiation or file a lawsuit with the WTO, but also depend on the product's prestige and quality to convince consumers from import country. We can make our exported aquatic product become "disburden food", "exempting from inspection food" through promoting quality. Therefore, we should continue to strengthen the development construction of a standard system for aquatic products, comprehensively carry out the HACCP management system, form and revise a batch of standards for quality, variety, production technology and the ecologi-

cal environment of aquatic products. Gradual networking with international circles and cultivating famous brand aquatic products are needed to enhance the products' competitiveness.

According to the actual and present situation of our country (bottleneck), the development level overseas (tendency) and the development needs of the 11th Five-Year Plan in technical industry (goal), processing and utilization of aquatic products had better conduct research and development of special topics as follows: processing technology of large-scale and ultra-low temperature freezing technology in distant seas to maintain freshness and the research of its equipment (the value storage in marine fish is of high priority, rapidly freezing large-sized fish is difficult, researching storage processing equipment in small-sized efficient ships is difficult); effective developed technology of small-sized but high production, fat-rich pelagic fish (small, fat-rich fish belonged to low value fish traditionally, however, in fact their healthy nutritive value is higher, so it is crucial to control the quality change caused by oxidative rancidity); processing and utilization of surimi (including the suitability of raw materials, new processing craft for surimi, making intermediate material into products, market management and development of surimi-based products); freshness-keeping and purification technology and equipment for shellfish (mainly referring to circulation and freshness, and the key technology is the guarantee of safety and effective purification that can reduce pollution); refined and deep processing technology for shrimp (nowadays convenience frozen preserved foods have changed the present situation of a sole variety of frozen shelled shrimp); comprehensive utilization technology of seafood wastes (the reason being that massive inedible wastes like fish heads, fish skin, internal organs, fish bones, carapace and shells, etc. are generated during processing, their full and

highly effective development and utilization may not only change waste into treasure but also reduce pollution, it may also clean production systems and increase benefit. Research and exploiting resources for collagen, highly unsaturated fatty acids, active peptide, polysaccharide and animal calcium are important); new effective development and utilization technology of algae (including functional food, new energy, technical drawings); effective development of marine micro-organism (including screening and utilization of superiority function fungi, control of harmful fungi, utilization of micro-organism metabolites, etc.); high-tech's application in keeping freshness, processing, and comprehensive utilization of marine products (including fast quality testing, hurdle technology, Ultra-high pressure technology,

supercritical CO₂ fluid extraction, ultra-micro pulverization, nano-technology, ohmic heating, microwave technology, freeze-drying in vacuum, and so on).

In conclusion, the processing and utilization of aquatic products are a new strategic opportunity period, which will also face more challenges. We expected to achieve greater progress and breakthroughs in some aspects: developing and utilizing aquatic biological resources with rich and high productivity, promoting and applying novel technologies for modern food processing, researching and developing new healthy foods, perfecting and enhancing the supervise and control level of quality and safety management for aquatic products, making a enormous contribution to sustainable development of fishery and food industry.

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Molecular Identification of Species and the Geographic Origin of Seafood

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Species and origin identification techniques are required for food labeling regulations to differentiate commercially important fish, shellfish, and seaweed species, because many of the distinguishing morphological features are no longer identifiable after food processing. In Japan, the Law Concerning Standardization and Proper Labeling of Agricultural and Forestry Products (JAS Law) has been establishing food quality labeling standards for foods and beverages. In perishable foods, product names, the production site, and country of origin must appear on the food label. In executing such functions, biochemical and DNA-based genetic techniques for rapid but critical identification of fish species and population have been investigated to determine whether the food labels describe regally correct information.

KEYWORDS species identification; origin identification; food labeling; trace element; mitochondrial DNA; mass peptide mapping

1. Introduction

Rapid identification techniques of species and geographical origin are the current requirement for the purpose of fraudulent labeling prevention and food regulation control with the development of quality assurance systems. Differentiation of commercially important fish and shellfish species of

various origins are of importance for the benefit of the product consumers. Identification of species used in preparing processed products is difficult because many of the distinguishing morphological features are no longer visible after processing. As price differences exist among various species and populations in manufacturing seafood products, negative substitution of cheaper materials from fish and shellfish with more

expensive ones in processed products is possible. Such undeclared substitution of the food constitutes in the processed products would not only be in contravention of food legislation but would mislead consumer interests as they choose products based on the labeling information.

In Japan, the Law Concerning Standardization and Proper Labeling of Agricultural and Forestry Products (JAS Law) has been establishing quality labeling standards for foods and beverages, and imposes them on manufacturers and others to assist consumers in selecting goods. In the case of perishable foods, the product name, production site, and country of origin particularly for imported products must be incorporated in the label messages. This rule states the definitions, requirements for consumer notification, product marking, and record-keeping responsibilities of retailers and suppliers with respect to production sites and country of origin for the labeling of raw materials for processed foods. Consequently, for imported marine products, country of origin labeling for processed foods is required. Thus, the prevention of fraudulent labeling of seafood

constitutes and their products is a reasonable challenge in line with the maintenance of food regulatory control and development of quality assurance systems. The development of biochemical and DNA-based genetic methods for species and product identification are of practical importance to certify correct food labeling practice presence in the marketplace (Table 1).

2. Species Identification Techniques for Food Labeling

Rapid and authentic species identification techniques are required to detect fraudulent labeling with the view of conferring better utilization of processed foods made by using commercially important fish species. It has recently been reported that more than 200 species are used as raw materials for marine products production in Japan, and closely related species harvested from in other countries are also used for marine products development. Since food labeling of the product name, production site, and country of origin is used for such marine products, analytical methods for examining the

Table 1. Species and origin identification techniques for marine products in Japan

Species and origin	Types of food or tissues used for analysis
mtDNA analysis	
- Japanese eel and Atlantic eel	grilled eel fillet
- Japanese horse mackerel and Atlantic horse mackerel	salted, dried fish
- Pacific mackerel, spotted mackerel, and Atlantic mackerel	salted fish and fermented fish
- Alaska pollack and Pacific cod, and Atlantic cod	seasoned egg
- Japanese sea basses and Nile perch	raw meat
- Red king crab and blue king crab	boiled and frozen-stored meat
- Pacific and Atlantic bluefin tuna, and other tuna species	raw meat or frozen-stored meat
- Sea urchin imported from North and South America	frozen-stored gonad
Peptide mass mapping	
- tuna species and skipjack tuna	raw meat, canned and others
Trace elemental analysis	
- Japanese eel from Japan, Taiwan, and China	bone of live fish
- Littleneck clam from Japan, Korea, and China	legs and shells of live shellfish
- Chinook salmon from Japan, Canada, Chili, and Russia	muscle from fresh or frozen fish

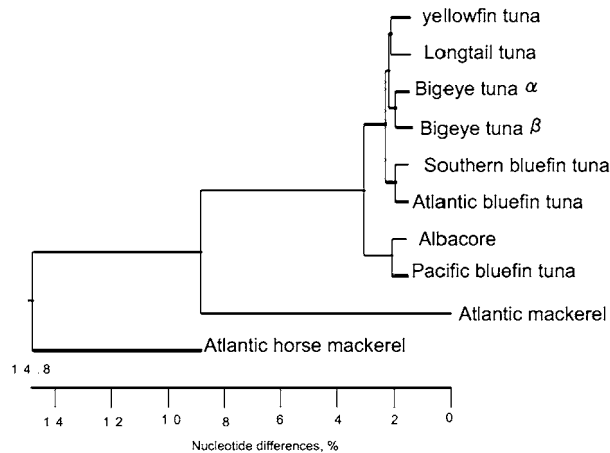


Fig. 1. Molecular phylogenetic tree of tuna species based on complete mtDNA sequences. The complete nucleotide sequences of mitochondrial genome of every tuna species were determined, and their phylogenetic relationship was analyzed using the CRISTAL W program. DNA analysis showed genetic differences and relationship among these tuna species. We used these data for species identification techniques.

authentication of species name and origin used are expected for food regulation and marketing surveys of marine products. Several fish species have been well investigated to develop the analytical methods (Table 1).

2.1. Eel

In recent years, Japan has been occupying the most important eel market in the world, with an annual demand of 120,000 tons. The country annually produces approximately 20,000 tons of Japanese eel (*Anguilla japonica*), which is still insufficient to reach up-to the mark of domestic demand (National Marine Fisheries Service 2005). The difference in the form of the amount between eel demand and national production is subjected to be imported from China and Taiwan as either live fish and processed foods (e.g., grilled eel fillet *kabayaki*). Over the last four decades, however, the juvenile *A. japonica* catch in Asia considerably fell down and prices for juvenile eel rose enormously. In China, to meet eel farm requirements, juvenile *A. anguilla* are imported

from Europe. Thus, European eel are produced mainly in China and exported to Japan as a processed food. A PCR-RFLP technique using the mitochondrial cytochrome b gene (*cyt b*) was developed to identify *A. anguilla* produced in China (Wakao *et al.* 1999). This PCR-RFLP technique was used to survey the food labels of commercial eel products to discriminate against processed products made of *A. anguilla* imported from China. Thus, the PCR-RFLP technique can be applied to develop inspection programs for other species to verify correct labeling. Sezaki *et al.* (2005) reported a very simple method to distinguish the above two eel species by PCR with a species-specific primer constructed based on a single nucleotide polymorphism. PCR products show two bands for *A. japonica* and one band for *A. anguilla* in the agarose gel electrophoresis. Furthermore, Itoi *et al.* (2005) detected single-nucleotide polymorphisms with partial sequences of the mitochondrial 16S rRNA gene to distinguish between the two eel species *A. japonica* and *A. anguilla*. They developed species-specific TaqMan minor



Fig. 2. Salted, dried fish of two distinct horse mackerel species. *Trachurus japonicus* is caught in Japan, the annual catch is about 210 thousand tones. Atlantic horse mackerels are imported from European countries in frozen state, annually 43 thousand tones.

groove binder (MGB) probes showing different fluorescence intensity between two eel species.

2.2. Tuna

Price differences exist among the various species of tuna, therefore there are many possibilities for substitution with cheaper species with more expensive one. Such undeclared substitution would not only be in contravention of food legislation by the JAS Law but would mislead consumers' right as they buy the content of products. In line with this, Chow *et al.* (2003) developed a PCR-RFLP method to identify *Thunnus* species using *cyt b* and the flanking regions of the mitochondrial ATPase and CO III genes. This method was successfully used in a market survey for the identification of fresh tuna products by the Ministry of Agriculture, Forestry and Fisheries following the JAS Act of Japan. We determined and compared complete mitochondrial (mt) DNA sequences for tuna species and found various nucleotide substi-

tutions between two pairs of tuna species (Fig. 1). Species-specific DNA sequences could be used to develop novel species identification techniques using DNA array analysis.

2.3. Horse mackerel

Japanese horse mackerel (*Trachurus japonicus*) and Atlantic horse mackerel (*T. trachurus*) are the most commercially important fish among the 14 *Trachurus* species, and Japan occupied its main global market. The *T. japonicus* is caught around Japan, and frozen *T. trachurus* is imported from the Netherlands, Norway, and Ireland as raw materials for salted and dried fish products production (Fig. 2). To characterize and identify mtDNA nucleotide sequence variation in these two *Trachurus* species, the complete mtDNA sequence of *T. trachurus* was determined (Takashima *et al.* 2006). Comparing the determined nucleotide sequences, a PCR-RFLP method was developed to differentiate between these two species (Fig. 3). The primer pair corresponding to ND5 was designed to amplify a 360-bp fragment, and following digestion with *Eco* RI, the PCR product for *T. japonicus* resulted in 93- and 267-bp fragments, whereas *T. trachurus* lacked a restriction site for *Eco* RI. In contrast, after digestion with *Hin* FI, the *T. trachurus* PCR product yielded 44-, 84-, and 232-bp fragments, while the *T. japonicus* product was not digested.

2.4. Alaska pollack and related fishes

A salted and seasoned fish roe product, karashimentaike is processed from the Alaska pollack (*Theragra chalcogramma*) ovary according to the fair trade competition agreement authorized by the Fair Trade Commission of Japan. However, ovaries of other fish species, such as Pacific cod (*Gadus macrocephalus*), Atlantic cod (*Gadus morhua*), Southern blue whiting (*Micromesistius australis*), and blue whiting (*Micromesistius poutassou*) are mixed in

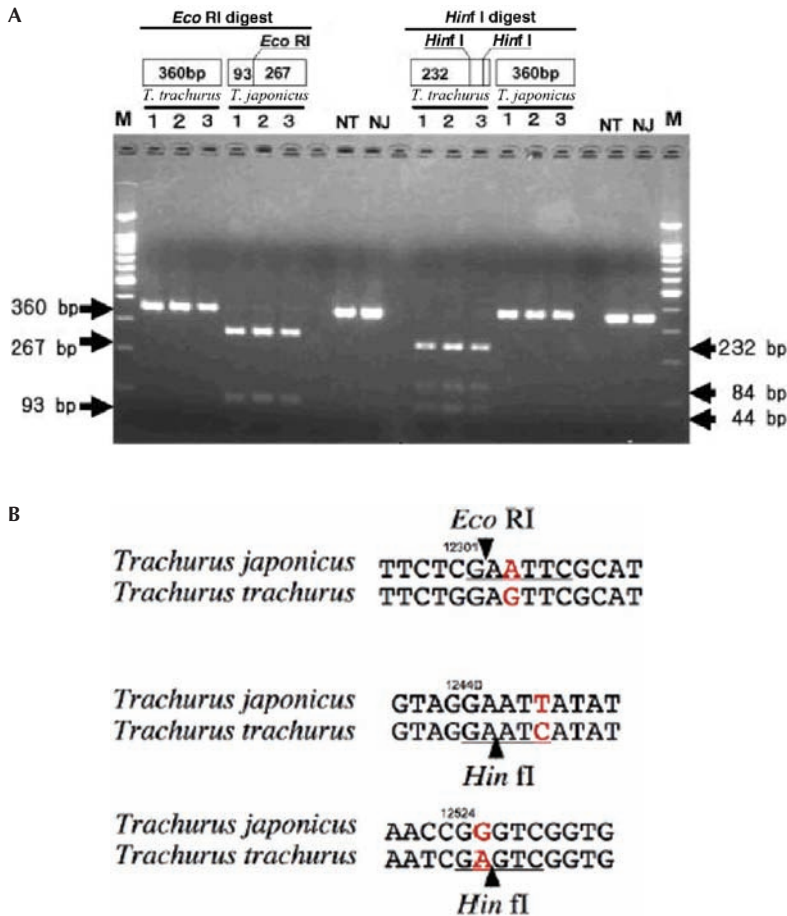


Fig. 3. Species identification techniques for two commercially important horse mackerel species. Three species-specific nucleotide differences at restriction enzyme recognition sites were found in the mtDNA. DNA was extracted by protease digestion and amplified by following polymerase chain reaction with specific DNA primers. After cutting by restriction enzyme of DNA, digested DNA was applied to an agarose gel electrophoresis. Lane M: 100 bp ladder DNA marker; lane 1–3: individual numbers; lane NT: undigested DNA of *T. trachurus*; lane NJ: undigested DNA of *T. japonicus*.

such products. To determine the concerned fish species of these products, we designed a species-specific primer set for Alaska pollack by PCR amplification of a 255-bp fragment encoding the mitochondrial ATP synthase F_0 subunit-6 gene. We also designed a species-specific primer set for *Micromesistius* species (*M. australis* and *M. poutassou*) by PCR amplification of a 223-bp fragment encoding the mitochondrial

cyt b. These PCR-based methods for Alaska pollack and *Micromesistius* species were successfully used for species identification of eight commercially important cod fishes and six fish roe products; the food labeling of raw materials was confirmed to be correct. Therefore, fish species identification techniques for cod roe products were developed using species-specific PCR primers to identify fish species even for a single egg.

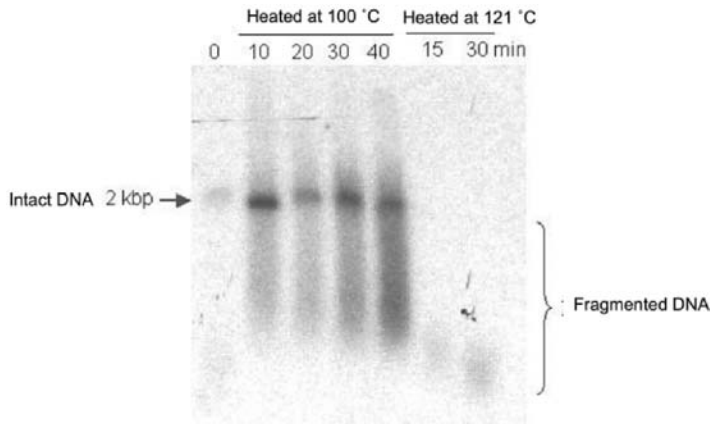


Fig. 4. Southern blotting of 2 kbp DNA fragment in processed fish meat. After the muscles of Japanese horse mackerel were processed at 100°C or 121°C, DNA was extracted, hydrolyzed with *Bam* H1, and then applied to gel electrophoresis. A specific mtDNA fragment DNA was detected with a DNA probe for the *cyt b* gene. The mtDNA was decomposed to small fragments by heating at 121°C for 15–30 min. Therefore, small fragmented DNA is applicable for DNA based analysis.

DNA-based species identification techniques are useful for processed marine products as well as fresh raw materials. The examined degradation patterns of 2 kb-DNA under various heating conditions of food processing, resulted in decomposed smaller sized fragments during heating at 121°C for 15 or 30 minutes (Fig. 4). Therefore, DNA analysis using short mtDNA sequences (<500 bp) was used for steamed, baked, and canned fish products identification. Furthermore, such DNA-based species identification technique using short mtDNA sequences was adapted to identify two related eel species according to the JAS Act of Japan.

3. Elemental and Other Chemical Composition Analyses

Recently, false labeling problems have been encountered in existing eel marketing in Japan. Live Japanese eels imported from Taiwan were illegally sold under Japan originated labeling. Biochemical analytical techniques using multiple trace elemental composition and vitamin K and its metabolites

concerning origin identification was practically used to differentiate the geographical origin of these Japanese eels.

Multivariate elemental analysis is increasingly used as a technique to differentiate the geographic origins of plants and wines. Differences existing in specific element composition among regions of origin are important to determine provenance. In the case of fish, otolith (ear stone) chemistry is used as a recorder of time and environmental conditions (Thorrold *et al.* 1998; Campana 1999; Rooker *et al.* 2003). Otolith chemistry is useful for identifying natal origin and assessing the relative contribution of different nursery areas to mixed adult stocks. Thus, in addition to DNA-based species identification techniques, multivariate trace elemental analysis may be useful to determine the geographic origins.

Eels collected from different regions in Japan, Taiwan, and China examined by analyzing the trace and heavy metal contents in the muscles to determine their origin; whether they originated from fish farms or from rivers caught as wild eels (Yamashita *et*

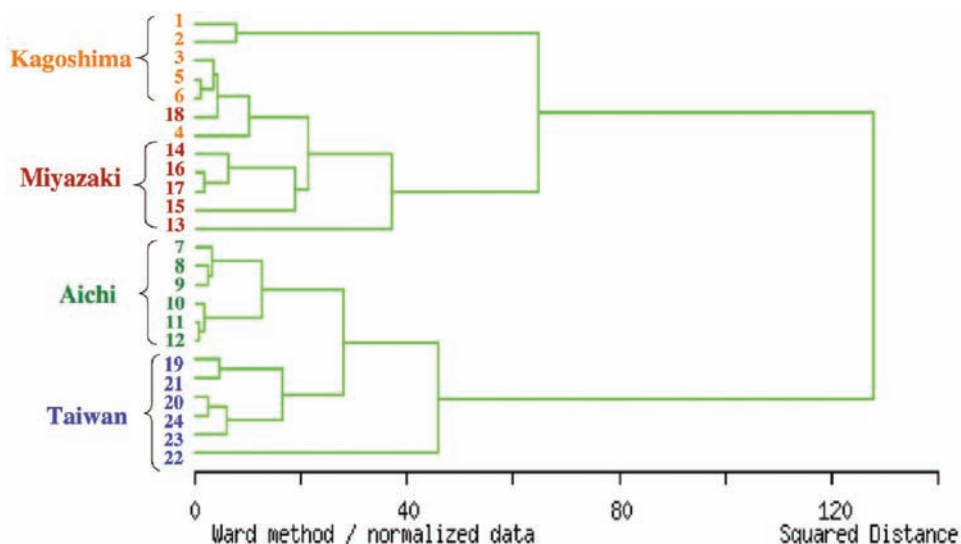


Fig. 5. Origin identification by multivalent trace metal analysis of Japanese eels. Cluster analysis of 12 element contents in eel bone (V, Mn, Co, Cu, Zn, As, Se, Sr, Rh, Cd, Pb, U) showed that the trace elemental patterns were different among distinct geographical origins.

al. 2006). Recently, inductively coupled plasma mass spectrometry (ICP-MS) is useful for the multiple trace elemental analysis. With the ICP-MS detection techniques, a very high sensitivity and selectivity function can be attained for determining rare trace metal concentrations at nM to pM levels. Fish culture systems are very different in Japan, Taiwan, and China. In Japan, most eel farms have tank-based advanced systems using running warm groundwater. In Taiwan and China, eel farms use traditional ponds for aquaculture. Thus, we hope to identify specific trace metal elements accumulate in the muscles and bones of eels derived from groundwater. Rare trace elements taken from the environment, such as uranium, lead, cadmium, and vanadium, are important for determining the origin of eels (Fig. 5).

Menadione (Vitamin K3) is used as a feed additive for fish production in aquaculture (Udagawa 2001; Udagawa and Yamashita 2008). After its intake and metabolism, menadione is converted to menaquinone-3 and this compound is accumulated in the

hepatopancreas of eels. Thus, eels grown in tanks in Japan accumulate menaquinone in the hepatopancreas and fat tissues. In contrast, eels are cultured in traditional ponds in Taiwan and southern China. The eels produced in such traditional ponds contain higher levels of phyloquinone and long-chained menaquinone derived from phytoplankton in the culture pond. Therefore, phyloquinone and long-chained menaquinone are detected in fish imported from Taiwan.

The trace element composition of littleneck clam was also examined by Yamashita *et al.* (data unpublished). Japan is occupied an important market for Japanese littleneck clam, with an annual demand of 90,000 tons. However, Japanese production of the species was 37,000 tons in 2003, which is still insufficient to meet domestic demand, and the remainder is imported in live from China, Korea, and North Korea. Due to the recent mislabeling scandals, origin identification techniques based on multivariate analysis of elemental contents have been expected to be applied to determine the

geographic origins (i.e., exporting countries) of clams.

The correlations between the site of origin of Japanese littleneck clams collected from Japan, Korea, and China were examined using trace element content in the muscles and shells of the clams. Distinctive patterns were found in clams originating from Japan, and from Korea and China. The levels of 13 trace elements in muscle tissue varied among the origins. A factorial analysis of the elemental patterns showed that Factor 1 was attributable to cobalt, copper, and strontium levels and Factor 3 to manganese and vanadium levels. Multivariate analysis showed that differences in elemental composition in the muscle between Japanese and imported clams were mainly due to these two factors. In addition, cadmium and arsenic levels observed in the muscles of clams originated from China and Korea were higher than those of clams from Japan, with one exception. Clams from Miyagi, Japan had higher arsenic content compared with those from China and Korea. Therefore, multiple elemental analysis can be used to identify imported clams from China and Korea.

4. Peptide Mass Mapping

DNA and other biological markers are often destroyed in food products during processing, and therefore it is difficult to identify the materials contained in processed food products with DNA-based standard laboratory techniques. Such difficulty running with the DNA-based techniques are limited to apply to processed food products and can be overcome by applying biochemical analysis using mass spectrometry (MS) based on the presence of species-specific amino acid sequences in meat, fish, and plant proteins. In contrast to DNA and other biological markers in processed food which are often decomposed during processing, proteins presence is the sufficient quality in MS.

One possible approach for species identification by protein structure is MS peptide mapping (Fig. 6). Our recent studies repre-

sent a method for characterization of species-specific peptide sequences (Yamashita, data unpublished). MS and electrophoresis are powerful tools that can provide microscale analysis of the primary structure of proteins. The coupling of one- and two-dimensional polyacrylamide gel electrophoresis (PAGE) with MS is experiencing a surge of activity, particularly in the areas of protein identification, using database-searching algorithms and characterization of posttranslational modification of proteins. These applications in the field of protein identification employ various types of gel separation and MS techniques that depend on *in situ* chemical or proteolytic digestion of the protein of interest, followed by elution of the digested peptide fragments for MS. This analytical procedure is useful to identify myosin and other muscle proteins in raw materials contained in various marine products such as canned, salted, and dried fish, and *surimi* products (Yamashita *et al.* data unpublished).

5. Future Prospects

The quality of fish and processed products might depend on the quality of the raw materials and geographic origin of their species, which determines their commercial value in the marketplace. Information and databases on the chemical components concerning the determination of food quality, functionality, and safety are very important to assist consumers in selecting foods. Therefore, authentic food labeling regarding species contents and their principle origins should be combined with the labeling information so as to maintain the quality and chemical components of processed food with keeping consumer rights.

Acknowledgements

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Strategy for application of peptide mass analysis on species identification techniques

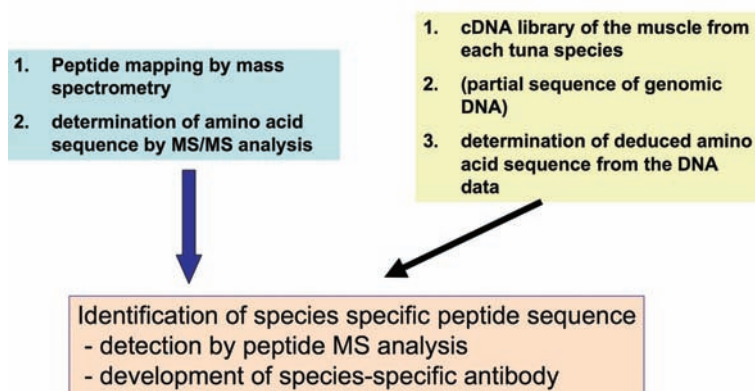


Fig. 6. The scheme of strategy of peptide mass mapping for fish species. Each peptide mass and its amino acid sequence are analyzed by MS/MS analysis after trypsin or other protease digestion of fish meat samples. Nucleotide sequences for the myosin heavy chain and myoglobin are also determined by cDNA and genomic DNA cloning techniques. By comparison with peptide mass and deduced amino acid sequences, species specific amino acid sequence substitutions will be identified. These species specific peptides can be used for species identification by mass spectrometry. A species-specific antibody and enzyme assay kit for species identification will be developed according to the species-specific amino acid residues.

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Effects of Climate Change on Marine Ecosystems

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Climate change will fundamentally alter the structure of oceans and directly impact marine ecosystems and human societies. Recent assessments of the global climate have concluded that ocean temperature, sea level and acidity have been increasing (IPCC report). Further, summaries of recent climatic data indicate that the intensity and frequency of ocean storms are increasing as well.

Climate induced changes and other less-understood anthropogenic changes will be superimposed on other impacts resulting from human activities such as over fishing, pollution, damming of rivers and habitat loss in coastal areas. Consequently, the fundamental characteristics of marine ecosystems, some already under stress, will be altered. Whether overall global yield from marine fisheries will decline due to climate change remains unclear; however, regime shift within individual marine ecosystems and trends in fish landing for certain species will likely occur. Calcareous plankton and coral are already suffering because of more acidic and warmer seawater. Combined with the potential loss of primary and secondary producers and important habitats, detrimental effects of climate change to fisheries are a matter of concern.

KEYWORDS climate change; ecosystem; fisheries; ocean acidification; coral

1. Introduction

Empirical data suggest that oceanic environments and marine ecosystems are changing as a result of natural or man-made variations in climate and river run-off (Turner and Rabalais 1994; Fogarty 2002; Rebstock and

Kang 2003; Lin *et al.* 2005; Gedney *et al.* 2006; Halpern *et al.* 2008). Any effects of such climate change on fisheries will compound the existing problems of massive over-capacities of usage, and conflicts between fishing fleets and among competing uses of marine ecosystems (IPCC 2001; Halpern *et al.* 2008). Increasing greenhouse gas concentrations

are expected to lead to substantial atmosphere warming during this century (IPCC 2007). As a consequence of atmospheric warming, we expect the warming of oceans and shifts in habitat ranges in addition to changes in algal, plankton and fish abundance in high latitude oceans to follow (IPCC 2007). In some locations ocean temperature has increased (e.g., Scranton *et al.* 1987; Freeland 1990; Bethoux *et al.* 1998); whereas, at other locations, oceans are cooling (Read and Gould 1992).

In marine ecosystems, slight changes in environmental variables, such as temperature, salinity, wind speed and direction, ocean currents, upwelling strength, UV-B radiation, pollution, diminishing wetlands and nursery areas as well as predation patterns, can sharply alter the food chain and the abundance of fish populations (IPCC 1996, 2001; Ji *et al.* 2007). Some of the effects of climate change-related warming on fisheries are summarized in Table 1 (IPCC 2001).

Of note is that the above mentioned changes impact the entire ocean water column. For example, Whitney *et al.* (2007) identified a warming trend and a concomitant decrease in oxygen in waters of the subarctic Pacific Ocean down to 370 ± 44 m (Fig. 1). Additionally, the bottom waters of

the Sea of Japan have increased in temperature and oxygen content has decreased (Fig. 2; Gamo *et al.* 1986; Kim and Kim 1996). Chen *et al.* (1999) suggested that bottom waters of the Sea of Japan might even become anoxic as early as 2200. Various organisms can adapt to a wide range of oxygen concentrations. However, a wide range of detrimental effects to marine animals occur as oxygen levels decline, progressing from slowed growth rates to metabolic impairment, and death. What follows concentrate on three types of effects of climate change on marine ecosystems.

2. Effects of Sea Surface Warming and Sea Level Rise

Variations in ocean temperatures will undoubtedly impact marine ecosystems and reduce fishery productions (IPCC 2001; Fogarty 2002). For example, the harvest of pink salmon off the Alaskan coast co-vary with the Pacific Decadal Oscillation Index, a measure of sea surface temperature in the North Pacific (Mantua *et al.* 1997). Temperature changes are expected to be greatest at high latitudes (IPCC 2001). For instance, warmed seawater and shifting winds have caused the Arctic ice pack to shrink such that

Table 1. Effects of climate change-related warming of fisheries (taken from IPCC 2007)

-
- longer growing seasons and increased rates of biological processes—and often of production;
 - greater risk of oxygen depletion;
 - species shift to more tolerant of warmer and perhaps less-oxygenated waters;
 - redeployment or re-design and relocation of coastal facilities;
 - coastal cultures may need to consider the impacts of sea-level rise on facilities and the freeing of contaminants from nearby waste sites;
 - changes in precipitation, freshwater flows, and lake levels. Strong regional variations are likely;
 - introduction of new disease organisms or exotic or undesired species;
 - establishment of compensating mechanisms or intervention strategies;
 - a longer season for production and maintenance;
 - modification of aquaculture systems, e.g. keeping them indoors under controlled light, may be needed more often to protect larvae from solar UV-B. In addition, several of the above and other factors, such as competing demand for coastal areas, may argue for technological intensification in ponds and non-coastal facilities.”
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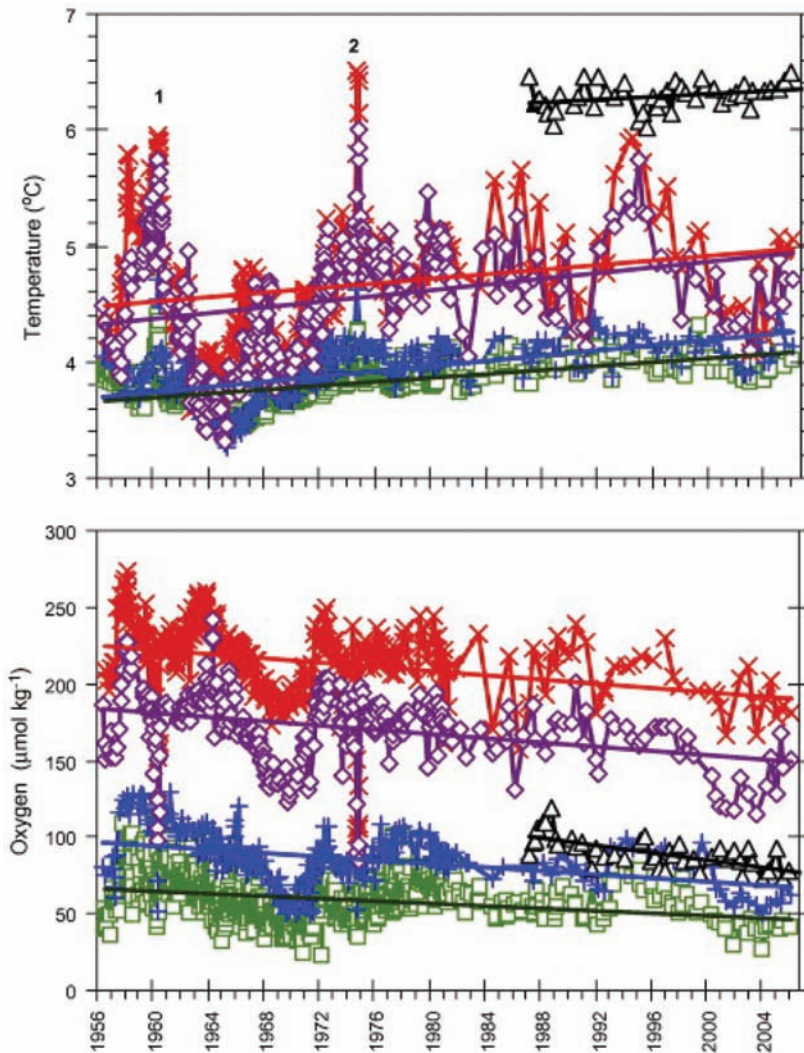


Fig. 1. Temperature and oxygen trends at Ocean Station P (50°N , 145°W) on the 26.5 ($\times$; 140 ± 15 m), 26.7 ($\diamond$; 168 ± 17 m), 26.9 ($+$; 278 ± 27 m) and 27.0 ($\square$; 370 ± 44 m) isopycnal surfaces and at station P4 ($\triangle$; 48.66°N , 126.67°W) on the 26.7 surface. P4 is warming at $0.0084^{\circ}\text{C y}^{-1}$, with O_2 declining at $1.22 \mu\text{mol kg}^{-1} \text{y}^{-1}$. Two mesoscale eddies are labelled 1 and 2 (taken from Whitney *et al.* 2007).

the size and number of floating ice-bergs have declined to an extent unparalleled over a century (Revkin 2007). Consequently, 2.6 million km^2 of ocean has lost ice cover in the Arctic and Antarctic Oceans beyond the long term average since satellites first started measurements in 1979 (Fig. 3).

Since most marine species have particular thermal preferences with a well-defined optimal temperature range, important northern fish species, such as broad whitefish (*Coregonus nasus*), Arctic char (*Salvelinus alpinus*), Arctic grayling (*Thymallus arcticus*) and Arctic cisco (*Coregonus autumnalis*), are

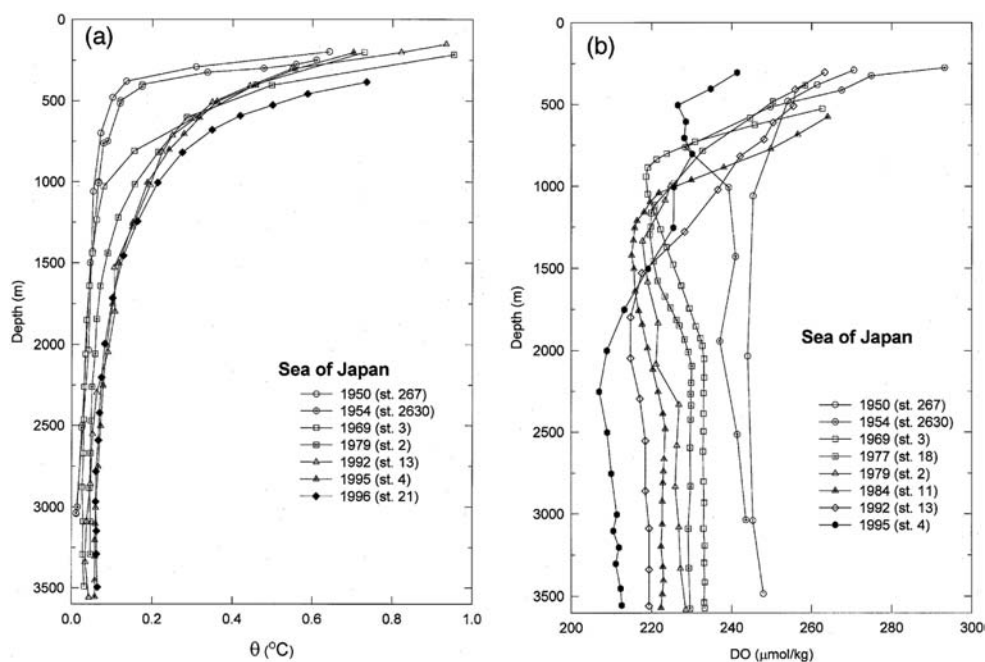


Fig. 2. Vertical distribution of (a) potential temperature and (b) dissolved oxygen in the Sea of Japan from 1950 to 1996 (taken from Chen *et al.* 1999).

likely to decline due to warming seawater and changing habitats (IPCC 2007). Predatory species in the Pacific and Atlantic Oceans, perhaps carrying new diseases, may move to warming Arctic waters as climatic barriers to species invasions are lowered (IPCC 2007). By the same token, with higher temperatures, invasion by non-native species is expected to occur, particularly on mid- and high-latitude regions (IPCC 2007).

Satellite data show phytoplankton biomass and growth generally decline as the oceans' surface waters warm up (Behrenfeld *et al.* 2006). Changes occurring in the expansive stratified low latitude oceans influence the availability of nutrients for phytoplankton growth, hence a prolonged decrease of net primary productivity of 190×10^{12} g C/yr since 1999. Such a decrease may be a harbinger of the future for marine ecosystems (Doney 2006a; Behrenfeld *et al.* 2006).

In the Southern Ocean, the decline in sea ice has been linked to a decline in krill populations, a keystone species in the Antarctic food chain (Loeb *et al.* 1997). Species that have adapted to life at the sea ice edge, such as crustaceans, may also experience population declines, thereby decreasing the available food sources for penguins, seals, polar cod and narwhals (IPCC 2007). Conversely, fishery production may increase in mid- to high-latitude regions due to increases in growth and survival of exploited species (Everett 1995; Fogarty 2002; IPCC 2007).

Open Arctic waters could experience a boon in fishing (Revkin 2007). However, as the Arctic ecosystem changes from ice-covered to ice-free, understanding how storm surges will affect coastal habitats, and how much of the fishery will be sustainable are questions yet to be answered. Increased fishing activities is likely from the modern industrial fishing fleet because of their ability to fish in harsh environments far from home.

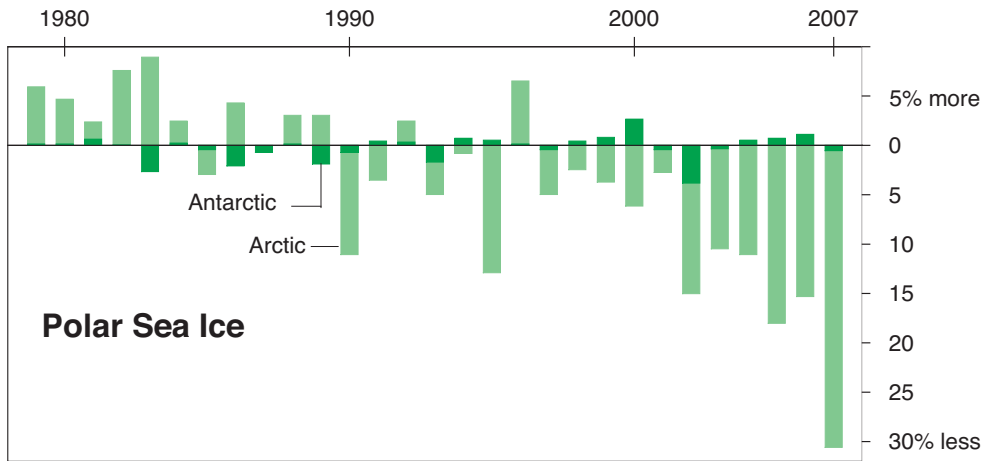


Fig. 3. Sea ice in the Arctic and Antarctic Oceans: Sea ice has declined in the Arctic in both summer and winter, and slightly increased around Antarctica in summer. Above is average deviation from August (taken from Revkin 2007).

It is speculated that developed nations will likely be the major players in the Arctic in the future, which is beyond the reach of most developing countries.

Many countries with extensive inhabited coastlines, especially island nations, are likely to suffer most from rising sea levels (IPCC 2007). Pressures will be greatest on developing countries as they are least able to afford mitigation measures. Rising sea levels may submerge important coastal habitats such as mangrove forests, sea grass beds and salt marshes, especially where they are constrained on their landward side, or starved of sediment because of construction of dams upstream which traps sediments. Approximately 30% of global coastal wetlands may be lost (Everett 1995; IPCC 2007). Further, warming surface oceans in the tropics will likely generate an increased number of hurricanes or tropical storms of increased magnitude (Emanuel 2005). As a result of increased coastal storms, damage to coastal cities and settlements and the submerging of coastal habitats may occur (IPCC 2007).

Habitats that have received the most attention as a consequence of climate change

are coral reefs. In 1998, El Niño and the concomitant failure of the Indian Monsoon resulted in bleaching and death of over three quarters of coral reefs in the Indian Ocean (Wilkinson *et al.* 1999). In fact, coral reefs elsewhere fared no better as they are vulnerable to thermal stress and have poor adaptive capacity (IPCC 2007).

Seawater has warmed by roughly 1°C over the past 50 years; thus, in El Niño years, temporary increases of a degree on top of this long-term temperature change may push ecosystems to their limits. The frequency and intensity of El Niño events have been predicted to increase (Steffen *et al.* 2002). These increases will adversely impact the Peruvian Anchovetta fisheries, and have other detrimental effects (Fogarty 2002). Fishery production is a critical issue of global food security as over 1 billion people are strongly dependent on fish as an integral component of their diet (FAO 2001). Yet, many of whom are poor and will adapt poorly to the decline or shifts in fish stocks (Sheppard 2001; Montaigne 2007). Effects of ocean acidification to be discussed below, are likely to compound the issue.

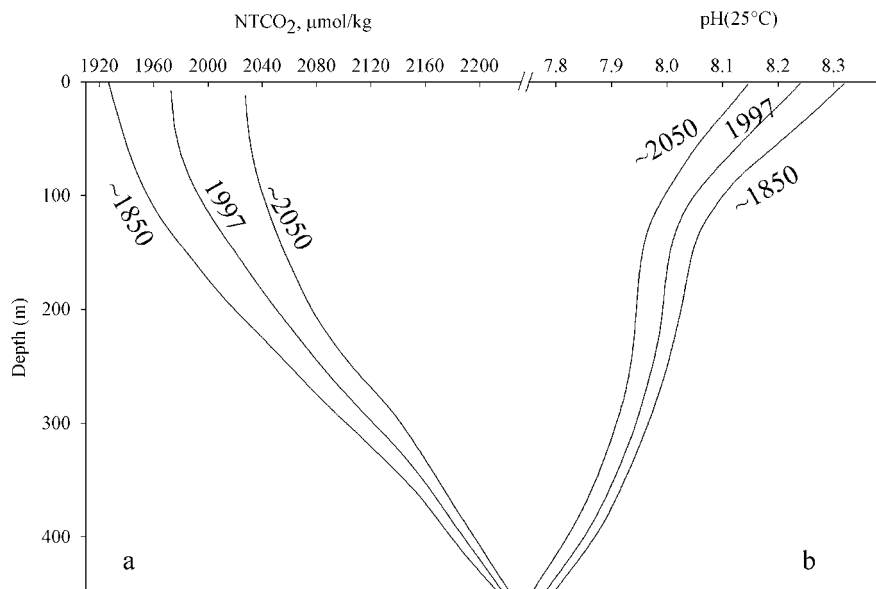


Fig. 4. Typical pre-industrial (~1850), present (1997) and projected (~2050) (a) NTCO₂ and (b) pH vertical profiles of seawater in the SCS (taken from Chen et al. 2006).

3. Effects of Ocean Acidification

In addition to increasing greenhouse gases in the atmosphere, a significant amount of CO₂ humans emit ends up in oceans. Figure 4 presents the typical pre-industrial (~1850), present (1997) and projected (~2050) normalized total CO₂ (NTCO₂: total CO₂ concentration at a salinity of 35) and pH vertical profiles of seawater in the South China Sea. To date, anthropogenic CO₂ emissions have already decreased surface water pH by roughly 0.1 units of pH. By 2050, pH is predicted to decrease further by approximately an additional 0.1 unit. Of course, ocean acidification in addition to seawater warming will make CaCO₃-based systems increasingly fragile (Sheppard 2001; Doney 2006b; IPCC 2007).

Predictions from ocean models suggest that ocean pH several centuries from now will be lower than at any time in the past

300 million years (Caldeira and Wickett 2003). Although these pH reductions may seem small, they provide ample cause for alarm. When oceans absorb CO₂, they become increasingly acidic, and calcite and aragonite dissolve with increased ease. Beyond a specific acidity threshold, organisms using calcite and aragonite for shell and skeletal growth, such as coral, mollusks, crustaceans, zooplankton and phytoplankton, start to suffer (Doney 2006a, b). Mesocosm experiments have demonstrated that at expected CO₂ concentrations in 2150, calcification of species such as the single-celled plants coccolithophores, the most productive calcifying organisms on the planet, will be reduced to almost half of that at the current ambient CO₂ concentration (www.carboschools.org). Ocean acidification therefore threatens the survival of a whole suite of marine species and the entire ocean food chain (Doney 2006b).

4. Effects of Changing the Stability of the Surface Mixed Layer

Climate change-induced warming and freshening of seawater on the surface layer will stabilize the water column in the global oceans. For instance, the temperature of bottom water in the Sea of Japan has been rising (Fig. 2(a)) because the surface layer has warmed, and thus become increasingly stable. As a result of sea surface temperature increases, the ability of the oxygen-laden surface water to sink to the bottom to replenish bottom oxygen has become more difficult (Chen *et al.* 1999). Additionally, studies have determined that some parts of oceans may be seeing decreases in salinity due to increased river runoff (Gendey *et al.* 2006). Further, wind patterns may change, thereby altering upwelling; a principal source of nutrients that support new production (Everett 1995; Chen 2004).

Changes in upwelling patterns and decreases in salinity can be illustrated best in small seas. Take the Baltic Sea as an example, it has been demonstrated that mean annual sea surface temperatures may increase by 2–4°C by the end of the 21st century (BACC Lead Author Group 2006). Sea ice would then decrease by 50–80% along with a decrease between 8–50% in average salinity. All these changes may increase phytoplankton production and shift the phytoplankton community toward species, such as cyanobacteria, that favor high temperatures. Cold-water species may be eliminated from much of their present habitat while cool- and warm-water species expand northwards. Stratification of the water column may also lead to accelerated eutrophication, putting pressure on bottom-dwelling species and a loss of biodiversity in the Baltic (BACC Lead Author Group 2006).

Decreases in salinity in the Baltic Sea will alter the sea's ecology. A decrease in

marine fauna is predicted to occur first in the northern Baltic Sea because of increased predicted rainfall (BACC Lead Author Group 2006). As an example, the common starfish and shore crab are among the species expected to decrease when salinity drops to below 15–25 (BACC Lead Author Group, 2006). When the salinity drops to <11.5, buoyancy would be too low for cod eggs to remain floating (BACC Lead Author Group, 2006).

Even in larger seas such as the Sea of Japan, changes to temperature and salinity mixed layer depth have been observed. For example, the mixed layer there was thinner and less variable (18.2 ± 4.9 m) in 1961–1975 compared with those (26.1 ± 15.1 m) in 1976–1990 during the March–May period (Fig. 5). The shallower mixed layer depth in spring during the earlier period before the regime shift around 1976 resulted in higher chlorophyll a concentrations and zooplankton biomass (Kang *et al.* 2000). Responses of some fish species to these changes can be large. For instance, the catch of saury crashed after 1976, while the sardine catch skyrocketed (Fig. 6).

As mentioned above, climate change-induced warming and freshening of the surface layer will stabilize the water column in the global oceans. Examples given above for the Baltic Sea and the Sea of Japan may serve as a basis for further studies on climate changes in other oceans.

5. Conclusion

Climate change will exacerbate the stress on living resources already impacted by pollution, over fishing and other anthropogenic activities. Even if the net impact on fishery production is neutral, shifts in regional production are likely to alter local availability of preferred species and food supply, and may have significant local effects on fishery-dependent communities. Although climate change science has improved rapidly

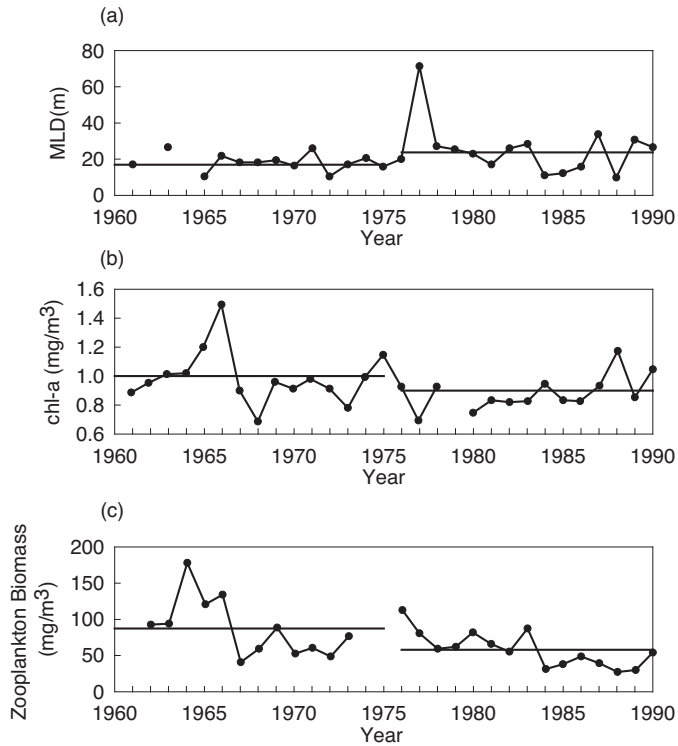


Fig. 5. Variations of (a) mixed layer depth, (b) chl-a concentration, and (c) zooplankton biomass in the Sea of Japan during spring (March–May, but mostly April) of 1960–1990 (taken from Kang *et al.* 2000).

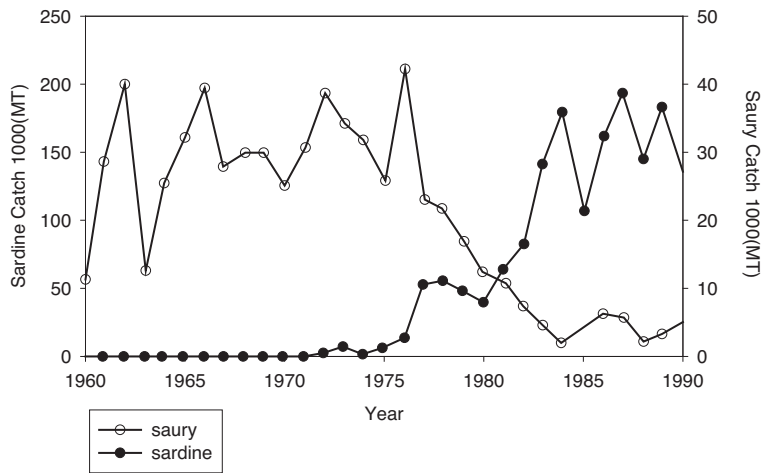


Fig. 6. Annual commercial catches for sardine and saury from the Sea of Japan (taken from Kang *et al.* 2000).

over the last few decades, the responses of marine ecosystems to the compounded effects of climate change and anthropogenic activities remain poorly understood. Consequently, little data for adaptation and mitigation measures are available. Further observations and research are clearly warranted.

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Harmful Algal Blooms and Ocean Observing Systems: Needs, Present Status and Future Potential

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Harmful algal blooms (HABs), commonly called “red tides” are increasingly common worldwide. These are caused by the growth and aggregation of microscopic algae, leading to negative impacts of many types, including illness and death in human consumers of contaminated fish and shellfish, as well as mortalities of wild and farmed fish, marine mammals, and other animals. The diversity of HAB species and their impacts, as well as the oceanographic complexity of these phenomena all present significant challenges to those responsible for the management of coastal resources and the protection of public health. A promising development in this regard is the advent of ocean observing systems (OOSs)—arrays of moored and mobile instruments that can collect and transmit data continuously from remote locations to shore-based scientists and managers. The potential benefits from ocean observing systems are many, and improved monitoring and management of HABs are frequently cited as example benefits to justify the investment of resources in these systems. During this era of accelerated instrument development and deployment through national and international OOS programs, it is instructive to examine the needs, present status, and realistic potential of ocean observatories as tools for HAB monitoring and management. HABs represent a biological component of coastal waters that challenges present technologies, in part because of the need for species- or toxin-specific detection capabilities. This paper discusses the observing system capabilities or assets that are needed for effective HAB monitoring and management, highlighting and evaluating new technologies and future capabilities. Examples are given of HABs worldwide, with special emphasis on paralytic shellfish poisoning (PSP) outbreaks in the northeastern United States as a HAB system with representative challenges in observatory design and capabilities.

KEYWORDS HABs; ocean observing systems; monitoring; management

1. Introduction

The Integrated Earth Observation System (GEO 2007) is becoming a reality, as satellites, ocean buoys, weather stations and *in-situ* earth observing instruments are being deployed worldwide and their data assimilated into advanced numerical models to provide the analysis and understanding needed to address critical societal issues such as climate change, drought, fisheries management, or pollution, to name but a few. This reflects the growing recognition that decision making benefits from access to a wide variety of environmental, biological, economic, statistical, and other data, and more importantly, the ability to integrate, analyze and evaluate these types of data within a common framework shared by many countries and end users.

Within this large and visionary context, ocean observing systems (OOSs) are being planned and deployed on a major scale. Oceanography has entered a new era—one in which the traditional ways of obtaining data from the ocean using research vessels are being replaced by arrays of moored and mobile instruments that can collect and transmit data continuously from remote locations to shore-based scientists and managers. Just as networks of meteorological stations and numerical models of atmospheric dynamics revolutionized our understanding of the weather and greatly improved our ability to provide accurate forecasts of weather events, OOSs and their associated numerical models of ocean dynamics have the potential to document long-term patterns and changes in the sea, to detect infrequent events that previously went unobserved, and to make predictions or forecasts about these and other phenomena that directly affect human populations and marine ecosystems. Advances in communications, robotics, computing, platform design, power systems, and sensor technology now make it possible to

get broader and deeper views of the oceans over longer periods and to share that information in real time among scientists, policymakers, resource managers, educators, and students.

The potential benefits from OOSs are many, and include the detection and prediction of climate variability, facilitation of safe and efficient marine operations, ensuring national security, preserving and restoring healthy marine ecosystems, mitigating natural hazards, managing living resources, and ensuring public health (NORLC 1999). Under the last three topics, harmful algal blooms (HABs) are frequently cited as phenomena that can be better understood and managed using ocean observatories (e.g., ORION Executive Steering Committee 2005). HABs are highly visible phenomena that affect the general public and coastal resources in many ways, and clear economic and managerial benefits would accrue from advance warning and forecasting capabilities. This potential is based on a need to understand the biological, physical, and chemical factors controlling the dynamics of individual HAB species at appropriate scales, but it is also a recognition that management of HABs can benefit from improved cell and toxin detection capabilities, coupled with modeling and forecasting of bloom transport and landfall (Ramsdell *et al.* 2005). However, HABs represent a biological component of coastal waters that challenges present technologies, in part because of the need for species- or toxin-specific detection capabilities.

In recent years, progress has been made in molecular and immunological cell and toxin detection technologies, opening the door to an era where remote, subsurface, near real-time detection of specific HAB taxa and toxins can be envisioned. Given this potential, a logical conclusion would be that instruments and observatory systems currently being planned or deployed would include some that will detect HAB cells or toxins, and that these systems are being deployed

in areas where HABs are a serious and recurrent problem. This, unfortunately, is generally not the case, as other scientific priorities have been used in observatory siting decisions, and available funds are being used to build and deploy instruments that are well proven for oceanographic measurements, but which do not provide the species-specific data needed for HABs. In effect, HAB-specific observatory instrumentation is not yet ready for operational deployment (Sellner *et al.* 2003; Paul *et al.* 2007). Here the objective is to examine the capabilities that are needed specifically for HAB detection and forecasting in regional monitoring and management programs, and to assess where we are with new technologies, and where we need to go if we are to meet the expectations of those who have funded the OOSs. It is hoped that this review will help to guide research and funding programs, and that it will inject a level of realism that moderates the lofty expectations and claims about future capabilities.

2. Harmful Algal Blooms

Over the last several decades, countries throughout the world have experienced an escalating trend in the incidence of “harmful algal blooms” (HABs; Anderson 1989; Hallegraeff 1993). HAB events are characterized by the proliferation and occasional dominance of particular species of toxic or harmful algae. When toxic algae are filtered from the water as food by shellfish, their toxins accumulate in those shellfish to levels that can be lethal to humans or other consumers. Another type of HAB impact occurs when marine fauna are killed by algal species that release toxins and other compounds into the water. HABs also cause mortalities of wild fish, seabirds, whales, dolphins, and other marine animals. Non-toxic blooms of algae can cause harm, often due to the high biomass that some blooms achieve, and the deposition and decay of that biomass, lead-

ing to anoxia.

A poorly defined but potentially significant concern relates to sublethal, chronic impacts from toxic HABs that can affect the structure and function of ecosystems. Adult fish can be killed by the millions in a single outbreak, with long- and short-term ecosystem impacts (Okaichi *et al.* 1989; Kim *et al.* 1999). Likewise, larval or juvenile stages of fish or other commercially important species can experience mortalities from algal toxins (White *et al.* 1989). Chronic toxin exposure may have long-term consequences that are critical with respect to the sustainability or recovery of natural populations at higher trophic levels (Ramsdell *et al.* 2005).

2.1. Paralytic shellfish poisoning in the Gulf of Maine

It is useful to view the issues involving HABs and observing systems in the context of a real-world problem—i.e., recurrent HAB outbreaks in an important site that has been well studied and characterized, and that offers representative challenges and constraints to the successful deployment of a HAB observing and forecasting capability. The example selected is the Gulf of Maine in the northeastern United States, the site of widespread outbreaks of PSP caused by the dinoflagellate *Alexandrium fundyense*.

A dominant feature underlying *A. fundyense* regional bloom dynamics is the Maine Coastal Current or MCC (Fig. 1; Lynch *et al.* 1997)—a composite of multiple segments and branch points. The two major transport features in this system are the eastern and western segments of the MCC, hereafter termed the EMCC and WMCC. Conceptual models of *A. fundyense* bloom dynamics have been provided by Anderson *et al.* (2005b) and McGillicuddy *et al.* (2005). Key features in the models are two large cyst “seedbeds”—one in the Bay of Fundy and the other offshore of mid-coast Maine (Fig. 2; Anderson *et al.* 2005b). Cysts germinate from the BOF seedbed, causing

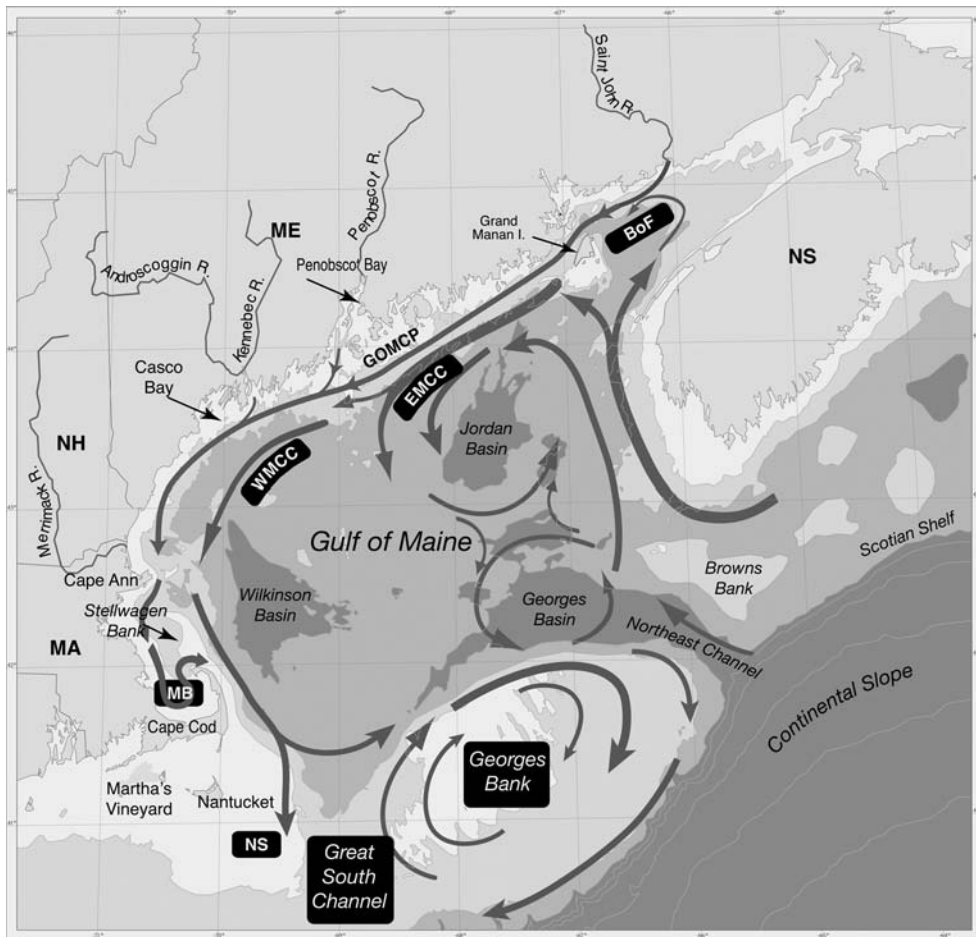


Fig. 1. Gulf of Maine surface circulation, with eastern and western segments of the Maine Coastal Current system identified (EMCC and WMCC). Other abbreviations: GOMCP—Gulf of Maine coastal plume (from Keafer *et al.* 2005); MA—Massachusetts; ME—Maine; NH—New Hampshire; NS—Nova Scotia (Modified from Pettigrew *et al.* 2005).

recurrent coastal blooms that are self-seeding with respect to future outbreaks in that area. The blooms also contribute to populations in the EMCC, as some cells escape the Bay of Fundy and enter the EMCC where they bloom. Some cells travel south and west with the EMCC, while others deposit cysts in the mid-coast Maine seedbed. In subsequent years, these latter cysts (combined with cells from the EMCC) inoculate WMCC blooms that cause toxicity in western por-

tions of the Gulf and possibly offshore waters as well.

Numerical modeling of *A. fundyense* and PSP dynamics in the Gulf of Maine utilizes a hierarchy of physical–biological models. The current *Alexandrium* sub-model formulation follows Stock *et al.* (2005) and McGillicuddy *et al.* (2005) and includes germination, growth, mortality, and nutrient limitation. Each year, germination from cyst seedbeds provides the inoculum for subsequent

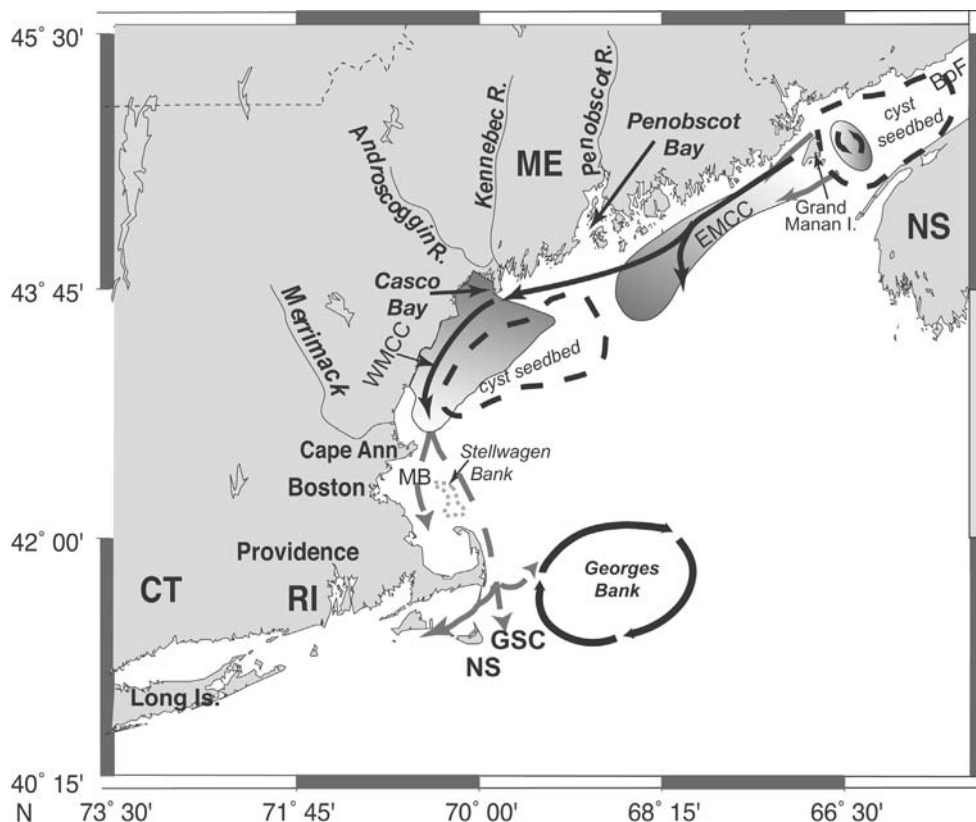


Fig. 2. Conceptual model of *A. fundyense* bloom dynamics and PSP toxicity. Solid black lines denote the eastern and western segments of the Maine Coastal Current system (EMCC and WMCC, respectively). Solid black lines also depict the circulation around Georges Bank. Short, dashed black lines delimit the cyst seedbeds in the BOF and mid-coast Maine. The shaded areas within the Gulf represent portions of the EMCC, WMCC, and BOF where *A. fundyense* blooms tend to occur with the highest color intensity denoting areas with higher cell concentrations. Dashed grey lines show the transport pathways of these water masses and their associated *Alexandrium* cells. Modified from Anderson *et al.* (2005b).

growth in the overlying waters, while wind events influence delivery onto the coast. Currently, realistic nowcasts of bloom development are possible using observed cyst distributions, cyst germination and vegetative cell growth rates, and continuous real time river flow and hydrographic data (He *et al.* in press). Forecasts are also being generated during the bloom season, but only on an experimental basis at present.

PSP outbreaks in the Gulf of Maine are sufficiently well characterized and modeled (McGillicuddy *et al.* 2005; Stock *et al.* 2005; He *et al.* in press) that they could benefit from automated observations through an ocean observatory system. In the sections that follow, the technologies and instrumentation that can be used to achieve this goal will be highlighted, and the challenges to realization of this potential discussed.

3. Observational and Analytical Needs for HAB Monitoring and Management

Anderson *et al.* (2001) highlight the different approaches adopted by countries and commercial enterprises worldwide to monitor and manage HABs in coastal waters. This is typically accomplished through the establishment of programs for toxin and cell detection (and quantitation) in water, aerosols, shellfish, fish, etc., development of bloom forecasting and early warning capabilities as well as medical intervention and therapeutic strategies, and to a growing extent, bloom prevention and mitigation strategies. There are, however, many challenges associated with these activities, due to the complexity and diversity of HAB phenomena. Resource managers and regulatory officials must deal with multiple toxins and multiple toxic algal species, multiple toxic fisheries resources, and large- and small-scale HAB events that occur intermittently. Many new technologies are emerging that can address these management challenges, however, as discussed herein. A comprehensive review on this topic is provided by Sellner *et al.* (2003), and a more general review by Paul *et al.* (2007) in the context of biological sensors that are not HAB-specific.

3.1. Sampling platforms

There are many possible ocean observatory configurations, varying in geographic scale as well as in the manner in which instruments are deployed, powered, and utilized. Instrument packages can be located at specific sites and depths using surface moorings, they can be sequestered on the ocean bottom, rising through the water column to obtain profile data, and they can be mounted on underwater vehicles of various types that travel through the water in a given area on pre-programmed missions. They can also be

mounted on surface vessels or simple platforms that house instruments that collect water at depth for automated analysis at the surface. Each of these design features has advantages and disadvantages with respect to HAB monitoring. For example, sampling position in the water column is a critical design constraint, as there can be considerable variability in the vertical distribution of HAB species. Some are often found in subsurface, thin layers that are difficult to detect and sample (e.g., Xie *et al.* 2007), but others are predominantly confined to the surface mixed layer, and thus would be amenable to detection using an instrument package moored within that layer. In the Gulf of Maine, for example, considerable information has been obtained from observations of the surface distribution of *Alexandrium fundyense* (e.g., Townsend *et al.* 2001; Anderson *et al.* 2005a). Thin, subsurface layers of *Alexandrium* have also been detected in some areas of the Gulf (Townsend *et al.* 2001) but the importance of these accumulations remains unknown in the context of shellfish toxicity and general bloom dynamics. A surface-deployed instrument package would therefore be highly informative in that region, but one that could obtain profiles of cell distributions would be even more so. In this latter instance, the value of the vertical profile data must be balanced against the additional costs incurred. The higher resolution of the vertical profile would require more reagents, filters, sensors, or arrays, and the instrument package would therefore need to be serviced more frequently than an instrument that samples at a single depth. This is an important consideration given the cost of supplies as well as personnel and vessel time for the servicing operations.

Horizontal coverage is an equally important factor in HAB monitoring, and again, the Gulf of Maine provides a good example of the issues that need to be considered in instrument siting. As described above, *A. fundyense* is transported to the

south and west in two coastal currents—the EMCC and the WMCC (Fig. 1). Not only are the *Alexandrium* distributions non-uniform within these currents (Townsend *et al.* 2001), but the location of these water masses varies through time as well (Pettigrew *et al.* 2005). With appropriate wind and hydrodynamic forcings, the EMCC and its associated *A. fundyense* populations can be carried offshore into the central Gulf of Maine, and/or delivered toward shore (Luerssen *et al.* 2005; Keafer *et al.* 2005). This variability in alongshore transport has direct implications to the patterns of PSP toxicity in nearshore shellfish (Luerssen *et al.* 2005). A single observatory mooring placed in these coastal waters would therefore not provide accurate cell abundance information. In this instance, multiple instruments arrayed along a cross-shore transect would be an expensive, but much more informative configuration.

These concerns about vertical and horizontal resolution can be addressed with mobile autonomous underwater vehicles (AUVs), but for most HABs, considerable development is needed if these vehicles are to provide species-specific or toxin-specific data. With the exception of *Karenia brevis* and the BreveBuster described by Robbins *et al.* (2006), most HAB species are not amenable to optical detection due to their lack of distinctive pigments or other features that can be distinguished optically. As a result, water samples must be collected, manipulated and complex chemistries performed, and this in turn requires power, space, and robotic capabilities that are not possible in AUVs at present. For most HABs, and for the near future, it therefore appears that AUVs will be used to supply contextual data (e.g., salinity, temperature, turbidity, chlorophyll) that will help in the understanding the patterns of HAB abundance and toxicity that are observed with other instruments. This limitation in HAB detection capabilities will change when miniaturized mass spectrometers are configured to detect HAB

toxins dissolved in seawater (see below), or when other features of these cells or toxins are identified that can be readily measured with the relatively simple type of robotics and detectors that can be deployed within power- and space-limited AUVs.

The foregoing discussion highlights one of the major obstacles that has slowed progress in the detection of HAB cells and toxins at ocean observatories—the need to process water samples through filters or other concentrating devices, and to manipulate those samples for extraction and analysis of toxins or the cellular targets needed for species identification and enumeration. Technologies are available for many of these analyses, but they need to be incorporated into an instrument that can be deployed underwater and that can perform the series of robotic functions needed for each analysis.

One instrument that provides these capabilities and that can be configured for use for HAB cell and toxin detection in ocean observing systems is the Environmental Sampling Processor (ESP; Goffredi *et al.* 2006; Scholin *et al.* 1998, in press). The ESP (Fig. 3) autonomously collects discrete water samples from the ocean subsurface, concentrates microorganisms (particulates), and automates application of molecular probes to identify specific microorganisms and their gene products (Scholin *et al.* 2006). The current prototype is the second generation ESP or 2G ESP. It consists of three major components: the core sample processor, analytical modules, and sampling modules. The core ESP is designed to collect and process small- to moderate-sized samples (mLs to several liters) at depths to 50 m. Analytical modules are stand-alone detection systems that can be added to the core ESP to impart different analytical functions downstream of common sample processing operations (e.g., PCR, capillary electrophoresis, competitive immunoassays, etc.).

The ESP currently utilizes DNA probe and protein arrays to detect target molecules

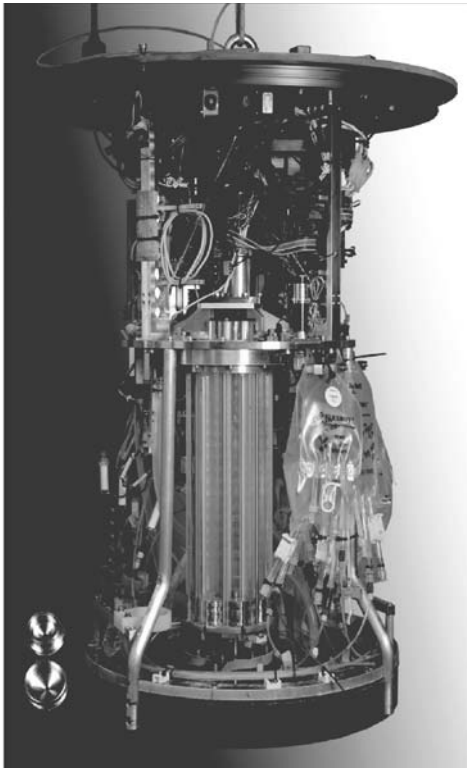


Fig. 3. Second generation ESP instrument (16" d × 30" h). bottom left: puck magnified ~2x relative to those loaded in the carousel. See also: http://www.mbari.org/ESP/esp2G_compare.htm

indicative of species and the substances they produce. As described below, within the core ESP, DNA probe arrays specific for target HAB species capture ribosomal RNA (rRNA) from a crude sample homogenate using a quantitative sandwich hybridization assay (SHA; Fig. 4A). A quantitative PCR module is under development as an alternative approach to cell enumeration. Protein arrays utilize a competitive ELISA (enzyme-linked immunosorbent assay) technique for detecting target substances such as domoic acid (e.g., Fig. 4b). The ESP can also archive samples for laboratory analyses after the instrument is recovered, including fluorescent *in situ* hybridization (FISH), various nucleic

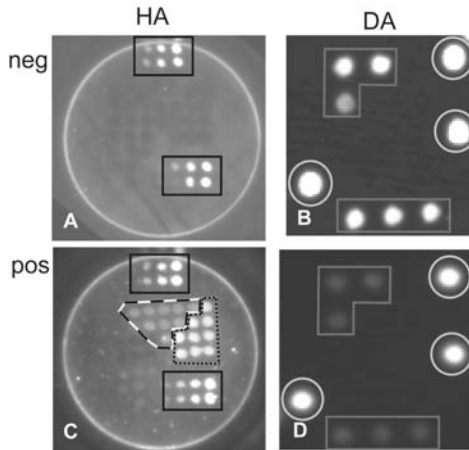


Fig. 4. *In situ* detection of *Pseudo-nitzschia* spp. and domoic acid (DA) in Monterey Bay, March 2006 using the ESP. **A)** DNA array with probes for *Pseudo-nitzschia australis*, *P. multiseriis/pseudodelicatissima*, *Alexandrium catenella*, and *Heterosigma akashiwo* (HA array). Only control spots are outlined as targeted species are below detection level. **B)** Protein array for DA, corresponds to (A). Circled spots are IgG controls confirming consistency of detection chemistry. Spots in red rectangles are DA conjugate. Maximum intensity in DA conjugate spots indicates DA levels are below detection limit, whereas decreasing spot intensity indicates presence of DA (competitive ELISA). **C)** HAB array from a later time indicates the presence of *P. australis* (spots bounded by dashed lines) and *P. multiseriis/pseudodelicatissima* (spots bounded by dotted lines), both of which can produce DA. **D)** DA array that corresponds to (C); circled spots are IgG controls; spots in rectangles are DA conjugate; the DA conjugate spots are considerably dimmed, indicating the presence of this toxin (from Doucette *et al.* and Greenfield *et al.*, in prep.).

acid analyses (cloning, sequencing) and algal toxin measurement (e.g., Greenfield *et al.* 2006).

Sample manipulations are carried out in reaction chambers called "pucks" that are loaded into and removed from various stations by robotic mechanisms. The automated

process from collection of a live sample to broadcast of an imaged DNA or protein probe array takes ~2 hours and can occur subsurface. The instrument can perform ~30–40 of those operations before servicing is required. The current limitation is the number of pucks stored in the carousel. There are, however, design options for increasing the number of sampling/analytical events and decreasing power consumption.

The instrument can be bundled with contextual sensors such as a CTD, fluorometer, transmissometer, and nutrient analyzer. Data from the external sensors along with results of the probe assays are uploaded periodically from the deployed instrument to a shore station for analysis and interpretation. Two-way communication allows for rescheduling of mission sampling profiles if desired. Further details on the instrument's design and operation are described elsewhere (Greenfield *et al.* 2006; Scholin *et al.* 2006; Roman *et al.* 2007; Paul *et al.* 2007; see also <http://www.mbari.org/esp>).

As promising as this instrument is, it is not yet commercially available, and thus testing has been predominantly through research grants to the developers and collaborators. To date, the ESP has been deployed multiple times in surface waters for periods of several weeks to a month, during which time it has successfully automated application of three classes of DNA probe arrays (HABs, bacteria/archaea, invertebrate larvae) and the domoic acid assay (Goffredi *et al.* 2006; Greenfield *et al.* 2006; Paul *et al.* 2007; Jones *et al.* in press; C. Scholin, G. Doucette, unpub. data). Further details on the instrument's design and operation are described in these citations and at <http://www.mbari.org/esp>.

At the present time, the only other advanced robotic instrument capable of *in situ* water collection, processing, and sophisticated molecular and biochemical analysis is the Autonomous Microbial Genosensor (AMG), designed to detect specific micro-

bial targets in coastal or oceanic waters (Paul *et al.* 2007). The current prototype of the AMG collects water samples, filters cells and extracts RNA, and performs amplification autonomously. A second generation AMG will incorporate microfluidic liquid processing, array technology, and intensified light detection.

3.2. Toxin detection

Of paramount importance to many HAB monitoring programs are methods to detect and quantify the toxins produced by HAB species, which include a broad spectrum of compounds ranging in size, potency, and solubility. In all cases, the marine HAB toxins that cause the human poisoning syndromes consist of families or groups of structurally related compounds, with individual derivatives exhibiting potencies that can significantly differ from other congeners (Van Dolah 2000). During food web transfer, HAB toxins can also be metabolized or biotransformed into structurally different compounds. The broad chemical and structural diversity of algal toxins and their derivatives and metabolites, coupled with differences in their potency account for many of the challenges associated with their detection in ocean observatory programs.

Traditionally, biotoxin monitoring programs have relied on measurements of toxins in shellfish samples collected weekly or bi-weekly from key locations in areas affected by HABs (e.g., Shumway *et al.* 1988). This procedure works well and provides appropriate public health protection if the stations are well sited, and the toxin assays are run at relatively frequent intervals. Toxin measurement methods can be grouped into three main types: chemical, *in vitro*, and *in vivo* assays (Hallegraeff *et al.* 2003). The latter (bioassays) have had a long history in HAB toxin detection, but are obviously not amenable to automation and high-throughput analysis in ocean observatories, so the

only options in that context are measurements of toxin in seawater using either chemical analyses or *in vitro* assays. This immediately introduces some concerns, as considerable work will be needed to relate measurements of toxins dissolved in seawater, or in particulate form in that water, to the risk to human consumers of shellfish or fish. In the Gulf of Maine example, where measurements of toxicity in shellfish tissues are used for regulatory decisions, measurements of *Alexandrium* toxins in the water column at ocean observatories will likely be used as supplementary information in assessing risk, both current and future. It will be many years before sufficient data are accumulated to allow such measurements to be used for regulatory purposes for nearshore shellfish.

Chemical methods for toxin analysis include high performance liquid chromatography (HPLC), and mass spectrometry coupled to liquid chromatographic separation (Quilliam 1996). Of these two alternatives, only mass spectrometry shows the potential for use in ocean observatories, and there the challenges remain significant due to the diversity, size, and solubility of the toxins, as well as the matrices in which they occur (e.g., particulate versus dissolved). Another constraint is the need to perform spectrometry in a vacuum and underwater, which poses significant engineering challenges. Progress has been good, however. For example, a small, modular mass spectrometer has been developed and mounted in an AUV (Wenner *et al.* 2004). That system consists of an *in-situ* membrane-introduction linear-quadrupole mass spectrometer capable of detecting dissolved gases and volatile organic compounds at sub parts-per-billion concentrations. This instrument is still under development and has not been configured for HAB toxins, but future designs may permit the analysis of HAB toxins that occur dissolved in seawater (e.g., brevetoxins, domoic acid, okadaic acid). Analysis of toxins in particulate form will

require a different approach, such as Laser Desorption Mass Spectrometry (LDMS), which is widely employed in analytical laboratories due to its simplicity of operation and rapid analysis times. One benefit of LDMS is that many different types of materials can be vaporized and ionized by a tightly focused laser beam (Cotter 1997). This can avoid sample purification or preparative techniques, which is critical to deployment of such technologies in a moored or mobile configuration in an OOS, as it will greatly reduce sampling and handling requirements, and thus power drain, space needs, and reagent needs as well. LDMS has been used for the detection of bacterial spores, vegetative cells, viruses, and toxins in aerosol environments (Fenselau and Demirev 2001), and efforts are underway to apply this method to HAB cells and dissolved toxins in seawater (A. Place, pers. comm.).

Another important and rapidly developing group of HAB toxin detection methods comprises the *in vitro* assays. One subgroup—the functional assays—relies on detection of a toxin's biochemical activity while the other—structural assays—depends on recognition of chemical structure at the molecular level (reviewed in Cembella *et al.* 2003; Van Dolah and Ramsdell 2001). A variety of functional assays have been developed for the detection of HAB toxins, including cytotoxicity assays (e.g., Manger *et al.* 1995), enzyme inhibition assays (e.g., Della Loggia *et al.* 1999), and receptor binding assays (e.g., Van Dolah *et al.* 1994). Nevertheless, retention of the biological activity of a cell line or a receptor preparation outside the laboratory remains a significant, and thus far, insurmountable obstacle to *in situ* use of these assays (Sellner *et al.* 2003).

In contrast, structural assays show considerable promise for automated deployment in an observatory system. These assays rely on the structural or conformational interaction of a toxin with a recognition factor such as an antibody. Antibody-based assays have

been developed for a variety of HAB toxins and many of these tests are now commercially available (Laycock *et al.* 2001; Cembella *et al.* 2003). In many ways, the procedures used for toxin immunoassays are similar to those used for HAB cell detection using oligonucleotide probes (described below), and thus these technologies have the potential to be combined in a single instrument that can detect HAB cells and toxins simultaneously. Doucette and co-workers (unpub. data) are developing an immunoassay-based method for detection of domoic acid in robotic fashion on board the ESP, described above. This analysis utilizes a known quantity of domoic acid-antibody conjugate immobilized on membranes as replicate spots. These membranes are then exposed to a simple extract of filtered plankton, and a competitive ELISA performed. The resulting arrays can be imaged (Fig. 4B) and the data sent to shore electronically. This is an example of a toxin-detection technology that can be automated and performed *in situ* in a moored instrument.

Other investigators are developing alternative immunosensors that also have the potential for *in situ* deployment, though most have only been configured for laboratory-based, bench-top formats at present. One novel immunoassay utilizes surface plasmon resonance (SPR) in a portable system developed for rapid field quantification of toxin levels in both shellfish and seawater. (Stevens *et al.* 2007). The SPR assay had a limit of detection of 3 ppb domoic acid and a quantifiable range from 4 to 60 ppb. Comparison of analyses with standard HPLC protocols gave an excellent correlation. This same technology should also function for detection of domoic acid (and other algal toxins for which antibodies are available) in concentrated algal extracts or high dissolved levels in seawater. With refinement of the extraction protocols and generation of higher affinity monoclonal antibodies, detection of much lower levels of toxin should be possible, leading to eventual application of automated SPR biosensors on moorings.

Another novel and potentially useful approach for *in situ* observations is a competitive immunoassay using screen-printed electrodes (SPEs; Kreuzer *et al.* 2002; Micheli *et al.* 2004). Excellent sensitivity and accuracy has been achieved with HAB toxins such as okadaic acid, brevetoxin, and domoic acid. For all toxins investigated, results compared favorably with other toxin analysis techniques. The advantages of speed of analysis, simplicity of design, *in situ* measurement capability, stability (storage up to four weeks prior to use), and disposability make SPE immunosensors good candidates for observatory instrumentation. Adaptation of this and other immunoassay technologies to robotic systems and deployment in remote locations is thus possible, but will require further development effort.

3.3. Cell detection

Two approaches have been followed to improve on traditional light microscope counts of HAB species in field programs. One utilizes optical characters that are unique to the target organism. The only success in this regard is for *Karenia brevis*, the Florida red tide organism, which produces a pigment called gyroxanthin-diester. This carotenoid is found in other fucoxanthin-containing dinoflagellate species as well, but in some areas, such as the Gulf of Mexico, it is sufficiently unique to be a useful biomarker for *K. brevis* and other toxic or potentially toxic *Karenia* species (Kirkpatrick *et al.* 2000; Richardson and Pinckney 2004). Instruments have been developed that can quantify this pigment in water samples, and these have been mounted on board research vessels (Kirkpatrick *et al.* 2003) and inside an AUV called the BreveBuster (Robbins *et al.* 2006). This approach thus has great potential for monitoring of those HAB species that have this unique pigment, but for the vast majority of other species, alternative approaches to cell detection are needed.

The second approach involves the development of species- or strain-specific “probes” which can be used to label HAB cells of interest so they can then be detected visually, electronically, or chemically. Progress has been rapid and probes and assays of multiple types are already available for many of the HAB species. The most promising of these approaches in the context of ocean observing systems are short pieces of synthetic DNA (probes or primers) that bind to complementary portions of those molecules in the target HAB species. These targets can be visualized and/or quantified using a variety of techniques such as whole-cell fluorescent *in situ* hybridization (FISH; Anderson *et al.* 2005b; Hosoi-Tanabe and Sako 2005), sandwich hybridization assays (SHA; Scholin *et al.* 1996; Diercks *et al.* 2008), and a variety of PCR-based assays (e.g., Penna and Magnani, 1999; Guillou *et al.* 2002). Of these, the FISH technique is not amenable to *in situ* use in observatory systems, so future applications will likely utilize either the SHA or quantitative PCR (qPCR).

The SHA involves chemical lysis of the algal cells to release ribosomal RNA target molecules that are then “captured” by a probe immobilized on a surface, and visualized using a colorimetric, fluorometric, or chemiluminescent reporting system linked to a second (“signal”) probe in solution. The SHA allows for rapid, high throughput sample analysis and has been effectively automated in a variety of formats, including in the ESP (Fig. 4A; Scholin *et al.* 2006; Greenfield *et al.* 2006). One advantage of this approach is that it utilizes a crude plankton lysate for analysis—i.e., no RNA purification is needed. Another is its sensitivity—with detection limits of a few thousand cells/L of *Pseudo-nitzschia* species, and 100 cells/L with *Alexandrium* species, given the present pumping and filtering specifications (C. Scholin, pers. comm.). With higher volume sample concentration, currently under devel-

opment, detection limits can drop to levels of a few cells/mL, sufficient to identify the earliest stages of blooms. For example, STMicroelectronics offers the In-Check® platform, a microfluidic chip that combines PCR amplification and probe array detection functions. Integrated devices like this could find application in an ocean observatory setting.

Another rapidly emerging approach to the detection of HAB species is qPCR (e.g., Bowers *et al.* 2000, 2006; Galluzzi *et al.* 2004; Coyne *et al.* 2005). With respect to *in situ* applications in robotic or moored systems, it is of note that quantitative PCR procedures require extraction and purification of nucleic acids from samples, an enzymatic reaction mixture, and application of one or more thermocycling protocols. Sample handling and processing are thus important considerations for *in situ* measurements. Nevertheless, portable instruments suitable for field applications are being developed and show promise for inclusion in ocean observatory-based HAB monitoring and research programs in the near future.

One example of the manner these constraints are being addressed is the portable sensor technology developed for detection and enumeration of the HAB species *K. brevis* using nucleic acid sequence-based amplification (NASBA; Casper *et al.* 2004). NASBA is an isothermal method for the amplification of RNA, so this simplifies the power and manipulation requirements of the assay which otherwise would require multiple heating and cooling steps. To address the problems with extraction of RNA from water samples, a simple procedure was developed that requires no special equipment or training, and that performs as well as expensive, commercial kits (Casper *et al.* 2007). Further progress was made with the development of a handheld sensor that provides real-time fluorescence plotting of the amplification. Results using the handheld NASBA analyzer compare favorably to

laboratory-based technologies. This extraction protocol and detection sensor are now being incorporated into an autonomous platform called the AMG, described above.

3.4. Modeling and forecasting

The value of data from ocean observatories is greatly enhanced by numerical modeling techniques that can lead to forecasts of HAB transport and dynamics. A region in which HAB-specific instruments are deployed should therefore take steps to develop and validate numerical models that incorporate local HAB species into hydrodynamic models of the region (physical-biological models). The ultimate goal is to obtain data on HAB cells and toxins through instruments in an observatory system, assimilate these data and contextual meteorological and oceanographic observations into the models, and provide continually updated forecasts of bloom behavior. The first step in this process is the formulation of conceptual models that explain in words and simple diagrams how HABs occur in a given area. Examples of conceptual models developed for *Alexandrium* blooms in the Gulf of Maine are given in Anderson *et al.* (2005b) and McGillicuddy *et al.* (2005). If a verbal description of a model can be formulated that is consistent with observations and data over an extended interval of time, it is much easier to formulate a numerical model that captures the same dynamics (McGillicuddy *et al.* 2005). For many areas of the world, there are significant challenges to achieving this goal, as neither conceptual models nor numerical models exist for regional HAB problems.

A significant constraint to numerical model development is the need to identify initial conditions for the biological fields (i.e., the HAB species' distribution). In the Gulf of Maine example, cyst maps in bottom sediments are used as the initial condition, with germination of those cysts producing

the vegetative cells that ultimately grow and form the bloom (McGillicuddy *et al.* 2005; He *et al.* in press). In other HAB systems, and in particular those without cyst populations, cell concentrations measured by instruments in an observing system may well provide the initial conditions for subsequent model runs and forecasts of bloom dynamics.

In addition to forecasting, numerical models can help in the identification of key locations at which instruments capable of detecting HAB cells and toxins can be deployed. Figure 5 demonstrates one of the challenges associated with siting decisions for observatory instruments. Given the conceptual model described earlier for *A. fundyense* in the Gulf of Maine (Fig. 2) it is possible to identify key locations in the different transport pathways that would facilitate HAB detection and forecasting in this large region. With a hypothetical set of 9 moored instrument packages, scientists would place instruments at key locations and branch points, with horizontal variability in the coastal currents and *Alexandrium* distributions addressed through cross-shore arrays of instruments at each of the key locations (Fig. 5A). However, shellfish managers would choose to locate the same number of instruments along a line just offshore or upstream of important shellfish growing areas (Fig. 5B), providing alongshore resolution, but none in the cross-shore direction. In this instance, the managers opt for early warning through direct cell detection rather than through numerical model forecasting. Resolution of the difference in viewpoints depicted in Fig. 5 would require a demonstration that a scientifically or hydrographically based mooring configuration, coupled with a numerical model, can provide early warning information suitable for management purposes.

Another justification for model development in an area where an ocean observatory is planned is that the model can be used to identify the locations where data on HAB

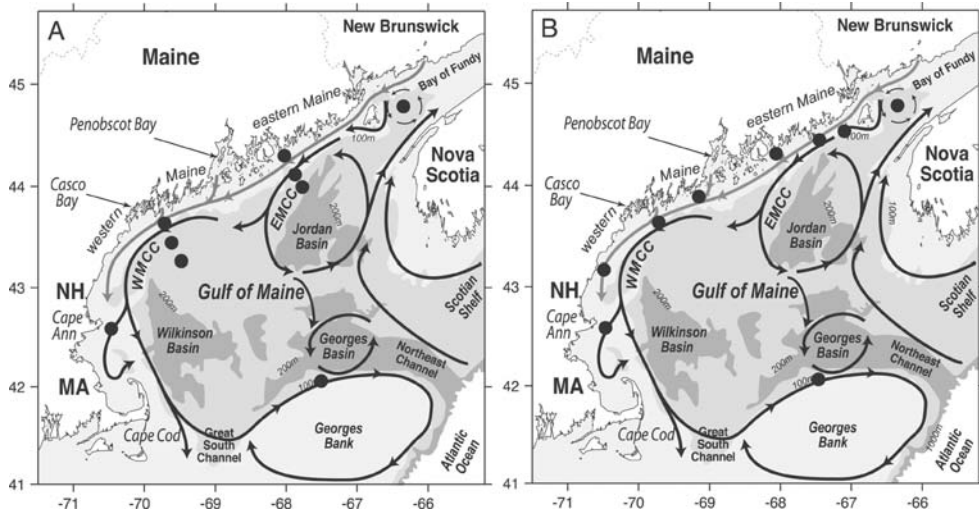


Fig. 5. Map showing possible locations of ESPs or other instruments capable of detecting *Alexandrium fundyense* cells and/or toxins in the Gulf of Maine. **A)** Configuration suggested by scientists to capture horizontal variability in cell distributions and water masses along key transport pathways. **B)** Configuration suggested by managers, who simply want early warning at major shellfish growing areas.

species abundance or toxicity would be the most useful. Termed observing system simulation experiments (OSSEs), these numerical analyses have been used in dynamic meteorology (e.g., Charney *et al.* 1969) and are becoming an important tool in the planning of oceanographic sampling systems (e.g., Robinson *et al.* 1998; McGillicuddy *et al.* 2001). OSSEs can help to optimize the number and location of instruments needed to provide a necessary level of coverage. The ultimate goal is to have an array of instruments located in strategic spots to capture the information that can then be assimilated into numerical models through time, greatly increasing their accuracy and utility. One cannot over-emphasize the importance of proper instrument siting or validated numerical models if the true potential of ocean observing systems is to be realized in HAB research and monitoring programs.

4. Summary

Improvements in HAB monitoring and forecasting are frequently cited as justifications for the deployment of ocean observing systems, yet few of the observatory systems being deployed worldwide have any HAB components. This is largely because the technologies needed to achieve HAB cell or toxin detection are still under development. Only a small number of HAB species can be detected using optical measurements, either *in situ* or remotely from space, and therefore instruments that can detect the vast majority of HAB species need to have capabilities for sample collection, concentration, and manipulation. The chemistries and procedures for cell identification and enumeration using molecular probe assays of various types are well established, so the challenge now is to incorporate these assays into autonomous instruments that are capable of

sample collection and processing while moored or deployed within an AUV. Several instruments have been developed that have this capability in moored configurations (i.e. the ESP and AMG) and others will surely be developed in the future given the need for this type of capability in many other types of environmental monitoring.

In a similar manner, methods for toxin detection have been developed that can be incorporated into moored instrument packages, typically based on structural assays such as immunoassays. Here again, incorporation of these methods into instruments capable of the appropriate processing has only just begun. Development is rapid on all of these fronts, but will be greatly accelerated if research and development funding is targeted to technologies for species-specific detection of HAB cells and metabolites.

All too often these days, ocean observa-

tory resources are dedicated to existing, proven technologies that are ready for deployment. If the development of HAB-specific instrumentation lags too far behind the pace of observatory infrastructure construction, observatory assets will be located in areas or at sites that are not optimal for HAB detection or will not have the appropriate power or mooring capabilities, and thus the goals of improved HAB monitoring and management through ocean observatories will not be realized.

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Coastal Artificial Habitats for Fishery and Environmental Management and Scientific Advancement

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Benthic reefs are the principal type of artificial habitat deployed in coastal waters worldwide to achieve fishery, ecosystem and other purposes. Ancient techniques to attract fishes to submerged natural objects for subsistence food harvest continue to be used, especially in tropical areas, while larger and more complex reefs commonly built of manmade materials and designed to meet an ecological life history requirement or limiting factor have been used in commercial fishing over the past 50 years, and more lately in marine ranching. Geographic centers of long-term artificial reef research and development include eastern Asia, the Mediterranean basin, India and North America. Trends in the use of this technology include a wider number of non-fishery applications, such as protection of habitat, conservation of biodiversity and socio-economic development, at a growing number of sites in over 50 countries. This paper presents significant findings and trends concerning the ecology of artificial reefs, their effects on fisheries and ecosystems, and appropriate applications of the technology. The maturation of long-term biological datasets has enabled quantification of ecological processes such as production of biomass at artificial reef sites, characterization of functional equivalence for artificial and natural reefs, and determination of sustainability of certain localized fishery harvests.

KEYWORDS artificial reefs; fisheries; environmental enhancement; habitat conservation; ecosystem restoration; eco-technology

1. Introduction

Artificial habitats are used in coastal waters worldwide for a variety of purposes concerning fishery and environmental management and sustainability. The most common application of this technology is in the form of artificial reefs, which include structures of natural or manmade origin intentionally deployed on the sea floor usually to modify ecological processes. Principal topics concerning artificial reefs addressed in this paper include their ecology and influences on fisheries and ecosystems, appropriate use of this technology, and progress toward their scientific understanding. The focus of this article is on common and somewhat larger benthic structures, so that small, less frequently used items such as plastic seagrass are not addressed. Also, suspended fish aggregating devices are not covered. Because they are prominent in certain situations, due to their role as *de facto* artificial reefs, very large objects including ships, petroleum production platforms, and rock breakwaters are included.

A growing body of evidence supports the argument that artificial reefs have merit as both natural resource management tools and research platforms in aquatic environments, giving them an appropriate place in mainstream Fishery Science. This gradual acceptance reflects a promise inspired by earlier practical successes and research advances reported only in the last decade, tempered by justified concerns for environmental and economic compatibilities still needing resolution.

2. Overview of Trends

In the past 20 years the use, modification and expansion of artificial reef practices have spread to all inhabited continents, and now include a more diverse set of practitioners than the earliest interests that, over millennia, have sought to increase subsistence fish-

ing harvest. Modern applications of artificial reef technology include not only the earlier purposes of artisanal and commercial fishing to produce food, but also newer and broader objectives including recreational fishing and diving, eco-tourism, aquaculture and marine ranching, conservation and management of biodiversity, habitat restoration, education and research (e.g., Jensen 2002; Relini *et al.* 2007). (Some of these aims are beyond the scope of this paper.)

The historical approach of clustering rocks or weighted logs in shallow waters to attract fishes for easier harvest has been supplemented by use of opportunistic materials (e.g., derelict ships) as well as design, development and testing of larger modules and fabricated structures of concrete, fiberglass and steel that can approach the volume of small buildings as much as four or five stories tall (Fig. 1). Globally there is neither a formal compilation of physical locations and attributes of reefs, nor a directory or network of the interests that build and use reefs. The history of the field remains scattered in individual reports. It would not be possible to determine total fishery landings from all reefs worldwide. It can be estimated that the coastal waters of over 50 nations and territories contain artificial reefs, based on participation of 48 in at least one international conference since 1987 (Appendix 1); additional nations are recent entrants in the field. The spatial extent of reefs in coastal waters worldwide is not documented, although the largest relative coverage in any nation appears to be in Japan where, as of 1987, 9.3% of seafloor shallower than 200 m was dedicated to "fishing ground area" (Yamane 1989).

The emergence of a small number of private businesses that deal with the technology of artificial reefs is one anecdotal indicator of the growth of this field in recent years. Meanwhile, numerous university research centers and governmental ministries, agencies and laboratories at national, regional and

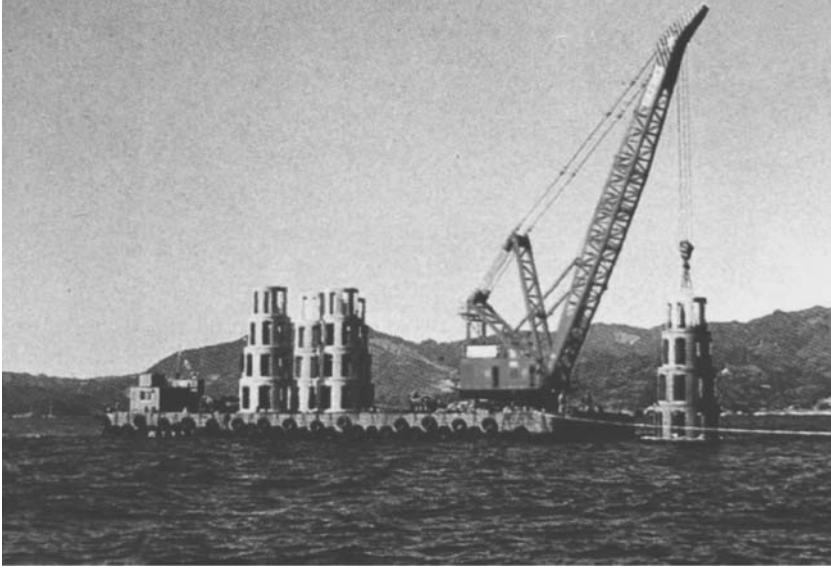


Fig. 1. Some of the largest artificial reefs, as used in commercial fishing in Japan (photograph courtesy of S. Otake).

local levels have engaged, for decades, in development, testing, inter-disciplinary research and deployment of artificial reefs. The earliest technological advances for reef development concerned physical attributes. Japanese interests pioneered this field in order to build and deploy large, manufactured structures in open-ocean, heavy-seas conditions. A series of engineering handbooks document this work (e.g., JNCFDA 1984), along with some of the earliest biological studies (e.g., Kakimoto and Okubo 1985).

More recently a notable trend has been the increasing use of science-based information in the biological aspects reef planning, design, construction, evaluation and management. Thus, it is worth investigation to determine if newer reefs, at least in some cases, have an overall ecological structure and function that more closely “mimics” natural reefs than older reefs. In other cases, newer reefs meet more closely the life history needs of at least some selected “target” species (Fig. 2). Manipulative ecological experiments have become more common in this field, as re-

search has evolved beyond observational and monitoring studies. More long-term investigations are in progress.

Since the Fourth World Fisheries Congress (2004), which included a session on artificial reefs, artificial reef technology has been implemented in additional countries not identified in Appendix 1, such as Egypt, Malta and Tunisia; another in a series of global conferences on the subject has been held (2005); and the first formal, technical, international course on design and management of reefs for fisheries was conducted (2007).

Finally, significant resolution of perhaps the most contentious (and publicly visible and misunderstood) issue in this field—the so-called “attraction/production” debate—seems to have occurred, at least in much of the scientific community. The rigid either-or nature of the debate has been set aside in the sense that research results reported in peer reviewed journal articles and conference papers help quantify the spectrum of levels of biological production (versus aggregation) originally proposed by Bohnsack (1989). The



Fig. 2. Representative of efforts to incorporate biological design criteria into the physical structure of artificial reefs is the Box Reef, with open space in its upper level and confined space below (photograph courtesy of C. Kim).

longer term rigorous investigations urged by observers in the 1990s are now coming to fruition, yielding a rich database relevant to some of the trends introduced above, and others, as discussed in the following sections.

3. Key Sources of Information

In some aspects, artificial reef technology has been advanced considerably by the applied efforts of competent practitioners on-site in the field, while the theoretical and scientific sector initially lagged behind in its thinking. Much of the development of this field has been reported in conferences and journals. As many as 28 nations have been represented at such meetings at one time. As a proxy for a directory of reef-related research and development worldwide it is possible to use the literature provided by these meetings. Table 1 summarizes the record of contributions by authors from 48 countries and territories, who in any one conference presented as many as 180 oral reports and posters (1991)

and published as many as 85 peer-reviewed journal articles (1994) and 101 non-reviewed proceedings articles (1995).

As a basis for analysis and synthesis of some recent trends and findings in research and management most recently reported at international conferences and in reviewed journals, the 55 papers published in the *ICES Journal of Marine Science* (2002, volume 59 [from 1999 conference]) and 20 papers in the *Bulletin of Marine Science* (2006, volume 78 [2005 conference]) were evaluated for content (Table 2). In addition to principal subjects covered, attributes of research including spatial and geographic locations, reef age and composition, and duration of study and overall database were noted, in part as an aid to forecasting future directions. It is informative to note that 34 of all the articles (75) reviewed for Table 2 address reef ecology primarily (45%), while 18 articles (24%) cover influences of reefs on fisheries, etc. and 23 (30%) cover planning and management-related issues. The analysis of literature is part of the basis for defining the

Table 1. Levels of effort for research and management related to artificial reefs indicated by number of presentations and published articles associated with international conferences (locations given in Appendix 1).

Conference Numbers				Publication Numbers			
Year	Countries of authors	Posters	Oral presentations	Year	Countries of authors	Abstracts	Papers
1983	7	14	43	1985*	7	26	29
1987	24	37	94	1989*	17	19	53
1991	25	(180 Combined)		1994*	25	127	85
1995	28	No Data	No Data	1995	27	0	101
1999	20	40	96	2002*	13	0	55
2005	20	25	68	2006*	9		20

*Articles peer reviewed

Table 2. Subjects addressed in peer-reviewed articles in journal compilations from two most recent international conferences on artificial reefs.

Overall theme discussed in paper and general subject area	Number of articles published	
	<i>ICES Journal of Marine Science</i> , 2002 (1999 conference)	<i>Bulletin of Marine Science</i> , 2006 (2005 conference)
Reef ecology:		
Species abundance, distribution, diversity	17	2
Behavior and movement	3	1
Diet and feeding	2	1
Productivity and production of biomass	6	2
Reef-environment interactions:		
Physical and chemical effects	4	1
Biological design criteria	3	2
Ecological and fishery effects	3	2
Ecological analysis and modeling	3	0
Reef use and management:		
Planning, policy, stakeholders	9	7
Reviews and methods	5	2
Total articles	55	20

following three sections. As much as possible, longer term studies are emphasized in this paper.

Time and space constraints did not permit extensive reviews of journals containing individual articles (i.e., included as “stand-alone” contributions), such as *Marine Ecology Progress Series* and *Hydrobiologia*. It is noteworthy that such journals are publish-

ing more articles on artificial reefs in recent years, which may reflect growing emphasis on hypothesis-driven and manipulative investigations.

4. Artificial Reef Ecology

An ecological premise of responsibly deploying artificial reefs is that they target

limiting factors, such as space for settlement, shelter or reproduction, and food. Bohnsack (1989) stated the rationale for reefs intended to increase biological production as providing "additional critical habitat that increases the environmental carrying capacity and eventually the abundance and biomass of reef fishes." Scientific documentation of the ecological structure and function of artificial reefs—often as objects worthy of study in their own right—began in earnest in the 1980s, subsequent to detailed engineering studies and ahead of socio-economic research. In a description of the progression of study in ecology, generally, from observational and descriptive to process-oriented and more experimental, Miller (2002) noted that "artificial reef research is an applied field and process-oriented study has often been given low priority" (p. S27). She also observed that artificial reef goals are "underpinned by ecological processes" including: recruitment, competition and trophic interactions (which bear on fisheries production); biogeochemical cycling and organism physiology (which bear on water quality enhancement); and life history/recruitment and species-habitat interactions (which bear on ecosystem restoration). Linkages between ecological processes and applied goals (such as the three indicated above) are part of the framework for this paper. Also, some of the methodologies used in research, and characteristics of reef sites are presented below.

There is not a universally used standard design of artificial reefs, for which commonly agreed measures of defined attributes might be made by the international research community. However, in numerous locations cubic modules of various designs are used in scientific studies and management trials and applications (Fig. 3).

Historically, early research worldwide has focused on species distribution and abundance, often at young sites just one or two years old. The purpose has been to characterize biodiversity using observational meth-

ods. Generally, the work has been in relatively shallow waters, performed by trained divers, whose techniques constitute a small body of literature derived from research on coral reefs and artificial reefs. The importance of long-term biological study has been recognized increasingly. For example, databases of 14 years from Portugal (Santos and Monteiro 2007) and 15 years from Italy (Relini *et al.* 2007) augment even longer term reports from the United States with a duration of as much as 25 years (Stephens and Pondella 2002). Fishes are the principal subjects of research, whereby of the 43 papers dealing with biological topics published in two journals after the two most recent international conferences, 29 dealt with fishes, 11 dealt with benthic invertebrates, three with plants.

Adding reef structure to the coastal benthic environment has been documented repeatedly to increase species abundance and diversity at the reef site. The rapid increase of adult fishes (via attraction) at newly constructed reefs is commonly observed. Also, patterns of colonization of increasing numbers of plant, invertebrate and fish species, to some plateau or equilibrium, have been documented; microbes remain unstudied. A 10-year database of 105 visual censuses at the Loano artificial reef (built of 8 m³ concrete cubic modules) indicates that colonization and maturation of the site was a slow process (Relini *et al.* 2002), in which a nucleus of 15 fish species (present in over 25% of surveys) was joined at various times by 29 other species. The authors compared the Italian artificial reef site to a nearby natural rocky area in France, finding principal fish families in common (Sparidae, Labridae, Serranidae), and concluded that the artificial reefs "act in the same way as natural rocky seabed, increasing both species diversity and fish biomass" (p. S136).

In a nine-year study of a *de facto* reef created by disposal of 2,000,000 tonnes of waste steel slag in 40 m of water off southern

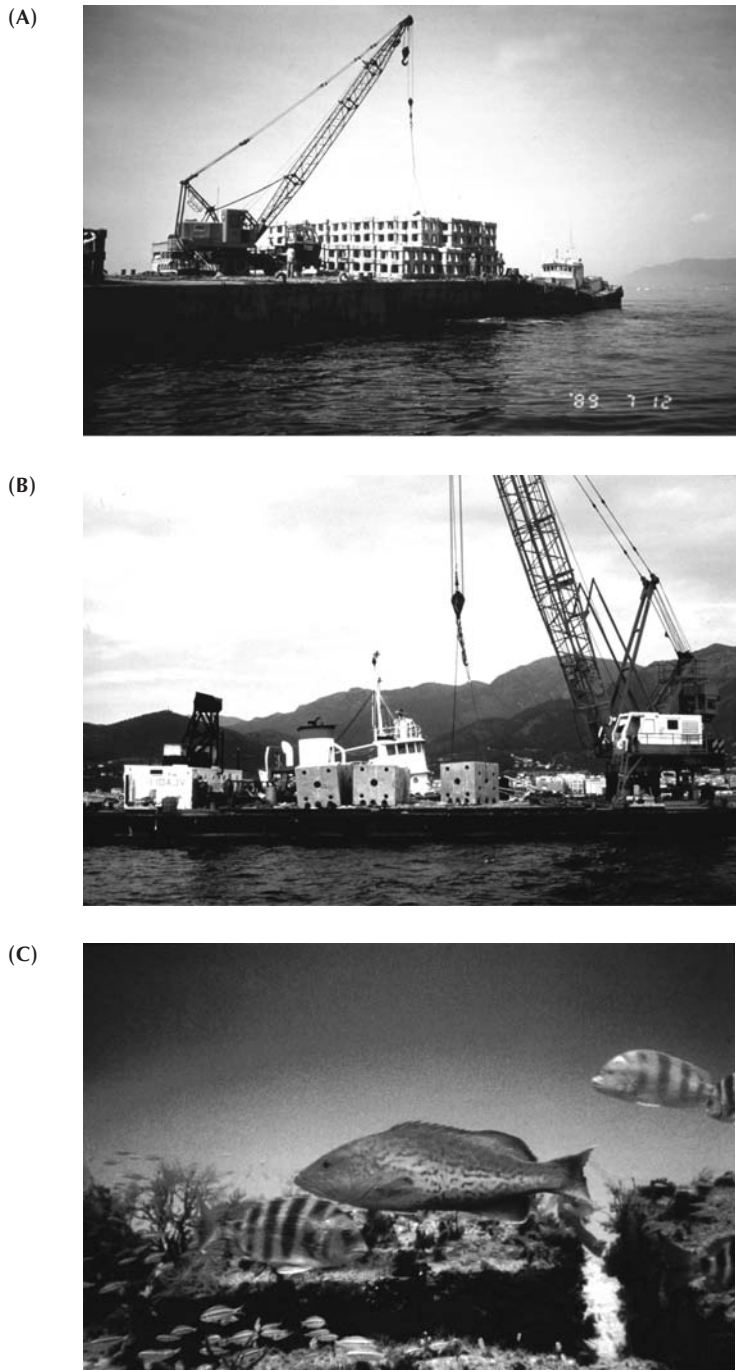


Fig. 3. Cubic modules with side dimensions of one to two meters are the closest structure used as a “standard” in artificial reef research and application. Convergence toward common design is illustrated by cubes used in (A) Taiwan, (B) Italy, and (C) United States (photographs courtesy of K. Tsao, G. Relini and W. Lindberg, respectively).

Taiwan from 1984 to 1989, Chou *et al.* (2002) determined by means of bottom trawling that number of fish species present at disposal area sampling sites (mean = 15.5, Shannon diversity index [H'] = 1.91) was significantly higher than at control sites (mean = 9.8, H' = 1.56). These authors report significantly different fish community structures as well, with the 12 km² slag disposal area favored by Bothidae and Scorpaenidae, for example. The nearby sandy control area favored Callionymidae and Mullidae. Overall number of individuals was not significantly different between the two areas. The conclusion of this study is that the material placed on the bottom increased habitat complexity.

Patterns of residence and site fidelity by red snapper, *Lutjanus campechanus*, on artificial reefs were determined by Schroepfer and Szedlmayer (2006). Using ultrasonic tags and arrays of remote receivers they determined that (1) for 99% of total time 87% of individuals stayed within 200 m of their original release site, and (2) median residence time was 373 days. They suggest "that artificial reefs provide suitable habitat for *L. campechanus*."

Artificial reefs can provide food for fish assemblages, and in turn contribute to production of biomass. A 25-year study of two embiotocid species, *Embiotoca jacksoni* (black surfperch) and *Rhacochilus vacca* (pile surfperch), is unique not only for its duration and rich database, but also for the viviparous life history of these species, whereby their entire ontogeny could be observed on the artificial reefs (Pondella *et al.* 2002). The site was 50 years old when the research began. The King Harbor artificial reef (a rock breakwater) supported significantly higher mean densities of both juveniles and adults of black surfperch (1.3 and 5.6, respectively, per 100 m²) and also pile surfperch (0.39, 2.4) than did the nearest natural rocky-reef 9 km away (0.1 and 3.1, 0.08 and 0.13, respectively). The authors

conclude that the artificial reef had a higher carrying capacity, and that its populations were self-maintaining and did not draw from natural reefs. Typically, trophic studies are much shorter. For example, in 1997–1999 Fabi *et al.* (2006) studied the established Senigallia artificial reef (built 1987, 11 m depth) and based on summer and autumn data determined that it "provided the main source of food" for the species of concern (i.e., *Sciaena umbra*, *Diplodus annularis*, *Lithognathus mormyrus*). Items in the diets included organisms from soft bottom (mollusks, polychaetes) and hard substrate (crustaceans).

The difficulties of evaluating (and comparing) production at artificial and natural reefs were identified by Pondella *et al.* (2002) as including lack of information about life history parameters (e.g., birth rates, mortality, immigration/emigration, growth), fluctuation of populations, and high variance over time. In a study of King Harbor breakwater (built in 1950–1958) and a control that lasted for 24 years, Stephens and Pondella (2002) not only documented long-term changes in environmental conditions, and corresponding change in fish assemblage composition, but also determined that the breakwater artificial reef contributed "generously" to the reef fish larval pool of the region, and at a magnitude comparable to natural reefs. The report of these findings (in 2002) addresses some of the information gaps identified in 1989 by Bohnsack.

Studies of epibiota on artificial reefs are relatively few. Of 17 articles concerning "species abundance" in the ICES 2002 document (Table 2), for example, only Collins *et al.* (2002a) address epifauna. Using an incubation chamber, over four summers (1995–1998) these authors determined respiration and oxygen production on a 12-m deep concrete block reef built in 1989 in Poole Bay, England. Research on macro-invertebrates and plants is somewhat more extensive and is addressed in a discussion of reef design criteria and ecosystem influences, below.

5. Artificial Reef Influences on Fisheries and Ecosystems

The effects of artificial reefs are broad, including physical, chemical and biological influences upon the natural environment, and they rightfully should be of concern. Leaching of deleterious chemicals, for example, must be evaluated in considering the use of so-called waste “materials of opportunity” such as tires, or ashes from combustion in electric power stations (e.g., Collins *et al.* 2002b). Also, entire reefs or their components may sink into substrate or else unexpectedly modify or be moved by hydrodynamic features such as prevailing currents and episodic storm surges (e.g., Sheng 2000). In this section the emphasis is on ecosystem and fishery influences of artificial reefs, consistent with the theme of the 2008 World Fisheries Congress. An important question to address is “What is the ecological response to the physical design of a reef?” More recent socio-economic issues are in the following section.

Japan’s fishery reef-building enterprise and industry is the oldest and most extensive in the world, with large corporations having units that focus exclusively on design, fabrication and deployment of structures. Governmental, academic and private laboratories there and in several other nations have initiated research and development for artificial reef materials and construction. Kim *et al.* (in press) compare Japanese and Korean efforts. Reports from the two most recent international reef conferences include only one engineering paper, however, in which Duzbastilar *et al.* (2006) report on the use of concrete scale models (1:30) of reefs tested in laboratory wave channels for stability and scour performance; this work is incorporated in a “stability chart” used to match design, installation and settling parameters to water depth and wave conditions in Turkey. Both physical and mathematical

modeling are distinct advantages to engineering research.

In contrast to physical and engineering research, ecological modeling and forecasting have been infrequently addressed in studies of artificial reefs. Recent work by Pitcher *et al.* (2002) considered over 250 species of fish at Hong Kong to develop an ECOPATH model of trophic flows among functional groups in the ecosystem and an ECOSIM simulation of biomass fluxes in response to fishing. This in turn led to an ECOSPACE evaluation of biomass and fishery responses to various possible management scenarios, with one conclusion being: “While small protected areas with human-made reefs achieve little to avert collapse of the fisheries or a shift towards catches of low-value species, larger protected areas can do much to restore valuable fisheries for reef-associated fish” (p. S17). Osenberg *et al.* (2002), meanwhile, proposed both a mathematical model concerning the effects of multiple processes on reef fish dynamics, and experimental approaches to address what they define as a gap in information: “We know of no study that has quantitatively compared production (or fish abundance) of replicate natural reefs with and without nearby artificial reefs and partitioned the total production between them” (p. S218).

The lack of study of the effects of artificial reefs on surrounding benthic ecosystems was indicated by Fabi *et al.* (2002), whose research in 1997–1999 on a group of 29 five-module (8 m³ each) concrete reefs built in 1987 and a nearby sandy-muddy area of the central Adriatic Sea (11 m depth) documented 166 invertebrate taxa. Infauna were removed by a suction sampler operated along transects internal and external to the reefs. Differences included accumulation of organic matter inside the reef, which favored deposit and suspension feeders such as polychaetes; molluscs were dominant externally.

A small body of recent literature specifically concerns the influence of attributes of habitat upon biological assemblages at artificial reefs. The subject of design is particularly amenable to manipulative experiments. One of the most expensive and largest (61 ha) artificial reefs in the United States is intended to mitigate for loss of kelp (*Macrocystis pyrifera*) in Southern California. Deysher *et al.* (2002) summarize a set of design specifications for establishing sustainable kelp populations derived from a five-year pilot study at a 9-ha site. The attribute of relief, for example, is most conducive to kelp cover when it is "low" instead of either "very low" or "high." Optimal depth is 12–14.5 m. Importantly, these and other studies (e.g., Reed *et al.* 2006) draw heavily on ecological knowledge of the species of concern, to account for effects of disturbance and grazing upon plant survival as a means of designing reef profile. Finally, this work is notable for establishing two success criteria, i.e., four adult kelp plants per 100 m², and "similarity" of invertebrate and fish assemblages to natural reefs, as a means of evaluating performance of the artificial reef to meet its objectives.

Broadly speaking, "complexity" of habitat is repeatedly identified as a key attribute that in turn dictates biological diversity on an artificial reef. Miller (2002) gave an example of experimental reefs with relatively "greater availability and heterogeneity of refuge space" as supporting more fishes, in a situation where predation was a mechanism limiting fish abundance (Eklund 1997).

In studies of concrete module-based control (deployed 1986) and experimental (deployed 1987, modified 1991) reefs, Charbonnel *et al.* (2002) in 1987–1989 and again in 1997–1998 conducted visual censuses which determined that total number of fish species doubled (to 36) on the experimental reef after addition of complexity, as did the mean number of species per census

(18.9), while total wet weight biomass increased significantly. The authors suggest that increased availability of shelter may be the most important factor involved, and note that increased food is available at the reef and in surrounding habitat. They further conclude that the original design of the reefs lacked effectiveness in meeting the ecological requirements of the local demersal fish fauna, an indication of the need to carefully consider reef design before deployment to enhance fisheries.

One prominent *de facto* artificial reef in the nearshore Pacific Ocean off Santa Barbara, California, U.S.A. is an accumulation of metal debris left on the seabed after drilling of exploratory and production oil wells. Based on analysis of videotapes from 130 objects Caselle *et al.* (2002) assessed fish assemblage composition as related to morphology of structure. They found that vertical profile positively affected abundance for two of four rockfish (*Sebastes*, Scorpaenidae) species studied, and that shelter positively affected species richness.

A study specifically addressing artisanal fisheries in Algarve, Portugal used nighttime gillnet samples (256 net sets over 14 years) to compare yield at artificial reefs (of 2.7 m³ units) and control sites (Santos and Monteiro 2007). Overall, catch at artificial reefs always exceeded control sites, with benthic species more abundant than nekto-benthic and then pelagic fishes. The authors note that ecologically the artificial reefs support the same "relative proportion" of these three functional groups as is found on natural/control reefs. The study concludes that the artificial reefs contribute to improved local artisanal fisheries. The occurrence of benthic species on the Algarve reefs meets one of the characterizations for species likely to inhabit artificial reefs listed by Bohnsack (1989); other life history characteristics include territoriality, philopatry and habitat limitation.

6. Artificial Reef Applications in a Management Context

The use of artificial reefs in mainstream fishery and aquatic science has become more accepted, even as healthy skepticism remains. The historical lack of evaluative studies and experimental manipulations of course have justified concerns for applying reef technology. Fabi *et al.* (2002, p. S343) state that “Artificial reefs are commonly used around the world as fishery-management tools and to replace habitat losses caused by human impacts.” Santos and Monteiro (2007) state that they “have become important elements of integrated fisheries management plans” (p. S25). In both cases these authors have been studying reef systems for over a decade. Conversely, Jensen (2002) states: “Artificial reefs are still seen by most managers of the marine environment and/or fisheries in Europe as an ineffective and expensive technology” (p. S9). He refers to concerns for overexploitation of aggregated adult fishes, but also to gaps in communication by experts to explain actual and potential applications.

Artificial reefs are deployed in coastal ecosystems for aims such as physical protection against illegal trawling, restoration of habitat, enhancing biodiversity, improving fishing catch, research on materials and designs performance, and environmental observation, as enumerated by Relini *et al.* (2007). These authors reported on one of the richest datasets for artificial reefs anywhere: From research over 30 years in the Gulf of Genoa, Italy, which has resulted in about 100 technical publications, they conclude that reef deployment successfully achieved increase of species richness and biomass, and protection against otter trawling in sensitive habitat. The long time-series of data contributes to a finding of functional equivalence between artificial reefs and natural rocky reefs.

Because of the localized and seemingly small scale of many deployments of artificial reefs it is important to keep in mind the community and cultural characteristics of key groups of stakeholders. A response to destruction of habitat and overfishing in many areas of the world is typified by artisanal and subsistence fishing interests in India to revitalize longstanding utilization of small artificial reefs. The situation described for Kerala by d’Cruz *et al.* (1994) is noteworthy because the local populace was strongly motivated to regulate fishing activity and access to sustain long-term harvest, and because the investigations closely incorporated local citizens into project planning and data gathering. Understanding the social framework of the community was a prime concern of the study. Similarly, in an urban setting, Ditton *et al.* (2002) observed a trend in natural resource management “towards an understanding of, and planning for, resource users and their recreational experiences rather than simply being concerned with biological enhancements” (p. S186), and reported the response of sport divers in Texas, U.S.A. that they would most prefer large naval ships as a reef material, instead of other structures such as concrete blocks. In fact 82% of divers with this preference indicated that they would increase their diving if a ship was deployed at their favorite dive site. Sensitivity to local needs is reflected in commercial fishing development, as well, as reported for Japan by Simard (1995).

Despite a trend toward increasing use of specially designed and fabricated reef structures worldwide, certain “materials of opportunity” (i.e., surplus and waste manufactured items of considerable size) will continue to be proposed. Managers of natural resources need to be prepared to deal with them objectively. Of particular note is the situation in which obsolete oil and gas production platforms are already used, or proposed for decommissioning and redeployment, as fishing enhancement structures. Eight of 55

papers published from the 1999 international conference on artificial habitats address petroleum platforms, as examined in a conference session, and offer benchmark information about fisheries potential. One of the papers, by Cripps and Aabel (2002), identifies 39 possible impacts of "rigs" being used as reefs including biological factors such as redistribution of biomass, overfishing and changes in infauna, as well as legal and operations issues. This approach is relevant to planning of artificial reefs generally. (For the Norwegian reef addressed by these authors, a protection strategy for platform use was deemed to have more benefit than a fishing strategy.)

A key element of understanding how artificial reefs can be used as an integrated management tool (Jensen 2002) is the ability to evaluate their performance. Preceding sections have focused on physical and biological aspects. Socio-economic assessment is also important, but its implementation has lagged. Milon *et al.* (2000) decry the "general lack of reports or studies about the demand for artificial reefs and the socio-economic efficiency of these projects." Such studies remain limited. Positive effects from the sinking of a naval ship in the Florida Keys National Marine Sanctuary (USA) were determined in testing of a hypothesis by Leeworthy *et al.* (2006) to include both reduction of diver pressure on nearby natural reefs and expansion of the local economy as measured by growth of income and employment in dive charter businesses. In a progression from biological to economic assessment at artificial reefs in Portugal, Ramos *et al.* (2006) found that commercial fishing regularly occurred at the Olhao Reef System and that an income significantly higher than the national minimum wage was realized,

Marine ranching has been emphasized in eastern Asia, and the additional concept of stocking hatchery-reared fishes onto artificial reefs developed there. The concept has been extended to southern Europe, where

trials with two species of Sparidae show promise as part of an integrated plan to enhance local artisanal fisheries (Santos *et al.* 2006).

7. Discussion and Outlook

As with other practices of Fishery Science, artificial reefs must be used in a science-based fashion and administered, evaluated and enforced realistically. A precautionary approach toward artificial reef development is common among scientists and administrators. The advent of a multi-disciplinary research base has enhanced the adoption of some reef practices, and the dismissal of others. Key advances in this field include maturation and subsequent analyses of long-term databases to address fundamental ecological issues (e.g., production); specification of physical, biological and socio-economic design criteria (e.g., habitat complexity) to enhance performance of reefs consistent with measurable resource management aims and success criteria; and in a few cases the "scaling-up" of reef size either for research (e.g., Loch Linne, Scotland reef of 42,000 tonnes) or to influence larger spatial areas for fisheries (e.g., Algarve, Portugal reefs involving 35 km²; Jensen 2002).

Scientifically, advancement of this field will be furthered by: (1) understanding of the basic biology of reef organisms; (2) tying design criteria to life histories of reef organisms; (3) development of long-term databases from individual sites; (4) comparison of reef sites across appropriate spatial gradients; (5) continuing expansion of manipulative experiments; (6) pilot studies as cost-effective precursors to large reef developments; (7) inter-disciplinary studies; (8) cooperative studies across regional and national boundaries; (9) fishery and ecosystem modeling; and (10) specification of success criteria and evaluation of reef performance against measurable objectives. Meanwhile, communication between scientists and the managers of resources

is essential to foster understanding of potential and limits to reef technology, and expedite information transfer. A model of expansive regional cooperation is seen in the European Artificial Reef Research Network, which has promoted formulation of common research priorities and documentation of scientific and management progress (e.g.,

Jensen 2002). Continuing the series of international conferences, expanding technical education courses for professionals on reef design and management, and conducting a review and synthesis of published reviewed technical literature on reef ecology and influences all would strengthen this field.

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Appendix 1. Identity of 48 countries and territories with artificial reef development as reflected by oral and poster presentations at international conferences, 1987–2005.

Country/Territory	Year and location of conference				
	1987	1991	1995	1999	2005
	United States	United States	Japan	Italy	United States
Australia	X	X	X		X
Bangladesh			X		
Belgium					X
Brazil				X	X
Canada	X	X	X	X	
Chile		X			
China			X		X
Colombia					X
Costa Rica	X				
Cote d'Ivoire	X	X	X		
Cuba		X	X		
England		X	X	X	X
France			X	X	
Germany	X			X	X

Appendix 1. (cont.)

Country/Territory	Year and location of conference				
	1987	1991	1995	1999	2005
	United States	United States	Japan	Italy	United States
Greece				X	
Guatemala	X				
Hong Kong			X	X	
India	X	X	X		
Indonesia	X				
Israel	X	X		X	X
Italy	X	X	X	X	X
Jamaica	X				
Japan	X	X	X	X	X
Korea		X	X		X
Malaysia		X	X		
Maldives	X	X			
Mexico	X	X			X
Monaco	X	X		X	
Nigeria		X	X		
Norway			X	X	
Oceania	X	X			
Philippines	X	X	X		X
Poland				X	
Portugal		X	X	X	X
Puerto Rico			X		
Romania	X				
Russia		X	X		
Scotland	X		X	X	X
Senegal			X		X
Spain		X	X	X	X
Sri Lanka	X	X	X		
Sweden			X		
Taiwan	X	X	X	X	X
Thailand	X				
Turkey				X	X
Ukraine			X	X	
United States	X	X	X	X	X
Virgin Islands	X	X			
TOTAL	24	25	28	20	20

“Sato-Umi”—A New Concept for Sustainable Fisheries

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A new concept for sustainable fisheries called “Sato-Umi”, is defined as “High productivity and biodiversity in the coastal sea area with human interaction”. To establish Sato-Umi, it is necessary to realize comprehensive material cycling in coastal sea area. In order to achieve high aquatic biodiversity, we have to keep nutrients concentrations moderate within coastal sea area to provide good habitats for marine biota. Proper management of aquatic resources is important to obtain high fish productivity in Sato-Umi.

KEYWORDS sustainable fisheries; material cycling; environmental conservation

1. Introduction

Some people say that “Nature takes its best state without mankind.” Would it be true that no environmental problems would exist if mankind was not present on Earth? However, there would be no meaning to a discussion regarding environmental problems without the presence of mankind.

Nature does exist that takes its best state under mankind’s interaction. In Japan, an example of such nature is called “Sato-Yama”. In Japanese, “Sato” means the area where people live and “Yama” means the forest. Sato-Yama is thus the forest near where people live, which will be explained

in the next section. The area of Sato-Yama in Japan is about 4,500,000 ha making up about 20% of total area of forest of 25,000,000 ha, which occupies 67% of the total area of Japan.

In this paper we discuss a new concept for sustainable fisheries based on the ideas of Sato-Yama.

2. Sato-Yama

Sato-Yama forests are dominated by deciduous trees such as oak and these trees are cut every 20–30 years for use in mushroom cultivation or for making charcoal. Fallen leaves are used as fertilizer in rice fields near Sato-Yama forests. Insects such as butterflies and

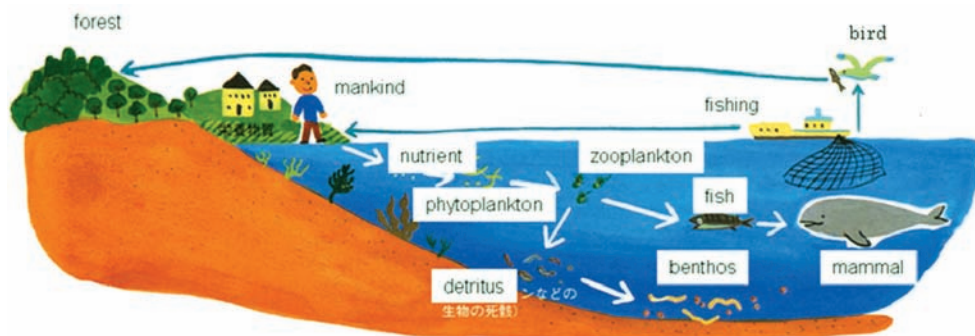


Fig. 1. Comprehensive material cycling in the coastal sea area.

beetles come to Sato-Yama for honey from flowers and trees honey, respectively, and small animals such as squirrels come for acorns. Many flowers bloom in the spring because there have been no leaves.

The Sato-Yama forests are managed through periodical cutting of plants and trees. Periodic harvest of plants and trees are actually good for the maintaining high biodiversity within Sato-Yama. A beautiful and successful relation between nature and mankind has thus been maintained in Sato-Yama. We may call such relationship a kind of symbiosis between nature and mankind, and can conclude that sustainable forest practices are realized in Sato-Yama.

Parts of Sato-Yama have decayed in recent years because many people have abandoned the life near Sato-Yama in the countryside and have moved to the city. The periodic disturbances have been lost in much of Sato-Yama and so the high productivity and biodiversity have also disappeared.

However, some city residents have recently started to go to Sato-Yama to work part-time to re-establish the nature of Sato-Yama (Takeuchi *et al.* 2003).

3. Sato-Umi

Would it be possible to create a “Sato-Umi” similar to Sato-Yama? In Japanese, “Umi”

means the sea, so “Sato-Umi” we define as “High productivity and biodiversity in the coastal sea area with human interaction” (Yanagi 1998).

To establish Sato-Umi, we first need to understand quantitatively the material cycling in the coastal sea area. We need to know the quantity of nutrients that are discharged from the coast, and the primary, secondary and tertiary productions in the Sato-Umi area. Understanding the comprehensive material cycling in Sato-Umi area is a necessary first step (Fig. 1).

Successful establishment of Sato-Umi will also need to identify permissible coast, shoreline and coastal sea area manipulations to increase fish production (human’s benefit from the coastal sea area) and biodiversity (many kinds of biota in the coastal sea area).

For example, a red tide (abnormal increase of phytoplankton biomass) which has a large material flow is not comprehensive material cycling because the large biomass of dead phytoplankton sinks to the bottom creating an oxygen-deficient water mass (hypoxia or anoxia). Therefore, elements such as nitrogen and phosphorus are not transferred up to zooplankton. Successful implementation of Sato-Umi will prevent the occurrence of red tides and oxygen-deficient water masses in coastal sea area.

Successful implementation of Sato-Umi in eutrophic coastal sea area must reduce

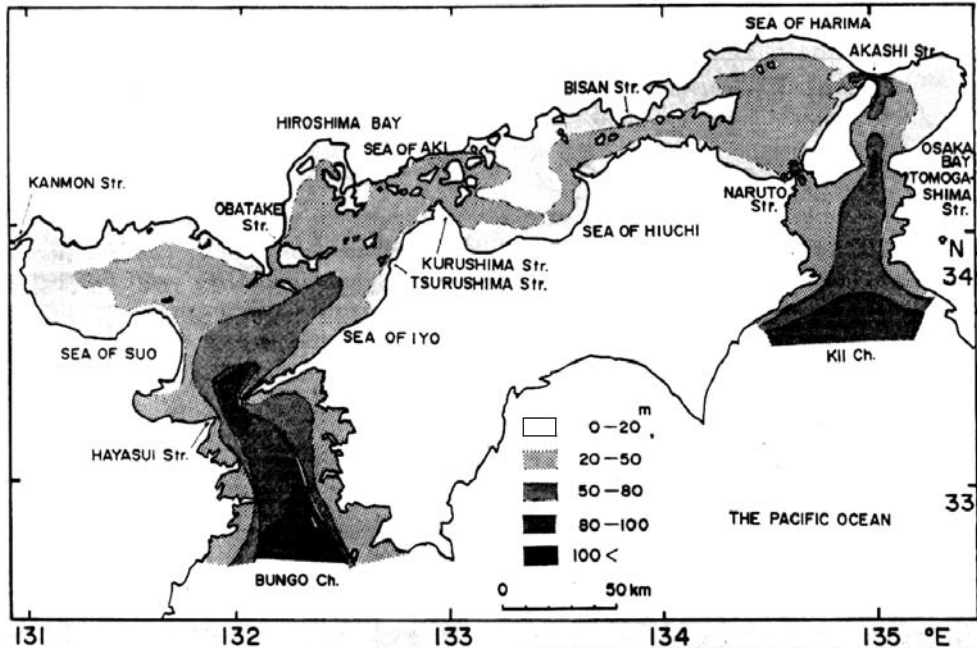


Fig. 2. Seto Inland Sea, Japan

nutrient loads from the land to prevent red tides. For example, in the Seto Inland Sea, Japan (Fig. 2), not only COD (Chemical Oxygen Demand) load but also TN (Total Nitrogen) and TP (Total Phosphorus) loads have been reduced since 1972, as shown in Fig. 3(a). The number of red tides has been reduced since 1978, six years after the reducing of COD load, as shown in Fig. 3(b).

In oligotrophic coastal sea area, we need to increase nutrient concentrations in the euphotic layer, through creation of a man-made upwelling structure. A field experiment using a man-made upwelling structure, made of concrete, was conducted in the Bungo Channel (water depth of 50 m), the western part of the Seto Inland Sea, Japan in 1987. A strong tidal current comes across a short wall of type-S structure and generates two eddies with a horizontal axis (Fig. 4(a)). Eddies, created from these structures, flow down, come across a long wall of type-L structure,

change their axes from horizontal to vertical and upwell into an euphotic layer with a thickness of about 30 m. Two walls of type-S and two walls of type-L structures were arranged as shown in Fig. 4(a). We expect generation of two upwelling eddies during flood and ebb tidal currents from the placement of these two types of structures. After these structures were installed, the chlorophyll-*a* concentration has increased 1.5 times compared to that before the setting of the structures (Fig. 4(b)). The local fishermen say that the fish catch has also increased there after 1989 (Yanagi and Nakajima 1991).

Material cycling in coastal sea area is governed by physical processes such as current and diffusion and also by chemical-biological processes such as primary production and predation (Fig. 5; Hayashi and Yanagi 2002). We know that within coastal sea area biochemical processes have a greater effect than physical processes, because the

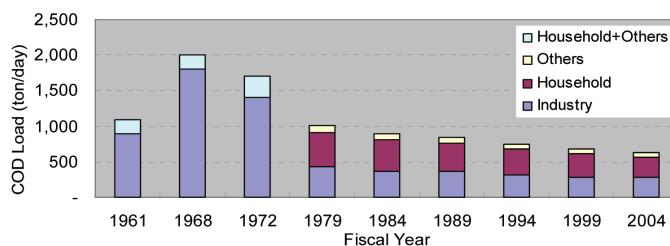


Fig. 3(a). Year-to-year variation in the COD load to the Seto Inland Sea, Japan.

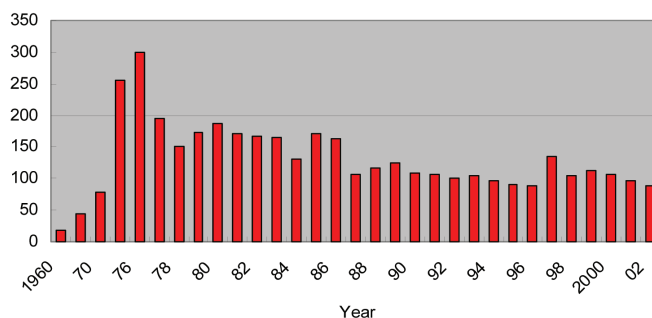


Fig. 3(b). Year-to-year variation in the occurrences of red tides in the Seto Inland Sea, Japan.

biochemical flux from nutrient to phytoplankton is larger than the physical flux from rivers (Fig. 5).

Therefore it is important to assure a living environment for many kinds of life from phytoplankton to dolphin (shown in Fig. 1) in Sato-Umi. For example, a gentle sloped coast is much better than a straight uplifted coast for the coastal environment because many kinds of biota can live along a gentle sloped coast where the environmental gradient is small (Fig. 6).

4. Discussion

The process of establishing Sato-Umi has just started in the Seto Inland Sea, the largest semi-enclosed coastal sea in Japan. We need to accumulate many experiences of the processes and occurrences related to comprehensive material cycling in the coastal sea area, in order to establish Sato-Umi (Okaichi

and Yanagi 1997; Research Institute for the Seto Inland Sea 2006).

Establishment of Sato-Umi will require a correct regulation of the nutrients loading from the land to prevent red tide occurrence and to prevent oxygen-deficient water masses in order to increase primary production. Regulations could follow the special law for the preservation of marine environment of the Seto Inland Sea which was enacted in 1973. The second method to preserve and rehabilitate the shallow sea area with a water depth less than 20 m is important to assure the sustainable recruitment of fish in the coastal sea area. Large shallow sea areas have been lost to reclamation in recent years due to the expansion of mankind's activities in the Seto Inland Sea (Fig. 7). Further, tidal flats and sea grass beds, which are also very important for primary production and as a nursery ground for fish, have been lost due to the reclamation and the

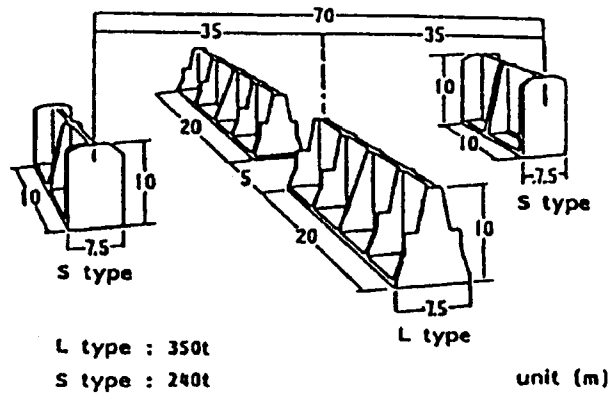


Fig. 4(a). Man-made structure for upwelling.

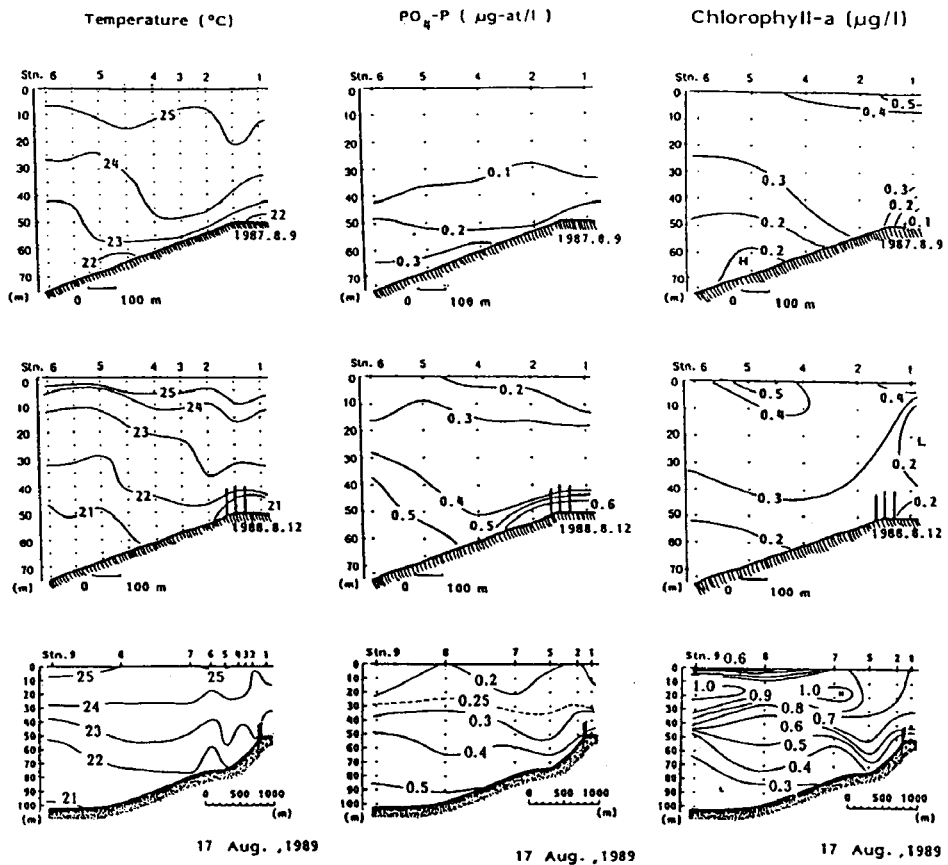


Fig. 4(b). Change in vertical distributions of water temperature, phosphate and chl.a from 1987 (upper; before construction) to 1988 and 1989 (middle and lower; after construction of the man-made structure). Three bars in the central panels show the positions of the structures (Yanagi and Nakajima 1991).

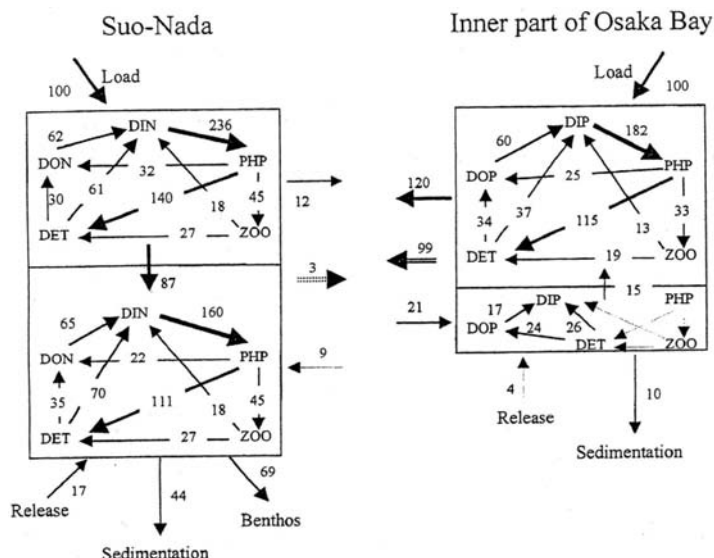


Fig. 5. Biochemical element flux in Suo Nada (left), where DIN a limiting nutrient for primary production, and Osaka Bay (right), where DIP is a limiting nutrient, in the Seto Inland Sea, calculated by box ecosystem models in which the flux is normalized by the river load as 100. Primary production flux in Suo Nada is 236 and that in Osaka Bay is 182, which are larger than river flux of 100. PHP: phytoplankton, ZOO: zooplankton, DET: detritus. (Hayashi and Yanagi 2002).

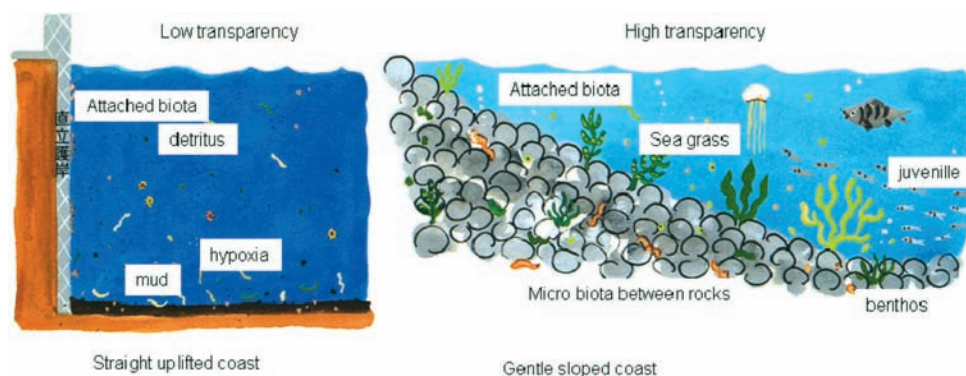


Fig. 6. Straight and uplifted coast (left) and gentle sloped coast (right).

decrease in water clarity (Fig. 8). Devastated coastal sea environments have to be recovered by proper management applying Sato-Umi concept.

Appropriate management of fishing effort is important for the preservation of fish resources, though this can be very difficult.

We can turn to some successful examples of fish resource management by Fishermen's Unions in the Seto Inland Sea, Japan such as Himeshima (Yanagi 2004) and Misaki (Yanagi 2005) to guide implementation of Sato-Umi. Fish management in these fishermen unions is community-based and all

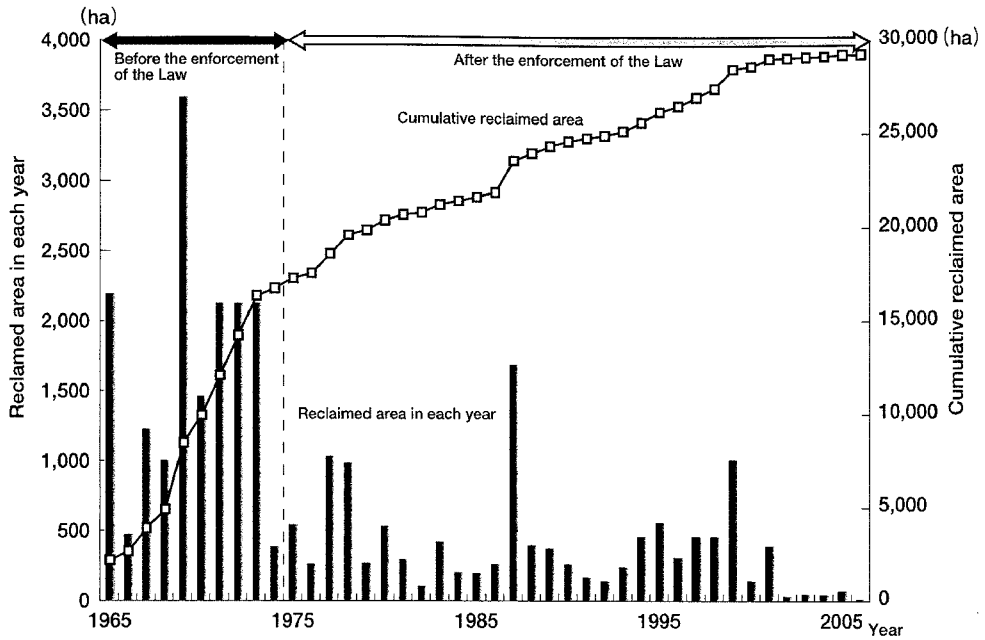


Fig. 7. Trends in reclaimed areas in the Seto Inland Sea, Japan.

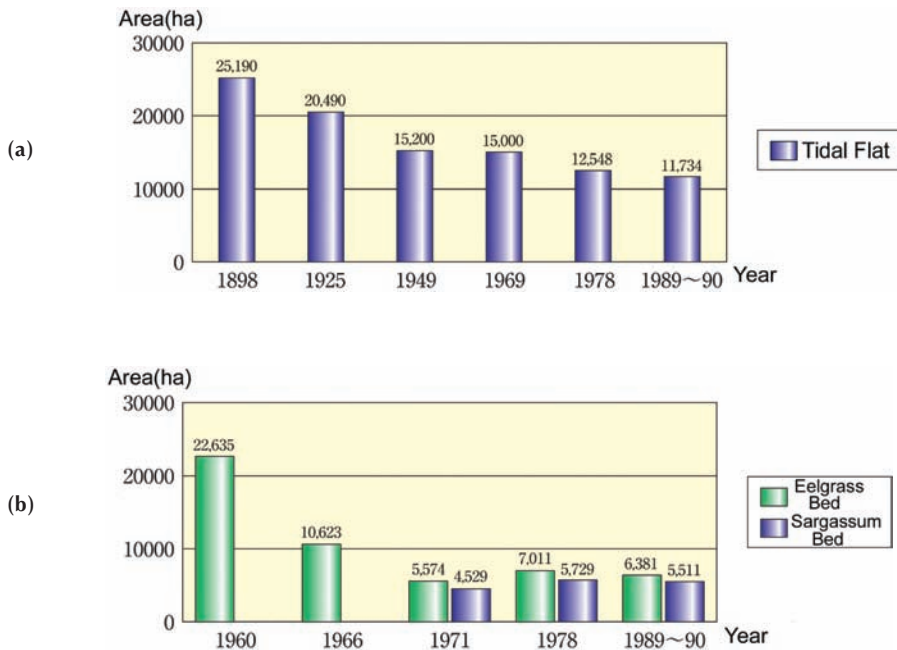


Fig. 8. Trends in areas of tidal flats (a) and sea grass beds (b) in the Seto Inland Sea, Japan.

fishermen in the Union cooperate to sustain the fish resources in their fishing ground. We will have to expand such successful examples of sustainable fish management to the whole coastal sea area in order to establish Sato-Umi. Finally, the sales strategy of har-

vested fish for the consumers in the city area is important to ensure a successful fishing economy (Yanagi 2007a).

The details of the Sato-Umi concept and trials for its realization are introduced in Yanagi (2007b).

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Optimal Fishing Policies That Maximize Sustainable Ecosystem Services

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The classic theory of fisheries management seeks a maximum sustainable yield from a target species. There are several variations that include uncertainty, fluctuation, and species interactions. The knowledge that sustainable fisheries do not always guarantee conservation of a diversity of species is well known. Ecosystems provide several categories of ecosystem services to human well-being: supporting, provisioning, regulating, and cultural services. Fishery yields belong to provisioning services. The existence of living marine resources may maintain these services, and certainly a much larger contribution from regulating services than that from fishery yields. Therefore, we define an optimal fishing strategy that maximizes the total ecosystem service instead of a sustainable fishery yield. We call this the fishing policy for “maximum sustainable ecosystem service” (MSES). The regulating service likely depends on the standing biomass, while the provisioning service from fisheries depends on the catch amount. We obtain fishing policies for MSES in a single species model with and without uncertainties and in multiple species models. In any case, fishing efforts are usually much smaller than those for a maximum sustainable yield (MSY). We also discuss the role of fisheries in sustaining ecosystem services, and the nature of ecosystem comanagement.

KEYWORDS maximum sustainable ecosystem service; MSES; uncertainty; ecosystem comanagement; maximum sustainable yield

1. Introduction

The theory of maximum sustainable yield (MSY) takes into account the long-term yield from living marine resources. This theory implicitly assumes that a fishing ban does not produce any benefit from marine ecosystems, although this theory explicitly assumes a negative relationship between yield and standing stock abundance. However, ecosystems give us a variety of benefits (World Resource Institute 2005). Harvests from agriculture, forestry and fisheries are just a small part of the ecosystem service (Costanza *et al.* 1997; Satake and Rudel 2007). Ecosystem services include supporting services such as soil formation, photosynthesis, and nutrient cycling, provisioning services such as food, water, timber, and fiber; regulating services that affect climate, floods, disease, waste, and water quality; and cultural services that provide recreational, aesthetic, and spiritual benefits (World Resource Institute 2005). Fishery yields belong to provisioning services. The existence of living marine resources may maintain these services and certainly make a larger contribution to the total ecosystem service than fishery yields (Costanza *et al.* 1997).

There are some criticisms of the theory of MSY (Matsuda and Abrams 2008). The MSY fishing policy does not reflect uncertainty in stock estimates (measurement errors) and in the relationship between the spawning stock and recruitment (process uncertainties). The MSY policy also ignores the complexity in ecosystem processes because the theory uses single stock dynamic models (Matsuda and Katsukawa 2002). Matsuda and Abrams (2006) analyzed the maximum sustainable yield from entire food webs with an independent fishing effort for each species. They concluded that MSY policy does not guarantee the coexistence of species and proposed the concept of "constrained MSY" that maximizes the sustain-

able yield under which all species coexist. However, Matsuda and Abrams (2006) did not incorporate ecosystem service into the optimal fisheries policy.

In this paper, we consider an optimal policy that maximizes the total ecosystem service under some mathematically simple assumptions. We call this the maximum sustainable ecosystem service, or MSES. We assume that an ecosystem service other than fishery yields depends on the standing biomass, while the fishery yield depends on the catch amount. Regulation of fishing efforts usually enhances the standing biomass, while it usually decreases the fishery yield. We compare the MSY and MSES policies in (1) a single deterministic stock dynamic model, (2) a stochastic model for a single species with measurement errors and process uncertainty and (3) food web models consisting of six species. We also discuss the role of comanagement in the ecosystem approach.

2. Optimal Fishing Policy That Maximizes Ecosystem Service

To take account these ecosystem services, we assume that the total ecosystem service from a target fish resource, denoted by $V(N, C)$ is given by

$$V(N, C) = Y(C) - cE + S(N) \quad (1)$$

where $Y(C)$ is the yield from fisheries with catch amount C , cE is the cost of fisheries with fishing effort E , and $S(N)$ is the ecosystem service other than fishery yields with stock abundance N . Hereafter, we simply call $S(N)$ the utility of standing biomass.

We assume the following fish stock dynamics :

$$\begin{aligned} dN/dt &= f(N)N - C, \\ f(N) &= r - aN, \\ C &= qEN, \\ Y(C) &= pqEN \end{aligned} \quad (2)$$

where t is an arbitrary time unit, $f(N)$ is the per capita reproduction rate, r is the intrinsic rate of population increase, a is the magnitude of density effect, q is catchability, and p is the price of fish. Although we have assumed linear functions for cost (cE) and yield ($pqEN$), the nonlinear relationship for either cost or catch may produce a more complex result. The carrying capacity, denoted by K , is given by r/a .

The utility of standing biomass, $S(N)$, is likely to be a convex or sigmoid curve because the utility usually saturates when the stock is sufficiently abundant. We assume the following specific function:

$$S(N) = S^\infty N^2 / (B^2 + N^2) \quad (3)$$

where S^∞ is the limit of $S(N)$ when N is the positive infinity, B is the stock abundance where $S(N)$ is the half of S^∞ . We assumed a sigmoid function of N as shown in Fig. 1.

In the first step we obtain the optimal fishing effort that maximizes the total ecosystem service $V(N, C)$ given by Eq. (1) at equilibrium stock abundance of Eq. (2). The equilibrium stock abundance, denoted by N^* , is given by

$$N^* = (r - qE)/a \quad (4)$$

The total ecosystem service at the equilibrium, denoted by V^* , is

$$V^* = pqEN^* - cE + S(N^*) \quad (5)$$

If the utility of the standing biomass is negligible ($S(N) = 0$) as is usually assumed in classical fisheries theory, V^* is a uni-modal function of E and the optimal fishing effort, denoted by E_{opt} , is well known by

$$E_{\text{opt}} = (pqr - ac)/2pq^2 \quad (6)$$

If cost c is negligible, E_{opt} becomes the effort at the maximum sustainable yield (MSY). The quantity V^* when $E = E_{\text{opt}}$ is known as the maximum economic yield.

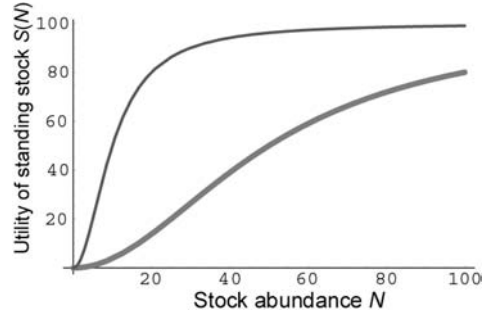


Fig. 1. The relationship between stock abundance and its utility given by Eq. (3). Bold and thin curves represent the cases where B is 50 and 10% of K , respectively.

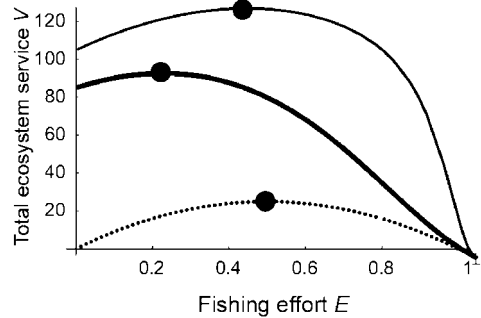


Fig. 2. The relationship between the fishing effort and the total ecosystem service for three cases of parameters. Bold, thin and dotted curves represent the cases where $(S^\infty, B) = (100, 10)$, $(100, 50)$, $(0, -)$, respectively. Other parameters are chosen as $a = 0.01$, $r = 1$, $q = 1$, $p = 1$, $c = 0.1$. The optimal effort for each case is indicated by closed circles.

If $S(N)$ is an increasing function of N , the optimal effort is always smaller than E_{opt} without the utility of standing biomass. However, the distance between E_{opt} with positive $S(N)$ and E_{opt} without $S(N)$ depends on the magnitude and curvature of $S(N)$. We numerically obtained the optimal effort for various parameters of S^∞ and B as shown in Fig. 2. Despite the fact that the maximum $V_{\text{opt}}(N^*, C^*)$, when $(S^\infty, B) = (100, 50)$ is larger than $V_{\text{opt}}(N^*, C^*)$ when $(S^\infty, B) = (100, 10)$,

the latter optimal effort is smaller than the former.

The derivative of V^* with E is

$$dV^*/dE = pqN^* - c - [pqE + s'(N^*)]q/a \quad (7)$$

because $dN^*/dE = -q/a$.

The optimal effort is smaller when the derivative of utility of the standing resource with respect to the stock is of a larger magnitude ($|dS/dN|$ is larger). If $S^\infty > (Ba^2 + r^2)^2(rpqa - ac)/2Bqra^3$, dV^*/dE at $E=0$ is negative and the fishing ban is optimal. For parameter values given in Fig. 2, the fishing ban is optimal when $S^\infty > 47595$ if $B = 10$ or when $S^\infty > 9995$ if $B = 50$.

3. Optimal Fishing Policy with Process Uncertainty and Measurement Errors

Equation (1), that includes the value of ecosystem service, is simple because any types of uncertainties, intrinsic instability, complexity and conflict between stakeholders are ignored. We incorporate these factors into the model to build the comprehensive theory of fisheries management. In the second step we consider a time-discrete model with the uncertainties in measurement and dynamic processes:

$$\begin{aligned} N_{t+1} &= f(N_t - C_t, \xi_t)(N_t - C_t) \\ Y(C_t) &= pC_t = pqE_tN_t \\ \tilde{N}_t &= G(N_t, \zeta_t) \\ V(N_t, C_t) &= Y(C_t) - cE_t + S(N_t - C_t) \\ E_t &= F(\tilde{N}_t)/q \end{aligned} \quad (8)$$

where N_t is the stock abundance in year t just before the fishing season, ξ_t and ζ_t are normally-distributed random variables with standard deviations σ_t and σ_m , $f(N_t - C_t, \xi_t)$ is the per capita reproduction rate with process uncertainty $\xi(t)$, $\tilde{N}_t$ is the estimate of stock abundance N_t in year t that is a function G of the true abundance N_t and measure-

ment error $\zeta(t)$. In this case, the fishing effort E_t likely depends on time because the stock fluctuates with process errors. In the decision making, fishers just use the estimate $\tilde{N}_t$, therefore E_t is a function F of $\tilde{N}_t$.

We here assume one of the simplest ways that is similar to Reed (1979):

$$f(N_t, \xi_t) = \exp(r + \xi_t - aN_t) \quad (9)$$

We also incorporate measurement uncertainty:

$$G(N_t, \zeta_t) = N_t \exp(\zeta_t) \quad (10)$$

The optimal harvesting policy is obtained by the dynamic programming theory (Mangel and Clark 1988) although the solution is still analytically unknown if a measurement error exists (Kotani *et al.* 2008). Here we simply assume that the allowable effort rule in actual fisheries management in the USA, Japan, and other countries as shown in Fig. 3:

$$F(\tilde{N}) = \begin{cases} 0 & \text{if } \tilde{N}_t < N_{\text{ban}} \\ F_{\text{target}} \left(\tilde{N} - N_{\text{ban}} \right) / \left(N_{\text{limit}} - N_{\text{ban}} \right) & \text{if } N_{\text{ban}} \leq \tilde{N}_t < N_{\text{limit}} \\ F_{\text{target}} & \text{if } \tilde{N}_t \geq N_{\text{limit}} \end{cases} \quad (11)$$

where N_{ban} and N_{limit} are the stock abundances of the upper threshold for a fishing ban and the lowest threshold that maintains a fishing effort F_{target} , F_{target} is the target fishing effort under which the maximum sustainable yield is theoretically achieved. The conventional fisheries management under the United Nations Convention on the Law of the Sea (UNCLOS) usually determines the total allowable catch (TAC) instead of the fishing effort. Therefore, the actual catch should have been the minimum of qE_tN_t and $qE_t\tilde{N}_t$.

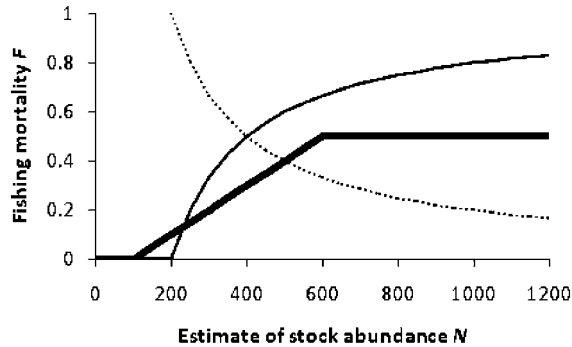


Fig. 3. Rules of fishing effort that depend on the estimate of stock abundance. Bold, thin and dotted curves represent the ABC rule given by Eq. (11), constant escapement given by Eq. (12) and constant catch given by Eq. (13), where $q = 1$, $N_{\text{crit}} = 100$, $C_c = 200$, $N_{\text{limit}} = 600$, $F_{\text{target}} = 0.5$.

Here we simply assumed that C_t is given by qE_tN_t or that the control of fishing effort depends on the estimate of stock abundance (see also Katsukawa 2004). The fishing mortality coefficient F is approximately given by qE .

If we ignore the utility of standing biomass ($S(N) = 0$) and measurement error ($\sigma_m = 0$), the constant escapement policy is well known as the optimal policy that maximizes the long-term yield:

$$E_{cs}(N) = \begin{cases} 0 & \text{if } N_t < N_{\text{crit}} \\ (N - N_{\text{crit}})/qN & \text{if } N_t \geq N_{\text{crit}} \end{cases} \quad (12)$$

where N_{crit} is a positive constant. The catch $qE_{\text{opt}}N_t$ is $(N_t - N_{\text{crit}})$ and the stock abundance after the fishing season is N_{crit} . The optimal escapement level depends on the magnitude of process uncertainty (σ_r). The fishing effort for the constant catch policy is

$$E_{cc}(N) = C_c/qN \quad (13)$$

where C_c is the constant catch amount.

If the measurement error exists ($\sigma_m > 0$), Katsukawa (2004) numerically obtained a suitable management policy that keeps a

larger average yield and a larger minimum stock with a smaller variance. This solution implicitly incorporates the utility of standing biomass into the performance of fishing policy. Katsukawa (2004) concluded that the fishing effort that achieves the maximum sustainable yield is a bad performance. He recommends a simpler rule given by Eq. (11) that is similar to the New Management Procedure of the International Whaling Commission.

Kotani *et al.* (2008) considered the optimal control of exotic species and numerically obtained an optimal harvesting policy when the utility of standing population is negative. Even for the optimal control of exotic species, the constant escapement is optimal.

Figure 4 illustrates a resultant stock abundance, catch amount from MSY, and MSES policies. We obtained the optimal policies by grid search for F_{target} , N_{ban} , N_{limit} as 0.1, 100, 100, respectively, to maximize the average yield or ecosystem services over 100 years from 100 simulations. The fishing effort under the optimal policy for MSY was higher than that for MSES, while the stock abundance under the optimal policy for MSY was lower than that for MSES. We chose parameters $(S^\infty, B, r, a, \sigma_r, \sigma_e, p, c) = (100, 1000, 0.5, 0.001, 30\%, 50\%, 1, 0)$. We obtained

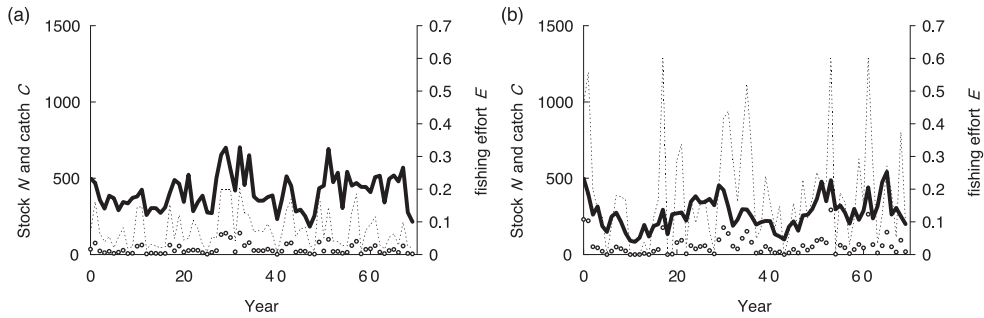


Fig. 4. An example of stock and catch fluctuation under fishing effort given in Eq. (11). Policies for MSY and MSES are shown in panels (a) and (b), respectively. Bold and dotted curves represent the stock abundance and fishing effort. Circles represent the catch.

a fishing policy characterized by $(F_{\text{target}}, N_{\text{ban}}, N_{\text{limit}})$ in Eq. (11). The policy for MSY was $(F_{\text{target}}, N_{\text{ban}}, N_{\text{limit}}) = (0.6, 100, 700)$, whose average yield and average total ecosystem services were 77.6 and 165.7, respectively. The policy for MSES was $(F_{\text{target}}, N_{\text{ban}}, N_{\text{limit}}) = (0.2, 100, 1000)$, whose average yield and average total ecosystem services were 43.3 and 216.4, respectively.

4. Optimal Policy from Food Webs

Ecosystems are characterized by uncertainty, dynamic properties and complexity. We have analyzed the effects of uncertainty and dynamic properties previously. Matsuda and Abrams (2006) used simple food-web models to investigate the nature of yield- or profit-maximizing exploitation of communities including a variety of six-species systems with as many as five trophic levels. These models show that, for most webs, relatively few species are harvested at equilibrium and that a significant fraction of the species is lost from the web. They also considered a constraint that all species must be retained in the system usually increases the number of species and trophic levels harvested at the yield-maximizing policy. The reduction in total yield caused by such a constraint is modest for most food webs.

Matsuda and Abrams (2006) did not explicitly evaluate the ecosystem service of standing biomass but added a constraint of species persistence. Here we incorporate the utility of ecosystem service from standing biomass into their model that includes s species:

$$\frac{dN_i}{dt} = (r_i + \sum_j a_{ji} N_j - q_i E_i) N_i, \quad \text{for } i = 1, 2, \dots, s \quad (14)$$

where $N_i(t)$ is the stock abundance of species i at time t , r_i is the intrinsic growth rate of population increase of species i , a_{ji} is the magnitude of intra- and inter-specific competition from species j to i , and E_i is the fishing effort on species i .

We define the total ecosystem service from the food web:

$$\begin{aligned} Y(\mathbf{E}) &= \sum_i E_i (p_i q_i N_i - c_i) \\ V(\mathbf{E}) &= Y(\mathbf{E}) + \sum_i S_i(N_i) \end{aligned} \quad (15)$$

where p , q , c and S differ between species. Matsuda and Abrams (2006) ignored the utility of standing biomass $S_i(N_i)$. There may be some multiplicative effects of interspecific interactions on the ecosystem service. It is difficult to quantitatively evaluate the total ecosystem service from the web. Here we

assumed the simple sum of the contribution of each species on the total ecosystem service.

$$S_i(N_i) = S_i^\infty N_i^2 / (B_i^2 + N_i^2) \quad (16)$$

We evaluate the total ecosystem service at the equilibrium. The equilibrium, denoted by $\mathbf{N}^* = (N_1^*, N_2^*, \dots, N_s^*)^T$, is given by

$$\begin{aligned} \mathbf{N}^* &= -\mathbf{A}^{-1}(\mathbf{r} - \mathbf{q}\mathbf{E}) \\ \text{or} \\ \begin{pmatrix} N_1^* \\ N_2^* \\ N_3^* \\ \vdots \\ N_s^* \end{pmatrix} &= \begin{pmatrix} a_{11} & a_{12} & a_{13} & \cdots & a_{1s} \\ a_{21} & a_{22} & a_{23} & \cdots & a_{2s} \\ a_{31} & a_{32} & a_{33} & \cdots & a_{3s} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ a_{s1} & a_{s2} & a_{s3} & \cdots & a_{ss} \end{pmatrix}^{-1} \begin{pmatrix} r_1 - q_1 E_1 \\ r_2 - q_2 E_2 \\ r_3 - q_3 E_3 \\ \vdots \\ r_s - q_s E_s \end{pmatrix} \end{aligned} \quad (17)$$

where $\mathbf{r} = (r_1, r_2, r_3, \dots, r_s)^T$; $\mathbf{q}\mathbf{e} = (q_1 E_1, q_2 E_2, q_3 E_3, \dots, q_s E_s)^T$ and $\mathbf{A}$ means the community matrix, the (i, j) -th elements of which is a_{ij} .

We obtained two types of solutions of fishing efforts $\mathbf{E}$ that maximize $Y(\mathbf{E})$ and $V(\mathbf{E})$ in Eq. (15) for the same food web with cost and prices, $\mathbf{A}$, $\mathbf{c}$, $\mathbf{q}$, $\mathbf{p}$, $\mathbf{r}$. We call these solutions “MSY policy” ($\mathbf{E}_{\text{MSY}}$) and “maximum sustainable ecosystem service (MSES) policy” ($\mathbf{E}_{\text{MSES}}$), respectively. We compare between the total ecosystem service $V(\mathbf{E})$ at MSY and $V(\mathbf{E})$ at MSES, denoted by V_{MSY} and V_{MSES} , respectively. The total yield using the MSY policy is always larger than that for the MSES solution, while the total ecosystem service for the MSES solution is always larger than that for the MSY solution.

We obtained MSY and MSES policies for 1000 randomly structured food webs of a six species food web with positive equilibrium. We assumed that the diagonal elements a_{ii} are -1 for bottom-level species ($i = 1$ and 2) and 0 for consumer species ($i = 3$ through 6). We ignored interspecific competition between species 1 and 2 ($a_{12} = a_{21} = 0$). We also assumed a_{ij} is 0 with a probability

of 50% and its absolute value is between 0 and 1 with a probability of 50% for any pair of predator species i and prey species j ($j < i$). We also assumed that q_i is 1, because q and p similarly affect the optimal solution. Other parameter values are chosen by independent draws from a uniform distribution between 0 and 1 for r_i , p_1 and p_2 , and between 0 and 3 for p_3 to p_6 because predators usually fetch a higher price than prey species. We ignored the cost of fishing effort, so $c_i = 0$. Finally, we assumed that S_i^∞ and B_i are 10 and $0.2N_i^{**}$ for each species, where N_i^{**} means the equilibrium stock abundance without fisheries ($\mathbf{E} = 0$).

Figure 5 shows four typical examples of food webs. When the total ecosystem service decreases with an increasing fishing effort of any species, the MSES policy does not fish any species at all, as shown in Fig. 5(a). Among 1000 randomly connected food webs, about 85% of the webs have the same food webs with the same set of exploited species. About 15% of the webs have the same food webs with the same fishing efforts between MSY and MSES (Fig. 5(b)).

The total yield at MSES (denoted by Y_{MSES}) ranged 0–100% of the total yield at MSY (denoted by Y_{MSY}) whose average was 40%. The total ecosystem service at MSY (V_{MSY}) ranged between 21 and 100% of the total ecosystem service at MSES (V_{MSES}) whose geometric average was 69%. There was a positive relationship between $Y_{\text{MSY}}/V_{\text{MSES}}$ and $Y_{\text{MSES}}/Y_{\text{MSY}}$ (Fig. 6), while a fishing ban at MSES appeared in 1% of the 1000 food webs when the fishery yields were the smaller part of the total services ($Y_{\text{MSY}}/V_{\text{MSES}}$ is small) and the MSES policy was identical to the MSY policy when the fishery yield was the larger part of the total services.

All species rarely coexisted under the MSY policy for 1000 randomly structured food webs, while these six species coexist for about 91% of the webs using the MSES policy. The number of exploited species at MSES was usually much larger than the

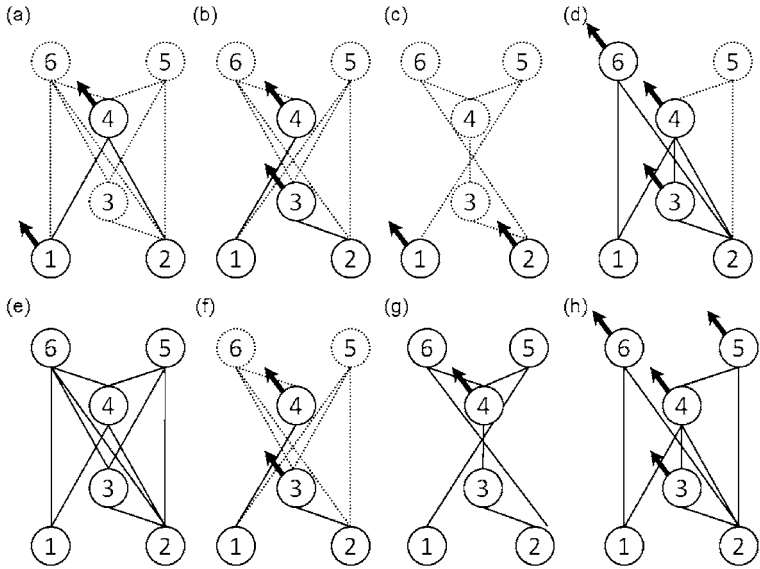


Fig. 5. Resultant food web and optimal fishing policies that maximize the total sustainable yield (panels (a)–(d)) and the total ecosystem service (panels (e)–(h)). The MSES policy for four examples of the six-species systems; circles and arrows represent species and fishery, respectively. Lines between circles represent trophic links from smaller numbered species to larger numbered species. Dotted circles mean that these species became extinct. Panels (e)–(h) are the MSES policies for four systems that are identical to panels (a)–(d), respectively.

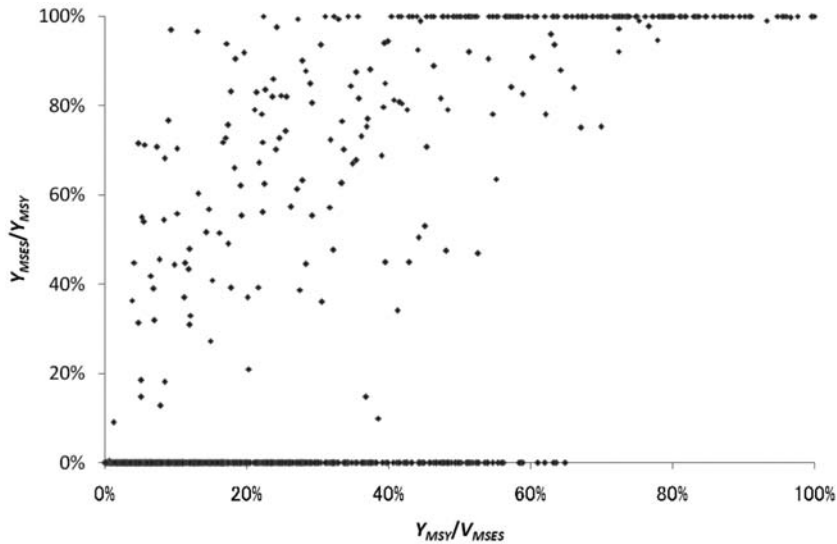


Fig. 6. The relationship between the relative ecosystem service and the relative total yield under the MSES policy from 1000 random food webs.

Table 1. Resultant food webs and fishing efforts from 1000 randomly constructed six species systems. Each column shows the frequency distribution of the numbers of extant species using MSY and MSES policies, the number of exploited species using MSY and MSES policies.

	No. of extant species at MSY	No. of exploited species at MSY	No of extant species at MSES	No of exploited species at MSES
1	31	160	0	0
2	139	828	178	201
3	48	10	431	512
4	56	0	220	256
5	18	0	24	28
6	6	0	144	0

number at MSY (Table 1). The ratio $Y_{\text{MSY}}/V_{\text{MSES}}$ implies the ratio between the maximum sustainable yield and the maximum sustainable ecosystem service by respectively optimal policies. This ratio widely ranged between 0 and 97%. If the total ecosystem service without a fishery yield is small, the MSES policy did not guarantee the coexistence of species.

5. From Fisheries Comanagement to Ecosystem Comanagement

In order to increase the fishery yield $Y(E)$, monitoring activities of status of stock are critically important. Even more so for sustaining the utility of standing biomass $S(N)$. Government should play an important role in these monitoring activities. However, in reality, it is almost impossible for the government to monitor all the detailed ecosystems along the coast and within exclusive economic zones (EEZ). Therefore, the knowledge of fishers and data from fishery activities should be fully utilized.

As Table 1 shows, yield-maximizing fisheries are likely to take only a small number of species from one trophic level. So, under the yield-maximizing policy, fishing gear and vessel type will converge on the most efficient, and we can gain information about very limited aspects of the ecosystem. Our analyses also indicate that yield-maximizing fisheries would invoke the loss

of a significant fraction of species in the web. It will inevitably lead to the degradation of $S(N)$, and easily set off the benefit from $Y(E^*)$, and ultimately reduce the total ecosystem services, $V(E)$. To avoid these situations, government has to monitor the rest of the ecosystem, and regulate the yield-maximizing fisheries in a top-down way. The reality is, again, these costs would be beyond the budget of many countries, especially developing countries. To sum up the above discussions, yield-maximizing, economically-efficient fisheries are rational for enjoying fishery rent, but not always so in sustaining total ecosystem services for society.

From the viewpoint of sustaining ecosystem services, one reasonable alternative is to conduct responsible fisheries targets for a wide range of species with a variety of gear. For example, in the Shiretoko World Natural Heritage area, local fishers have conducted various kinds of operations and caught most of the keystone species of the local ecosystem. They have accumulated the catch data of these species for over 50 years. In addition, local fishers have autonomously monitored the resource status every year, and adaptively modified the local rules for sustainable resource use. These data and knowledge is now one of the most important foundations for monitoring the changes in the ecosystem structure and functions in the heritage area. As this case shows, responsible fisheries can significantly contribute to

the sustainability of ecosystem services. Like the Shiretoko case, a fisheries management approach in which government and local fishers share the responsibilities and authorities for the use of sustainable resource is called fisheries comanagement, the strongest argument against the conventional top-down approach (Makino and Matsuda 2005).

Fisheries comanagement will not always lead to the ecosystem management. As this study showed, the total ecosystem service from MSES policy is always larger than that from MSY policy, and E_{MSY} has a tendency to be greater than E_{MSES} . In order to increase the total ecosystem services, interests from other sectors than fisheries, such as an environmental ministry or non-government organizations (NGOs), should be included in the decision-making arena (Makino 2005). Then, fisheries comanagement will evolve into an ecosystem comanagement. Under this ecosystem a comanagement framework, co-existence of small-scale, artisanal fisheries and large-scale, efficient fisheries is the rational solution for sustaining ecosystem services.

6. Discussion

In the three types of models we have analyzed in this paper, the concept of maximum sustainable ecosystem services results in a more conservative policy than MSY. A fishing ban is optimal if the ecosystem service from standing biomass is sufficiently larger than the fishery yields, despite the fact that MSES do not always prevent any species from becoming extinct. These results are intuitively understandable.

It is difficult to quantitatively evaluate the magnitude of ecosystem services. Eco-

system processes, including food web interaction, are complex and not well known. The assumptions and parameter values used in this paper should be improved in the future. Our analyses have the following problems.

First, we do not know the appropriate magnitude of ecosystem services (S_i^∞). We assumed that the ecosystem services from a standing biomass is a simple sum of the service from each species with the same magnitude of S_i^∞ . Even for such simple models, we obtained a variety of MSES policies from a fishing ban of all species to the extinction of some species. The regulating services are at least 10-fold larger than fishery yield (Costanza *et al.* 1997). Our analyses suggest that a fishing ban is optimal, even when the fishery yield using the MSY policy is much more than 10% of the total ecosystem services using the MSES policy. If the regulating services do not significantly decrease through little fishing effort, a fishing ban is less likely, but a significant reduction of the standing biomass is strongly discouraged.

Second, we do not include implementation errors. This is a big problem in consensus building. Depending on the countries or areas, food supply and job creation by greater fishing efforts are considerably important factors from a social security point of view. Therefore, relative weights between ecosystem services and fishery yields might be the societal choice.

Acknowledgement

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Ecosystem-Based Sustainable Conservation and Management of Pacific Salmon

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Pacific salmon (*Oncorhynchus* spp.) play an important role as a keystone species in North Pacific ecosystems, where their populations are influenced by natural factors and human impacts. Carrying capacity for Pacific salmon is related to the long-term climate change, and also to density-dependent effects. For example, the residual carrying capacity of chum salmon (*O. keta*) was positively correlated with body size of adult salmon but negatively correlated with age at maturity. The abundance of wild chum salmon in the North Pacific in the 1990s declined to about 50% of what it was in the 1930s, despite significant increases in introduction of hatchery-produced salmon. This indicates that fisheries management has limitations at the population level, and that biological interactions between wild and hatchery-produced populations of Pacific salmon should be considered. Global warming has affected growth and survival of Asian chum salmon since the 1990s, and has had a positive effect on Hokkaido populations but negative effects for more southern populations in Iwate Prefecture and in Korea. Predictions about global warming effects on chum salmon suggest that their area of distribution will change resulting in a displacement to the northern area such as the Arctic Ocean, and the loss of migration routes such as the Okhotsk Sea. This paper presents a framework for ecosystem-based sustainable conservation and management of Pacific salmon, which takes account of climate change and interactions between wild and hatchery fish.

KEYWORDS ecosystem-based sustainable conservation and management; Pacific Ocean; carrying capacity; climate change effects

1. Introduction

Marine food should be reproducible resources for human beings. However, world fish catches have peaked since the 1990s despite increase in aquaculture production (Fig. 1). Tuna (*Thunnus* spp.) abundance extremely decreased by overfishing since the 1980s (Myers and Worm 2003). Bluefin tuna (*T. thynnus*) is already "critical species" in the IUCN. Although production from aquaculture is increasing world-wide, many aquaculture programs also cause the destruction of aquatic ecosystem such as vanishing mangrove forests caused by the shrimp aquaculture over the last 20 years in the Eastern Asia (Primavera *et al.* 2005), marine pollution, and threats to marine food security (e.g., contaminants in farmed Atlantic salmon; Hites *et al.* 2004).

Traditional fisheries science consider only fisheries, some consequences of which include fishing down marine food webs (Pauly *et al.* 1998), the over fishing by the tuna laundering, the tragedy of commons, the food mileage, ecosystem crashes, and food pollution. A paradigm shift is needed from the traditional fisheries science to a new fisheries science and oceanography for the protection of marine ecosystems and human food resources.

Analyses of the nitrogen stable isotope concentration in animals in the Gulf of Alaska have demonstrated that Pacific salmon occupy the fourth and fifth trophic levels in this ocean ecosystem (Kaeriyama 2003). Pacific salmon play an important role as a keystone species in the Subarctic Ocean and the freshwater ecosystems. Pacific salmon are also a key species for sustaining the biodiversity and productivity in the riparian ecosystem because they supply marine-derived material into river systems and the adjacent watersheds (Kline *et al.* 1990; Hilderbrand *et al.* 1999; Helfield and Naiman 2001). Therefore, Pacific salmon are important not only as fisheries resources but also as keystone species in the Subarctic aquatic ecosystem.

The objectives of this paper are to consider the following issues in relation to Pacific salmon: 1) carrying capacity, 2) global warming effects, and 3) sustainable conservation and management.

2. Carrying Capacity

Changes in the catches of Pacific salmon have a 30- or 40-year periodicity that coincides with long-term climate indices such as the Pacific Decadal Oscillation (PDO; Mantua *et al.* 1997) and climate regime shifts

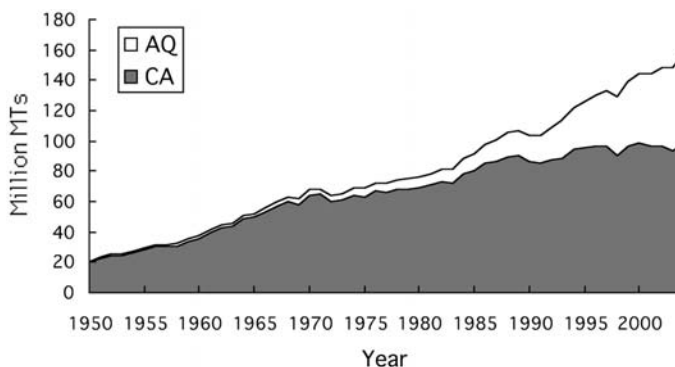


Fig. 1. Time trend of capture fisheries (CA) and aquaculture production (AQ) in the world during 1950–2001 (from FAO Fisheries Statistics).

(Fig. 2; Kaeriyama and Edpalina 2004). Kaeriyama (2003) defined the carrying capacity (K) of Pacific salmon as the replacement level of the Ricker recruitment curve. The carrying capacity of sockeye (*O. nerka*), chum (*O. keta*), and pink salmon (*O. gorbuscha*) since the 1976 regime-shift year has increased approximately by 100% compared with that in the 1947–1975 year classes. Statistically significant correlations were consistently detected between the mean Aleutian Low Pressure Index (ALPI; Beamish and Bouillion 1993) and the carrying capacity (K) of these three salmon species, but were not always significant for the α and β parameters of the Ricker curve. Thus, it appears that the carrying capacity of Pacific salmon is significantly synchronized with long-term changes in climate variation (Kaeriyama 2003).

Annual changes in biomass, which include catch and escapement, of wild and hatchery chum salmon indicate that the mean population biomass of both wild and hatchery chum salmon in the 1990s (132 million individuals) was roughly the same as that in the 1930s (140 million individuals). However, the abundance of wild chum salmon in the 1990s (67 million individuals) was only 50% of that in the 1930s (136 million individuals) despite the significant increase in the biomass of hatchery populations (Fig. 3; Kaeriyama and Edpalina 2004). This phenomenon in chum salmon suggests that the hatchery chum salmon recruited into the vacant ecological niche in the North Pacific Ocean left empty through recruitment failures in wild salmon stocks, that were linked to mass poaching (Korolev 2001). Wild populations were replaced with hatchery derived individuals such as occurred for pink salmon in Prince William Sound (Hilborn and Eggers 2000). The residual carrying capacity (RCC) was defined as $RCC = (K - \text{biomass})K^{-1}$ (Kaeriyama 2003). Relationships between the RCC and the fork length of adult Hokkaido chum salmon indicated a

positive correlation. In contrast, the mean age at maturity of adult Hokkaido chum salmon negatively correlated with the RCC (Fig. 4). This indicates a density-dependent population effect reduces the individual growth in Hokkaido chum salmon population with a resulting decrease in the residual carrying capacity (Kaeriyama and Edpalina 2004). The same result was observed in the relationship between the RCC of total chum salmon in the North Pacific and the individual growth reduction of Hokkaido chum salmon (Kaeriyama and Edpalina 2004). These results suggest that the carrying capacity of chum salmon in the North Pacific would be closely related to changes in climate change, but also with density-dependent population effects. Therefore, biological interactions between wild and hatchery populations should be an important consideration in sustainable fisheries management that operates at the ecosystem level.

3. Global Warming Effect

After spending their early marine life in coastal waters, Hokkaido chum salmon migrate to the Okhotsk Sea and then move to the Western Subarctic Gyre for their first-wintering. Thereafter, they migrate between the summer feeding grounds in the Bering Sea and the overwintering grounds in the Alaska Gyre. After about four years, they return to their natal rivers for spawning (Urawa 2000; Yatsu and Kaeriyama 2005). There are two hypotheses concerning the periods of critical mortality in Pacific salmon: (1) size-selective (predation) mortality in the early marine life period (the first few months after seaward migration; Healey 1982) and (2) size-related mortality over the first marine fall and winter which is dependent upon the salmon achieving sufficient growth by the end of first marine summer (Beamish *et al.* 2004). As the survival rate of Hokkaido chum salmon is significant—positively correlated with the mean

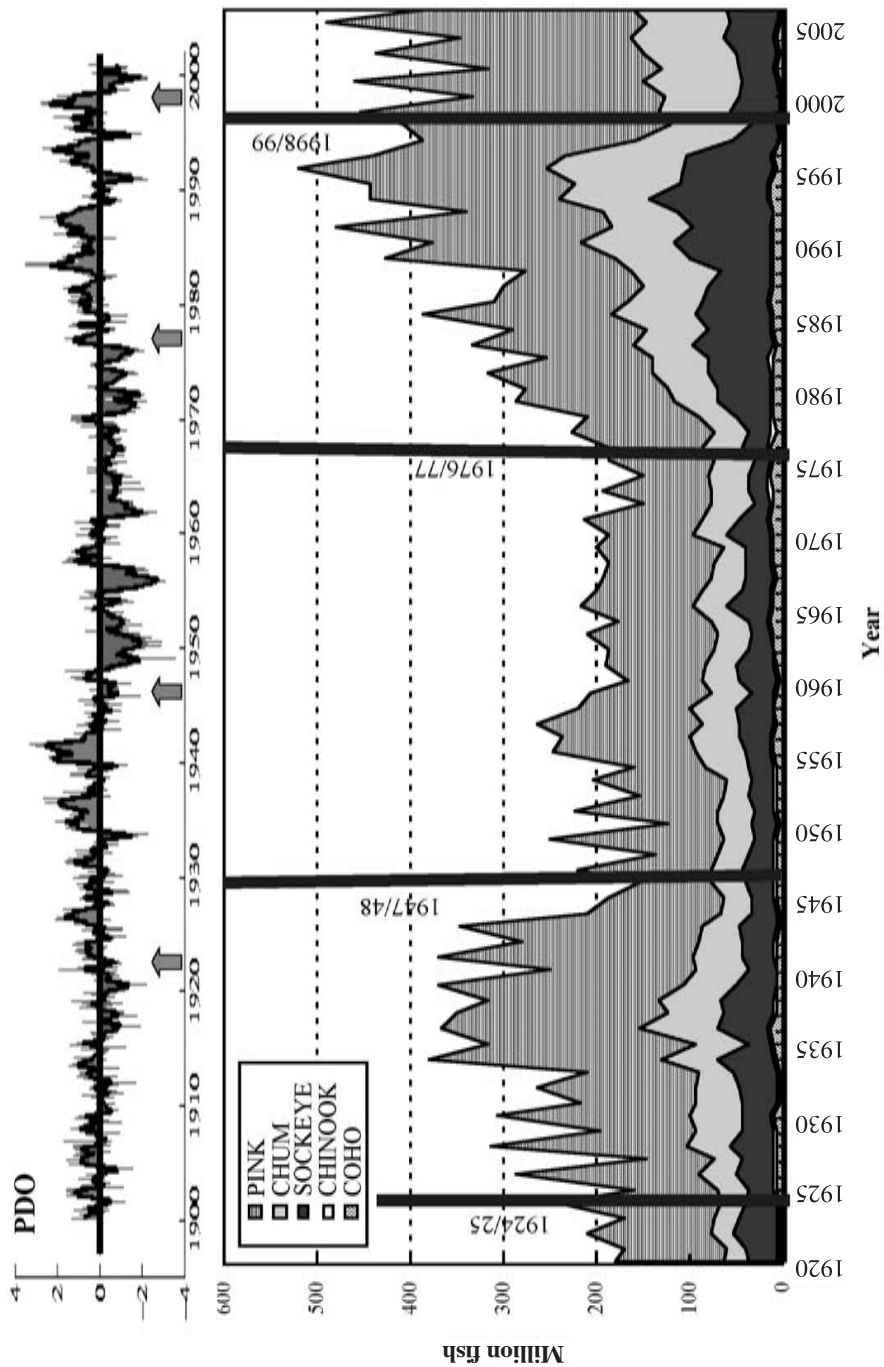


Fig. 2. Annual changes in the catch of Pacific salmon and the Pacific Decadal Oscillation (PDO) in the North Pacific Ocean in 1920–2006. Bars and arrows indicate the regime-shift year. The PDO is based on Mantua *et al.* (1997).

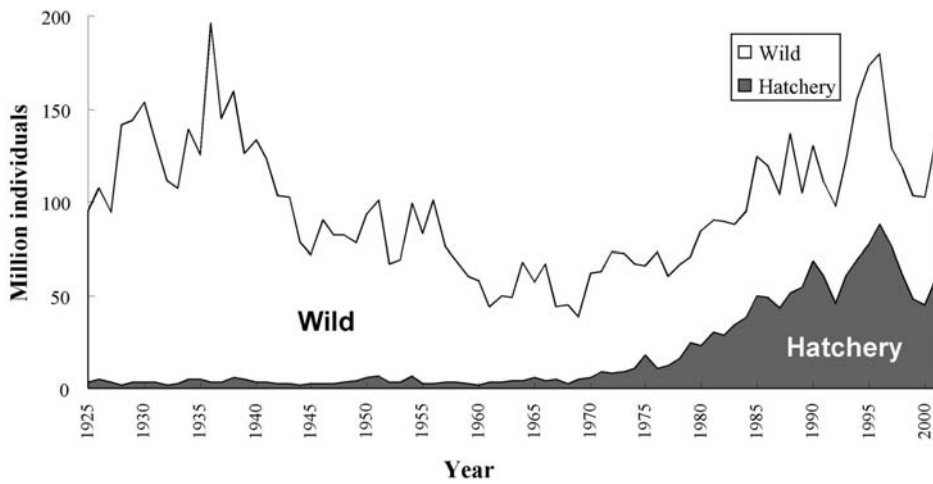


Fig. 3. Temporal change in biomass of wild and hatchery populations of chum salmon in the North Pacific during 1925–2001 (Kariyama and Edpalina 2004).

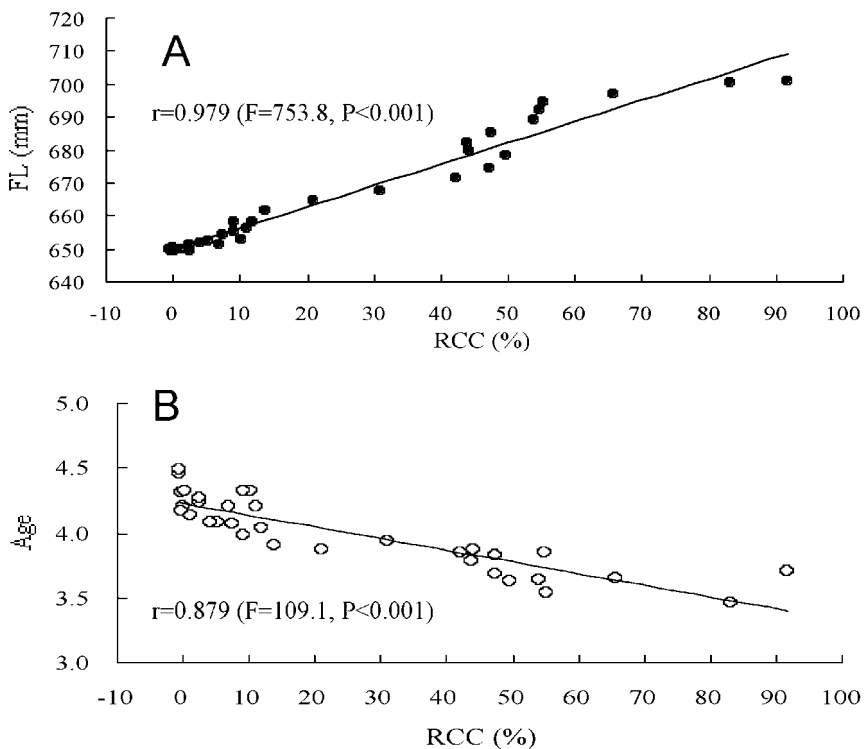


Fig. 4. Relationship between residual carrying capacity (RCC) and mean fork length (FL, **A**) of age-4 female adult-returning to 11 rivers or mean age at maturity (**B**) of Hokkaido chum salmon.

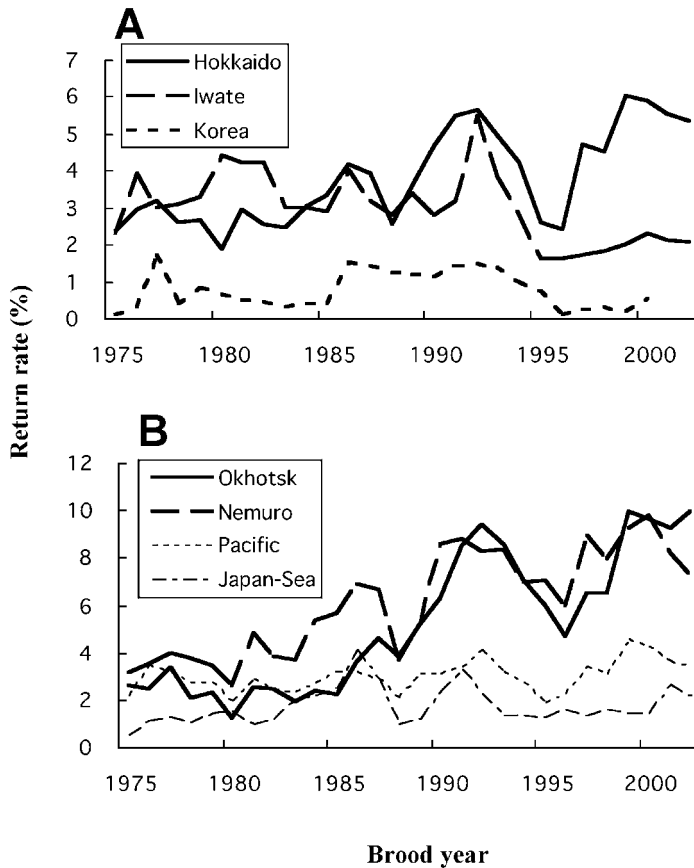


Fig. 5. Temporal changes in return rate of Hokkaido, Iwate, and Korean chum salmon populations (A), and of local populations in Hokkaido (B).

body-size of juveniles released and the growth anomaly of juvenile in the Okhotsk Sea, they would be affected by size-related mortality in the first marine winter after their growth period in the Okhotsk Sea, although mortality rates in the early marine period would be higher than those during their first marine winter (Kaeriyama *et al.* 2007).

Using scale analyses, back-calculated growth rates from Hokkaido chum salmon show that their growth anomaly is strongly and positively correlated with sea surface temperature (SST) during summer and fall, but is negatively correlated with the rate of sea-ice-covered area during winter in the

Okhotsk Sea (Kaeriyama *et al.* 2007). Zooplankton biomass in the Okhotsk Sea has also been decreasing since the 1980s (Shuntov and Dulepova 1996). Therefore, the increase in growth of Hokkaido chum salmon during the 1990s appears to have been affected by the increase in SST and not by a decrease in zooplankton productivity, relating to the sea ice concentration in the Okhotsk Sea (Kaeriyama *et al.* 2007). The extent of sea ice concentrations have been decreasing during the last 100 years as air temperatures on the Okhotsk Sea coast of Hokkaido have increased. Aota (1999) suggested that this phenomenon would be one

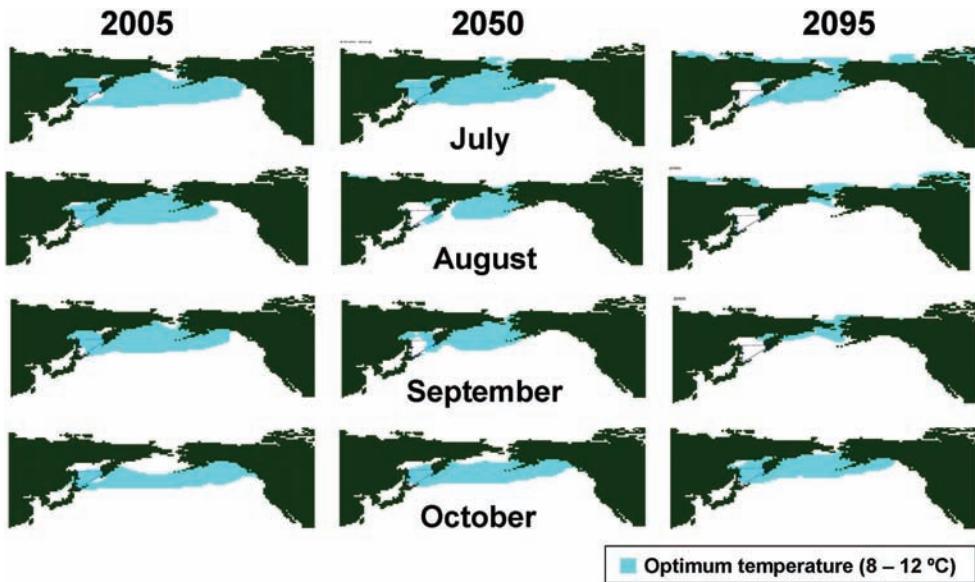


Fig. 6. Prediction of the global warming effect on chum salmon in the North Pacific Ocean based on the SRES-A1B scenario of the IPCC.

of the symptoms of global warming. Therefore, increases in the growth of Hokkaido chum salmon in the Okhotsk Sea since the 1990s may be related to the effects of global warming on sea ice cover.

Return rates of Korean and Iwate chum salmon have decreased since the 1990s, although that of Hokkaido chum salmon has increased since the 1976 regime-shift year (Fig. 5a). In Hokkaido, the chum salmon populations of Nemuro and Okhotsk Sea coasts, located near the Okhotsk Sea, have had return rates markedly higher than other populations (Fig. 5b). Korean and Iwate chum salmon are distributed in southern water and are affected by the Tsushima Warm Current during their spring offshore-migration period. Declines in their return rates did not coincided with the years of the climate regime shifts (1976, 1988, and 1998). These results suggest that global warming can be expected to have positive effects on Hokkaido chum salmon populations in the Okhotsk Sea, but negative effects on Korean and Iwate populations because of improving

force of the Tsushima Warm Current.

In the near future, what and how will Pacific salmon be affected by the global warming? Using the SRES-A1B scenario of the IPCC, it is possible to infer the global warming effect on chum salmon based on their optimal temperature for growth (8–12°C; Kaeriyama 1986; Ueno and Ishida 1998). This leads to the suggestion that chum salmon would be brought into direct competition with other salmon populations leading to a decrease in survival rate and population density-dependent effects because of the reduction of distribution area, displacement to the north (e.g., the Arctic Ocean), and the loss of migration routes (e.g., the Okhotsk Sea; Fig. 6).

4. Ecosystem-Based Sustainable Conservation and Management

The population dynamics of Pacific salmon is directly affected by a number of stresses (climatic and human impacts) that need to

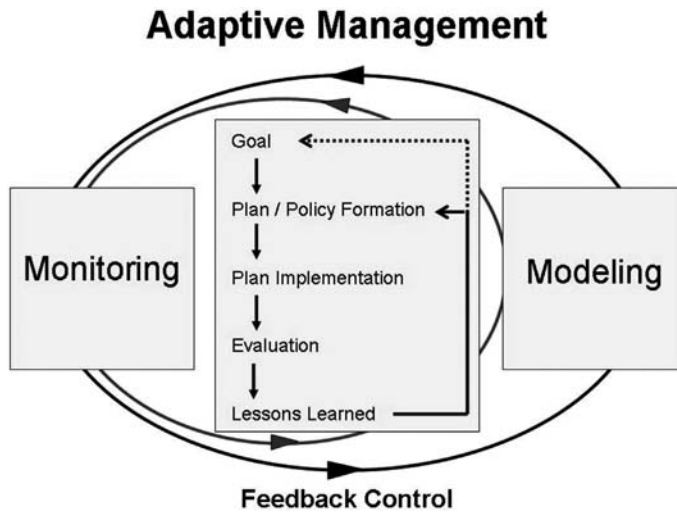


Fig. 7. Conception of the adaptive management for the sustainable fisheries management of Pacific salmon based on the ecosystem approach.

be considered within an ecosystem context. The structure and function of the ecosystem includes the interaction between the abiotic environment and the organism, and biodiversity, respectively. The aquatic ecosystem is subject to disturbance by natural factors and human impacts. Recently, human impacts have strongly affected the aquatic ecosystem (e.g., global warming, overfishing, habitat loss, artificial river channelization, dam construction, and negative effects of aquaculture and hatchery programs; Kaeriyama and Edpalina 2004). We need to recognize the limitations of fisheries management that is focused at the population level, and establish sustainable conservation and management based on the integration of population level approaches within a wider ecosystem-based approach.

Definitions of an ecosystem-based approach to fisheries management have been proposed by several authors (NRC 1999; Witherell *et al.* 2000; FAO 2003; McLeod *et al.* 2005; Murawski and Matlock 2006; Marasco *et al.* 2007). In this paper, I have used the definition of McLeod *et al.* (2005):

“Ecosystem-based management is an integrated approach to management that considers the entire ecosystem, including humans. The goal of ecosystem-based management is to maintain an ecosystem in a healthy, productive, and resilient condition so that it can provide the services human want and need. Ecosystem-based management differs from current approaches that usually focus on a single species, sector, activity or concern. It considers cumulative impacts of different sectors.” In this century, the ecosystem conservation and the stable production of food from marine sources are the most important issues for human beings in the global earth system, taking into account increases in human population and impacts such as global warming. Sustainable conservation management based on the ecosystem approach (SCMEA) for Pacific salmon should be part of the sustainability science of fisheries and oceans. Three aspects of the structure and function of the ocean ecosystem should be monitored, in particular for Pacific salmon;

- 1) spatial and temporal changes: carrying capacity, food web and trophic level,

- 2) climatic oceanic conditions: global warming, regime shifts,
- 3) biological interactions: between wild and hatchery populations, density-dependent effects, and inter- and intra-specific competition.

For the SCMEA of Pacific salmon, adaptive management and the precautionary principle are important. In particular, adaptive management should be conducted based on the feedbacks between monitoring, model-

ing, and adaptive learning, which includes learning by undertaking risk analyses and consensus building (Fig. 7).

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Some Lessons from Implementing Management Procedures

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The processes needed to implement Management Procedures (MPs) successfully are considered, with particular reference to the International Whaling Commission and South Africa which have the longest experiences with the MP approach. The three most important aspects in implementing MPs are: i) agreement of a Protocol which details specifications concerning data quality and availability, and the special circumstances under which the recommendation provided by an MP might be changed or the regular review of the MP brought forward; ii) structures for regular interaction between scientists and stakeholders during MP development to facilitate consensus buy-in to the outcome; and iii) adherence within the overall development schedule to strict deadlines for completion of the sequential steps of finalisation of past data and then of operating models for use in simulation testing of MPs. Difficulties in the definition of risk are discussed briefly, together with certain aspects of decision rules. For the latter, the importance of TAC variability constraints is emphasised, and a preference for empirical over model-based approaches is suggested based upon reader understanding by lay stakeholders.

KEYWORDS empirical; deadline; management procedures; management strategy evaluation; model-based; review process; risk; robustness; simulation test; uncertainty

1. Introduction

A Management Procedure (MP) is defined as the combination of pre-specified methods of data collection and analysis, and a simulation-tested decision rule which calculates a management recommendation (e.g. a To-

tal Allowable Catch—TAC) for a fishery (Butterworth *et al.* 1997). This process for providing scientific advice contrasts with the traditional approach of basing such a recommendation on a “best” assessment of the resource at the time, coupled to some biological reference point such as a target fishing mortality. (The Appendix provides more

details of this Management Procedure approach.)

The MP approach (alternatively termed Management Strategy Evaluation—MSE; Rademeyer *et al.* (2007) provide a glossary of the terminology that has developed in the field) was pioneered in the International Whaling Commission (IWC) (Punt and Donovan 2007), and its application has spread slowly but steadily. Punt (2006, Table 1) lists 17 resources for which the approach has been, is, or is under consideration for use. In addition the MP approach is the basis for management recommendations for two aboriginal subsistence whaling operations in the IWC, and is planned for North Atlantic bluefin tuna (*Thunnus thynnus*) in the International Commission for the Conservation of Atlantic Tunas (ICCAT) (ICCAT 2006), Southern bluefin tuna (*T. maccoyi*) in the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) (CCSBT 2005), krill (*Euphausia superba*) in the Commission for Conservation of Antarctic Marine Living Resources (CCAMLR) (CCAMLR, 2006), and Greenland halibut (*Reinhardtius hippoglossoides*) in the North Atlantic Fisheries Organisation (NAFO) (Miller *et al.* 2007). Furthermore, De Oliveira *et al.* (2008) detail initiatives under way to use the MP approach in Australia, Europe and the USA, while a recent FAO Expert Consultation in relation to Ecosystem Modelling (FAO 2008) considered the approach best practice for the move towards taking account of such models in fisheries management by using them as models of the possible underlying realities (Operating Models—OMs) against which decision rules are simulation tested.

A core motivation for the MP approach is to take formal account of uncertainties as required under the Precautionary Approach (FAO 1995). It relies on feedback to adjust management controls to attempt to move

resource abundance towards target levels, even if the current best assessment of the resource is in error. Schnute *et al.* (2007) consider MSE to be a dominant issue in the future of fisheries stock assessment.

There have been many contributions providing details of the MP approach/MSE, how OMs are best selected and specified, and what are seen as the overall advantages and disadvantages of the approach (such as the positive aspect of providing a structured framework for taking proper account of uncertainties in line with the Precautionary Approach, and the negative aspect of a lengthy development time) (e.g. Butterworth and Punt 1999; Smith *et al.* 1999; Kell *et al.* 2006; Punt 2006; Butterworth 2007; Plagányi *et al.* 2007; Rademeyer *et al.* 2007; Punt, this volume). However, rather less has been said about the associated processes necessary to see MPs successfully implemented in practice.

The IWC and South Africa have the longest experiences with the approach. For the three major South African fisheries, scientific TAC recommendations for hake (*Merluccius* spp.) and for the small pelagics, sardine (*Sardinops sagax*) and anchovy (*Engraulis encrasicolus*), have been provided on this basis for over 15 years, and those for west coast rock lobster (*Jasus lalandii*) for over 10 years. Since the late 1990s, South African decision makers have not modified recommendations based on such procedures unless motivated by scientific justification based on new information, which is suggestive of the approach's success.

This paper seeks to distill some of the lessons learnt concerning implementation, especially in the IWC and South Africa. The paper concentrates primarily on aspects of process, but also includes discussion of issues related to risk and decision rules.

2. Overarching Process-Related Aspects

2.1. Protocols

Conceptually, once an MP is implemented, it can be left to run indefinitely like “clock-work” (Hilborn and Luedke 1987) or an autopilot, to provide future management recommendations; these recommendations will, when implemented, ensure through feedback control that long term objectives for the resource and fishery are met. In practice however, future reviews and possible revisions are necessary to take account of scientific advances in resolving some of the uncertainties against which the original MP was designed to be robust. Furthermore, allowance must be made for possible intervention if circumstances arise which are beyond those for which this “autopilot” was designed to cater.

For the successful implementation of an MP over time, the agreement and documentation of a Protocol setting out procedures associated with these practical aspects is almost as important as agreement on the details of the MP itself. MPs for the South African fisheries function under a set of “Procedures for deviating from OMP [Operational Management Procedure] output for the recommendation for a TAC, and for initiating an OMP review” (see Rademeyer *et al.* (in press), Appendix II). These were developed from similar proposals for such a protocol associated with the MP planned for Southern bluefin tuna (Basson *et al.* 2004; Basson and Polacheck 2005). The specification document for the IWC’s Revised Management Procedure (RMP) for baleen whales (IWC 1999) also contains some such provisions.

The justification for pre-specifying a regular series of reviews to update MP evaluations in the light of new scientific inputs is not contentious. IWC (1999) specifies a five-year interval between these reviews under

the RMP, while South Africa intends a four-year period and the CCSBT (which conceived TAC adjustments only every three years) plans for nine years.

However, allowing for deviation from MP recommendations or bringing MP reviews forward seems dangerous, as it provides an opening for the very tinkering with recommendations that the MP approach is intended to avoid (Butterworth 2007). Consequently it is important to specify that such action may be considered only provided that there is first compelling evidence presented for its necessity. The primary criterion to be satisfied in justifying such *Exceptional Circumstances* is that the resource has moved outside the range over which earlier simulation testing had shown the MP to be robust. To allow such checks to be made, it remains necessary to carry out regular (typically annual, except for very long-lived species) baseline assessments of a resource between the regular MP reviews. Indeed, during the fairly lengthy periods of development of both the SBT (CCSBT, 2004) and the most recent South African hake (Rademeyer *et al.* in press) MPs, a demonstration that an updated assessment showed the resource to be outside the range predicted by the OMs guiding the MP development led to the revision of those OMs before the final MP simulation testing process was conducted.

Reaction to *Exceptional Circumstances* can take two forms. One is an *ad hoc* adjustment to the MP’s recommendation, coupled perhaps with bringing the next scheduled MP review forward. However, for short-lived and highly fluctuating resources (such as the South African sardine and anchovy), simulation-tested metarules may be pre-specified to prescribe the action to be taken in such circumstances. These metarules may take the form of more conservative catch control laws and a suspension of limitations on the extent to which the TAC can be reduced from year to year (e.g. Cunningham and Butterworth 2004). One rationale underlying such

metarules (which correspond to the “jacket” concept in control theory—see Jacobs 1989) is that the MPs for such highly fluctuating resources are designed to secure a low probability of abundance falling below some threshold level, but if that situation nevertheless appears to have occurred, different management measures need to be put in place. These different measures must ensure that there is high probability of resource recovery, while still if possible avoiding the draconian measure of fishery closure with its concomitant negative socio-economic consequences.

In practice, instances of *Exceptional Circumstances* have occurred more frequently than predicted over the period of applying MPs to the major South African fisheries. This outcome is not entirely unexpected, as the range of uncertainties against which MPs are tested is typically based on the past behaviour shown by the resource in question or similar resources elsewhere (for example along the lines of the plausibility ranking scheme of Butterworth *et al.* 1996—see discussion in Punt, this volume), but the resource is not guaranteed to maintain such behaviour in future.

Kolody *et al.* (in press) raise the concern that more frequent instances than expected of *Exceptional Circumstances* may compromise the credibility of MPs, and mention one possible remedy of deliberately expanding the range of uncertainties considered in the simulation testing process to make greater allowance for the associated “unexpected” future events. However, the difficulty with such an approach is that the greater such expansion, the more conservative the MP that will result (almost certainly TACs will be lower in the short term), and stakeholders from industry are consequently virtually guaranteed to take issue with the somewhat arbitrary nature of the choice of the extent by which to expand this range.

On balance, I would argue that risk of a greater frequency of *Exceptional Circum-*

stances declarations when the range of uncertainties is determined by “past behaviour” is worth taking, if only to get an MP accepted by consensus. Formal consensus constitutes buy-in to accept the future TAC changes (whether positive or negative) that feedback control will bring. (Indeed, in South Africa MP parameters have sometimes been set to ensure no substantial TAC change in the first year of implementation as a deliberate “sweetener” to obtain buy-in to such acceptance, which has been seen as the most important strategic goal of the process.) Industry stakeholders in particular, given their financial investments, can be reluctant to tie themselves down to application of the same formula over a lengthy period; the specifications of a regular review process and *Exceptional Circumstances* provisions make the MP package all the more saleable. Over time there seems to have been an increasing appreciation in South African fishing industry circles of the value of the security and transparency provided by MP-based management. The well-defined processes involved are seen as an important counter to possible sudden maverick interventions by interest groups seeking to influence decisions.

2.2. Robustness

The concept of an MP providing recommendations that take account of uncertainties in ensuring management objectives are met is easily advocated, but there are associated practical problems. The source of the difficulty is that no MP can offer such robustness under *every* conceivable hypothesis for resource and fishery dynamics. Thus some allowance for the relative plausibility of the hypotheses underlying the various uncertainties has to be made when selecting a MP.

In practice in South Africa, final selection of MPs has been based primarily on performance under either:

- a Reference Case OM reflecting the “best assessment” of the resource which

takes account only of estimation error in current numbers-at-age estimates and some demographic parameters (sometimes within a Bayesian framework), together with future observation errors in monitoring data and recruitment fluctuations (process error), or

- more recently often a Reference Set of a limited number of OMs which span the major axes of uncertainty and over which performance statistics for the MP are integrated (Plagányi *et al.* 2007; Rademeyer *et al.* 2007). The SBT MP evaluation process followed this same approach (CCSBT, 2004).

Consideration of the results of other robustness tests has been at a qualitative level only (to check in particular that resource abundance projections do not differ dramatically from those under the Reference Case/Set). Furthermore, except in the case of the highly fluctuating sardine and anchovy resources, attainment of resource recovery targets has been assessed in terms of projection medians, with only qualitative consideration given to the associated probability intervals.

As emphasized by Punt and Donovan (2007), typically errors in structural assumptions can have a greater impact on simulated MP performance than process and observation error. Consequently, there is a need for greater emphasis in simulation tests on the implications of alternative stock structures, spatial dynamics and ecosystem effects. Recent MP refinements in South Africa are moving in this direction, with the rock lobster MP taking account of spatial effects by providing zone-specific TAC recommendations. Further, there are plans to examine alternative pelagic fish harvesting strategies so as to investigate their implications for African penguins (*Spheniscus demersus*), which feed on sardine and anchovy, within the MP framework.

Punt and Donovan (2007) also call for the MP Protocol to include a well-specified

process for selecting an MP after simulation tests have been completed, where such a process would take quantitative account of the results of robustness tests and their relative plausibilities. At present only the IWC is well advanced in this respect, with a scheme that effectively “lowers the bar” on target levels for population size at some future date when the hypothesis underlying the trial is accorded a lower plausibility (IWC 2005).

2.3. Scheduling

The process of finalizing an MP is a much more extensive exercise than a typical stock assessment. A particular difficulty is that arguments can arise in mid-process to return to the beginning (backtrack) so that new data or hypotheses can also be taken into account. Both the IWC and CCSBT have experienced such difficulties (Punt and Donovan 2007; Kolody *et al.* in press). The severity of these difficulties has led to the IWC specifying a schedule that sets specific deadlines within a two year time frame from commencement to MP selection (IWC 2005), within which no backtracking is allowed.

Similarly in South Africa, a sequence of deadlines is set to finalise each of these steps, again with no subsequent backtracking, for:

- i) the data to be used in conditioning OMs,
- ii) the OMs and their conditioning, and
- iii) candidate MP testing leading to MP selection.

In practice, however, slippage on the first two of these deadlines has unfortunately proved very difficult to prevent. The second step of OM specification usually leads to the longest overrun, with consequent extreme pressure of time on completing the third step for which the deadline is immovable (given requirements for management advice for the forthcoming season). This slippage has at times led to inadequate opportunity to fully explore alternative options for an MP, with only the robustness tests judged as likely to have the greatest impact being completed in

time for consideration in the selection of the MP.

The reasons that underlie the pressure to extend deliberations for the second step are understandable. Final MP selection is frequently determined primarily by achievement of abundance targets over the medium to long term (10–20 years, or longer for whales) under the Reference Case/Set of OMs. With TACs over the next few years usually highly dependent on this selection, and limited scope for adjustment of the MP in the short term, industry stakeholders and their scientific consultants on the one hand, and NGO interests on the other, will contest Reference Set selection vigorously to try to advance their respective foci on short and longer term goals, and it is difficult to take the overall process forward successfully until consensus has been reached on this aspect.

The broad advice to be given is to ensure that adequate time is allowed for the whole process (15 months has been typical in South Africa), and to err on the generous side in the allocation for the third step given that some slippage at the second stage will probably be unavoidable in practice.

2.4. Objectives

De Oliveira *et al.* (2008) state that a major benefit of the MP approach is that it requires the identification and quantification of management objectives while forcing a longer-term view of resource utilization on decision makers. However, this does not mean that these objectives need to be precisely articulated at the start of the process (Kolody *et al.* in press).

The process of finalizing objectives should be an iterative one, particularly as broad objectives (e.g. maximize catch, minimize risk) often conflict, so that trade-off choices will need to be made. Typically, it is only as computations develop, and the key trade-offs come to be identified and quanti-

fied, that decision makers can become more fully aware of the constraints within which they are limited to operate, and hence become able to meaningfully quantify their objectives.

2.5. Data aspects

MP implementation is based on the premise that resource monitoring data of an adequate quality (e.g. abundance estimates within the levels of bias and precision considered in the simulation testing) will continue to be forthcoming as time proceeds. However assured this continued provision might seem, unanticipated events (e.g. mechanical problems with a research vessel forcing cancellation of a survey) can lead to nonavailability of such data.

Thus the MP Protocol must include clear specifications of the defaults to apply in circumstances where a datum anticipated is not forthcoming, with performance under these defaults preferably having been simulation tested. Depending on the nature of the data concerned, such defaults may be as simple as assuming the value in question to be the same as observed the previous year. Defaults may also incorporate a carrot-and-stick approach, such as the IWC's RMP (IWC, 1999) under which surveys that are more frequent or intensive (hence leading to more precise estimates of abundance) will lead to larger TACs, whereas TACs are continually phased down from the values that would otherwise apply the longer the period that elapses (after a specified minimum) before a further survey takes place.

Another difficulty that can arise is if TACs are undercaught (not reached), particularly if the reasons are administrative delays in finalising and advising quota allocations and quota holders request roll-overs to the following season. MPs do react to undercatches by increasing future TACs above the levels which would otherwise have eventuated. However, typically this "compensation"

is only partial and slow in coming. Once again, the Protocol should specify in advance how such circumstances are to be handled to preempt difficult discussions and *ad hoc* adjustments at the time.

2.6. Organisation

Transparency and standards can be enhanced through the practice of keeping the process of coding simulation tests independent of the use of this code to test candidate MPs. Punt and Donovan (2007) suggest that major gains may be made by having more than one group contribute to the process of developing and testing candidate MPs (as has occurred in both the IWC and CCSBT), and the availability of user-friendly code can facilitate this occurrence.

However, this multi-group involvement may be more readily achieved only at an international level, where the Secretariat of an RFMO can undertake or oversee the coding process, and scientists from member states offer alternative candidate MPs. At national level, lack of resources and expertise make this more difficult, though Schnute *et al.* (2007) suggest that the development of a common conceptual framework, from which standardized open source software for MP testing might emerge, could ameliorate this problem. The development of Fisheries Libraries in R (FLR, www.flr-project.org, Kell *et al.* 2006) is an initiative in this direction.

3. Risk

“Minimising risk” is a readily agreed broad objective for fisheries management, but as Punt (this volume) bemoans, decision makers are seldom willing to provide an agreed definition for “risk” and an associated threshold for acceptability.

One problem is that development of definitions to which lay stakeholders can readily relate has proved difficult. Often the approach used scientifically is to express risk

in terms of the probability of abundance dropping below some threshold level (typically motivated by concerns that below this level, recruitment success could be jeopardized) over a specified period of time (e.g. as used for Antarctic krill (CCAMLR 1994, paras 5.18–5.26) and the South African sardine and anchovy resources (De Oliveira and Butterworth, 2004); in the former case the risk criterion applied is the probability of spawning biomass dropping below 20% of its pre-exploitation median level over a 20 year period of harvesting not exceeding 10%).

Even if one leaves aside the question of whether the computation of such probabilities should be extended beyond a Reference Case OM to integrate over, for example, structural uncertainties and their relative probabilities, it is even then not straightforward to maintain consistency over time with this approach. This is because estimates of key demographic parameters can change over time as further data become available. Figure 1 shows the series of at times rapidly changing estimates of biomass from annual hydroacoustic surveys of the South African sardine resource, and associated recruitment plots. As time progressed, best estimates of adult natural mortality (M) and the extent of variability (σ_R) about a fitted stock-recruitment relationship have changed (Table 1). The lower M or the larger σ_R , the greater the extent to which abundance will fluctuate naturally in the absence of exploitation; hence the lesser the concern that arises if harvesting reduces the resource to a particular level.

Thus acceptable risk thresholds for sardine (and similarly for anchovy) have had to be re-specified over time, because maintaining the same estimated probability of the resource dropping below a particular level (such as 20% of the average abundance in the absence of exploitation) would not correspond to an unchanged biological risk (as it is perceived at any time given the demographic

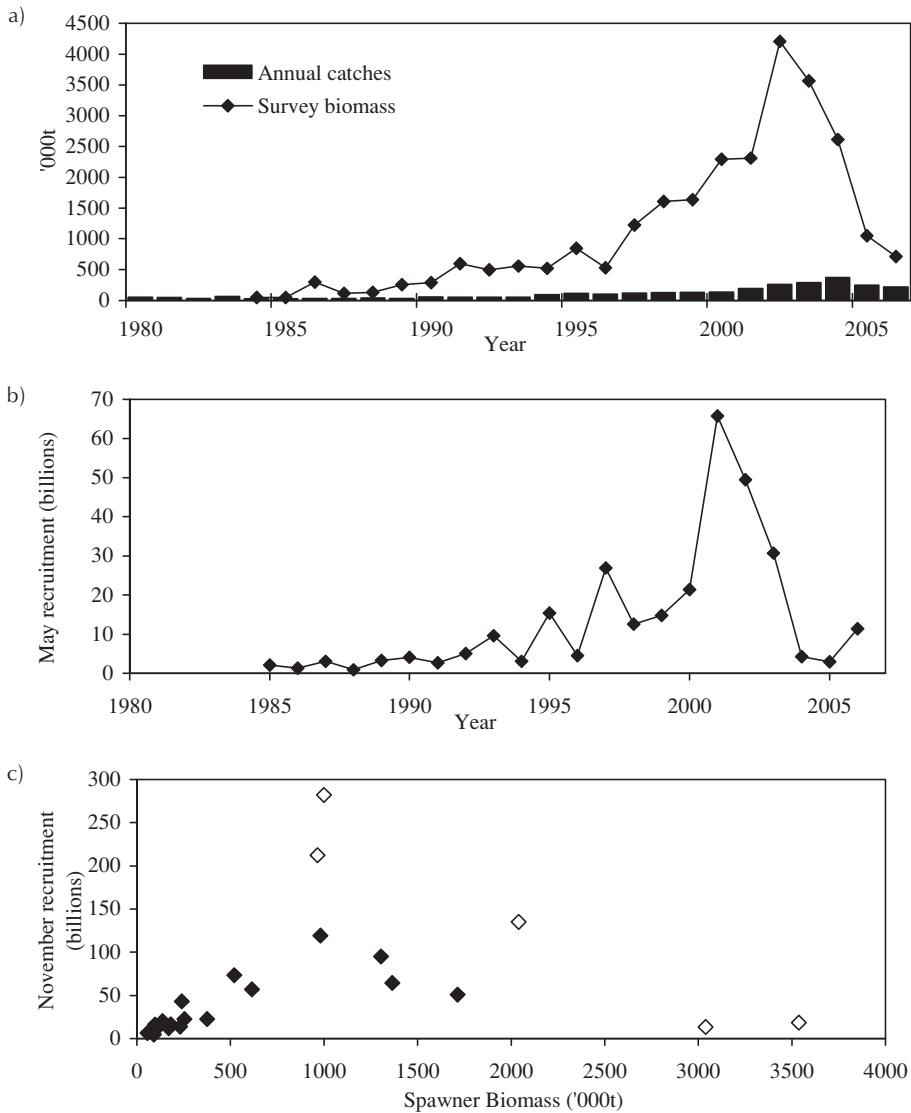


Fig. 1. Time series of **a)** total annual catches and November hydroacoustic survey estimates of biomass and **b)** population model estimates of annual recruitment for the South African sardine, together with **c)** a stock-recruitment plot based on model estimates. The last plot distinguishes points during the period of the recent peak and then decline in abundance.

estimates then available). The changes in estimates of M and σ_R for sardine were mainly increases, suggesting that the acceptable probability of falling below a particular level should be set higher, but by how much? The approach adopted at present to attempt

to maintain a consistent approach to risk given these changing estimates, is to adjust this probability threshold so that under the corresponding MP, the anticipated biomass distribution below the median is moved to the left compared to the pristine distribution

Table 1. The evolution of estimates of demographic parameters to which risk evaluation is particularly sensitive in the Reference Case OMs (denoted by the year of the associated assessment) used in selecting the sequence of MPs adopted for the South African sardine resource. M is adult natural mortality and σ_R the standard deviation of the residuals of log recruitment about a stock recruitment relation, which has been assumed to have a hockey-stick form. (Note that the value of M was initially guessed and later poorly estimated, until the rapid recent decline in abundance (see Fig. 1) provided the contrast for improvement in circumstances where ageing information is not yet considered reliable.)

Assessment year	MP name	M (yr ⁻¹)	σ_R
1993	OMP-94	0.8	0.33
1996	OMP-97	0.6	
1998	OMP-99	0.6	0.51
2001	OMP-02	0.4	0.50
2004	OMP-04	0.4	0.49*
2007	OMP-08	0.8	0.50*

*Bayes posterior median

by about the same relative proportion as was the case for the previous MP under their respective Reference Case OMs (see Fig. 2) (Cunningham and Butterworth 2008).

4. Decision Rules

4.1. Form

Decision rules in MPs frequently take the form of catch control laws which provide TAC recommendations based on estimates of current biomass (B). Figure 3 provides examples of some common forms: constant catch, constant fishing mortality (F) and constant escapement, in the form of both the TAC and of F as a function of B .

Interactions with industry in South Africa have served to emphasise the importance they attach to limiting TAC variability to the extent possible in the interests of enhanced

socio-economic stability. Figure 4 illustrates the rather more complex catch control law for sardine that has evolved in consequence. Notable features of this and the associated limitations on TAC variability are:

- The rule is based on modifications to the constant F approach.
- Over a range of lower values of B , a constant “minimum” TAC applies, as the industry would need substantial restructuring if the TAC fell below this level; note that this means that over this range of B , F increases as B decreases to contain the socio-economic risk.
- The lower end of that range is the *Exceptional Circumstances* threshold; below this F decreases linearly and the TAC quadratically with B , with the limitations on annual reductions in TAC that would otherwise apply falling away.
- There is a maximum TAC, both because of limits on the industry’s catch and processing capacity, and because simulations indicated that a constant F rule when biomass is estimated to be high (and this estimate could be considerably above the true abundance because of survey sampling error) led to greater risk.
- Over an intermediate biomass range, there is a maximum proportional amount by which the TAC can be reduced from the previous year’s value.
- When the previous year’s TAC is above a certain relatively high level, this maximum proportional reduction limitation falls away and is replaced by a specific level to which the TAC can be reduced; this feature of the decision rules is to allow advantage to be taken if sudden peaks in abundance occur by increasing TACs substantially, while still containing risk by also allowing for large and rapid TAC decreases on the downside of such peaks.

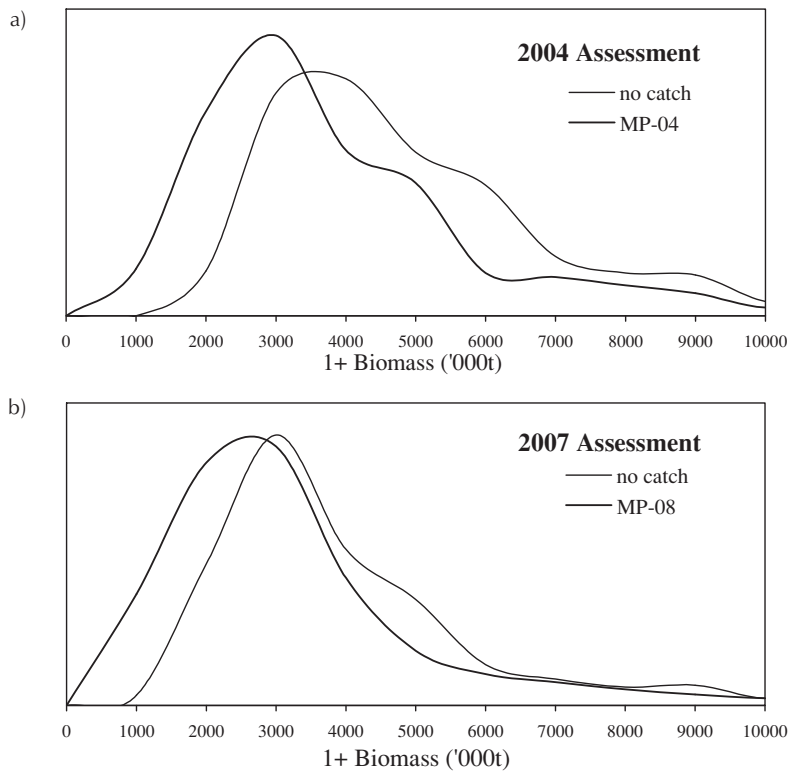


Fig. 2. Biomass distributions inferred from Reference Case OMs for the South African sardine resource under zero catches and under the MP implemented for **a)** the 2004 MP and **b)** the 2008 MP.

4.2. Continuity

Continuity is a necessary and important feature of control rules. Circumstances where a small change in an abundance estimate would lead to a large change in TAC serve only to invite endless haggling over revision of an abundance estimate slightly below (or above) the level at which the discontinuity occurs. An exception to this may be the maintenance of the current TAC unless the change indicated by the control rule is above a certain smallish threshold, simply to avoid the administrative inconvenience brought about by a small TAC change of no real biological consequence. Care needs to be exercised to ensure that the property of continuity is main-

tained when constraints on annual TAC adjustments may alter (e.g. when *Exceptional Circumstances* apply).

4.3. Model-based vs. empirical

MPs may be split into two categories:

- “model-based”: where typically the current abundance of the resource is estimated by applying some assessment procedure to the available data, with that abundance then input to a control rule to provide a TAC recommendation; and
- empirical: where resource monitoring data are entered directly into the control rule (e.g. the TAC is adjusted in proportion to the short-term trend with time of an index of abundance).

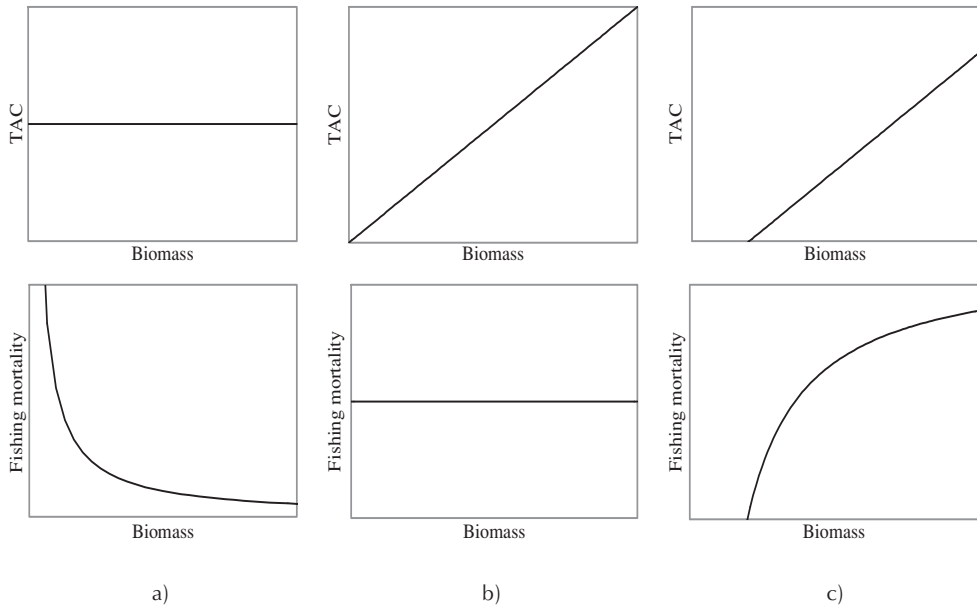


Fig. 3. Illustrative plots of catch control laws that relate TACs (upper panels) and fishing mortality F (lower panels) to biomass B for **a)** constant catch, **b)** constant fishing mortality and **c)** constant escapement approaches.

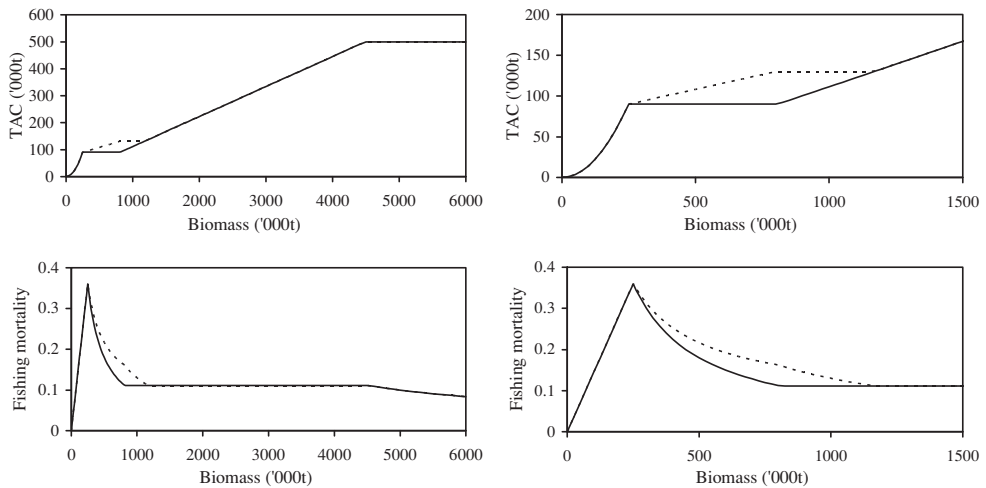


Fig. 4. The catch control law for the South African sardine under the 2008 MP, with the upper panels showing TAC and the lower panels fishing mortality F . The full line reflects the basic law, while the dashed line includes a year-specific adjustment (here applying to 2008) that arises from variability constraints linked to the previous year's TAC.

Model-based MPs tend to result in lesser inter-annual variability in the TAC than empirical approaches at comparable risk and catch levels. This property follows because model-based procedures can make their estimates using more data, as they are able to take account of the complex trends in abundance of the underlying population over a long period. Empirical approaches on the other hand are usually restricted to linear trends because abundance indices typically exhibit high variability, and hence must limit the data used to estimate this trend to a period for which the trend in the true abundance is likely to be well approximated by a straight line. Model-based procedures also make use of improved estimates of resource productivity as more data become available, i.e. they “learn” in a manner that diminishes the extent of a core uncertainty. For these reasons, model-based approaches have been preferred in the IWC (IWC 1992) and CCSBT (CCSBT 2005).

In contrast, however, the recent trend in South Africa has been towards empirical procedures, with the most recently adopted MP for hake being a particular example, succeeding the use of model-based approaches in the past (Rademeyer *et al.* in press). Among the reasons for this change are that model-based approaches are often in practice restricted to relatively simple age-aggregated production model approaches as testing complex models with many estimable parameters would be computationally infeasible. Given long time series of data, these simpler models can prove incapable of reproducing past behaviour, and so may also exhibit multi-modal likelihoods as well as an inability to reflect (and hence have the MP react adequately to) recent changes in abundance index trends.

The particular advantage of empirical approaches is that they are easily comprehended by lay stakeholders, which enhances the transparency and saleability of the MP. Arguably the disadvantages compared to the

model-based approaches can be offset by explicit imposition of limitations on the extent of interannual TAC changes, and having the updating of OM (and hence also possibly of MP control parameters) in the regular review process take care of the “learning” aspect of improving estimates of demographic parameters.

An apparent disadvantage is that target and limit reference points are emergent properties of empirical approaches, whereas they can be explicitly included in the design of the control rules for model-based MPs. However, this “advantage” of the latter category of MPs is really illusory, as even for those MPs, it is performance statistics under simulation that determine the reference points likely to apply in practice, and these points may differ substantially from those implied by the deterministic form of the control rule because of the many stochastic aspects within the process.

5. Discussion

While the material presented above paints a fairly positive picture regarding MP implementation to link with affirmative appraisals of the advantages in principle of the MP approach itself as referenced earlier, one should not be blind to some reality checks.

- In ICCAT and CCSBT, the work towards MP development for bluefin tuna is on hold, having been overtaken by more pressing imperatives related to establishing effective bluefin tuna catch controls in the Eastern Atlantic and Mediterranean and to evidence of substantial under-reporting of catches over the past 10–20 years (CCSBT 2006).
- The IWC RMP is yet to be applied in practice (although Norway is implementing a variant thereof domestically to provide catch limits for its Northeast Atlantic minke whale harvests). The continuation of the IWC’s moratorium on commercial whaling allows its Scientific

Committee the luxury of considerable time to spend on a very thorough MP evaluation process at the analysis level. However it is unclear whether this degree of attention to detail would be sustainable under the typical pressures of time and requests for analyses that would arise if a large number of whale populations again came under commercial harvest.

- Proper and continued application of the approach need resources and a relatively high level of expertise, which are not available in many countries.
- The South African “success story” of a decade of unaltered implementation by decision makers of MP-generated TAC recommendations may be an artifact of pre-occupation during this period with a process of rights allocation in local fisheries (DEAT 2002, 2004a, b; Moola and Kleinschmidt 2008). With disputes focusing on whether past rights holders had too much of TAC taken from them, or whether new rights holders were entitled to a greater proportion of the TAC, scientific working groups constituted by the responsible arm (Marine and Coastal Management) of the Government’s Department of Environmental Affairs effectively took over a structural vacuum that had developed in the process of recommending what the size of the TAC should be. However, with this re-allocation process now near completion, there are signs of this (science-centrally-speaking) honeymoon period coming to an end, with some of those unsuccessful in the process seeking alternative approaches to getting a share of the TAC, and supposedly short-term *ad hoc* enlargement of the TAC seemingly being contemplated as a possible solution.

Nevertheless, one benefit of the somewhat atypical *modus operandi* for TAC advice generation that developed over the last

decade in South Africa was that the larger fishing companies, noting where the key decisions were being developed regarding TAC advice, made full use of their options for attendance as observers with speaking rights at meetings of scientific working groups. While initially turbulent, and sometimes still involving robust discussions, this mix did provide the opportunities for the effective interactions needed to develop improved mutual understanding and consensus buy-in by stakeholders to MPs, the associated processes, and the subsequent TAC recommendations arising from these MPs.

The South African situation may be atypical of that in a number of first world countries in that non-governmental conservation groups have to date played hardly any role in this aspect of marine resource management. These groups’ impact could be similar to that ascribed to the industry above, except that they would typically exert pressures in the opposite direction, with a greater long and lesser short term focus together with a more risk-averse emphasis. Nevertheless, conclusions above about the importance of structures for effective interactions and potential advantages from the MP approach would seem to apply in similar manner to conservation groups.

6. In Summary

- Agreement of a Protocol which covers matters such as data quality, action to take if anticipated resource monitoring data fail to become available, and the specification of regular reviews and the circumstances under which there may be deviations from recommendations output by an MP, or its review brought forward, is a necessary and important component of successful MP implementation.
- Structures for regular and effective interactions between scientists and stakeholders are essential for achieving

consensus buy-in to the MP eventually adopted.

- There must be strict deadlines to complete first the data and then the OM finalisation steps within the MP development process, with no backtracking allowed, to ensure that a timetable for eventual adoption will be met.
- The readier lay understanding of empirical compared to model-based MPs may prove important in securing consensus acceptance of an MP; the negative aspect of absence of explicit refinement of resource productivity estimates in empirical MPs may be offset by adjustment of OMs and MP control parameter values in the regular MP review process.
- TAC variability constraints are important to industry. Catch control laws need to be continuous in terms of abundance

estimates, and may need to be relatively complex particularly to dovetail with TAC variability considerations.

- Areas requiring further attention are appropriate measures of risk to which lay decision makers can readily relate, and the development of approaches to better incorporate the results from robustness tests in MP selection processes, while also expanding these tests to encompass spatial and stock structure together with ecosystem aspects to a greater extent.

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Appendix: Basic Elements of the Management Procedure Approach

The traditional approach to providing a scientific recommendation for the TAC for a fishery starts with the development of an assessment. All available information with a bearing on the dynamics of the resource harvested is analysed within the framework of a model which is able to integrate this information to provide estimates of resource productivity and current abundance. The results from what is considered to provide the best representation of the actual situation from amongst a number of alternative hypotheses (models) of the dynamics of the resource and fishery—the “best assessment”—are then fed into a catch control law (decision rule). This law could, for example, reflect a constant fishing mortality approach—e.g. harvest the resource at a fishing mortality F that will (in due course) yield the maximum sustainable yield (MSY) on average. This rate is termed F_{MSY} , and taken together with the results from the best assessment for the current abundance of the resource, would yield a TAC recommendation.

This traditional approach gives rise to a number of difficulties. For example, it takes no direct account of uncertainty—that the model selected may not constitute the best representation of the actual resource dynamics. Furthermore, while projections of abundance based on the “best assessment” can take account of model estimation error, to-

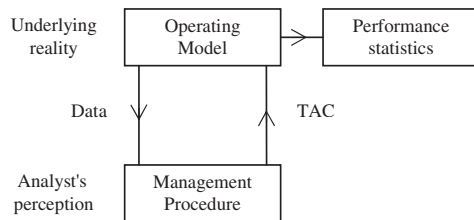


Fig. A1. Schematic representation of the process of simulation testing of a Management Procedure.

gether with future recruitment variability, they typically have to be based on constant catch or F assumptions. These overestimate risk, as they take no account of the information from resource monitoring that will become available in the future. This information will “feedback” into decisions to modify TACs (e.g. downwards if the trend in resource abundance indices is down) to attempt to change that trend back towards a management reference point such as a target abundance (an approach generally known as “feedback control”).

The Management Procedure (MP) approach seeks to rectify this by simulating the annual process of TAC determination, and checking whether it can be expected to attain management goals if kept in place over a period of time (typically 10–20 years for other than very long-lived populations).

Figure A1 illustrates the calculation process used. An Operating Model (OM) is developed to represent the possible underlying dynamics of the resource (e.g. the “best

assessment” could be used for one such OM). This model generates resource monitoring data (e.g. abundance survey results, catch per unit effort values) of the type (and with the same error structure) as would be available in practice. These data are fed into a formula (the MP) which determines the TAC recommendation. The formula may be complex (e.g. replicating the standard annual assessment plus catch control law basis for TAC recommendation), or simple such as a fixed fraction of the most recent survey estimate of abundance. Importantly, computer evaluations are structured so that the formula (MP) is allowed no knowledge of or direct input from the actual underlying dynamics of the resource (current values of variables of the OM) except as reflected in the data available for assessment purposes, as would be the case in practice.

The TAC calculated is input to the OM, so that the dynamics can be updated by one year, and then the loop within Fig. A1 is repeated until the end of the projection period under consideration. The “performance” of the MP is then summarised by performance measures drawn from the underlying reality, the OM, such as the average annual catch achieved over the period, and the final population abundance compared to an intended management target level. To take account of estimation imprecision, random noise in future data generated from the OM, and future variation in recruitment, this whole process is repeated typically 100 times, so that performance outputs take the form of statistical distributions, rather than single values.

The choice of a final formula from amongst a number of candidate MPs is determined by a consideration of trade-offs amongst such performance statistics, e.g.

which formula provides the greatest average annual catch without a lower percentile for resource abundance dropping below some limit value which could result in a threat to successful future recruitments.

However, the issue of uncertainty about the underlying dynamics remains to be addressed (typical examples might involve the choice of functional form for the stock recruitment relationship, or alternative assumptions about the time series of catches which could, for instance, be uncertain because of illegal activities or incomplete records of discarding practices). This is done by repeating the set of simulations described above for other OM—ones that represent different, but still plausible representations of the resource and fishery dynamics. To be acceptable for implementation, a candidate MP must not only achieve management targets in simulations where the OM represents the “best assessment”, but also come reasonably close to doing so under these other OM, i.e. it must demonstrate robust performance. In this way the concerns of the Precautionary Approach—taking due account of uncertainties—are addressed.

This process of “simulation testing” of a decision rule is the critical component required to consider it classified as a MP. MPs are able to perform robustly because of their feedback nature—the decision rule self-corrects by, for example, typically decreasing TACs if abundance trends are downward and *vice versa*. This can result in management targets for abundance still being near attained, even though the underlying reality differs from the best assessment which may have guided the choice of control parameter values for the MP decision rule.

Reconsidering the Contribution of Fisheries to Society and Millennium Development Goals

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The assertion that fisheries in developing countries can contribute to society and the Millennium Development Goals (MDGs), principally through jobs, income and human nutrition, needs reassessment. Two aspects of a reassessment are examined here, the globalized and integrated seafood business, together with the resultant north–south relationships, and better models for managing tropical nearshore fisheries. Fisheries can make simultaneous contributions to both societies in developing countries and directly to MDGs 7 and 8, provided that (1) models for tropical nearshore fisheries include simultaneously managed linked ecosystems, and (2) developed and developing countries collaborate to better manage the global fish trade and industrial fisheries.

KEYWORDS fish trade; globalization; industrial fisheries; linked ecosystems; alternative management models; North–South relations; small-scale fisheries; tropical countries

1. Introduction

Fisheries in developing countries have long been assumed to create employment and generate income, thereby contributing to society through poverty alleviation, as well as playing an important role in human nutrition (e.g., FAO 1997; Thilsted *et al.* 1997). In the same ways they might also contribute to at-

taining the eight Millennium Development Goals (MDGs, Table 1), both directly, via specific goals, and indirectly to all the goals, through enhanced livelihoods (World Fish Center 2005, Table 2).

However, those assumptions about income, employment and nutrition might be inaccurate. Based on a study of some 300 documents on various aspects of poverty in fisheries, Macfadyen and Corcoran (2002)

Table 1. The Millennium Development Goals.

MDG Goal Number	Objective
1	Eradicate Extreme Hunger and Poverty
2	Achieve Universal Primary Education
3	Promote Gender Equality and Empower Women
4	Reduce Child Mortality
5	Improve Maternal Health
6	Combat HIV/AIDS, malaria, and other diseases
7	Ensure Environmental Sustainability
8	Develop a Global Partnership for Development with developing countries.

Table 2. Hypothesized indirect ways in which small-scale fisheries could contribute to the Millennium Development Goals (The World Fish Center 2005).

MDG Goal Number	Activity
(Goal 2)	If through fishery activities incomes increase, then school attendance is likely to improve.
(Goal 3)	Women are further empowered through trading in fish (which is very often already in their hands!), and by facilitating various kinds of enterprise (many of which are already operated by women!).
(Goals 4 & 5)	Child and maternal health conditions would improve if fisheries can contribute either directly or indirectly to reducing hunger and improving nutritional levels.
(Goal 7)	Properly managed fisheries ensure that environmental capital and services are preserved for future generations.

concluded that there is nothing to substantiate the abundance of largely unsupported statements that fishing communities are either poor or the poorest level of society. More than the harvest of fish or other resources, local institutions and power structure that control access to resources might be the major determinants of levels of poverty (Ruddle 1987, 1989, 1994a, 1998; Ruddle *et al.* 1992; Béné 2003).

Like any other sector of rural development, small-scale fisheries could provide the opportunity for more people to participate in the supply and demand chains of regional and local economies. However, alleviation of poverty, improvement of nutrition levels

and provision of food are not straightforward tasks. Further, because capture fisheries and aquaculture communities have had little examination of either nutritional status or of the characteristics, causes and dynamics of poverty, claims about the actual or potential contribution to both societies and fulfillment of MDGs remain assertions in need of verification.

In order for fisheries to make a contribution to both societies, especially in developing countries, and to the attainment of the MDGs, a major rethinking of the concepts applied to fisheries management is required. In addition, re-arrangement of priorities among the MDGs should be considered.

Two major issues complicate any analysis of the contributions of fisheries to societies and the MDGs. The first concerns the numbers and economic condition of the people involved, and levels of fish production. The second is the complex linkages that characterize both capture fisheries and aquaculture. These are dealt with in the following section of this article. The old assumptions about the potential contribution of fisheries to societies in developing countries and to the attainment of the MDGs must now be viewed against the globalization of the fish trade and the resultant North–South relationships. That is addressed in Section 3. The assumptions also need to be reviewed in terms of alternative approaches for small-scale tropical fisheries in rural development. In Section 5 first I suggest a re-arrangement of the priorities of the MDGs. Then the contribution of fisheries to the MDGs and societies is re-considered in terms of (1) near-shore tropical fisheries and the attainment of MDG 7, and (2) the management of the globalized fish trade and the attainment of MDG 8.

2. Two Major Difficulties

Because data on all national fisheries are based on a compilation of estimates provided by national governments, measurement of the amount of global employment and income generated by fisheries is difficult. Those estimates suggest that employment in fisheries doubled from 1970 to 1990, when they provided the primary income of some 28.5 million persons (FAO 1997). That figure balloons to an estimated 60 to 100 million when employment in upstream (e.g., boat-building) and downstream (e.g., processing) activities and those based on the local expenditure of fishers and their families are included (Delgado *et al.* 2003), and to over 200 million people when dependents are considered. Similarly, global fish consumption is thought to have approximately

doubled in the last 35 years (Delgado *et al.* 2003). Further estimates conclude that fish now provide some 2.8 billion people around the world with nearly 20% of their protein requirements, and in many tropical countries are the sole source of animal protein available to the poor (Delgado *et al.* 2003). The annual average per capita global consumption rate of fish in 2005 was thought to be about 16.6 kg. Regardless of accuracy, it is these estimates that shape international assistance programs.

A second difficulty arises from the ramified upstream and downstream linkages that characterize capture fisheries and aquaculture, and which might also be important to regional economies and household incomes. However, in general, little is known of the role of the various linkages, despite a few case studies in limited areas, or how these linkages change, often quite rapidly, in response to alterations in general economic, social and technological conditions. For example, a case study done 20 years ago in Guangdong Province, South China, demonstrated that changing rates of inputs to integrated farming systems that included a large aquaculture component are reflected in altered labor demand and household income (Ruddle, 1985, 1986; Ruddle *et al.* 1986; Ruddle and Zhong 1988). The contribution of upstream and downstream activities to poverty alleviation and food security, and the impact on poverty of technological change, community and fishers' organizations, and management systems, remain to be systematically studied by both specific case studies and comparative research.

Linkages at the global scale are also problematical. For example, assigning the benefits of a large, international industrial fishery and its downstream employment has become extremely complicated, in large part owing to consolidation and vertical integration of the international fishing business during the last two decades. Several large companies control much of the world fish trade,

and the resultant patterns of benefit from the fishery are complex and fragmented. For example, it becomes difficult to track resource benefits when a Korean company catches Pollack in Russian waters, processes it in low-wage China, and sells the *surimi* products largely to Europe and North America (Won 2003).

3. The Old Assumptions in New Contexts

Continued repetition of old and unproven assumptions about employment, poverty alleviation and human nutrition based on fisheries, as sustainable contributions to either society or to attaining the Millennium Development Goals (MDGs), or both, is particularly pointless when viewed against contexts that have changed drastically in recent decades. This is now considered briefly in terms of globalized trade links and approaches to development.

3.1. The context of a globalized fish trade

Fish production—or, rather, extraction—has metamorphosed during the last three decades from what was essentially a local industry into the present globalized, high-value, corporate activity, in which developed and developing countries are now inextricably linked. As a result, industrial fisheries within the waters of developing countries are now more an aspect of national policy and foreign affairs than a fisheries issue. This has been examined in detail for the Pacific Island nations (Hunt 2003; Petersen 2003) and Sénégal, among other Western Africa cases in which EU nations are heavily involved (UNEP 2002).

An estimated 40% of world fish production enters international trade, with 2005 exports of US\$ 77 billion representing a 40% increase over those of 2000 (FAO 2001). The main flow is from developing countries to the developed nations, the former having

57% of the trade volume and 77% of its value (FAO 2001). However, the trade has led to some serious problems. Particularly deplorable is that potential benefits for developing countries have not always been realized. On the contrary, some results have been perverse.

For example, the fish exported by developing countries are obtained mainly by their domestic fleets operating within their own EEZs, and by selling EEZ access rights to foreign fleets. With donor assistance and foreign advice, many domestic fleets of small craft were expanded to profit from the growth of international trade (FAO 1998; Alder *et al.* 2006). Rather than profit, however, over-exploitation of nearshore fisheries, owing to poor management advice and to excess capacity stemming from subsidies, has been a common result (Porter 1997, 2001).

Potential benefits to developing countries have been further diluted by a great expansion of developed country fleets operating in developing country waters. Scrapping their large domestic fishing fleets would have entailed major economic and social woes for the developed countries, whose governments were unwilling to pay the heavy political price that would naturally have ensued (Kurien 1998). Since their domestic waters were already overfished and unable to sustain increased effort, they usually elected to negotiate access agreements to the EEZs of developing countries, rather than work out joint ventures and involve themselves in the troublesome development of local fishing industries. As a consequence, despite some local crewing and processing, a major source of fisheries and fisheries-related employment is usually lost to the developing countries. The “fleets to the South and fish to the North” policy, based on large fleets subsidized, yet barely regulated, by developed country governments (Dommen 1999) has often led to overexploitation of fish resources in the developing countries (Kurien 1998), and is a lost opportunity to create local skills and jobs.

Selling access rights and servicing foreign fleets are not always valuable sources of foreign exchange for developing countries. Although in a country like Tuvalu, with few other sources of income, 50% (1991) of the government revenue was generated by selling fishing rights (Hunt 2003), in other cases the amount paid does not necessarily reflect the real resource rent of the fish caught (Petersen 2003). Although developing country foreign exchange revenues from fish exports increased from about US\$ 5.1 billion (1985) (Delgado *et al.* 2003) to 16 billion (2002) (FAO/GIEWS 2002), sometimes that growth was obtained by increasing exploitation of fish, as in Ghana (Atta-Mills *et al.* 2004), or by reducing domestic fish supply, as in Sénégal (UNEP 2002). Further, in some instances, as in the Pacific Islands, the heavy dependency on foreign aid has resulted in their governments giving sharply discounted access rights in exchange for aid flows (Petersen 2003). The aid and monies generated by access rights are often poorly invested—commonly in domestic fisheries, which have mostly given poor results (Petersen 2003).

Illegal, unreported and unregulated fishing (IUU) is an integral part of the global trade in fish and fish products, but one that severely undermines fisheries management, as has been noted recently for Pacific Island nations (FFA, 2000; Hunt 2003). Worldwide, an estimated annual total of at least US\$ 4 billion of fish are caught illegally (WWF 2006). Developing countries suffer the most; for example, Africa south of the Sahara is estimated to lose annually US\$ 1 billion, or the equivalent of 25% of the total annual legitimate fishery exports from all of Africa (WWF 2006). Additional revenue is lost when various fees and taxes are avoided by illegal operators. Illegal fishing also affects livelihoods directly, as when potential employment and income in upstream and downstream activities is foregone. Small-scale fishing communities also suffer further as

their catches are reduced by the combined impact of IUU, weak surveillance, and competition from unsustainable industrial fishing. In the Pacific Basin the Forum Fisheries Agency (2000) stated that the most urgent fisheries management tasks are (1) combating IUU, and (2) reducing the widespread under-reporting of catches by licensed fleets.

A principal driver of IUU is the global overcapacity of developed country fishing fleets, mainly as a result of subsidies (Sumaila 2001). Earlier worldwide estimates of US\$ 10–20 billion for the value of IUU (World Bank 1998) were recently re-estimated at US\$ 30–34 (Sumaila and Pauly 2006). That is equivalent to about one-third of global fisheries sector revenues.

By 2010 it is estimated that the developed countries will import 10 million tons of fish (Delgado *et al.* 2003). On the other side of the equation this means that poor developing countries with food deficits and undernourished populations must seek to supply rich developed countries under both increasingly stringent access conditions and without depleting their fish stocks, and thereby compromising domestic food supplies. In other words, developing countries must try to earn the much needed foreign exchange without further impoverishing their own people's food supply; an almost impossible task when the scenario is unfolding within the massive constraint that further expansion in global marine capture fisheries is unlikely.

A closely related and inseparable part of the global industrialization process is that the potential contribution of fisheries to society and development is now severely constrained by the existing and likely future realities of the global environmental condition and the state of fisheries resources. Although now largely obscured in the public consciousness by the mass media emphasis on global warming and related environmental catastrophes, nevertheless crises in fisheries have been well documented (e.g., FAO 1995, 1998, 2000;

Myers and Worm 2003). Around the world, marine capture fisheries are in a critical condition, and probably have now exceeded their capacity to fulfill the demand for fish. This is exacerbated because fishing activities, particularly industrial fisheries, have major negative impacts on the marine ecosystems (Watling and Norse 1998). That, in turn, can eventually be expected to have negative repercussions for societies in developing countries.

3.2. The context of unsuitable approaches and models

Small-scale, nearshore marine fisheries, together with most inland fisheries, are best understood as an integral part of domestic rural development (Ruddle 2006). As such, an emphasis placed solely on nearshore small-scale fisheries and their management as a distinct sector is clearly misplaced; fisheries cannot be managed independently of other resource uses and their environmental impacts (Ruddle and Hickey 2008). During the last 60 years, when rural development was led by agricultural policies, it was amply demonstrated that reducing poverty had little to do with resource management *per se*. Rather, poverty reduction is intimately associated with access to resources and the alternative employment opportunities provided by widened rural economic bases, which occur as an integral part of an expanded national economy (World Bank 2000; Dixon *et al.* 2001).

Industrial fisheries have been the long-standing policy emphasis of all international agencies and most national fisheries services. In contrast, small-scale fisheries, particularly those in tropical developing countries, are still not well understood, despite increased study since the mid-1970s. Policy, administration and management have usually been based on sectoral development programs focused on making small-scale fisheries more efficient economically while conserv-

ing fish stocks. Further to their detriment is that during the last 60 years small-scale fisheries have not usually been included within comprehensive rural development programs, when activities focused on small farms and related infrastructure and institutions (Ruddle and Hickey 2008).

However, a fundamental and largely unacknowledged reason for management failure in tropical small-scale fisheries is the implementation of policies and programs based on Western developed country models and approaches, coupled with an inability and/or unwillingness to consider non-Western alternatives of empirically proven value, such as those epitomized by many pre-existing management systems in the Asia-Pacific Region, and elsewhere (Ruddle 2007a). This inability or unwillingness is embedded in the behavior of many donors and development agencies, and is manifested in a relative lack of understanding of tropical milieux and a persistence of various prejudices, in particular a temperate bias in conventional approaches to fisheries education and management (Johannes 1994; Pauly 1994; Ruddle 2007a). Further, there is an extremely negative connotation to the term "tropics" among fisheries scientists based in temperate latitudes (Pauly 1994). Not surprisingly, scientists commonly fail to appreciate differences between the temperate zone industrial fisheries with which they are familiar from their own training and research experience, and tropical small-scale fisheries (Johannes 1981, 1994; Pauly 1994; Ruddle 2007a, 2007b; Ruddle and Hickey 2008). As a consequence, during education, via the literature and through consultation, erroneous interpretations are passed to those who fund and make development policies and design development programs (Ruddle 2007b; Ruddle and Hickey 2008). Nor has the situation been helped by fragmentation and insularity within fisheries and allied sciences (Pontecorvo 2003).

4. Reconsidering Contributions

In addition to major reforms in the management and exploitation of both industrial and small-scale fisheries, realizing the potential contribution of fisheries to both societies and the MDGs requires that national governments also have some vision of the potential. However, it is not easy to be optimistic that such a realization is widespread, based on an examination of the Poverty Reduction Strategy Papers (PRSPs), organized by the IMF and produced by national governments to orient comprehensive national policy, planning and investment.

Only exceptionally do the national PRSPs identify fisheries as even a rather narrow investment sector with some potential contribution to attaining the MDGs. Most, such as that of Cameroon, for example, give no indication of plans for including fisheries within the MDGs of the nation (IMF 2006a). Further, in some cases where a narrow sector is identified, as in Nicaragua, for example, “prioritized strategic guidelines” for fisheries development under MDGs seem to concentrate on activities that would benefit just a small and already comparatively well-off minority of the population. In Nicaragua, fisheries development proposed to meet MDGs is focused narrowly on improving “shrimp farming” “...via a better regulatory framework and improved electricity and water services, and with government aid to lower private sector production costs through research, the dissemination and adoption of better practices for larvae quality, growth diet, pond water quality and the implementation of good crop management practices” [*sic*] (IMF 2005a:38).

However, some countries, among them Bangladesh, Cambodia and Guinea, for example, appreciate the need for wider approaches. The PRSP of Bangladesh, where fisheries provide an estimated 6% of the GDP and employ 10% of the total labor force

(IMF 2005b), specifies a mix of both detailed technological approaches combined with strategies for tackling underlying social issues of land ownership for fishing communities on floodplains (IMF 2005b). Similarly, in Cambodia, where crucially important fisheries provided an estimated 9% of the GDP in 2005, the goal is to ensure sustainable access to fisheries resources for the poor, and empower communities to participate directly in fisheries planning and management (IMF 2006b).

The PRSP of the West African nation of Guinea places fisheries development squarely within the context of rural development (IMF 2006c:40): “Since over 80 percent of the poor are living in rural areas in the interior of the country or on the coast, any investment in the rural economy can have a major impact on poverty by creating jobs and increasing incomes.” Were major constraints removed, it is claimed that fisheries development could improve food security, increase per capita fish consumption from 13 to 17 kg/yr by 2010, and play a role in poverty reduction (IMF 2006c).

5. Reconsidering the MDGs

Prior to discussing the potential contribution of fisheries to fulfilling the MDGs, a reconsideration of the eight MDGs is important, because their present order is misleading. Were there only two main goals—(1) environmental sustainability and (2) development of a global partnership—underlying issues would be better emphasized. All MDGs depend on these two critical goals, without which none of the others now listed as 1–6 could be achieved. These two goals need to be at the top of the agenda, and not in the lowest positions, as at present.

Although the goals as stated at present recognize that human well-being depends basically on environmental sustainability, as is explicitly covered by MDG 7, a greater emphasis is required. Further, MDG 7 needs

urgent re-consideration because it is the only goal for which the global situation is worsening (WWF 2007). That puts all the others at risk. This is discussed briefly below with reference to the use of pre-existing models of tropical resource management for both managing fisheries and implementing "The Ecosystem Approach" to attain MDG 7.

Since nothing will be achieved toward any of the MDGs unless there is a meaningful partnership between the developed and the developing countries, a much greater emphasis must also be placed on MDG 8. This is discussed below, with particular reference to the globalization of fisheries.

5.1. Nearshore tropical fisheries and MDG 7, ensuring environmental sustainability

Although much environmental damage in the marine environment can be pinned on the global reach of industrial fisheries, in contrast a reconsideration of the environmental context of some tropical small-scale fisheries may provide concepts for reconfiguring aspects of MDG 7. Policy solutions to global problems must be complemented by local actions, for which concrete plans can be drawn up and implemented, and local benefits quickly appreciated. Such local level efforts, especially for small-scale tropical fisheries, require a different level of understanding and skills than are demanded at the policy level. Local efforts also demand a reconsideration of the approaches and models designed by Western scientists, practitioners and donors, including the now popular community-based and co-management models in fisheries, which were designed to overcome the weaknesses of centralized governance (Ruddle and Hickey 2008). An unbiased evaluation of pre-existing and alternative models for resources management in nearshore marine environments and linked ecosystems is also required (Ruddle and Hickey 2008).

5.1.1. Pre-existing alternative models of fisheries management

An enormous gap separates the concepts that underlie many pre-existing marine resource management systems from the predominant Western thought on the subject. Whereas Western models of fisheries management focus on fish stocks and stock externalities and assume an open access resource regime, pre-existing systems in many tropical regions, as has been well-documented in the Pacific Islands, for example, take a different approach (Ruddle 1994a, 2007b). In pre-existing systems management is based on the three interrelated factors of stock externalities, gear externalities and allocation problems, and implementation is based on defined geographical areas to which access is controlled (Ruddle 1994a, 2007b).

The core difference, which has generally never been widely appreciated (Ruddle 1994a, 2007; Ruddle and Hickey 2008), is that Western fisheries management has focused on modelling the biological and physical flow of fish resources onto and through fishing grounds, and, in implementation, on attempting to manage the resultant stock externalities. In other words, it focuses on trying to manage what is unknown, and perhaps inherently unknowable, and thus unmanageable (cf. Larkin 1977, 1978; Ruddle 1994a, 2007b). In striking contrast, pre-existing Pacific Island management systems, for example, make no such attempt. Rather, they focus on the interaction among "stock externalities," "technological externalities" and "allocation problems," human problems which are inherently manageable, although still not without their particular complexities, depending on economic, political, social and cultural conditions and changes within them (Ruddle 1994b). This implicitly accounts for the complex multi-species and multi-gear nature of the resource, thereby avoiding inherently irresolvable issues.

5.1.2. The pre-existing tropical resource estate and “The Ecosystem Approach”

In many tropical regions there exists the concept of a “resource estate”, a territory held jointly by a kinship-based group (Ruddle 1994a). These are particularly evident on high islands in the Pacific, where such “resource estates” usually extend (or extended) in a wedge-shape from a central watershed along lateral ridges into inshore marine waters, and formed self-contained units with the complete set of the resource areas and habitats required to provision local communities (Ruddle 1994a). Until the advent of monocultural agriculture and plantation systems, rural economies in the tropics were never about just fishing or farming. Rather, a non-specialized approach that included farming, fishing and exchange systems was traditionally adopted to spread risk and provide a balance of subsistence goods in uncertain environments with limited resources (Ruddle 1991, 2006).

A further important attribute of the “estate concept” of management is that it brings fishing communities into an intimate relationship with upstream and *in situ* users of other resources (e.g., farmers and foresters), and with the impacts that those resource users have on the aquatic environments on which nearshore fisheries depend (e.g., coastal turbidity levels) (Ruddle 2006). This attribute is important because resource enhancement and habitat protection are two inter-related management functions; stock enhancement being pointless if the habitat(s) on which it depends cannot simultaneously be protected. The attribute is also important because it can be applied locally in support of MDG 7, “Ensuring Environmental Sustainability.”

It is noteworthy that some of the concepts manifested in such pre-existing resource management systems, which can also be regarded as alternative ecosystem ap-

proaches, have recently been re-packaged by Western donors and academics as “The Ecosystem Approach,” but without recognizing the practical examples of the tropical antecedents (Ruddle and Hickey 2008). The Ecosystem Approach to Fisheries (EAF), for example, is basically an extension of the conventional principles for sustainable fisheries development and management to deal explicitly with such ecosystem issues as resources conservation, habitat protection, and fishery and non-fishery impacts, among other things (Garcia *et al.* 2003). However, it is far less comprehensive in formulation than the concepts embodied in pre-existing tropical resource estates (Ruddle and Hickey 2008).

Programs to attain MDG 7 that involve fishing communities would have to adopt an ecosystems approach, whether it be EAF directly, or a more sophisticated variant developed from the concepts of pre-existing systems. Doing so would constitute a major paradigm shift that would demand a fundamental change of the Western-style fisheries management institutions. It would require the phasing out of sectoral policies for the marine environment and resources. Fisheries management would then become part of a broader ecosystem management strategy, with new and longer time horizons.

5.2. Present MDG 8: The globalized fish trade and industrial fisheries

Finding solutions to problems that have arisen for both developing and developed countries from the now globalized fish trade and industrial fisheries that sustain it is central to the scope of MDG 8, i.e., to establish a global partnership for development between the developed countries and developing countries (Table 1). Developed countries are a principal driver of natural resource loss and climate change, through their production and consumption patterns (WWF 2006).

Therefore developed countries, and particularly the EU, Japan and the USA, as the world's largest seafood markets and importers, together with China, now a major importer, must coordinate efforts among themselves and in concert with the developing countries, to ensure: (1) that their agents (corporations, businesses and governments) cease exploitative activities; (2) that international trade and investment structures do not prey on weak governance; (3) that they assist in developing the capacity and resources to implement the international agreements to which they are party; (4) that they take action to reduce the negative impacts of their fleet subsidies; and (5) that they ensure their markets do not launder illegal catches. Realistically, these tasks must be shared between the developed and the developing countries, as envisaged in MDG 8, if anything concrete is to be achieved.

For example, developed countries should consider assisting developing country governments to take military policing and enforcement actions for their fisheries. In the worst cases that might have to occur within a broader framework, whereby general security and then comprehensive governance are re-established as pre-requisites to securing marine waters. A test case could well be in East Africa, off Kenya, Somalia and Tanzania, where rewarding fisheries attract heavily armed illegal foreign fishers. Off Somalia, for example, unmarked foreign boats have been poaching for many years; "Once, they [local fishermen] could scare them off with a shout and a rocket-propelled grenade-launcher. These days, the fishermen say, the unmarked vessels carry 23 mm anti-aircraft guns" (Anon 2006:44). The problems of IUU are compounded in failed states, where foreign vessels are captured and massively "fined" by local "coastal patrols"; "Some longliners used to buy fishing licenses in the past, but invariably from the wrong warlord" (*ibid.*). Clearly, under such conditions, something more than negotiated deals

to buy a share of fishing rights are required to regulate the industry!

The complex issues raised by the globalization of the fish trade have major implications for the redesign of national fisheries administration. International fisheries relationships and general affairs is now a highly important aspect of the work of many fisheries departments, regardless of their perceived status within a nation's administrative framework. Such work is usually highly political in nature, and generally beyond the competence of fisheries technical specialists, since it involves diplomacy, international law and treaties, and financing. As such, it is best left to ministries of foreign affairs, to which deep and distant water fisheries personnel should be re-assigned as technical specialists.

6. Conclusions

Fisheries do make a contribution to employment, income and nutrition, but, in the absence of rigorous study and more precise databases, nothing useful can be said beyond the usual unsubstantiated assertions. In order for them to make a contribution to both societies in developing countries and the MDGs, two main issues must be addressed.

The first is that the globalized fish trade which has emerged over the last three decades, together with the often acrimonious relationships that have resulted between developed and developing countries, must be addressed as a central issue. In particular, it must be accepted that serious impediments and inequities imposed on poor developing countries by an international fish trade that is controlled by terms dictated largely by the richer developed countries, and that the worsening condition of the global environment and the parlous state of resources, which also have serious impacts on fisheries resources, are in large part the result of overconsumption in developed countries. However, assigning blame is counter-productive and

detracts from the urgency of the need to achieve a meaningful and equitable partnership between the developed and the developing countries, which will focus on solving the problems within the framework of MDG 8.

The second main issue is that for small-scale, nearshore tropical fisheries to make greater contributions to societies and the MDGs in developing countries, unsuitable management systems, introduced mostly as a result of their imposition through inappropriate fisheries education and aid conditionalities, should be replaced with those that are more appropriate to the task of fisheries management, within the broader

framework of rural development. Greater contributions to society can be attained simultaneously with contributions to MDG 7, provided that the inappropriate models can be replaced with pre-existing alternative models long used by tropical societies, many of which are (or were) also used simultaneously to manage linked inshore marine and terrestrial ecosystems.

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Advantages and Disadvantages of the Fisheries Trade

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The spirit of trade is mutual benefits among all participants. However, current international trade is just like a zero-sum solution with winners and losers and developing a feeling of distrust among participants. Responsible international fisheries trade is an important tool to promote economic growth of many countries and the World Trade Organization (WTO) has been encouraging free trade as a part of globalization. Although free trade is ideal when the world is fair and equitable, current international trade in favor of capital has diverse effects and has both positive and negative aspects, but is not fair and equitable between participants in both producing and consuming countries. Profit-seeking capital behavior is different from community behavior requiring sustainability and the role of government is to balance these two extremes, continually maintaining vital communities. However, many governments including official development assistance (ODA) have been in favor of capital too much to sustain rural fishing communities and developing countries have been repeating the same problems as developed countries have experienced. This paper reviews cases of the most important fisheries trade commodities: shrimp, tuna and salmon. All cases indicate the existence of negative aspects of the fisheries trade. Governments as trustees of people are expected to remove or reduce these negative aspects of international trade and to think of the importance of the community.

KEYWORDS fisheries trade; shrimp trade; tuna trade; salmon trade; Japan's fish trade

1. Introduction

With development of the 3rd United Nations Law of the Sea Conference together with US dollar devaluation, increase in oil prices, and free trade movement in the 1970s, many

coastal countries took fisheries expansion policies for exports emphasizing foreign exchange earnings, increases of employment opportunities, improvement of sanitation/food safety/transparency of management/fishers' income and living standard (Matsuda 1984, 1987, 1992, 1995).

These policies were also supported by developed countries and further facilitated by the Plaza Agreement in 1985 which promoted a drastic appreciation of the yen. In many coastal countries, fishing fee incomes and fisheries joint-ventures were increased. This resulted in increases in conflicts between traditional coastal fisheries and new fisheries. At the same time, distant-water fishing suffered from it due to increases in cost associated with labor and fuel in particular. In the 1980s, environmental problems became obvious due to economic growth policies in many coastal countries. These fisheries production policies also faced over-investment, over-fishing and resource depletion problems (FAO 1992). Legal frameworks of international regulations were strengthened in the 1990s, and now fisheries are in transition (Willman 2006). FAO's Code of Conduct for Responsible Fisheries concerns all aspects in fisheries management, fishing operation, aquaculture, integration of fisheries into coastal area management, post-harvest practices, trade and fisheries research. Although the Code is voluntary and global in scope, certain parts of it are based on relevant rules of international law, including the UNCLOS and contains provisions that may be or have already been given binding effect by means of other obligatory legal instruments amongst the parties such as the Agreement to Promote Compliance with International Conservation and Management Measures by Fishing Vessels on the High Seas, 1993 (FAO 1995).

In 2005, world fisheries imports accounted for US\$ 81,529 million while exports accounted for US\$ 78,419 million. The fisheries trade is very important for developing countries in terms of exports while it accounts for a considerable proportion of imports in developed countries. The top 15 exporting countries include China (US\$ 7,519 million), followed by Norway, Thailand, the USA, Denmark, Canada, Chile, the Netherlands, Vietnam, Spain, Russian Federation,

the UK, Indonesia, Iceland and Taiwan. On the other hand, the top 15 importing countries include Japan with import values of US\$ 14,436 million, followed by the USA, Spain, France, Italy, China, Germany, the UK, Denmark, South Korea, the Netherlands, Hong Kong, Canada, Belgium and Sweden. The shares of these top 15 countries in total fish trade values in the world are US\$ 48,567 million or 61.9% for exports against US\$ 65,007 million or 79.7% for imports (FAO 2007). Thus, exports are more diversified as compared to imports concentrated on Japan, the USA and EU countries. China has become a new market for fisheries commodities. Traditional fish trades within a region have also been accelerated by free trade agreements (FTA) (Yamao 2006). Shrimp, tuna and salmon are still the major species in the fisheries trade. This paper focuses on these species.

Joint-venture is an ideal form of capital-intensive fisheries development in developing countries where the developed countries provide the capital and technology at the beginning while the developing countries provide the working environment and labor (Martin *et al.* 1981). These fisheries joint-ventures have increased rapidly since the 1970s. However, many failed within 10 to 20 years. This is because of distrust among partners in terms of joint-venture business management, difficulty in technology transfer pertaining to preparation, education, production, processing, marketing and manageability as well as hastened nationalization. These create joint-ventures high risk low return businesses (Matsuda and Ouchi 1984).

International trade has affected both fishing and aquaculture. In particular, capital intensive activities have been promoted by many countries. As a result, fisheries management and environmental and/or community problems have become serious. Regional tuna fisheries management organizations such as ICCAT suffer from false reporting and data collection problems from unreported

fishermen catching tuna (ICCAT 1996). FAO has tried to promote responsible fisheries worldwide since 1995 (FAO 1995). On the other hand, the Organization for the Promotion of Responsible Tuna Fisheries, an NGO, was established in 2000 and promoted the removal of IUU (Illegal, Unregulated and Unreported) vessels, capacity reduction of over-fishing vessels, promotion of responsible tuna aquaculture and reduction of by-catches and/or incidental catches. IUU/FOC (Flag of Convenience) vessels are pirate fishing vessels that operate and overexploit tuna resources, disregarding international rules for the conservation and management of the resources. With the introduction of the Positive List system, international trading of tuna caught by IUU/FOC vessels has been prohibited since November 2003 (OPRT 2008). The Marine Stewardship Council (MSC), established in 1997 (MSC 2008), is another international NGO which promotes responsible fisheries. The MSC recognizes, via a certificate program, well-managed fisheries and harmless consumer preference for seafood products bearing the MSC label of approval. Characteristics of these NGOs are strong concerns about whole situations from production to processing, marketing and consumption, rather than a part such as production.

2. Shrimp

Shrimp exports have been particularly important as a source of foreign exchange earnings, income generation and employment in developing countries such as Vietnam, Indonesia, India, Thailand, China, Burma, the Philippines, Bangladesh, Columbia, Ecuador, Honduras, Nicaragua and Mexico. World shrimp production in 2005 was 6,230,000 tonnes consisting of 3,650,000 tonnes from capture fisheries and 2,680,000 tonnes from aquaculture. Major shrimp exporting countries include Thailand with US\$ 1.2 trillion in 2001, followed by Indo-

nesia, India, Vietnam, Mexico, Argentina, Ecuador, China and Bangladesh while major importing countries include the USA with US\$ 3 trillion in 2001, followed by Japan, Spain, France Canada, Italy, the UK, the Netherlands, Belgium and Thailand (FAO 2007). Thailand imports shrimp for processing, then exports the final products to developed countries. Thus, shrimp has been a typical trade item produced in the south and exported to the north with the assistance from developed countries resulting in capital and technology transfer (FAO 2007).

The major investment for shrimp capture fisheries is the requirement for capital intensive shrimp trawl vessels. Since the 1970s, many coastal countries have promoted such industrial fisheries in ways of joint-venture, state enterprise, private enterprise or foreign fishing with fishing fee. This policy in developing countries attracted official development assistance (ODA) as well as private development assistance from developed countries. These arrangements contributed to coastal developing countries in terms of foreign exchange earnings, technology transfer, increases in employment opportunities for local people, income generation for people concerned and fostering domestic shrimp fisheries (FAO Regional Office for Asia and Pacific 2008).

However, this industry also created problems such as over-fishing and resource depletion, increasing by-catches, incidental mortality of sensitive species such as turtle, illegal fishing, encroachment of coastal fisheries resulting in conflicts with coastal fisheries, development of income disparity among fishermen and high risk. Thai trawl fisheries depleted most of the shrimp resources in the Gulf of Thailand during the 1960s. In Indonesia, trawl fisheries were banned at the beginning of 1980 except for improved trawl nets such as turtle excluding devices (Indonesian Gov. 1980). This is attributable to increasing by-catches, resource depletion and conflicts with local fishermen.

However, these improved gears were not practical. In the Philippines, the conflicts between shrimp trawlers and coastal fishers have continued. The situation is complicated. Coastal fishermen in San Miguel Bay have suffered from resource depletion due to the encroachment of industrial shrimp trawlers into the coastal fishing grounds. However, shrimp trawl vessel owners are often bosses in the villages and the young sons of coastal fishermen are employed by such industrial fisheries. Although fisheries officers recognize the problem, they never help small-scale coastal fishermen because they are afraid of their bosses (Lim *et al.* 1995).

Aquaculture is another way to meet demands for shrimp. Artificial propagation technology of *Penaeus japonicus*, a temperate species of Kuruma shrimp, was developed by Gensaku Fijinaga in Japan in 1964 and this technology was successfully applied by a Taiwanese scientist to *Penaeus monodon*, a tropical species of the Kuruma shrimp, in 1968. This was a breakthrough of shrimp culture in the world. In the 1970s, shrimp culture was paid much attention by many developing countries such as Ecuador, Indonesia, India, Vietnam and Taiwan. In the Philippines, the Southeast Asian Fisheries Development Center Aquaculture Department was established and emphasized shrimp production technology. This was a preparation period for the shrimp culture industry in the world. Shrimp culture for export purposes has grown rapidly since the 1980s.

Current shrimp culture includes more than 17 species, but are dominated by two major species: *Penaeus monodon* (Giant Tiger Prawn) in the Eastern hemisphere and *Penaeus vannamei* (Whiteleg shrimp) in the Western hemisphere. By 2000, cultured shrimp production exceeded 1.1 million tonnes as compared to 2.7 million tonnes in 2005. World production has been increased, but rise and fall fluctuations of this industry have been well documented due to the outbreak of disease problems caused by virus

diseases including the so-called white spot disease. Production dropped in Taiwan from 16,715 tonnes in 1985 to 2,459 tonnes in 2001 due to the outbreak of *Monodon Baculovirus* (MBV) in 1988; in the Philippines from 88,850 tonnes in 1995 to 36,859 tonnes in 1998; and in Ecuador from 129,600 tonnes in 1998 to 50,110 tonnes in 2000 due to the Taura Syndrome virus (TSV) in 1998. More than 20 virus diseases have been identified by now and caused high risk for the industry. Indonesia, Thailand, India, Vietnam and Mexico are also suffering (Fukano 2004). Thus, capital-intensive shrimp culture is a typical high-risk high-return farming, though shrimp culture in Vietnam and China has recently been growing rapidly.

The disease problem is also associated with problems of mangrove destruction, drug use and water pollution. During 1980 through 2005, 3.6 million hectares of mangrove were destroyed in the world. This is equivalent to 20% of the mangrove areas in 1980, though the speed of this destruction has slowed down from 185,000 hectares per year in the 1980s to 105,000 hectares per year after 2000 (Worldwatch Institute 2006). Some of these mangroves were destroyed for the development of shrimp culture ponds though a large portion of mangrove destruction is related to logging and charcoal production, local self-consumption as well as the Vietnam war before 1980. Due to the disease problem, 150,000 hectares of shrimp ponds converted from mangrove were abandoned in Asia from 1985 to 1995. Some of these abandoned ponds are currently used for extensive or semi-intensive polyculture of shrimp with milkfish, tilapia, rabbit fish, mud crabs, seaweeds and mangroves, as well as for mangrove rehabilitation projects in various countries such as Ecuador, Thailand, Indonesia, and the Philippines.

Apart from the disease problem, capital-intensive export-oriented shrimp culture has been criticized in many ways (Taya 2003). These include the following:

- 1) Government-supported industrial capital-intensive shrimp culture has worked at a cost to the local community. These include mangrove destruction resulting in land acquisition with extremely low prices for local residents, no trespassing, depletion of fisheries resources, flooding, increases of natural hazards such as damages from storms, high tide and earthquakes, and destruction of self-sufficiency;
- 2) Water and soil pollution due to heavy feeding, disease and drug use, resulting in abandonment of those ponds in Taiwan, the Philippines, Indonesia, Thailand, Ecuador;
- 3) Little contribution to the local economy since the industry's interest is in her own profits and not the benefits of the community;
- 4) Irreversible conversion of rice paddies to brackish water shrimp ponds in Vietnam and China in particular;
- 5) Hike of domestic shrimp prices. Shrimps used to be a common seafood for local people at reasonable prices. However, the price for exports has been set much higher than the domestic one. As a result, the availability of shrimps domestically has decreased and the price has increased.

Some of the abandoned shrimp ponds have been used by local residents for extensive or semi-intensive polyculture in the Philippines. However, people are very interested in shrimp culture because the average price is seven times higher than milkfish, though mortality rates are more than 90% due to the disease problem. The current marketing system is unable to separate healthy shrimps from those contaminated by virus and others.

In Vietnam, shrimp culture is a very diverse industry. Small-scale farms occupy 90% with an extensive production of 200 kg/ha as compared to an intensive production of 10–15 tonnes/ha and there are 439 process-

ing plants of which 300 are accredited by the HACCP for export. However, they have the following problems (Muroya 2006):

- 1) Difficulty of collecting both brooders and exportable shrimp: Availability of hatchery technology is limited and most farmers are small-scale so that each purchase is a small lot of several kilograms. Further, traceability is very difficult due to a mixture of shrimp from many farmers including all categories of farming such as extensive, semi-intensive and intensive culture;
- 2) Insufficiency of feed and drug management even among capital-intensive farms;
- 3) Insufficiency of understanding about water management, food safety and sanitation;
- 4) Lack of appropriate infrastructures such as electricity and roads;
- 5) Low investment capability;
- 6) Lack of technology and information concerned.

Lobster is an important export item in Nicaragua. Accordingly, diving fisheries for lobster are popular among natives (Masito). However, fishing grounds have been changing to deeper zones from 30 feet in 1988 to 120 feet in 1998. As a result, the occurrence of decompression sickness increased among divers. Some became physically handicapped and some lost their lives. To prevent such cases, there are many laws, rules and regulations, but there is no enforcement. The government is in favor of the industry but appears to have less concern about local residents (Acosta 2005).

3. Tuna

In the 1970s, many coastal countries introduced fisheries expansion policies to increase foreign exchange earnings (Matsuda, 1986, 1987). Although tuna had been regarded as a highly migratory species in the UNCLOS III, many coastal countries, in

particular South Pacific island countries, declared that those within their Exclusive Economic Zone (EEZ) belonged to the coastal countries. The Forum Fishery Agency established in 1979, took a leading role in regional fisheries management in the South Pacific, based on the Nauru Agreement in 1982. This was materialized after a multilateral agreement between the USA and 16 Pacific island countries in 1987 (Matsuda 1992, 1995). Their management concept is regarded as enforcement without force (Moore 1987). Most tuna caught in the waters were shipped to the tuna markets in Japan, the USA and EU, since local people did not customarily eat large tuna except small skipjack tuna.

Many countries developed tuna fisheries joint-ventures for export purposes in vein mainly due to distrust in business management between partners and hasty nationalization, and some resulted in ethnic violence such as in Solomon Islands in 1998. Solomon Taiyo in the Solomon Islands was regarded as a successful tuna fisheries joint-venture between the government of the Solomon Islands and the Taiyo Gyogyo Company in Japan, lasting from 1971 to 2000. The company was involved in fishing, processing and marketing. By 1984, the company exported Solomon\$ 29 million, 97% of the total fisheries export value in the Solomon Islands and equivalent to one-third of foreign exchange earnings, and employed about 1,000 people which is about 5% of total employment in the country (Matsuda 1986). Solomon Taiyo grew 1999, when it had an annual turnover of around US\$ 100 million, employed close to 3,000 Solomon Islanders on its fleet of more than 20 fishing boats, and had a large shore base with a canning factory. Barclay describes the neutral evaluation of Solomon Taiyo (Barclay 2007).

There are two extremes in the concept of fisheries management. One is complete government control, popular in Western countries (Rigg *et al.* 2003), and the other is

comanagement between government and fishermen, popular in Japan (Matsuda 2002, 2005) which has adopted by Organization for the Promotion of Responsible Tuna Fisheries (OPRT) and Marine Stewardship Council (MSC). Comanagement could also be facilitated by decentralization policies (Fernandes *et al.* 2000; Satria and Matsuda 2004; Kooiman *et al.* 2005; McClannahan and Castilla 2007). Under government control, fishermen are regarded as persons under surveillance while they are regarded as partners with the government under the comanagement approach. Assuming uncertainty in fisheries, monitoring, control and surveillance (MCS) costs without the understanding of the fishermen will be very high. Activities are limited by budgets or by the amount of benefits from the fisheries. The MCS cost could be reduced drastically under the comanagement approach.

Narita International Airport in Japan is also known as Narita fishing port which receives both fresh, frozen and processed fish. However, all cultured tuna are from a limited number of countries such as Australia, Spain, Malta, Turkey, Croatia, Italy, Tunisia, Cyprus, Greece and Libya. These fish used to be fresh for Sashimi market through fish wholesale markets in Japan, but nearly half of them are not handled through fish wholesale markets today (Yamamoto 2006). On the other hand, the National Federation of Tuna and Skipjack Fisheries Cooperative (NIKKATSUREN), the most active tuna fisheries cooperative federation in Japan, was forced to close in 2004 due to financial problems. A positive list system was introduced in the bluefin tuna trade by ICCAT in 2004. This is because production of tuna aquaculture in the Mediterranean Sea increased from 14,000 tonnes in 1994 to 21,000 tonnes in 2003. Under-sized juvenile fish are also used for this aquaculture and competition between capture fisheries and aquaculture has increased.

4. Salmon

Both natural and cultured salmon production has been increasing since 1975. In particular, capital-intensive cultured salmon produced by agri-businesses in the world increased rapidly from 17,000 tonnes in 1980 to 200,000 tonnes in 1990. This is attributable to increased production in Norway. During this period, demands for salmon changed from salted salmon to frozen salmon in Japan and canned salmon to frozen salmon in the United States and EU markets. Major producing countries are the United States, Japan, Russia, Canada for natural salmon: Pink salmon (*Oncorlynchus gorbuscha*), Sockeye salmon (*Oncorlynchus nelka*), Chum salmon (*Oncorlynchus keta*), Silver salmon (*Oncorlynchus kisutch*), and Chinook salmon (*Oncorlynchus tshawytscha*), while Norway, Chile, the UK, Canada, Feroe Islands, Finland, Ireland, the USA, Japan, Australia, Denmark, Sweden, New Zealand and Iceland produce cultured salmon: Atlantic salmon, trout, silver salmon, Chinook salmon. Since 1997, cultured salmon production has exceeded that of natural salmon. These cultured salmon are basically for export and not for domestic consumption (Sano 2003).

Alaska in the United States, Norway and Chile are the major salmon exporters in the world although Alaska is based on natural salmon production while Norway and Chile dominate cultured salmon production. Alaska is the major natural salmon producing area in the United States. The salmon industry provides one-sixth of all employment opportunities there. Major species are Sockeye salmon and Pink salmon in Bristol Bay and southwest Alaska caught by seine (Silver salmon), gillnet in Bristol Bay and purse seine in southwest Alaska. The market is Japan. They face a very short fishing season, marketing problems, resource management problems, low prices and over-investment.

In Norway, fisheries products are an important export item, second to oil and occupy 7% of foreign exchange earnings of the country. Most of them are exported to EU countries. The salmon culture industry contributes 3% of its exported value to the Fish Export Council and salmon export is regarded as a national interest and the government fully supports the industry. In 1979, the government enacted an aquaculture law and started a cooperative experiment with the Nichiro Gyogyo Company of Japan. In 1989, the government liberalized aquaculture capital. In 1991, it adopted an individual transferable licensing system and targeted a production system with a feed conversion ratio of 1.3:1, concentration on export up to 98% and introduction of a new technological system including breeding, prevention of epidemics with vaccine, feed quota system, computer-based management, waste treatment, fresh product production for EU countries, HACCP (Hazard Analysis Critical Control Point), mechanization, year-round marketing, and agri-business oriented management. In 2004, they produced 1 million tonnes of cultured salmon. Weaknesses of Norwegian salmon culture are as follows (Sano 2003; WWF 2004):

- 1) Oligopoly: More than 800 management units in 1992 were reduced to 270 in 1999 and the top six groups produce three-quarters of the total production, resulting in price-war situations;
- 2) No domestic market for cultured salmon, resulting in high risk;
- 3) Rising of the license price due to limited numbers of licenses;
- 4) Increasing international competition, lowering profit margins;
- 5) High-risk high-return to high-risk low-return industry;
- 6) Escaped farmed salmon resulting in a creation of genetic disturbance;
- 7) Depletion of wild Atlantic salmon by 80% between 1970 to the end of the 20th century in the North Atlantic Ocean. A

reason for this is suspected to be attributable to the salmon culture.

In Chile, there are many suitable places for salmon culture and high availability of low price feeds. With Japan's cooperation, a salmon culture experiment was started in 1980. At the same time, they introduced aquaculture technology from Norway. After trial and error, they adopted the capital-intensive Norwegian aquaculture method. The license is issued by the military government. A distance between two adjacent farms is restricted to more than 1.5 km. Enlargement of farm size is promoted and optimization of the production scale from 800 to 18,000 tonnes per year. As a result, the top ten companies produced half of the total cultured salmon in 1998. Further, a value addition for market differentiation was promoted for Atlantic salmon to the EU market and Silver salmon and trout for Japan. In 2004, they produced 440,000 tonnes of cultured salmon.

Although Chilean salmon culture learned a lot from its Norwegian counterpart, several problems have been identified (Sakuma 2007; Takahashi 2007; Hayashi 2007):

- 1) Use of wild caught fish for fish-feed with a low feed conversion ratio: 8.6 kg of wild caught sardine, anchovy, and horse mackerel are used as feeds to produce 1kg of cultured salmon;
- 2) Pollution: Coastal capture fisheries have become non-existence in 10 provinces where salmon culture is very popular;
- 3) Use of pesticides and antibiotics: There is no inspection on this matter;
- 4) Defective legal system resulting in victimization of health and the lives of laborers;
- 5) Weak enforcement capability;
- 6) Widening of income disparity;
- 7) Increasing abnormal cultured salmon due to feeding of heads and bones of cultured salmon.

5. Issues in Imported Countries: A Case of Japan

Japan experienced a rise and fall in fisheries after WWII. It used to be an exporting country of fisheries commodities. However, the situation changed in 1975 and it became an importing country, resulting in it becoming the top importer in the 1980s. In 1997, Japan imported 1.9 billion yen (3.4 million mt), next to oil imports of about 5 billion yen. This is the result of dollar devaluation beginning with the Nixon shock in 1971, GATT Tokyo round 1973–1979 reducing tariffs and treating fisheries commodities as non-agricultural commodities, just like automobiles and televisions, the development of the Third Law of the Sea Conference, export expansion policy for textile, automobile and electric commodities, and life-style changes. As a result, her self-sufficiency rate of fisheries commodities decreased from 100% in 1975 to 57% in 2005, even though her comparative advantages as a maritime country with 35,000 km of coastline, long history and experiences in fisheries, the sixth largest exclusive economic zone of 4.47 million km² including one of the three richest fishing grounds in the world, and both hard and soft fisheries infrastructures (Matsuda, 2006).

Fisheries production decreased from 12.8 million tonnes at its peak in 1984 to 5.8 million tonnes in 2005 in volume with a drop in value from 2.98 billion yen in 1982 to 1.60 billion in 2004. The number of fishermen decreased from 1,990,453 in 1952 at independence to 231,000 in 2004 and the number of fishing vessel construction licenses decreased from 1,521 in 1963 to 26 in 2005. As a result, expected contributions of fisheries and fishing villages to society, such as renewable resources utilization, environmental protection, protection of lives and the assets of people (like rescue, surveillance and environmental monitoring), places for marine recreation and education, succession of

culture such as festivities and economic and employment opportunities in remote areas have been reducing. Japanese fisheries face the unprecedented crisis.

On the other hand, people have been urbanized, life styles have changed from slow food to fast food and the installation of home refrigerators since the 1960s. There has been technological developments in the handling of food in cold storages with temperatures of up to -65°C . Supermarkets became popular and frozen fish transactions outside central fish wholesale markets increased. Convenience stores and fast food sushi restaurants became popular in the 1980s and sushi became one of the children's favorites, just like *carry* and rice, noodles, hamburgers and Kentucky Fried Chicken. These supermarkets and fast food sushi restaurants use imported frozen fish because of stability in quality, volume and price. Consumers have preferred ready-to-cook or ready-to-eat fish to fresh wholesome fish. The restaurant business grew to 29.1 billion yen by 1997 at its peak, then decreased to 25.6 billion yen in 2002. Sushi shares about 5% in restaurants (Torii 2006). Westernization of food has resulted in malnutrition problems. Together with BSE (mad cow disease), bird influenza, swine cholera, poisoned imported food and camouflage of labels, these have encouraged people to change their eating habits toward domestic food consumption and to become more conscious about the quality of food, food safety and reliable food.

6. Conclusion

Free trade is desirable in the long term, but too early to practice without market failures. The spirit of trade is for the mutual benefit of participants which creates mutual trust. These participants today are all related parties in the trade including not only sellers and buyers but also people concerned in production, processing, marketing and communities. However, issues raised here are com-

mon rather than specific and current trade supported by governments in fact is in favor of profit-seeking capital and not in a sustainability seeking community. As a result, winners are created for capital and losers for the community in the name of competition. This is a market failure with high risks for a country, resulting in high social costs and not a responsible trade at all. Thus, the role of governments is the reallocation of capital-biased benefit to mutual benefit in optimal ways, though not losing incentives. However, this government role is not functioning in many countries. Developing countries have also been repeating the same problems as developed countries experienced in the past and missing the opportunity to take advantage of starting late.

Advantages for fish exporting countries include foreign exchange earnings, employment opportunities, education in sanitation/food safety/transparency of management, higher fishermen's incomes, resulting in an increase in living standards of fishers, though levels of achievement are biased towards foreign exchange earnings and business profits. Those of fish importing countries include an increase in availability of cheaper primary species, benefiting consumers and related processing and marketing people and companies such as supermarkets; and an expansion of export opportunities for exporting industries of other commodities such as cars and electrical goods.

Disadvantages for exporting countries include the creation of a dual structure in fisheries, taking over land at low prices by the government for industrial development, environmental destruction such as the destruction of mangrove resulting in resource depletion, increases of natural hazards and destruction of self-sufficiency; water and land pollution; no contribution to the village communities concerned; high risk of industrial fisheries development, transfer of rice paddies to irreversible shrimp ponds; disease and drug use problems, and rise of domestic

prices of related species. Those of importing countries include the destruction of fresh-fish marketing and consumption, promoting an energy-intensive mass consumption society just seeking convenience and the destruction of rural fishing communities which

make critical contributions to society.

Governments as trustees of people are expected to remove or reduce these negative aspects of international trade and to think of the importance of communities.

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Japan's Fisheries Cooperation: Principle, Programs and Achievements

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Fisheries resources are renewable but limited natural resources. Therefore, sustainability is the key word when discussing anything relating to fisheries development. Japan, as one of the major fishing and fish-eating countries in the world, has been cooperating to promote fisheries in developing countries, which includes fisheries grant-aid projects and technical cooperation through JICA and OFCF Japan. Japan is also playing an active role in international organizations such as FAO and RFMOs to ensure proper management of fisheries resources. Since fisheries resources are common property resources with no ownership until captured, adjustments to address the interests of all stakeholders are necessary whenever discussing the management of fisheries resources and promotion of fishing activities.

KEYWORDS fisheries cooperation; sustainability; fisheries management; grant aid; technical cooperation; JICA; OFCF Japan

1. Introduction

Demand for fish products has been increasing due to several factors such as rapid expansion of the human population, improved diet thanks to increased incomes, growing concern about food safety caused by BSE and bird-flu epidemics, and the consciousness of human health. However, it appears that there is a ceiling of the production of

marine capture fisheries, which has been stable at around 90 million tons since the late 1980s, or decreasing if China's catch is excluded. The World Bank* characterizes the challenges now facing the fisheries sector as follows: "Approximately 25% of the world's marine fish stocks are considered

*<http://web.worldbank.org/WBSITE/EXTERNAL/TOPICS/EXTARD/0,,contentMDK:20451222~pagePK:148956~piPK:216618~theSitePK:336682,00.html>

overexploited and an additional 50% are fully exploited. The depleted state of wild fish stocks is due to overfishing and increasing degradation of coastal, marine and freshwater ecosystems and habitats, as growing coastal populations exert increasing pressures on natural resources. Aquaculture production has grown at almost 9% per year since 1970 and aquaculture now provides 40% of the world's fish supply for direct human consumption, and brings new challenges to sustainable use of aquatic resources and environments."

At the same time, many fishers in developing countries are still living in marginal conditions, which justifies the need for continuation of international cooperation in the field of fisheries. The World Bank* analyzes the situation as follows: "The livelihoods of about 200 million people rely on fisheries, aquaculture and associated activities and over 20% of the world's 38 million full-time fishers earn less than US\$ 1 per day. Many fishers live in the world's poorest countries where their communities are often marginalized and landless. As fishing is often the livelihood of last resort and fish often the only source of animal protein for the poor, the state of the world's fisheries can be critical in the fight against poverty in many parts the developing world."

Japan, as a country surrounded by waters with abundant fisheries resources, has a long history of utilizing these resources for human consumption. Because of this long history, Japan has developed techniques relating not only to the production and utilization of fisheries resources, such as harvesting, aquaculture, processing and consumption but also to the enhancement of stocks and stock management. Japan's production decreased to 5 million tons in 2005 from 11 million tons in 1989, when it hit its peak.

However, Japan is still one of the major fishing and fish-eating countries in the world.

Further, Japan can be characterized as the country with a unique history of enjoying the utilization of fisheries resources for hundreds of years, which has resulted in establishing community-based resources management as an activity of fisher's cooperatives. Therefore, Japan has the advantage of international cooperation in this field, especially for promotion of artisanal/small-scale fisheries which could be well addressed through community-based approaches.

Many developing countries, especially coastal countries including small island developing states, have a desire to promote their own fisheries, and Japan is expected to play a major role in this field. Sustainable development is the key word for the future development of fisheries. Taking account of the fact that fisheries resources are common property, development and utilization of these must also consider the interests of all stakeholders.

Japan, as one of the leading countries in the fisheries field, both in production and consumption, has been engaging in various kinds of international cooperation. This paper summarizes the principle, programs and achievements of Japan's cooperation in this field and discusses the challenges we are facing and possible course of action for the future.

2. Principle

2.1. Sustainable utilization

Fisheries resources are renewable natural resources. Therefore, they can be utilized in a sustainable way without causing any adverse effect on the conservation of resources through appropriate management measures. Sustainability is the fundamental principle for the utilization of fisheries resources which must be emphasized when discussing anything relating to the fishing activities.

*Refer to the footnote in the front page of this article.

2.2. Promotion of fisheries

Fisheries resources are valuable food resources for human consumption. In addition, they provide opportunities of employment and income, which is an important aspect of promoting fisheries especially in developing countries. Some products, which are popular in developed countries, bring foreign currency to developing countries through international trade. The utilization of fisheries resources creates the opportunity not only for production and consumption but also for processing and marketing activities as well.

Japan puts a priority on poverty reduction in accordance with the basic policy of its ODA Charter to ensure human security. Promotion of fisheries is one of the tools to achieve this objective and, therefore, an important element of Japan's cooperation.

2.3. Resources management

Fisheries resources, which are renewable but limited natural resources, could be exhausted without any appropriate and effective resource management in force. Therefore, resources management is indispensable when promoting fisheries. Further, since fisheries resources are common property with no ownership until captured, adjustments to address the interests of all stakeholders are necessary within the context of proper resources management.

2.4. Scientific research and study

All resource management should be based on scientific findings and no management could be deemed as proper management without a sound scientific basis. Therefore, scientific research and study for this purpose is essential for the management.

However, scientific research and study do not produce short-term and direct benefits to fishers and, since training of scientists, construction of laboratory, implementation of research and study, are cost con-

suming activities, it is often the case that inadequate financial resources are allotted to this field, especially in developing countries.

Scientific research and study are, therefore, an important area of Japan's international cooperation, although it is not directly related to the promotion of the industry and less priority is given to this aspect in many countries.

2.5. Consideration on environmental aspect

Recently, global environmental issues such as global warming and destruction of the ozone layer have attracted attention worldwide. As fisheries are activities which utilize natural resources, they are susceptible to changes in the environment. Deterioration of the environment, such as reduction or loss of critical habitats and pollution of waters, could have immediate adverse affects on the sustainability of fisheries.

On the other hand, fishing activities can affect the environment and cause undesirable influences on the environment. Attention should also be paid to this aspect. For example, considerable effort is made to solve the issue of over-fishing as challenges to resources management. Other environmental issues, such as leakage of fuel and dumping of used fishing gears, should also be addressed properly.

3. Programs

3.1. Multilateral approach

Although some fisheries resources are available only within a specific country's waters, others are utilized by several countries. Therefore, international cooperation for the management of such resources is important as an area of cooperation in the fisheries field in addition to the cooperation for the promotion of fisheries in developing countries. Japan is, therefore, making significant contributes to multilateral organizations such as

FAO (Food and Agriculture Organization of the United Nations) and Regional Fisheries Management Organizations (RFMOs) in this field.

3.1.1. FAO

FAO is a UN organization specialized in food, agriculture, forestry and fisheries. FAO has the Committee on Fisheries (COFI), under which the Sub-committee on Fish Trade and the Sub-committee on Aquaculture are established. FAO's undertakings include activities relating to the promotion of international cooperation on resources management, such as holding meetings of specialists, which concluded, for example, the Code of Conduct for Responsible Fisheries, as well as promotion of fisheries and proper management of fisheries resources in developing countries.

Japan, as an active member of FAO, is contributing by providing funds trusted to specific projects in addition to the contribution to the regular budget. Japan actively participates in the meetings of FAO and hosted, with the collaboration of FAO, the International Conference on Sustainable Contribution of Fisheries to Food Security in 1995 in Kyoto, which adopted the "Kyoto Declaration and Plan of Action".

3.1.2. RFMOs

In accordance with the UN Convention on the Law of the Sea (UNCLOS), all relevant countries should cooperate in the management of highly migratory species, fish stocks which occur both within the exclusive economic zone and in areas beyond and adjacent to the zone and resources of the high seas. For this purpose, many RFMOs and arrangements have been established targeting various kinds of stocks in regions all over the world.

Fishing fleets from Japan have been exploring the world's oceans and found many fishing grounds which are still utilized as important areas of operation. Japan participates in almost all RFMOs and arrangements

where the Japanese fleet is operating in order to contribute to the international resource management.

RFMOs discuss management measures to ensure sustainability of stocks based on scientific findings. Japan is contributing to the activities of these organizations by providing scientific data which are necessary to conduct scientific assessment of resources, undertaking scientific research and study. For example, the data from Japanese tuna fishing vessels are essential inputs for the stock assessments done by many tuna RFMOs.

Japan is providing funds trusted to such activities as data collection and tagging experiments by RFMOs in addition to contributing to the regular budget. Further, Japan hosted the Joint Tuna RFMOs Meeting in 2007 in Kobe, to strengthen cooperation and coordination of activities by five tuna RFMOs, which adopted the "Course of Actions for RFMOs from the Kobe Meeting of Joint Tuna RFMOs".

3.2. Bilateral approach

Japan is undertaking bilateral assistance programs as ODA, which includes cooperation in the fisheries field. This part briefly describes the kinds of such assistance.

3.2.1. Grant aid and Yen loan

As a program to assist developing countries, Japan is providing necessary funds for development projects. Grant aid is a program to provide funds without obligation of repayment, while Yen loan is a loan with eased conditions such as low interest rates.

As grant aid is without obligation of repayment, it is mainly targeted at relatively small projects and least-developed countries. The Ministry of Foreign Affairs is responsible for the grant aid and the Japan Bank for International Cooperation (JBIC) is responsible for Yen loans. However, JICA (Japan International Cooperation Agency) will be the implementation agency for both grant aid and Yen loans from mid-2008 to facilitate a unified approach to Japanese international cooperation.

3.2.2. Technical cooperation

Technical cooperation is a program for human resources development in developing countries. JICA is the major implementing agency of technical cooperation, although some public service corporations are also conducting technical cooperation in the field of their expertise with the support of the relevant ministries.

Technical cooperation includes projects with acceptance of trainees and dispatch of specialists, development studies and dispatch of Japan Overseas Cooperation Volunteers (JOCV).

3.2.3. OFCF Japan

The Overseas Fishery Cooperation Foundation (OFCF Japan) is an organization established with the approval of the Ministry of Agriculture, Forestry and Fisheries, and focuses on international cooperation on fisheries. It implements such technical cooperation as the development and promotion of overseas fisheries, and international resources management and research.

OFCF Japan was established as a unique organization to secure overseas fishing grounds for stable operation of the Japanese fishing fleet and then to facilitate development of the fisheries of Japan through implementing various kinds of international cooperation.

Cooperation by OFCF Japan includes projects with acceptance of trainees and dispatch of specialists as well as provision of low interest funds to Japanese organizations which execute cooperation projects.

4. Achievements

4.1. FAO

In addition to the regular activities such as COFI meetings and technical cooperation including holding workshops, FAO concluded various kinds of international instruments for promotion and management of sustainable fisheries including:

- 1993 Agreement to Promote Compliance with International Conservation and Management Measures by Fishing Vessels on the High Seas (came into force in 2003)
- 1995 Code of Conduct for Responsible Fisheries
- 1999 International Plan of Action for Reducing Incidental Catch of Seabirds in Longline Fisheries;
International Plan of Action for the Conservation and Management of Sharks;
International Plan of Action for the Management of Fishing Capacity
- 2001 International Plan of Action to Prevent, Deter and Eliminate Illegal, Unreported and Unregulated Fishing

4.2. RFMOs

In addition to Table 1, discussions are underway to establish new mechanisms for management of species other than tuna in the South Pacific Ocean and the Northwestern Pacific Ocean.

4.3. Grant aid and Yen loan

Yen loan projects for the fisheries sector are limited and mainly for the construction and rehabilitation of fishing ports.

Figure 1 shows the budgets for the fisheries grant aid. (Japanese fiscal year is from April to the following March.)

Major areas of the fisheries grant aid projects are summarized for the following six areas:

- Scientific research and study (e.g. Construction of fisheries research laboratory),
- Promotion of artisanal and/or small-scale fisheries,
- Technical training (e.g. Construction of training center for fishers),
- Construction and/or rehabilitation of fishing ports,
- Facilities relating to processing and marketing,
- Aquaculture and stock enhancement.

Table 1. Major RFMOs.

Name	Standard Nomenclature	Area of Competence	Established in	Major Species
Tuna Fisheries				
WCPFC	Western and Central Pacific Fisheries Commission (www.wcpfc.int)	Western and Central Pacific Ocean	2004	Tuna
IATTC	Inter-American Tropical Tuna Commission (www.iatcc.org)	Eastern Pacific Ocean	1950	Tuna
IOTC	Indian Ocean Tuna Commission (www.iotc.org)	Indian Ocean	1996	Tuna
ICCAT	International Commission for the Conservation of Atlantic Tunas (www.iccat.es)	Atlantic Ocean	1969	Tuna
CCSBT	Commission for the Conservation of Southern Bluefin Tuna (www.ccsbt.org)	n.a.	1994	Tuna
Other Fisheries				
NAFO	Northwest Atlantic Fisheries Organization (www.nafo.int)	Northwest Atlantic Ocean	1979	Redfish, Greenland Halibut, Shrimp
NEAFC	North East Atlantic Fisheries Commission (www.neafc.org)	Northeast Atlantic Ocean	1982	Redfish, Herring, Mackerel, Blue Whiting
SEAFO	South East Atlantic Fisheries Organization (www.seafo.org)	Southeast Atlantic Ocean	2003	Alfonsino, Orange Roughy, Oreo Dories
CCAMLR	Commission for the Conservation of Antarctic Marine Living Resources (www.ccamlr.org)	Antarctic Ocean	1982	Krill, Toothfish

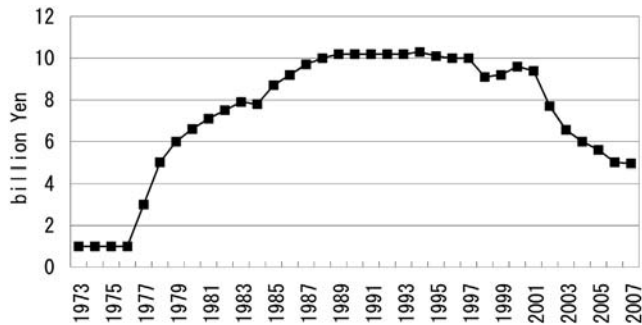


Fig. 1. Budget for fisheries grant aid.

Table 2. Record of JICA's technical cooperation in the fisheries field (source: JICA).

	2001	2002	2003	2004	2005
Acceptance of trainees	400	584	573	660	962
Dispatch of specialists	123	89	69	76	84
Survey mission	322	234	169	141	168
JOCV	11	13	10	9	11
Other volunteers	7	10	11	5	9

4.4. Technical cooperation

Table 2 summarizes the JICA's record of activities relating to the fisheries.

4.5. OFCF Japan

Figure 2 summarizes major programs implemented by OFCF Japan.

5. Discussion

Fisheries resources have two particular characteristics, namely limited but renewal natural resources and no ownership until captured. Therefore, we should not only ensure sustainability of resources but also adjust the interest among stakeholders when managing these resources.

As Japan has a long history of utilizing fisheries resources, it has established a unique system of management. It is said in short that "coastal resources are managed by

the local community while offshore stocks are the common property".

5.1. In the case of offshore stocks

When the fishing technology was primitive relative to the abundance of resources, there was no need for management and fishers enjoyed the freedom of operation. It was a concern over conflicts among stakeholders that made the government introduce the limited entry system for offshore resources. Although the central government or local government limits the number of permits, they were decided mainly to adjust the interest among stakeholders. However, the depletion of certain resources in the offshore areas required the government to consider the sustainability of resources, especially after the ratification of UNCLOS.

As technology evolved, fishers eventually expanded the operation area from the



Fig. 2. Major Overseas Fisheries Cooperation Programs

Programs Implemented by OFCF (2000~2004)



Implemented by OFCF Japan (2000–2004; source: OFCF Japan).

offshore to distant waters. It was partially because fishing vessels operating in the offshore areas should be reduced to adjust the interests of all stakeholders in the fishing grounds. Since the 200 mile zone regime was established in the late 1970s, the coastal countries started to exercise their rights to manage resources in their waters. Therefore, the management of stocks on the high seas is the major challenge for which international cooperation is most required.

The management of the offshore fisheries in Japan has been conducted mainly by restrictions of input such as the limited entry system. However, it initiated output restrictions (i.e., TAC) to supplement the conventional management scheme upon the ratification of UNCLOS. To the contrary, the international management measures have tended to use output restrictions such as TAC and allocation of allowable catch to member countries, although the necessity of input restrictions, especially the control of fishing capacity is to be recognized.

Input restrictions have the merit of less costs for administration, although it is sometimes difficult to propose specific conservation measures which all stakeholders can easily understand, for example, how to measure the fishing capacity of different fishing fleets. On the other hand, the output restriction require higher costs for administration especially in the case where many fishers are involved, but conservation measures such as TAC are easy to understand among stakeholders. Nevertheless, it is critically important to obtain the consent of all stakeholders concerned to ensure effective implementation of the conservation measures.

5.2. In case of coastal resources

The most significant feature of the management of the coastal resources in Japan is the system to delegate the management authority to the local community, which is realized as a fishing right-based fishery under the current law. Under the system, all the interest of stakeholders at the local community can be reflected on the management mea-

sures through fisheries cooperatives.

Although this system has the weakness of possibly excluding new entries from outside interests to the fisheries, it has successfully ensured stable production from the fishing grounds, even though the form of production might change from capture to aquaculture. Actually, the production of coastal fisheries in Japan has been stable for decades despite the fact that the long coast line of Japan has been subject to rapid development by other industries.

The key to success of the system is to ensure the involvement of all stakeholders to the management in the local community, which enhances the awareness of the necessity of proper resources management. Further, this would not only facilitate planning the conservation measures but also smooth and cost-effective implementation of these measures. However, because of the technological development in the field of coastal fisheries, concern about the deterioration of resources will also call attention to the sustainability of the stocks.

Based on the experiences described above, promotion of the proper management of fisheries resources is a very important area as the international cooperation of Japan in addition to conventional undertakings such as the construction of basic infrastructures and technical cooperation on fishing and processing technology. As one major area of interest of developing countries is to enhance their artisanal fisheries in the coastal areas, the knowledge on Japan's practices in the coastal area (i.e., fishing right-based fishery) should be used in this area.

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Institutional Capacity Development for Sustainable Aquaculture and Fisheries: Strategic Partnership with Local Institutions

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Many people living in the rural areas in the Philippines, as in other developing countries in Southeast Asia, depend on aquatic resources for their food and livelihood. For the past two decades, the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC-AQD) has been working with fishing communities and people's organizations, business sector, local government units, national government agencies, non-government organizations (NGOs) and academic and other research institutions to promote the efficient conservation, management and sustainable development of the country's fisheries and aquatic resources so that these may continue to serve the needs of the people today and tomorrow.

Using the lessons learned from those two decades of multi-sectoral and inter-disciplinary collaborations, SEAFDEC-AQD launched in late 2006 a project called Institutional Capacity Development for Sustainable Aquaculture (ICDSA) to hasten the transfer to and adoption by coastal villagers of appropriate technologies that would enhance the productivity of aquatic resources and at the same time safeguard the fragile balance of the aquatic ecology.

The experience of SEAFDEC in coastal resource management shows that it is important to engage the collaboration of the local government units and other "on-the-ground" institutions, such as NGOs and people's organizations, to be able to introduce effectively any social and technological interventions to target community-beneficiaries. However, before a fruitful collaboration among these institutions could be attained, there is a need to build their capacities, and those of the beneficiaries, for the vital roles that they play in the implementation of livelihood projects and environmental management programs.

As of January 2008, SEAFDEC-AQD is implementing ICDSA projects in four provinces—Antique, Capiz, Guimaras and Northern Samar in central Philippines. In the pipeline are similar projects for a province in southern Philippines and two provinces in the north.

KEYWORDS capacity building; aquatic resources management; sustainable aquaculture; Malalison, Philippines

1. Introduction

The aquatic resources of the Philippines provide food and livelihood to some millions of Filipinos in rural areas of the archipelago. As in other Southeast Asian countries, the Philippines' fishery resources have suffered alarming degradation due to overfishing to meet the demands of the burgeoning population, destructive fishing methods, industrial and domestic effluents, and irresponsible aquaculture practices. In response to warnings from different sectors since the mid-1960s, the Philippine government enacted laws and made policies for the protection, conservation and sustainable development of the country's fisheries and aquatic resources.

Two landmark legislations that have significantly supported the aquatic resources development thrust of the Philippines are Republic Act (RA) 7160, known as the Local Government Code, and RA 8550, the Fisheries Code of the Philippines. The Local Government Code sets the legal framework for the democratic decentralization—from the national executive branch of government to local government units (LGUs), i.e. provincial and municipal levels of government—of decision-making, planning and implementation of development programs, and promulgation and implementation of local ordinances in support of local development goals. The Fisheries Code spells out the government's goals and policies regarding fishery resources, and unequivocally states that LGUs plan and implement their own fisheries development programs, and calls for the empowerment of people's organizations (POs) to manage, conserve and protect, develop and utilize fishery resources within municipal waters (15 kilometers from shoreline). The LGUs shall grant demarcated fishery rights to fishery organizations/cooperatives for mariculture operation in specific areas.

The Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC-AQD), mandated to promote and undertake aquaculture research and development (R&D) relevant to Southeast Asia, has been collaborating during the past two decades with LGUs, POs, non-government organizations (NGOs), national government agencies, business sector, and academic and research institutions on community-based coastal resources management projects that foster the sustainable development and responsible utilization of fishery resources. Its mission is to provide dynamic and competent leadership in the generation of science-based technologies in order to strengthen stakeholder capacities in aquaculture and aquatic resources management.

From its experience, SEAFDEC-AQD has seen that the success of social and technological interventions to ameliorate the people's lives and conserve aquatic resources hinges on a strong collaboration among the stakeholders—the LGUs, concerned national government agencies, legislative and policymaking bodies, beneficiary-communities, POs, NGOs, fund donors, and academic and R&D institutions. Strong collaboration is dependent on the capacity of the different institutions to perform their roles in support of the projects.

A community-based development model provides for the return of the decision-making to the people and recognizes their abilities to manage the resources needed to meet their needs (Agbayani and Babol 2001). This development model requires the transformation of the community institutions to enable them to manage and control resources in response to local needs and preferences (Korten 1988). In an emerging paradigm shift of community-based resource management or co-management in a broader sense, the role of local government is to facilitate the development process through empowering and capacity building of local institutions.

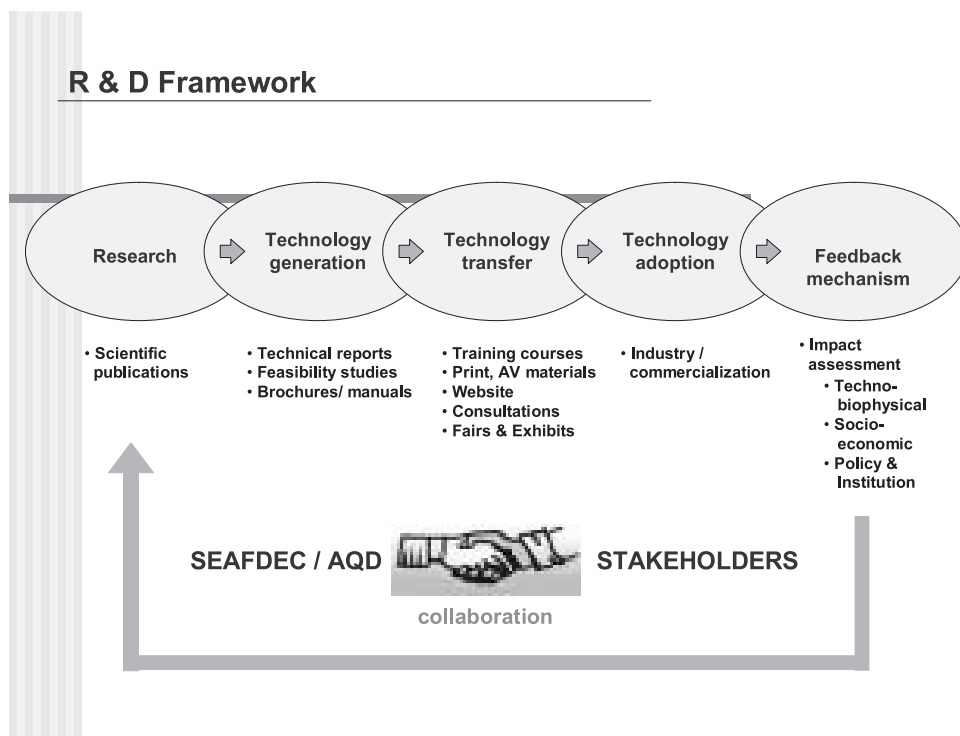


Fig. 1. SEAFDEC R&D framework

2. SEAFDEC-AQD R&D Framework

Figure 1 demonstrates SEAFDEC-AQD's R&D Framework (Agbayani *et al.* 2007). It integrates aquaculture research and development in a cycle of activities that is based on a strong collaboration between SEAFDEC-AQD and the different fishery resources stakeholders such as fishing communities, fishing and aquaculture business sector, LGUs, national government agencies, legislative and policy-making bodies, academic and research institutions.

Research, Technology Generation:

Strong science is the foundation of technology generation. Aquaculture technologies generated by SEAFDEC-AQD are the results of years of painstaking research done by scientists and technologists whose work have

been published in international, peer-reviewed scientific journals.

Technology Transfer, Technology Adoption:

Technologies generated by SEAFDEC-AQD go through commercial-scale runs to evaluate their technical and economic feasibility, and fine-tune them for eventual dissemination to end-users. After successful trial runs, information about the technologies is disseminated through the popular media, and how-to manuals and brochures are prepared for clients.

Farm demonstrations and training courses are conducted either at SEAFDEC-AQD facilities or on-site, whichever is more convenient to technology users.

Technology Impact Assessment:

Aquaculture technologies are assessed in terms of their techno-biophysical and

economic feasibility, social acceptability, and implications on institutional arrangements.

SEAFDEC-AQD Collaboration with Stakeholders: Throughout the R&D process, SEAFDEC-AQD maintains regular consultation and dialogue with stakeholder-collaborators to ensure the effective transfer of technology and information, and the accuracy of impact assessment.

3. The Malalison Experience: Community-based Fishery Resources Management

SEAFDEC-AQD's experience in working with fishing communities, LGUs, NGOs and POs started with an eight-year (1991–1998) community-based fishery resources management (CFRM) project on Malalison Island, Culasi, Antique Province, in central Philippines. The success of this project has made it a model for other CFRM projects in other parts of the country (Agbayani *et al.* 2000).

3.1. Background and objectives

The CFRM Project, funded by the International Development Research Center (IDRC) of Canada, was implemented by an interdisciplinary team of biologists, socioeconomists, social workers, engineers and aquaculture technologists. The project objectives were to: 1) develop the community into a strong organization that could manage its fishery resources; 2) teach livelihood skills to the fisherfolk and provide them capital to start livelihood projects; and, 3) regenerate fish habitats and increase fish stocks.

Biological and socioeconomic surveys were conducted in 1991–1992 to determine the people's preparedness to receive such interventions. Significant findings are presented below:

Malalison's municipal waters were fished by both the island's residents and those from other communities. The dominant fish

species caught were fusilier, surgeonfish, snapper and grouper. Dynamite and cyanide fishing were rampant despite the national laws banning and penalizing these destructive practices because of inconsistent law enforcement. The coral reefs were degraded and their fish yield was a low 5.8 tons/km²/yr (Amar *et al.* 1996).

The socioeconomic survey revealed that 98% of the Malalison residents depended on marine resources surrounding the island for their food and livelihood; 93% had an income below poverty level (Agbayani and Siar 1994). The islanders generally had low educational attainment: most had only four-six years elementary schooling with a few reaching high school level.

The islanders had an informal and unregistered organization called MICA (which stood for the island's location address; Malalison Island, Culasi, Antique) that coordinated community efforts mainly for the celebration of the annual feast day of their patron saint.

3.2. Capacity-building and social reform: preparations for socioeconomic, environmental and policy interventions

The findings of the surveys showed the need for capacity-building and social reforms to prepare the islanders for socioeconomic, environmental, and policy interventions. SEAFDEC-AQD, in close coordination with the village leaders conducted consultations with the islanders to determine the training needs and social reforms to be advocated.

3.2.1. Community empowerment through knowledge and skills training

SEAFDEC-AQD engaged the services of an NGO (PROCESS Foundation) as partner in the social preparation and values formation of the islanders. Starting in 1992, PROCESS conducted training courses on organizational development and management, cooperative development and management, financial management, environmental protection,

gender sensitivity, hog-raising, entrepreneurship development, project management, and funds accessing and sourcing. PROCESS also conducted seminars on para-legal work, lobbying and advocacy, and on the provisions of the Local Government Code on fisheries management.

3.2.2. Study-tours of potential models for development

SEAFDEC-AQD also brought the village leaders on a study-tour of coastal barangays in different provinces whose initiatives could serve as Malalison's examples for development.

3.2.3. Formal organization for formal link and community action

One important social preparation was the establishment of a formal organization for the community: the Fishermen's Association of Malalison Island (FAMI). Registered with the Securities and Exchange Commission in 1992, FAMI served as the formal link between SEAFDEC-AQD and the community and the platform for community action. Calls for community dialogues on contentious issues and consultations on the Project's activities were channeled thru FAMI.

FAMI also functioned as a cooperative, thus, the CFRM Project released thru FAMI loans for alternative livelihood such as hog- and poultry-raising, a cooperative store, and seaweed farming (Hurtado-Ponce 1992; Hurtado *et al.* 1998).

FAMI, guided by the Project, hosted dialogues on how to assert the islanders' rights over their territorial waters and protect their fishery resources from further destruction due to illegal fishing. They prepared resolutions asking their town council, through their barangay council to grant them territorial use rights in fisheries (TURF), declare a reef area as fish sanctuary, and approve the deployment of concrete artificial reefs (ARs) near their island and allocate an amount from their barangay funds to help defray costs for the deployment.

3.3. Project milestones

The following are the milestone achievements of the Malalison CFRM Project:

1) *Granting of TURFs:*

With TURF granted by Municipal Ordinance 5-90, the FAMI members were given the rights to and responsibility for the proper utilization, management and control of the territorial waters of their island.

2) *Declaration of a fish sanctuary*

With technical advice and scientific data provided by SEAFDEC-AQD, the islanders chose the Guiob reef area to be declared as a fish sanctuary. The area was a major fishing ground for the islanders; it is the habitat of various fish species that needed to be conserved; and it is near enough for surveillance purposes.

3) *Construction and deployment of concrete artificial reefs*

The ARs were designed and constructed by SEAFDEC-AQD engineers. They were used for coral reef regeneration, and not for fish aggregation. These were deployed in 1995 in two coral reef areas—Guiob and Kawit—with 26 modules per area (Tenedero 1995).

4) *Creation of the Fisheries and Aquatic Resources Management Council*

In accordance with the Local Government Code, the islanders formed a Fisheries and Aquatic Resources Management Council (FARMC) in June 1995. FARMC's functions are to: 1) prepare fisheries management plans and policies based on scientific assessment and socioeconomic considerations; and, 2) recommend to this local government and concerned national government agencies the issuance of permits for the appropriate use of fishery resources.

5) *Use of scientific data in support of fish sanctuary*

The value of scientific data for policymaking was demonstrated when the

town council approved the declaration of a fish sanctuary based on the data provided by SEAFDEC-AQD.

6) *Municipal Council approval of the declaration of a fish sanctuary*

The declaration of a fish sanctuary by the Culasi Municipal Council was a first in the province of Antique, and is considered as a groundbreaking initiative.

7) *Multiplier effect of the Malalison CFRM*

The Malalison CFRM project "spawned" 14 fish sanctuaries in the municipality of Culasi and in three nearby municipalities—Sebaste, Pandan and Libertad. Two unified coastal resources management councils were also established, patterned after the Malalison model. The first is the LIPASECU (Libertad, Pandan, Sebaste, Culasi) Council which was organized to coordinate and oversee the planning and implementation of an integrated coastal resources management in the four municipalities. The second is the Banate Bay Coastal Resources Management, Inc. which was established to coordinate and integrate the management and development of four coastal municipalities in the adjacent province of Iloilo.

8) *Enhancement of SEAFDEC-AQD training courses*

SEAFDEC-AQD's training courses were enhanced by the incorporation of topics on community-based coastal resources management. Most information incorporated in the lectures were from the Malalison experience.

9) *Impact assessment*

Fish landings. Fish landings on Malalison Island were monitored during June 1995–1997 to determine the species composition of catch, catch per unit effort and yield from different fishing areas. Reef and reef-associated fish yield was estimated at 22.44 tons/km²/yr. This was almost four-fold increase from the 1992 estimate of 5.8 tons/km²/yr. (Primavera, 2002).

Policy dialogue and development. The concept of TURF was acceptable to the fishers and was perceived as beneficial to them (Siar *et al.* 1992).

Resource co-management strategy. A case study revealed that fishery resources co-management was perceived to be successful based on the criteria of social equity, management efficiency and environmental sustainability (Baticados and Agbayani 2000).

4. Institutional Capacity Development for Sustainable Aquaculture

4.1. Background and rationale

The success of the Malalison project enabled opportunities for SEAFDEC-AQD to reach out to various stakeholders in other provinces.

Using lessons from the Malalison CFRM Project and guided by its R&D framework, SEAFDEC-AQD launched in mid-2006 a project called Institutional Capacity Development for Sustainable Aquaculture (ICDSA). The first project site is located in the province of Antique, in central Philippines. The next three are located in the provinces of Guimaras, Capiz and Northern Samar, all in central Philippines.

The project is based on the belief that strong collaboration among stakeholders underpins the effective introduction of social and technological interventions to improve the socioeconomic conditions of the people and promote the efficient management and sustainable development of natural resources. Strong collaboration may only be achieved if the stakeholders have the capacities to perform their share in communal initiatives.

The overall goal of ICDSA, therefore, is to build the capacities of aquatic resource users by providing them with knowledge and skills to become responsible resource managers and users.

ICDSA's specific objectives are to: 1) build the capacity of LGUs, community-based organizations, non-government organizations, fishery schools and other on-the-ground institutions for the practice of sustainable aquaculture; 2) demonstrate the technical and financial feasibility of aquaculture technologies in project sites; 3) ensure the sustainability of aquaculture as a livelihood for small-scale fishers; 4) provide stakeholders with scientific information on aquaculture and aquatic resources; 5) assess the Project's contributions to the improvement of the socioeconomic well-being of fishing communities, impacts on the aquatic ecology, and influence on local legislation for effective governance of aquatic resources; and, 6) evaluate the effectiveness of mechanisms for the transfer and adoption of aquaculture technologies.

4.2. Project strategies

4.2.1. Community-based and co-management approaches

SEAFDEC-AQD makes it a pre-requisite for its ICDSA projects that target beneficiary-communities are organized and are made responsible for the proper management and utilization of their aquatic resources. But for them to become efficient co-managers, they must possess the knowledge and skills for the job.

SEAFDEC-AQD conducts a rapid social assessment to establish a socioeconomic database of the community. The survey includes socio-demographic profile, income and livelihood options, and involvement in community activities, including resource management and conflicts resolution. The survey, together with community consultations, determines the social and technological interventions to be introduced and the social preparations needed for the effective introduction of such interventions.

Social preparation is critical in the success of community-based resource management and livelihood projects. NGOs and aca-

demic institutions are usually engaged to conduct seminar-workshops on basic environmental protection and management, organizational development and management, cooperative management, basic accounting and bookkeeping, gender-sensitivity awareness, lobbying and advocacy, fund accessing and sourcing, and basic laws and ordinances that have to do with the development and utilization of common properties.

4.2.2. Formal partnership with local institutions

For ICDSA projects, SEAFDEC-AQD enters into formal partnership with LGUs and other concerned government agencies, NGOs and POs. A Memorandum of Agreement defines the partnership's terms of references, and the roles and responsibilities of the parties involved in the project.

The budget for the project implementation is provided by a client (LGU, NGO, PO). It is either sourced internally (as in the case of LGUs) or from a donor. SEAFDEC-AQD normally shoulders expenses for pre-project activities, such as transportation, accommodation and incidental expenses of scientists and technical staff who conduct pre-project surveys needed in the project proposal preparation.

4.3. Project activities

Training, technology demonstration and research are the three main activities of ICDSA projects. The ICDSA projects, in effect, become: 1) an R&D platform for the demonstration of the technical and economic feasibility of aquaculture technologies; 2) a laboratory for the assessment of socioeconomic and environmental impacts of aquaculture technologies; and, 3) on-site training ground for beneficiary communities.

4.3.1. Season-long training courses

Season-long training courses are conducted on-site. A season-long course consists of a series of training modules conducted

throughout the production cycle of a cultured commodity. Each module is usually conducted over a two-three day period and is composed of lectures and hands-on practical sessions.

The purpose of season-long training courses—which can extend for four to six months or longer—is to enable participants to learn technical knowledge and skills by allowing them to participate in actual production activities such as pond/pen preparation, stocking, feeding, water quality management, fish health management, harvesting and marketing. The extended period of training gives participants more time to absorb and understand the course topics. Camaraderie among the trainees and familiarity with their trainers may develop over time; this friendly atmosphere is conducive to sharing observations and solving production problems collegially.

The general topics covered by the training courses are: 1) Aquaculture production systems; 2) stock sampling, feeding, water quality and fish health management; 3) farm-based feed formulation and preparation; 4) business planning and management; 5) harvest, post-harvest handling, and marketing, and other special topics.

The languages used during lectures are a combination of English and Filipino. The local dialects are also used if the lecturers are conversant in them. Visual aids are a combination of the modern and standard implements: PowerPoint presentations, and illustrations on chalkboards or manila paper.

4.3.2. On-site technology demonstration and production runs

The selection of species and culture systems that will be demonstrated on-site are determined by community consultation, expert observation and analysis, and economic viability. The community consultation helps determine the appropriateness of a technology based on the resources and capabilities of the beneficiaries. SEAFDEC-AQD scien-

tists analyze the techno-bio-physical characteristics of the aquatic resources to determine if the aquaculture technology that will be demonstrated is suitable for them.

The aquaculture farm design is prepared by SEAFDEC-AQD experts in consultation with the clients/beneficiaries/donors. Construction of farm facilities is done with selected beneficiaries before the start of or during the “training-run” production, whichever is more practical and appropriate.

Preliminary financial feasibility analysis is prepared by SEAFDEC-AQD economists using costs-and-returns and discounted financial projections. Financial indicators used are return on investments and payback period, net present value, internal rate of return, and benefit-cost ratio. The indicators are used as budgeting instruments in the production run.

The first production run is a “training run,” closely supervised by SEAFDEC-AQD trainers. Participants perform the actual production operations like stocking, feeding, sampling and monitoring, disease surveillance and prevention, cage/pond repair and maintenance, harvesting, and marketing. The succeeding production runs are operated and managed by the beneficiaries, if they are evaluated as ready and capable, with minimum supervision by SEAFDEC-AQD.

ICDSA project duration is usually three years—a period long enough for the beneficiaries to learn and gain confidence in operating and managing aquaculture farms.

4.3.3. Research studies

Baseline socioeconomic data are gathered through surveys and from secondary sources prior to or in the early months of the project implementation. At the start of the project, selected areas are studied to determine their carrying capacity as potential sites for aquaculture projects.

At the end of the project, assessments will be conducted to measure, quantitatively and qualitatively, the project impact on the

socioeconomic condition of the beneficiaries and on the aquatic environment. The information will be packaged into policy briefs and presented to LGUs to encourage legislations in support of sustainable aquaculture and fisheries development.

4.4. Highlights of on-going projects

As of January 2008, there are four ongoing ICDSA projects in four provinces—Antique, Capiz, Guimaras and Northern Samar—in central Philippines. The following are the key points of the projects.

4.4.1. Antique province

A three-year (2006–2008) ICDSA project is funded mainly by the Community Development Fund of the province's congressional representative (US\$ 10,000). The municipality of Pandan, one of the project sites, contributed US\$ 2,000.

1) Training courses

a. Seabass cage culture in ponds in the municipality of Hamtik

Held in June 2007, the training course was attended by 10 pond technicians. The trainees had a maximum of 10 years of formal schooling (elementary grades to high school level).

b. Seabass cage culture in Pandan River in the municipality of Pandan

Held in July 2007, the course was attended by 20 members of a fisherfolk cooperative, Mag-aba Multi-purpose Cooperative. Most participants are full-time small-scale fishers with educational attainment of up to high school.

c. Mudcrab culture in ponds in the municipality of Tibiao

Held in August 2007, the course was conducted for students and teachers of fishery courses at the Polytechnique State College of Antique (PSCA). SEAFDEC-AQD has an agreement with PSCA to conduct training courses for fisheries students and

teachers. The goal is to develop PSCA as a training and production center of selected marine and freshwater fishes in Antique Province.

2) On-site aquaculture technology demonstrations

a. Seabass cage culture in ponds in the municipality of Hamtik

For the nursery operation, four net cages ($3 \times 2 \times 1$ m; mesh size, 0.5 cm) were installed inside a pond. Hatchery-bred seabass fry were stocked in the first 2 cages at $130/\text{m}^3$ (780/cage). After 30–45 days, seabass fingerlings with average weight of about 20 grams were transferred to grow out compartments.

For the grow-out phase, a 1.25 ha.-pond was divided by nets into six compartments ($2080 \text{ m}^2/\text{compartment}$). Seabass juveniles (ave. wt, 20 g) were released from the first set of 4 units nursery cages to grow-out compartments at 5000 fish/ha (1040 fish/compartment). The second batch of fingerlings from the second set of 4 units nursery cages was released into 3 compartments two months later.

A strong typhoon hit the province midway through the production cycle and caused the overflow of water and escape of fish from the ponds. Fish harvest was done after eight months, when 350–400 g wt was reached. The low survival—less than 50%—was attributed to overflow of water during the typhoon.

Another production run is planned for 2008.

b. Cage culture of seabass in Pandan River

For the nursery phase, three units of nursery cages ($3 \times 2.5 \times 1$ m) were set up to grow 1600 fry (2–3 cm long) up to 10–15 cm long. Fry were stocked at $100 \text{ fish}/\text{m}^3$. After 30–45 days, 5–10 cm long seabass fingerlings were transferred to grow-out net cages.

For the grow-out phase, 10–15 cm long fingerlings from the nursery cages were

stocked in 8 units of stationary net cages ($4 \times 2.5 \times 1$ m) at 20 fish/m³. The fish were given feeds formulated by SEAFDEC-AQD.

Selected harvesting was done starting on the 6th month, when the fish reached 400 g. Harvest was completed on the 8th month. Survival was 60%.

3) Research

a. Abalone sea ranching in the municipality of Anini-y

Started in October 2006, this ongoing project aims to determine the technical and financial viability of the culture system. Sea ranching of abalone can be adopted by organized fisherfolk.

b. Socioeconomic survey of the municipality of Anini-y

The survey was undertaken in late September 2007 to establish a socioeconomic database that will be used in the assessment of the impacts of the abalone sea ranching and other future aquaculture projects.

4.4.2. Capiz Province

The three-year (2007–2009) project is funded by the Capiz Provincial Government amounting to US\$ 59,000. The Project has three components: brackishwater aquaculture, freshwater aquaculture and coastal resources management.

1) Training

a. Season-long training on brackishwater aquaculture of grouper and mudcrab in Roxas City

The training was conducted in the ponds of Capiz State University (CAPSU) in February–July 2007. There were 58 participants: 17 LGU personnel, 19 fishpond/hatchery operators, and 9 students and 13 teachers of CAPSU.

b. Season-long training on freshwater aquaculture of tilapia, catfish and prawn in the municipality of Dumarao

The first session was conducted in November 2007 for 20 rice farmers whose farms

are perennially flooded due to the unfinished construction of a dam.

2) On-site aquaculture technology demonstration

a. Grouper and mudcrab culture in brackishwater ponds in Roxas City

Renovation and preparation of 3 units of ponds started in November 2006. Grouper fingerlings and crab juveniles from SEAFDEC/AQD hatcheries were stocked in February 2007, right after the first training module on Aquaculture Production Systems. Stock sampling was done at 15-day intervals to monitor fish/crab growth. Water quality was monitored regularly to determine dissolved oxygen, water temperature and salinity. Mudcrabs were harvested after a four-month culture period.

Results of the mudcrab harvest showed a very low 12% survival. This was due to high water salinity and high water temperature (39°C) during the summer months (March–May). Crab molting is difficult in summer, resulting in slow growth and high rate of cannibalism. Another run is planned during the rainy seasons (May–October) and is expected to produce better results and demonstrate the economic viability of mudcrab culture.

For grouper culture, stock sampling showed encouraging results with fish attaining a 295 g average body weight (ABW) after 6.5-month culture period. Fish growth is within the acceptable range. The harvest was completed at the end of September 2007.

Results showed a 300 g ABW and 89% survival rate.

b. Freshwater cage culture of tilapia, catfish and freshwater prawn in the Badbaran River, in the municipality of Dumarao

This was part of the hands-on activity of the on-site training course cited above. In November 2007, net cages (8 units of $4 \times 4 \times 1.5$ m) were installed and stocked with tilapia, catfish and freshwater prawn (ulang).

3) Research

a. Socioeconomic survey of rice farmer-trainees in Dumarao

In January 2008, SEAFDEC-AQD will conduct a socioeconomic survey of the 20 rice farmers who participated in the season-long training course on freshwater culture of tilapia, catfish and prawn in November 2007.

The results of the study will serve as baseline data for the assessment of the socioeconomic impact of the technological intervention.

b. Ecological study of the Badbaran River in Dumarao

In early 2008, an ecological study of Badbaran River will be conducted to determine its carrying capacity.

4.4.3. Guimaras Province

The ICDSA project, called "Pilot Project on Milkfish Cage Culture as Livelihood Option for Guimaras Fisherfolk Affected by Oil Spill," is funded by Petron Corporation, the oil company that chartered the sunken oil carrier that leaked oil in the seawaters of Guimaras in October 2006. The project site is in the Mariculture Park of the SEAFDEC-AQD Marine Station in Barangay Igang in Guimaras. Started in October 2007, this one-year project is estimated to cost US\$ 78,000. Four fisherfolk organizations from four barangays (Igang, Rosario, San Antonio and Santo Domingo) are participating in the project. They are represented by 29 persons from 29 fishing households from the four barangays.

1) Training

a. Season-long training course on milkfish cage culture

This training course has five phases. Phase I was a 2-day lecture-cum-practicum module conducted in October 2007. All 29 representatives of the four fisherfolk organizations attended Phase 1. The lecture topics

were: Overview of Sustainable Aquaculture; Milkfish Culture in Cages, Pens & Ponds; and Water Quality & Methods of Monitoring. Practicum was on instrumentation and fabrication and installation of net cages. Phase II, conducted in November 2007, was on stock sampling, water quality monitoring, feed formulation and preparation, and cage maintenance. Phases III–IV will be completed in the first quarter of 2008.

2) On-site aquaculture technology demonstration

Three floating net cages were constructed and installed within the Mariculture Park in October 2007. The cages serve both as training and production facilities. Each cage has a capacity of 600 m³ and was stocked with 24,000 milkfish fingerlings per cage. Estimated culture period is four months. Commercial brands and SEAFDEC-formulated feeds are used. After 75 days of culture, fish ABW was 135g; estimated survival rate was 95%. The estimated profit of US\$ 1000/per cage will be equitably shared by the participating fisherfolk organizations. The money will be used to construct and operate fish cages in their respective areas.

3) Research

a. Socioeconomic survey of participating barangays

A socioeconomic survey was started in November 2007 to gather baseline data that will be used in the assessment of the impact of the milkfish enterprises on the participating barangays.

b. Ecological survey of the Mariculture Park

The survey will determine the carrying capacity of the Mariculture Park. Besides the Petron project, other aquaculture projects have been set up in the Park by private firms.

4.4.4. Northern Samar Province

Started in July 2007, the 2.5-year project called "Enhancing Adoption of Mud Crab Production Technologies in Northern Samar"

is funded by the Australian Center for International Agricultural Research (ACIAR). Funds are channelled through ACE, an NGO that implements Australian development projects in the Philippines. Estimated to cost US\$ 73,000, the project's beneficiaries are 325 fishing households from four municipalities (Rosario, Lavasares, Lao-ang and Pambujan). The households derive their income mainly from the sale of crabs caught from the wild.

All the training courses were held in the facilities of the University of Eastern Philippines in Catarman, the capital town of Northern Samar.

1) Training

a. Season-long training on mudcrab nursery in Catarman

The Phase 1 of the season-long training course was attended by 30 crab-catchers from the four municipalities. They were chosen by the fisherfolk organizations in their respective towns.

The training methodology was a combination of lectures and practical sessions. Languages used were English and Filipino as well as Waray, the local language. SEAFDEC-AQD brought the ingredients and utensils for the practicum in feed preparation. Exercises on the computations of feed ingredients were conducted to ensure that the participants understood the lectures and the practical sessions.

Phase 2 was on methodology on stock assessment in selected sites. This was attended by representatives of partner-NGOs. The training included lecture and actual mapping and test-run in selected sites.

2) On-site aquaculture technology demonstration

a. Nursery

Demonstration of nursery culture will start in January 2008.

Under Phase 1, "fly"-size crab juveniles (<1 cm carapace width) will be grown to ju-

veniles (2–2.5 cm CW) in 30 days using net cages in ponds.

In Phase 2, juveniles will be grown to match box size (4–5 cm CW) in 20–30 days in net cages or ponds. The crabs will be fed fish and snails.

b. Grow-out

Juveniles will be grown to marketable size of 300–500 g in 5–6 months.

c. Fattening

Lean crabs will be fattened in ponds or pens in mangrove areas in 3–4 weeks or until marketable size.

d. Feed formulation

To reduce the use of fish as crab-feed, low-cost diets, using locally available ingredients, will be formulated for the project.

3) Research

a. Mudcrab market survey

Started in November 2007, the one-year market survey will determine the supply and distribution channels of crabs from the province to crab farms all over the country.

b. Socioeconomic survey

The baseline data gathering which covers 325 household-participants will start in the first quarter of 2008.

c. Mudcrab stock assessment

Started in November 2007, the ongoing stock assessment is conducted in two sites—Rosario and Pambuhan—to determine the seasonal trends in relative abundance of mud crab by size, sex, volume (CPUE), stage of maturity, and condition of habitat. The research data will be used to improve local ordinances governing the management of mudcrab stock in the province.

5. Problems Encountered

Several problems were encountered during the first year of implementation of the ICDSA Project.

1) The “Scientist Box”

Some SEAFDEC scientists are still adjusting from a laboratory-type research setting to “real life” community projects. The mind set of some scientists is still confined to the rigid scientific methodology, something not attainable in real life situations. Scientists need to get out of the “scientist box” to be able to realistically address fisherfolk needs that are immediate and concrete.

2) Non-delivery of commitment

Institutional partners, including SEAFDEC, do not always deliver their part of the agreement—in kind or time. Such remissions disrupt work schedules and cause delays that jeopardize the project.

3) Cynicism of beneficiaries

Potential beneficiaries are cynical of development projects, such as ICDSA, because of past experiences where their expecta-

tations were not met. Project participants, particularly the impoverished fisherfolk, expect immediate and concrete economic returns for their effort. When this expectation is not met, the participants raise doubts on the validity of the projects. This cynicism causes lukewarm attitude and commitment. It will benefit the ICDSA Project to discuss frankly with the beneficiaries regarding their expectations at the onset of the Project. Furthermore, the role of the project beneficiaries should be clear to them so that they can deliver their part of the deal.

4) Risks: natural and technological

Aquaculture technologies may not always perform as projected due to many reasons; both controllable (inputs) and uncontrollable (natural causes). These eventualities should be factored into the financial projections of the project using sensitivity analysis.

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International Cooperation for Higher Education in Aquaculture and Fisheries Science —A European Point of View—

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Since the signing of the Bologna Declaration in 1997, European (higher) education has gone through probably the most thorough and swift reform in its history. The process aims at transparency and mutual recognition leading to unlimited mobility across the European Communities' universities for students and teachers. The Lisbon declaration emphasized the role of top-quality education as a prerequisite in developing Europe as the most competitive and dynamic knowledge-based economy in the world. In the process towards these Lisbon objectives, much effort is invested in initiatives for lifelong learning (LL) as lifelong learning is considered to be a cornerstone in achieving competitiveness and employability. Lifelong learning contributes to social inclusion, active citizenship and personal development.

In response to the diversity and increasing specialisation of the European fisheries and aquaculture sector, a range of higher and vocational education programmes has developed responding to this diversity. AquaTNET, a European Commission funded network, promotes harmonization of education programmes in the European Union and serves as a representative and advisor for the aquaculture and fisheries education providers.

Internationalization of education is high on the agenda of the European Commission. The European Commission's ERASMUS Mundus programme features various activities that promote mobility of students and staff, and develops partnerships between European and non-European education providers. This programme eventually improves the appeal of the European education. Besides the centralised initiatives, numerous valuable cooperation projects on education exist between European and non-European states, institutes and other parties such as NGO's.

KEYWORDS education; cooperation; policy; Europe

1. Introduction

Education is a cornerstone in the development of both individuals and modern societies. Education enhances social, cultural and economic development, active citizenship and ethical values. The maturity and quality of any society can be measured by the appeal that its culture has for other countries. Education is an excellent channel to promote intercultural appeal and understanding through bilateral exchanges with other countries.

2. Education Policy and Reforms in Europe

The European Community¹ in its effort to harmonize the education systems throughout its member states has experienced what is probably the most thorough educational reform in its history. The following paragraph describes the objectives of this reform and its implications for the educational sector.

2.1. The Lisbon declaration

The European Council¹, at the 2000 meeting in Lisbon (Portugal), launched a comprehensive process to develop the European Union into the most competitive and dynamic knowledge-based economy in the world, capable of sustainable economic growth with more and better jobs and greater social cohesion. To achieve this ambitious goal, European Heads of States or Governments asked for “not only a radical transformation of the European economy, but also a challenging programme for the modernisation of social welfare and education systems.” In 2002 it was specified that by 2010, Europe should be the world leader in terms of the quality of its education and training systems.

The drive to realize this objective has initiated an unprecedented transformation of

education and training throughout Europe. However, in Europe, education in general and higher education in particular are not subject to a “common European policy”: regulations concerning the content and the organization of education remains a competence of individual member states.

Therefore, the described educational reforms are implemented in each country according to national contexts and traditions. The European harmonization will be driven by cooperation between Member States at European level, through the sharing of experiences, working towards common goals and learning from what works best elsewhere.

Indeed, according to the Treaty of Nice (2001), the European Community <<shall contribute to the development of quality education by encouraging cooperation between Member States>>, through a wide range of actions, such as promoting the mobility of citizens, designing joint study programmes, establishing networks, exchanging information or teaching languages of the European Union. The Treaty also contains a commitment to promote life-long learning for all citizens of the European Union. Therefore, the European Commission¹, which has a triple role to play: to add a European dimension to education, to help to develop quality

¹ A brief glossary of Euro-speak

- The European Union (EU) is the economic and political partnership between 27 democratic European countries.
- The Council of Ministers of the European Union represents the member states. It is the EU's main decision-taking body. When it meets at Heads of State or Government level, it becomes the European Council whose role is to provide the EU with political impetus on key issues.
- The European Parliament, which represents the people, shares legislative and budgetary power with the Council of the European Union.
- The European Commission (EC), represents the common interest of the EU, it is the main executive body. It has the right to propose legislation and ensures that EU policies are properly implemented.

education and to encourage life-long learning.

2.2. The Bologna declaration and Bologna process

As a result of the historical rich diversity of European societies, huge differences in educational organisation and content among the European countries have developed. In order to harmonise European education, the Bologna Process was initiated. The Bologna process derives its name from the Bologna Declaration, which was signed on 19 June 1999 by higher education ministers from 29 European countries. Although the Bologna declaration precedes the Lisbon declaration, implementation converged seamlessly with the spirit of the Lisbon Declaration.

The Bologna process is an intergovernmental European reform process aimed at establishing the European Higher Education Area (EHEA) by 2010. Ultimately, the European Higher Education Area must be an area that allows students, graduates, and higher education staff to benefit from unhampered mobility and equitable access to high quality higher education. In this European-wide area, students are or will be able to choose from a wide range of high quality courses and benefit from smooth recognition procedures among the countries implementing the Bologna reforms. The cornerstones of such an open area are mutual recognition of degrees and other higher education qualifications, transparency based on readable and comparable degrees organised in a three-cycle structure (bachelor/master/doctorate) and European cooperation in quality assurance.

The Bologna process affects more than the EC member states further adding to its complexity. Having initially started with 29 countries; the Bologna process is now sweeping through the 46 member countries of the European Cultural Convention. The Bologna Declaration has put in motion a cascade of

drastic reforms which, to the surprise of supporters and sceptics alike, are occurring at all levels involved in the process at a breathtaking pace.

The key to success of the Bologna process is the underlying partnership approach, in policy-making and implementation. The process currently unites not only the 46 participating countries, but also relies on cooperation with various international organisations and European associations representing higher education institutions, students, staff and employers.

An important feature of the European Higher Education Area is the social dimension of European higher education with an emphasis on participative equity and employability of graduates. This social dimension is to be achieved primarily through the use of lifelong learning.

Finally, the European Higher Education Area will display openness to the world. European Higher Education must become more compatible and comparable, more competitive and more attractive for Europeans and for students and scholars from other continents. A European education system that is attractive and competitive beyond Europe is essential if Europe is to match the performance of the best performing systems in the world, notably the United States and Asia.

2.3. Implementation of the Bologna declaration

2.3.1. The European Credit Transfer and Accumulation System (ECTS)

An essential tool for transparency and comparability of study programmes is a uniform credit system. The European Credit Transfer (and Accumulation) System is a student-centred system based on the student workload required to achieve the objectives of a programme, objectives preferably specified in terms of the learning outcomes and competences to be acquired. Conventionally, a competency is a skill or piece of knowhow

a person has acquired that enables him/her to perform a task in his/her working or social environment. A learning outcome is then a very specific statement that describes exactly what a student will be able to do in some measurable way. A competency may therefore have several specific learning.

ECTS was introduced in 1989 and has ever since been the only credit system which has been successfully tested and used across Europe. Initially the system facilitated the recognition of periods of study in another European country and thus enhanced the quality and volume of student mobility in Europe. But ECTS can be used for all types of programmes, whatever their mode of delivery, and for lifelong learning purposes. It serves both mobile and non-mobile students: it can be used for accumulation within an institution and for transfer between institutions. ECTS helps learners moving between countries, within a country, town or region, as well as between different types of institutions; it also covers self-study and work experience. For these reasons the well-known acronym "ECTS" now stands for "European Credit Transfer and Accumulation System".

ECTS enables local and foreign students to read and compare study programmes. ECTS facilitates mobility and academic recognition, it helps universities to organise and revise their study programmes and can be used across a variety of programmes and modes of delivery. In the end ECTS makes European higher education more attractive for students from across Europe and beyond which is one of the key objectives of the Bologna process.

2.3.2. The diploma supplement

The Diploma Supplement is a document attached to a higher education diploma providing a standardised description of the nature, level, context, content and status of the studies that were successfully completed by the graduate. The Diploma Supplement provides transparency among different educa-

tion programmes and facilitates academic and professional recognition of qualifications (diplomas, degrees, certificates).

2.3.3. Lifelong learning

Following the adoption by the European Commission in 2001 of the strategy paper "Communication on Making a European Area of Lifelong Learning a Reality", lifelong learning has become the guiding principle for the development of an education and training policy. Lifelong learning is believed to form a core element to competitiveness and employability. It contributes to social inclusion, active citizenship and personal development and enables individuals at all stages of their lives to pursue stimulating learning opportunities across Europe. The Communication sets out concrete proposals that aim to make lifelong learning a reality for all.

Lifelong learning encompasses learning for personal, civic and social purposes as well as for employment-related purposes. Lifelong learning takes place in a variety of environments, in and outside the formal education and training systems. Lifelong learning implies raising investment in people and knowledge; promoting the acquisition of basic skills, including digital literacy; and broadening opportunities for innovative, more flexible forms of learning. The aim is to provide people of all ages with equal and open access to high-quality learning opportunities, and to a variety of learning experiences, throughout Europe. Education systems have a key role to play in making this vision a reality. Indeed, the Communication stresses the need for Member States to transform formal education and training systems in order to break down barriers between different forms of learning.

The Lifelong Learning Programme is the flagship European funding programme in the field of education and training. For the first time, a single programme covers learning opportunities from childhood to old age. The

Lifelong Learning Programme covers the period 2007–2013, and is the successor to the Socrates, Leonardo da Vinci and eLearning programmes that ended in December 2006. The Lifelong Learning Programme consists of four sub-programmes: Comenius (for schools), Erasmus (for higher education), Leonardo da Vinci (for vocational education and training) and Grundtvig (for adult education). A transversal programme complements these four sub-programmes in order to ensure that they achieve the best results. The transversal programmes consist of four key activities: policy co-operation, languages, information and communication technologies, effective dissemination and exploitation of results generated by projects in the sub-programmes.

2.3.4. The ERASMUS programme

Thus, ERASMUS is the overarching programme that covers all EC initiatives pertaining to higher education. Erasmus supports actions in the fields of mobility (period of study or placement abroad for student, teaching staff exchange), European projects and networks. ERASMUS Mundus is a more recently launched sub-programme of ERASMUS that specifically supports education initiatives that reach out beyond European boundaries (see further).

For the period 2007 to 2013; the EU has allocated a significant budget of nearly EUR 7 billion, to lifelong learning programmes. EU funding of more than € 2 million is being allocated to 17 regional networks for lifelong learning. The projects involve 120 regions which are committed to developing advanced lifelong learning education and training strategies.

3. Fisheries & Aquaculture Education in Europe

In order to meaningfully address fisheries and aquaculture education in Europe, some understanding of the European fisheries and

aquaculture sector is essential (Section 3.1). Pivotal in the EC policy towards this sector is the Common Fisheries Policy (Section 3.2).

3.1. Status of fisheries and aquaculture in Europe

Fishing and aquaculture provides a healthy and valued source of food, creates much-needed jobs in coastal areas and promotes the social and economic well-being of the European Union's fishing and culturing regions.

3.1.1. European fisheries facts & figures

Although the fishing sector's contribution to the gross national product of Member States is generally less than 1%, its impact is highly significant as a source of employment in areas where there are often few alternatives. In addition, it helps to supply fish products to the EU market, one of the biggest in the world. With a production of over 7 million tonnes of fish, in 2003, from fisheries and aquaculture, the EU is the world's second largest fishing power after China. Yet, while 6 million tonnes of fish products were exported, 10 million tonnes had to be imported to meet the needs of the EU. This imbalance between imports and exports resulted in a deficit of over € 10 billion in 2003.

Fisheries fleet capacity has declined over the past few years because it was too large for the available fish and had become uneconomic. The EU has facilitated the reduction of the fleet but further modernisation of the vessels is required.

3.1.2. European aquaculture facts & figures

According to the latest data available (FAO, 2006), EU aquaculture accounted for 2.3% of world aquaculture production, or some 1.38 million tonnes. This represents a value close to € 2.8 billion i.e. 7.5% of the value of world aquaculture production or roughly

30% by value of total EU fishery production (Fig. 1). However, these figures also vary considerably from sector to sector. EU production thus represents 5.7% of world shellfish production by weight, and only 1.3% for freshwater fish, but 10.9% for marine fish.

While global production rose by around 9% annually from 1995 to 2004, EU production grew by only 3 to 4% a year up to 1999, and may be considered to have stagnated since then. This stagnation itself disguises a decrease in mollusc and freshwater fish production, compensated by a continuing increase in marine fish.

The EU is well-placed to capitalise on the global growth in aquaculture. Europe has a strong market for seafood, a long tradition of freshwater and marine fish and shellfish cultivation, dynamic and advanced research, modern technology, qualified and trained entrepreneurs and fish farmers, suitable climatic conditions and sites for the species currently farmed. However, the EU aquaculture sector also faces a number of challenges which have an impact on production. These include limitation of space and of water of good quality, and measures to protect public health and the environment. The high EU standards put European aquaculture at the forefront of sustainable development in the world, both in terms of social and environmental impacts, but make it more difficult to compete price-wise with third-country producers especially in Asia and in South-America. A key issue to safeguard a competitive edge for the European fisheries and aquaculture sector is to provide the industry with well-trained workers, scientists and managers.

3.2. The common fisheries policy

The Common Fisheries Policy (CFP) is the European Union's instrument for the management of fisheries and aquaculture. Because fish and fisheries are located across country boundaries, fish and fisheries are

considered a common property. The Common Fisheries Policy ensures exploitation of living aquatic resources under sustainable economic, environmental and social conditions. To assure sustainability, the European Community applies a precautionary approach in adopting and implementing policies designed to protect and conserve living aquatic resources, to provide for their exploitation and to minimize the impact of fishing activities on marine eco-systems. This policy involves the progressive implementation of an eco-system-based approach to fisheries management that guarantees efficient fishing activities within an economically viable and competitive fisheries and aquaculture sectors. Finally, the CFP provides a fair standard of living for those who depend on fishing activities and takes into account the interests of consumers.

3.3. Specific education needs for the European fisheries and aquaculture sector

The trends in the European fisheries and aquaculture industry directly affect the European labour market. The diversification of the industry, that is mostly driven by new technological developments, calls for the training of a highly skilled and specialized workforce. Meanwhile, young entrants to the job market will be expected to be more flexible and mobile in the labour market. If education is to meet these labour market requirements than qualifications need to be more flexible, both in content and form, as well as more transparent (in accreditation and recognition). This means that a employer will be able to easily assess the qualifications of freshly graduated job applicants, wherever and whatever in Europe he/she has studied.

In traditional aquaculture facilities, farms are small and employees (and owners who tend to work on the site) usually are generalists who have to be capable of stock management, equipment maintenance and

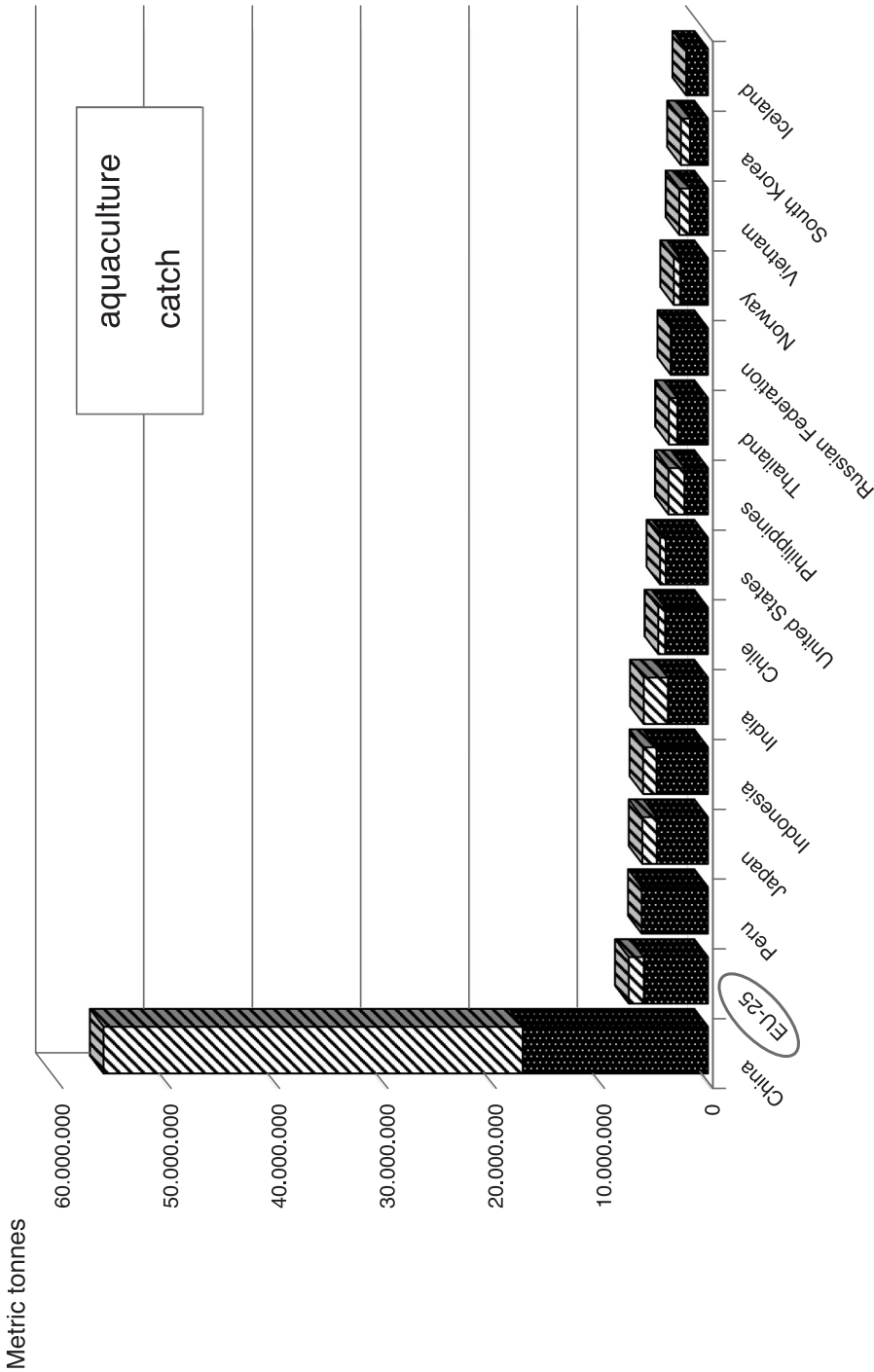


Fig. 1. Fishery and aquaculture production of 15 leading producing countries in 2005 (source: Facts and figures on the CFP 2006).

even selling the end product. The trend towards increased mechanization, specialization in production techniques and increased size of production units has led to the need for specialists. Recirculation units, used for the production of salmon smolts, eels, turbot and even some shellfish need both husbandry specialists and maintenance personnel familiar with the complex equipment needed to maintain water quality. Larger boats, cranes and forklift trucks, normal items of equipment on a sea pen farm, all require special training and certification for staff. Further, specialists are required in fields including bioengineering, biotechnology, fish biology, environmental issues, fish health, HACCP and food quality systems. Obviously, the increased complexity of aquaculture products and direct links with buyers also requires staff with special skills.

Traditionally, needs of the fishery and aquaculture sector for specifically trained employees were predominantly addressed by national or regional vocational and higher education. Since the recent European education reforms, nearly unlimited student mobility and improved accreditation has abolished local boundaries and students can now virtually 'shop' throughout Europe for study programmes that optimally meets their needs and interests. The range of study opportunities in aquaculture and fisheries has now extended to a European scale. This is a considerable asset for students, but also a challenge for universities that now face competition with an increasing number of similar programmes at universities that were previously too remote to compete. A number of European top-quality providers of aquaculture education have soon realized that it is wiser to cooperate than to compete. These universities (Universities of Algarve (Portugal), Bergen (Norway), Cork (Ireland), Trondheim (Norway) and Warmia & Mazury (Poland)) have formed a consortium and are working towards a modular European MSc programme in Aquaculture and Fisheries, called MAqFish.

3.4. AquaTNET

As a result of the reform of European education, a Thematic Network was established that specifically promotes harmonization of aquaculture education among all universities and institutes that provide aquaculture and/or fisheries education. AQUA-TNET is a multidisciplinary Thematic Network that unites the academic and vocational aspects of the Bologna reforms and the establishment of the European Higher Education Area in Aquaculture, Fisheries and Aquatic Resources Management. The network was established in 1996 and is funded by the European Commission. AquaTNET discusses and formulates recommendations on geographic and functional mobility of staff and students, trans-national placements, delivery of specialized and advanced education and training, joint development of specific courses, and accreditation and mutual recognition of learner² qualifications.

4. International Cooperation for Higher Education

Transeuropean education is becoming increasingly common in Europe. Many European countries are running exchange programmes via bilateral relations with non-European countries, and there are a number of transnational higher education initiatives within the European Union. However, in Europe there is a firm conviction that more should be done if European universities and learning centres are to derive the full benefits of internationalization in education.

European education ministers stated in the Bologna Declaration: "The vitality and efficiency of any civilisation can be measured by the appeal that its culture has for

² 'learner' is a term that is broader than 'student' in the sense that it includes all persons taking part in a learning process, irrespective of the age or career stage of that person.

other countries. We need to ensure that the European higher education system acquires a world-wide degree of attraction equal to our extraordinary cultural and scientific traditions” (Bologna declaration, June 1999). Later statements encouraged international collaboration on education with third countries³ outside the EU.

4.1. Perceptions of European higher education in other parts of the world third countries

During 2005, a major study was carried out entitled: ‘Perceptions of European higher education in third countries’ (Academic Cooperation Association 2004). The conclusions of this study served as a basis to shape instrument to improve the international position of European education, mostly through the ERASMUS Mundus programme.

4.1.1. What were the main findings of this study?

- Europe is regarded as a union in an economic and political respect, but not in terms of higher education. When it comes to higher education, the perception focuses on the continent’s individual countries, and mostly on the larger ones (Germany, France, UK, ...).
- Europe’s higher education institutions are perceived positively: Students coming to Europe cited the high quality, accessibility and long tradition of European universities. However, the study also showed that Europe is losing out to the US on a number of issues, such as the perceived prestige of institutions, labour-market acceptance of qualifications, and the dynamism and innovation capacity of our university campuses.
- The most important factors influencing the decision for a destination were quality of education, reputation and prestige of the institution and of the degrees earned, as well as affordability, and safety.

- A very clear signal emerged that there is a lack of information on higher education in Europe, the outstanding opportunities for study which it offers and the advantages of studying here compared with other parts of the world.

Based on the survey results, the following recommendations were formulated:

- A perception of European higher education as a whole should be created, by means of a “European brand”, with characteristics common to all European countries.
- Study opportunities in Europe must be have higher recognition outside of the European Union. Higher recognition of European educational opportunities can be achieved by way of a coherent and convincingly implemented information policy, including the creation of a single, well functioning web portal.
- Marketing alone is not sufficient: Europe must improve its educational programmes in order to become and remain attractive to students from elsewhere.

4.2. ERASMUS Mundus

As mentioned above, the ERASMUS Mundus programme is the ‘international’ (read: extra-European) section of the higher education programmes installed by the European Commission. Erasmus Mundus was first introduced in July 2001, as a response to a Communication by the European Commission on strengthening EU-third country co-operation in higher education. The Commission adopted a programme proposal, Erasmus World, in July 2002. The programme was then renamed Erasmus Mundus. “Mundus” is the Latin word for “world”.

³ In a EU context, ‘third countries’ is commonly referring to all non-EU countries.

The Erasmus Mundus programme is a co-operation and mobility programme in the field of higher education. It aims to enhance quality in European higher education and to promote intercultural understanding through co-operation with third countries. The Erasmus Mundus programme has earned political support from governments, policy-makers and higher education institutions all over Europe. The programme is seen as a useful means to respond to the challenges European higher education faces today, in particular the need to promote convergence of degree structures and to enhance the attractiveness of European higher education world-wide.

Erasmus Mundus supports European top-quality Masters Courses and provides EU-funded scholarships for third country nationals participating in these Masters Courses, as well as scholarships for EU-nationals studying at partner universities throughout the world. The budget of the programme is 230 million euros for 5 years (2003–2008), plus 66 million euros for student scholarships for citizens coming from a range of specific countries (China, India, etc.).

Inspired by the highly successful Erasmus programme (an internal EU programme supporting cooperation and mobility between European higher education institutions) Erasmus Mundus offers a framework for valuable exchange and dialogue between cultures. Erasmus Mundus is a global scheme, providing a distinct “European” variety in higher education programmes to those beyond EU borders. By supporting the international mobility of scholars and students, Erasmus Mundus prepares European and non-European participants for life in a global, knowledge-based society.

Erasmus Mundus complements the European Union’s existing regional programmes and bilateral agreements on higher education with third countries. Regional programmes, such as Tempus, ALFA and Asia-

Link, will continue to foster international co-operation in higher education between the European Union and its partners.

The specific aims of the Erasmus Mundus programme are:

- The support of joint programmes of outstanding academic quality at Master’s and Doctoral level, including a scholarship scheme for high-calibre EU and third-country students and academics;
- The promotion of partnerships between European and third-country universities in specific regions as a basis for structured co-operation, transfer of knowledge, and exchange and mobility at all levels of higher education;
- The support of measures which will help to enhance the world-wide appeal of Europe as an educational destination.

To achieve these objectives, four main actions were initially launched: Action 1 — Erasmus Mundus Masters Courses, comprising integrated courses at masters level offered by at least three universities in three different European countries; Action 2 — Erasmus Mundus scholarships for students and scholars from third countries; Action 3 — Partnerships with higher education institutions in third countries. The partnerships offer scholarships for students and scholars from EU countries for research and study mobility towards third countries; Action 4 — Projects to enhance the worldwide attractiveness of European higher education. In the second phase of the programme a number of changes were introduced:

- More opportunities and variety in the institutional cooperation activities between European and third-country universities and in the individual mobility scheme;
- Extension of Erasmus Mundus to doctoral studies and, partially, to the undergraduate level;
- Stronger financial support for European students through the offer of more attractive scholarships.

Table 1. Currently approved European Master programmes in the field of maritime and aquatic sciences.

Title	URL
EMBC (Erasmus Mundus Master of Science in Marine Biodiversity and Conservation)	embc.marbef.org
Comem Erasmus Mundus MSc (Coastal and Marine Engineering and Management)	www.comem.tudelft.nl
EURO-AQUAE (Euro Hydro-Informatics and Water Management)	www.euroaquae.org
European Joint Master in Water and Coastal Management	www.ualg.pt/EUMScWCM

Erasmus Mundus started in 2004. In the first three academic years (2004–2006), more than 2,300 students have participated in the programme and over 1,800 have been selected to start their studies in Europe in September 2007. The second phase of Erasmus Mundus builds on this by becoming the EU reference programme for cooperation with third countries in this area. Over a period of five years, just over 950 million euros will be available for European and third-country universities to join forces in joint programmes (masters and doctorates) or collaborative partnerships, and to grant scholarships to European and third-country students for an international study experience.

To date, 103 MSc programmes have successfully applied for the European Master label. Unfortunately, it is a competitive selection process and only 4 programmes related to marine sciences, fisheries or aquaculture have as yet been selected (see Table 1). This limited success does by no means imply that there is little international cooperation on fisheries and aquaculture education by European countries. Bilateral agreements and other large initiative provide several valuable approaches to cross boundary fisheries education. Individual agreements among countries and NGO's are much harder to trace and assess. Typically, cooperation projects with a distinct educational aspect involve student and staff exchange (in both directions!) for the reinforcement of

research-based educational capacity. Individual agreements also support infrastructure improvement ranging from construction of teaching facilities (classrooms, laboratories, ...) to computer and communication support. Currently, the growing maturity of the cooperation between partners increasingly allows for a more comprehensive approach including joint study programmes, mutual recognition of study modules and eventually joint degrees. For instance, Ghent University (Belgium) with Trondheim University (Norway) and Wageningen University and Research Center (The Netherlands), have launched an initiative with both a Chinese and a Vietnamese consortium of aquaculture and fisheries institutes and universities to exchange MSc students for thesis work and PhD students for research stays.

5. Conclusions

Internationalisation of education is a priority for Europe as it is believed to be the basis for better intercultural understanding. Education is an excellent instrument to spread European cultural richness and diversity and technological assets. Substantial efforts have been invested to increase attractiveness of European education for non-European students. Support for more comprehensive education ranges from support for student and staff mobility in between education institutions and the development of

integrated courses and eventually joint degrees.

Parallel to this process, European education is reforming towards pan-European harmonised study structures and complete transparency and mobility among European Universities. These internationalisation and harmonisation processes are interlaced and contribute equally to the Lisbon objective of making Europe the most competitive and dynamic knowledge-based economy in the world by 2010.

Aquaculture and fisheries education is characterized by rapidly increasing specialisation and diversity, both geographically and technologically. AquaTNET provides an example of an European network for higher

education in aquatic resources. AquaTNET attempts to harmonize education programmes and represent the interests of all fisheries and aquaculture universities and institutes on the European level.

International cooperation in aquaculture and fisheries education is still predominantly managed by individual institutes or local partners but is increasingly regulated on a European level for instance through the ERASMUS Mundus programme of the European Commission.

Finally, international education cooperation is evolving from pure development cooperation to cooperation with mutual benefits between equal partners, a reassuring notion.

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