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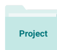

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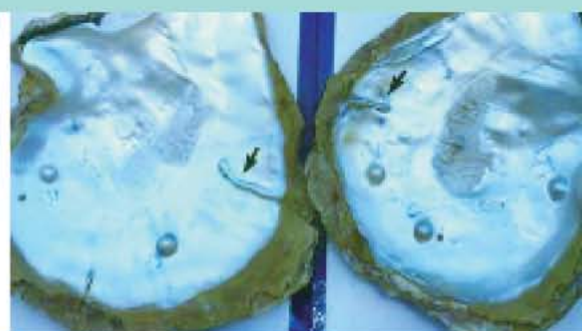

Cover photos:
Left: Pearl oyster farm in China (FAO/M.G. Bondad-Reantaso).
Right, top to bottom: Pinctada maxima with mudworms (FAO/M.G. Bondad-Reantaso); South Korean women cleaning and sorting mother-of-pearls before grafting (courtesy of F.C.J. Berthe/EFSA); South Sea pearls (FAO/M.G. Bondad-Reantaso).

Pearl oyster health management

A manual

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2007

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Preparation of this document

This document was prepared in an effort to determine what health management options can best support development and sustainability of the pearl oyster industry as well as collate past experiences in dealing with pearl oyster disease outbreaks and other health problems. This is the second occasion that FAO is publishing important information about pearl oyster. The first and pioneering publication was the Pearl Oyster Farming and Culture, an output prepared for the Pearl Oyster Farming Training Course (Training Manual 8) conducted by the Central Marine Fisheries Research Institute at Tuticorin, India and organized by FAO's Regional Seafarming Development and Demonstration Project (RAS/90/002).

This paper was prepared under the technical supervision of Dr Melba G. Bondad Reantaso, Fishery Resources Officer, Aquaculture Management and Conservation Service, Fisheries and Aquaculture Management Division, FAO Fisheries and Aquaculture Department.

Part 1 consists of two articles: "Why the interest in pearl oyster health?" by Dr Sharon E. McGladdery of the Canadian Food Inspection Agency (CFIA) and "Overview of the cultured marine pearl industry" by Prof. Paul Southgate of James Cook University. Part 2 on Pearl oyster health management was jointly written by Dr Sharon E. McGladdery of CFIA, Dr Melba G. Bondad-Reantaso of FAO and Dr Franck C.J. Berthe of the European Food Safety Authority. Part 3, consisting of experiences in dealing with pearl oyster mortalities and other health management options, was contributed, in alphabetical order by Dr Franck C.J. Berthe (France/Italy), Dr Jeremy Carson (Australia), Dr Melba G. Bondad-Reantaso (Philippines/Italy), Dr Ben Diggles (New Zealand/Australia), Dr Francis Mike Hine (New Zealand/France), Dr J. Brian Jones (Australia), Ms Daisy Ladra (Philippines), Dr Sharon E. McGladdery (Canada), Dr Jean Prou (France), Dr Katsuhido Wada (Japan) and Dr Wang Chongming (China).

Abstract

The pearl oyster industry is a growing multibillion dollar sector of mollusc aquaculture. Pearl farming occurs throughout Australasia, the Middle East and South America. Few species of molluscs possess the ability to produce pearls of gem quality. The South Sea pearl oyster is one of them. Pearl production in the wild is an unpredictable and uncontrolled event which human intervention, through pearl culture, has progressively overcome by improving culture practices. Farming mother-of-pearls shares commonalties with edible mollusc aquaculture. However, the endproduct, pearl production, is unique to this sector. In aquatic production, health issues are of utmost importance; pearl production is based entirely upon health. The pearl itself is a product of the oyster's immune defences as a response to soft-tissue irritation. Exploited stocks receive frequent handling stresses which often predispose farmed animals to infection and diseases. Therefore, the importance of health management for pearl oysters is paramount. Today, most disease problems are caused by opportunistic pathogens taking advantage of oysters weakened by the stress of handling, including pearl surgery and sub-optimal growing conditions. Except for the mass mortalities experienced in Japan, the pearl oyster industry have not yet faced the types of epizootics which has impacted mollusc culture elsewhere in the world. Development of the industry will, inevitably, lead to increased risk of disease introduction, spread or emergence. Against such an unwanted future, health management is the critical defence line.

The objectives of this technical paper are to: (i) review pearl oyster mortalities and disease problems in order to help design programmes aimed at reducing the risks from diseases; and (ii) provide technical guidance to pearl oyster farmers and the industry on management of pearl oyster health so that sector development will be sustainable not only in providing huge employment to communities where pearl farms are located but also contributing to maintain environmental integrity. Pearl oyster farming can serve as environmental sentinels recognizing the fact that pearl oysters thrive only in pristine environment.

This publication contains three parts. Part 1 consists of pearl oyster health – the current interest in it and an overview of the cultured marine pearl industry. Part 2 on pearl oyster health management consists of seven sections, namely: (a) introduction; (b) general information on husbandry and handling, hatchery production, introductions and transfers; (c) disease diagnostic protocols dealing with field collections of samples, gross external examination, gross internal examination and laboratory protocols; (d) health zonation; (e) disease outbreak protocols; (f) national strategies on aquatic animal health; and (g) references. Certain countries in the pearl oyster producing regions have acquired a great deal of experience in health management of cultured species. Experiences from Australia, the Cook Islands, Japan, the French Polynesia, the Philippines, China, the Persian Gulf and the Red Sea are included in Part 3 which also contains a general review of pearl oyster mortalities and disease problems.

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Pearl oyster health management: a manual.
FAO Fisheries Technical Paper. No. 503. Rome, FAO. 2007. 120p.

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Abbreviations and acronyms

ASBUMI	Indonesian Pearl Culturer's Association
ASE	accelerated solvent extraction
BFAR	Bureau of Fisheries and Aquatic Resources (Philippines)
BHIA	brain heart infusion agar
BOD	biological oxygen demand
BRD	Brown Ring Disease
CMFRI	Central Marine Fisheries Research Institute
DO	dissolved oxygen
EDTA	ethylenediaminetetraacetic acid
EM	electron microscopy
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
GC-FID	gas chromatography/flame ionization detection
ICES	International Council for the Exploration of the Seas
ICP-MS	inductively coupled plasma mass
LD50	lethal dose 50
MA	marine agar
MMR	Ministry of Marine Resources (Cook Islands)
MOP	mother-of-pearl
NT	Northern Territory
OIE	World Organisation for Animal Health
PCR	polymerase chain reaction
PPTA	phosphotungstic acid
QLD	Queensland, Australia
RFTM	Ray's Fluid Thioglycollate Medium
SOD	sediment oxygen demand
SOPAC	the Pacific Islands Applied Geoscience Commission
TCBS	thiosulphate citrate bile salt
TEM	transmission electron microscopy
TSA	tryptone soya agar
WA	Western Australia

Glossary

Abcess	an aggregation of haemocytes (blood cells) which contains necrotic (decaying) host cells
Akoya pearl	saltwater pearls cultivated from *Pinctada fucata*; the mainstay of the Japanese and Chinese cultured pearl industries
Bivalve mollusc	a mollusc, such as an oyster or a clam, that has a shell consisting of two hinged valves. Bivalves are members of the phylum Mollusca, class Bivalvia.
Blister pearls	a natural pearl, usually irregular in shape, which occurs when a parasite (or an irritant) enters a mollusc through its outer shell causing the mollusc to secrete nacre over the irritant, cementing it to the shell
Ceroid	non-staining metabolic by-product found in many bivalves. Abnormally high concentrations indicate possible environmental or pathogen-induced physiological stress
Conchiolin	nitrogenous albuminoid substance, dark brown in color, that forms the organic base of molluscan shells
Concretions	non-staining inclusions in the tubule and kidney cells of pearl oysters, produced during the digestive cycle. Similar inclusions are also found in the epithelia of other bivalves
Cultured pearl	pearls which are produced by the reaction of an oyster or molluscs to insertion of a foreign object (called a nucleus or a bead) into its tissue; this induces secretions to cover the nucleus. Culture pearls are formed when a pearl oyster secretes nacre over the nucleus
Fouling	accumulation and deposition of living organisms and certain non-living material on hard surfaces, most often in an aquatic environment
Gold-lip oyster	one of two varieties of *Pinctada maxima*; the other is the silver-lipped oyster. The names relate to the colour of the mother-of-pearl lining the shell
Gonad	the reproductive organ that produces either the sperm or the eggs. gonads in males are called testes; gonads in females are called ovaries
Grafting	also known as “seeding”, “nucleus implantation” or “nucleation” is a surgical procedure where a nucleus and a small piece of mantle tissue (from another oyster) are inserted into the gonad for cultured pearl formation

Mabé pearl — a pearl which is formed when a flat-sided nucleus is glued to the inside of a pearl oyster shell. Also known as "half-pearls" and "blister-pearls" they can be made in a variety of shapes determined by nucleus shape

Mantle — the part of a pearl oyster's soft tissue that lines the inside of the shells and secretes nacre

Mantle retraction/ recession — during periods of no growth in molluscs, the mantle retracts away from the edge of the shell. Prolonged mantle retraction leaves the inner shell edge open to erosion and fouling

Mikimoto pearls — a leading brand of pearls founded by the Japanese Kokichi Mikimoto, the Japanese credited for creating the cultured pearls

Mother-of-Pearl — the substance which is secreted by pearl oysters to line the inside of their shells. It is also called "nacre" and is the same substance which forms pearls. Mother-of-pearl is now used extensively as the nucleus in pearl cultivation. The shell of a mussel is cut into squares and then run through a process which rounds the pieces into beads. These beads are then implanted into the oysters which then secrete nacre upon the mother-of-pearl beads to form the cultured pearl

Nacre — also known as mother-of-pearl is the basic substance which is secreted by oysters and molluscs after a foreign substance (e.g. a grain of sand, a piece of rock or even a parasite) has entered the shell and caused irritation. Nacre is composed of layers of calcium carbonate (in a crystalline form) and conchiolin (an organic protein substance which provides bonding)

Natural pearl — pearls which are formed in nature, following the actions of a parasite or foreign body lodging itself in the gonad or mantle tissues of a host oyster

Nucleus — a bead or implant onto which nacre is secreted to form cultured pearls. They may be round (round pearl production) or flat sided (mabé production). Round nuclei are generally made of mollusc shell that has been cut, rounded and polished

Pearl — a hard, round object produced by certain animals (primarily molluscs) such as pearl oysters particularly valued as a gemstone and is cultivated or harvested for jewellery

Pearl formation — when a small irritant or parasite penetrates the shell and irritates the mantle tissue a pearl may be formed when nacre is secreted as a response. As nacre builds up in layers, it surrounds the irritant forming a pearl. Pearls that form within tissues generally do so when mantle epithelial cells are dislodged into the tissue

Pearl oyster — bivalve molluscs of the Family Pteridae (genera *Pinctada* and *Pteria*) all members of the Family share the physiological properties that lead to the production of large pearls of commercial value

Pinctada fucata	Akoya pearl oyster producing cultured Akoya pearls
Pinctada margaritifera	the black-lip pearl oyster producing the “black” South Sea pearls
Pinctada maxima	the gold-lip or silver-lip pearl oyster producing “white” South Sea pearls
Pteria penguin	species of pearl oyster also known as the “winged pearl oyster”, rainbow pearl oyster” or “penguin shell” used to primarily to produce mabé
Pteria sterna	species of pearl oyster from Central America also known as “concha nácar” or “rainbow lip pearl oyster” used to produce mabé and cultured round pearls
South Sea Pearls	pearls produced by both *Pinctada maxima* and *P. margaritifera* which are differentiated on the basis of their colour
Spat	young juvenile pearl oyster or other bivalve mollusc

PART 1

PEARL OYSTER HEALTH AND INDUSTRY

1.1 **Why the interest in pearl oyster health?**
Sharon E. McGladdery

1.2 **Overview of the cultured marine pearl industry**
Paul C. Southgate

1.1 Why the interest in pearl oyster health?

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McGladdery, S.E. 2007. Why the interest in pearl oyster health? pp. 3–6. In: M.G. Bondad-Reantaso, S.E. McGladdery and F.C.J. Berthe. Pearl oyster health management: a manual. *FAO Fisheries Technical Paper*. No. 503. Rome, FAO. 120p.

The multibillion dollar value pearl oyster industry, which is growing throughout southeast Asia, Australia, India, the Middle East and South America, is based entirely upon shellfish health. The pearl itself is a product of the immune defenses of the mollusc, which rally to wall off any indigestible irritation to the soft-tissues (Sparks, 1985; Awaji and Suzuki, 1995). All molluscs possess this ability, however, certain species, such as the Pteridae of the South Pacific, produce pearls of gem quality. Since significant energy is required to lay down this defensive pocket of calcium carbonate and protein, the most productive pearl oysters are those that are in good health with access to the energy reserves required (Numaguchi, 1996).

In the wild, pearl production is a random event, with environmental conditions and physiological stresses on individual oysters playing a key role in the quality of the end product. Human intervention, in the form of pearl culture, reduces the uncertainty and waste in searching for "wild pearls" and has evolved into a multibillion dollar industry, with various shapes, sizes and colours of pearls being produced with consistent quality (Fassler, 1998).

The pearl oyster industry shares commonalities with edible mollusc aquaculture world wide, on top of which the end product, pearl production, is unique to the sector.

Increasing pearl culture means an increased demand for pearl producing oysters, with certain species and stocks being exploited more heavily than others. It also means that cultured oysters receive greater handling than non-cultured oysters. This has led to greater awareness and monitoring of oyster health and survival – a positive step for selecting the fittest stocks or individuals for pearl surgery. However, the same handling and monitoring presents a physiological stress that the oyster would not normally face in the wild (Dybdahl, Harders and Nicholson, 1990).

The energy requirements for pearl production have to be carefully balanced against the need to handle and grow the oysters in conditions they did not evolve to fit (Dybdahl, Harders and Nicholson, 1990). This balancing act is simplified if no additional factors, such as overcrowding (Intés, 1994), overfishing (Sims, 1992 a,b) or disease (Dybdahl, Harders and Nicholson, 1990) enter the equation. Interestingly, pearl oysters have not yet faced the types of contagious disease agents which have plagued mollusc culture elsewhere in the world. Most disease problems center upon opportunistic pathogens taking advantage of oysters weakened by the stress of handling, including

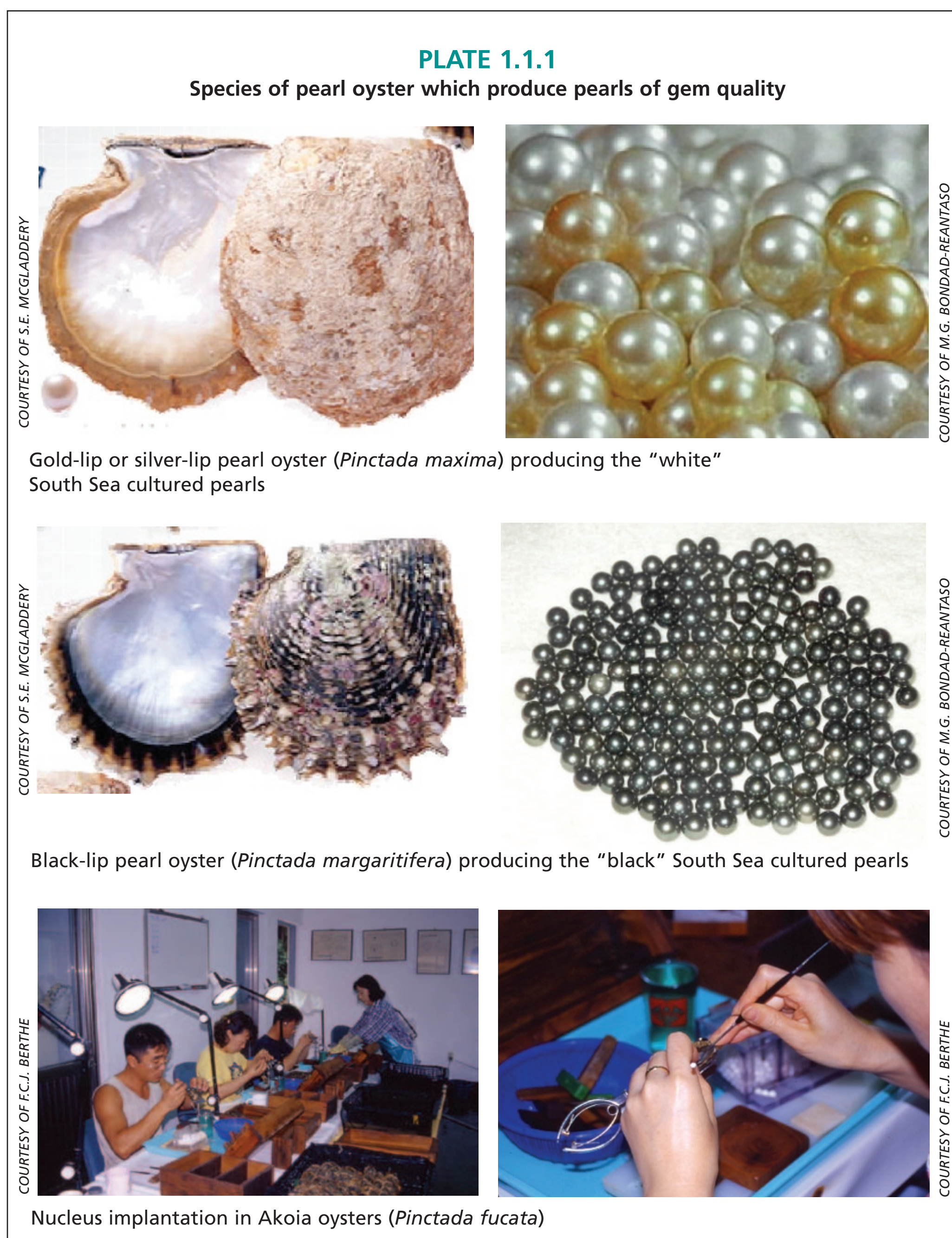

PLATE 1.1.1

Species of pearl oyster which produce pearls of gem quality

Gold-lip or silver-lip pearl oyster (*Pinctada maxima*) producing the "white" South Sea cultured pearls

Black-lip pearl oyster (*Pinctada margaritifera*) producing the "black" South Sea cultured pearls

Nucleus implantation in Akoia oysters (*Pinctada fucata*)

pearl surgery and sub-optimal growing conditions (Dybdahl, Harders and Nicholson, 1990; Sims, 1990; Rio-Portilla, Re-Araujo and Voltolina, 1992; Buestel *et al.*, 1995). The lack of contagious disease problems, although an unquestionable blessing, has also left the industry with relatively minimal pathology support or a good reference of information documenting normal versus abnormal parasites, pest and diseases for the various species cultured (Sims, 1990; Joll, 1992). Since increased development of the industry will, inevitably lead to pressure to select oysters from more and more remote sources (Wada, 1993, 1996; Benzie, 1994; Fassler, 1994, 1998; Sims and Sarver, 1994; Numaguchi, 1995) and sub-optimal growing areas (Gervis and Sims, 1992), this increases the risk of accidental disease introduction or induction. Both remote sources and mixed stocks enhance the chance of introducing a pathogen to a naïve or vulnerable (stressed) population (Sindermann, 1986; ICES, 1995) and the best defense against such an unwanted event is a solid knowledge of the health profiles of the animals on a culture site, as well as those from source sites.

Once an epizootic occurs in an aquatic habitat, the chances of eradication and control are limited. In fact, there are no examples, to date, of any molluscan disease agent being actively eradicated from an open-water system. This is important to remember when conducting risk-benefit analyses for new species, stocks, growing techniques or habitats. It is also an important fact to remember when mortalities are observed and quick health management action is required.

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INTRODUCTION

In the early twentieth century, the ability of pearl oysters to secrete mother-of-pearl (MOP) was harnessed for production of cultured pearls. Today this technique supports a global multi-million dollar industry, which utilizes a number of pearl oyster species in more than 30 countries. While the products of this industry adorn the jewelry shop windows of our major cities, it is also an industry that offers economic opportunities to coastal communities in less developed countries; an industry which involves individuals, cooperatives and families, as well as large multinational companies.

Global marine cultured pearl production and the major producing countries are shown in Table 1.2.1. The major species used to culture marine pearls are the silver-lip or gold-lip pearl oyster (*Pinctada maxima*), the black-lip pearl oyster (*P. margaritifera*) and the Akoya pearl oyster (*P. fucata*[1]). Species from the genus *Pteria*, commonly known as winged pearl oysters, also make notable contributions to global cultured marine pearl production. Table 1.2.1 shows the total cultured pearl production in 2004 had a farm gate value of around US$625 million of which freshwater pearls contributed

TABLE 1.2.1
Major cultured pearl producing species and the value of world cultured pearl production at the pearl farm level in 2004 according to Golay's estimates

Species	Pearl type	Major producers	Value US$ millions	Percentage of supply
Pinctada maxima	White South Sea pearls	Indonesia Australia Philippines Myanmar	220	35
Pinctada fucata	Akoya pearls	Japan China	135	22
Pinctada margaritifera	Black South Sea pearls or Tahitian Pearls	French Polynesia Cook Islands	120	19
Freshwater mussels	Freshwater pearls	China Japan	150	24
Total			**625**	**100**

Source: Anon. (2006)

[1] It should be noted that there is confusion regarding the taxonomic status of the Akoya pearl oyster which is probably best considered a complex including *P. fucata*, *P. martensii*, *P. radiata* and *P. imbricata*.

around 24 percent. Global marine pearl production in 2004 had an estimated value of approximately US$475 million of which white South Sea pearls from *P. maxima* contributed more than 46 percent.

Silver-lip/gold-lip pearl oyster, *Pinctada maxima*

Pinctada maxima is the largest pearl oyster species (Shirai, 1994) and is consequently used to produce the largest cultured pearls (approximately 10-20 mm in diameter). It is distributed within the central Indo-Pacific region, bounded by the Bay of Bengal to the west, Solomon Islands to the east, the Philippines to the north, and northern Australia to the south.

The terms "South Sea cultured pearl" and "South Sea pearl" are used for pearls produced in marine waters south of Japan. These names are associated with large cultured pearls produced from both *P. maxima* and *P. margaritifera* (Strack, 2006). The international market recognizes and distinguishes between "white" and "black" South Sea cultured pearls, produced by *P. maxima* and *P. margaritifera*, respectively.

The major producers of cultured pearls from *P. maxima* are Indonesia, Australia and the Philippines with approximately 40 percent, 32 percent and 20 percent of total production, respectively (Table 1.2.2). Total production of pearls from *P. maxima* in 2005 was more than 9.3 tonnes with a total value of US$248 million. Pearl production from *P. maxima* increased by approximately 260 percent between 1999 and 2005 (Henricus-Prematilleke, 2005) to become the leading pearl category.

COURTESY OF JOSEPH TAYLOR

South Sea pearls produced from *Pinctada maxima* range from white/silver through to gold in colour. They are the largest of the cultured pearls.

Australia

Pearl production began in Australia in the 1950s. Total pearl exports generally varied between 200 000 and 600 000 pearls per year from 1965 to 1995. There was a decline from 500 000 to 50 000 pearls per year in the mid-late 1980s resulting from high oyster mortalities (Pass, Dybdahl and Mannion, 1987). Exports increased from 200 000 to 2 million pearls per year between 1995 and 2006, however, the unit value of exported Australian pearls reached a 20 year low during 2004-2006. This decline probably reflected increased production, as well as external factors such as the Asian economic crisis of the late 1990s.

Australia enjoys an excellent reputation for the quality of its pearls.

TABLE 1.2.2
Production of cultured white South Sea pearls from Pinctada maxima in 2005

Country	Volume (kg)	Value (US$ millions)
Indonesia	3 750	85
Australia	3 000	123
Philippines	1 875	25
Myanmar	563	13
Malaysia	75	2
Papua New Guinea	75	unknown
Total	9 338	248 million

Source: Henricus-Prematilleke (2005)

This is demonstrated by the data in Table 2 showing that Australian pearls made up approximately 32 percent of total white South Sea pearl production in 2005 but accounted for almost 50 percent of the total value. However, Australia faces increasing competition from other producers who, one would assume, will be seeking to improve pearl quality. The Australian pearl industry is based primarily on adult oysters that are collected from the wild and used directly for pearl production (Wells and Jernakoff, 2006). The proportion of hatchery produced oysters used by the industry is therefore small (approximately 20 percent). Given that hatchery production provides the basis for selective breeding programmes, this strategy may, in the long term, favour other producers of white South Sea pearls, such as Indonesia, that rely on hatchery production.

Pearl farm workers clean nets containing cultured *Pinctada maxima* from a floating pontoon at a farm in West Irian, Indonesia. The nets are suspended from a long-line which is held on the surface using floats.

COURTESY OF JOSEPH TAYLOR

Indonesia, Philippines and other countries

The Indonesian cultured pearl industry began in the 1970s when new laws enabled foreign companies to invest in Indonesia. The 1990s brought much-needed modernization of pearl farms resulting primarily from investment by foreign companies, which entered partnerships in Indonesia. The Indonesian Pearl Culturer's Association (ASBUMI) was founded in 1995 to develop marketing strategies. By 1999, Indonesia supplied more than a third of the world's South Sea cultured pearls and by 2005 production had risen to more than 3.7 tonnes (Table 2). There are currently around 107 pearl farms in Indonesia. All commercial pearl production is hatchery-based and the industry is supplied by at least 36 hatcheries.

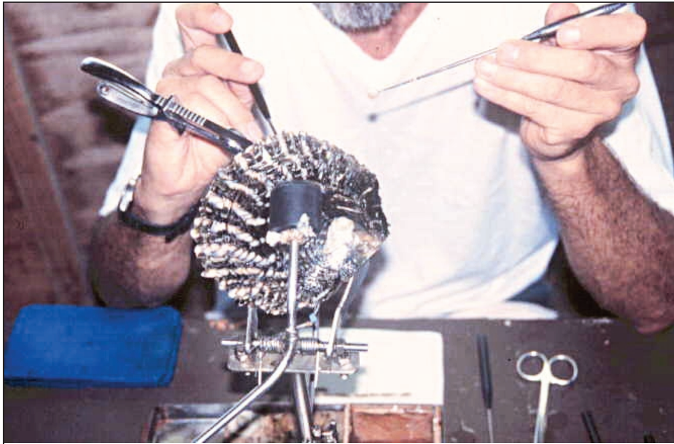
Production of South Sea pearls from around 30 farms in the Philippines has risen from approximately 0.5 tonnes to 2 tonnes a year since 1999. Many of the farms have Japanese partners and much of the crop is exported to Japan. The pearl farms are centered to the north of Palawan Island and the adjoining Calamian group, in Samar and Cebu Island around the southern tip of Palawan and in Mindanao Island. Only wild collected *P. maxima* were used for pearl production until about 1990; however, hatchery-produced oysters have played an increasingly important role since the end of the 1990s.

Other countries producing significant quantities of cultured South Sea pearls from *P. maxima* include Myanmar, Malaysia and Papua New Guinea (Table 2). Small-scale pearl production from *P. maxima* also occurs in Thailand (Bussarawit, 1995), northern Viet Nam and south-western China.

Black-lip pearl oyster, *Pinctada margaritifera*

Pinctada margaritifera has a wide geographical distribution from the Red Sea and east Africa to eastern Polynesia. Despite its vast range, this species is used for commercial cultured pearl production almost exclusively within the atoll lagoons of Polynesia, in French Polynesia and the Cook Islands. It is the second largest pearl oyster species and generally produces cultured pearls in the 9–20 mm size range.

Kokichi Mikimoto established a pearl farm at Ishigaki, Okinawa in 1914 and a second farm in Palau in 1923 from where he succeeded in producing round pearls from *P. margaritifera* (Hisada and Fukuhara, 1999). In 1951, there were nine companies



COURTESY OF JOHN LUCAS

A technician inserts a nucleus and a piece of mantle tissue from a donor oyster into the gonad of a host oyster (*Pinctada margaritifera*) for cultured pearl production. This process is called "seeding", "grafting" or "nucleation".

in Okinawa producing cultured black pearls. Only one of these survived and it reports annual production of approximately 2 000–3 000 pearls annually (Hisada and Fukuhara, 1999). Okinawan pearls provided the basis for market acceptance of cultured black pearls in Japan and, when French Polynesia became the dominant producer of these pearls in the mid-1970s, their product found a ready market.

French Polynesia

The cultured pearl industry in French Polynesia is based on collection of wild pearl oyster spat (juveniles), which are grown to a size suitable for pearl production (approximately 100 mm). Spat are easily collected by immersing a suitable substrate into lagoon waters when pearl oyster larvae are abundant. The larvae attach to the "spat collectors" and grow into juveniles, which are removed to become culture stock when required. The geomorphology of the atolls of eastern Polynesia, and their limited flushing by oceanic water, support abundant aggregations of pearl oyster larvae and high rates of spat collection. Using natural spat collection, it was easy for island residents to develop their own farms throughout the archipelagos of French Polynesia and there was a rapid increase in the number of authorized leases for pearl farms throughout the 1980s and into the late 1990s. By 2001, the number of pearl farms in French Polynesia had reached more than 2 500.

The first 71 cultured round black pearls were harvested in French Polynesia in 1972 and by 1977 the harvest had risen to 28 000 pearls. The rapid increase in the number of pearl farms during the 1980s and 1990s supported an exponential rise in pearl production, which peaked at approximately 11 tonnes in 2000 with a value of approximately US$170. However, over- production, declining pearl quality and a flood of lower grade pearls brought prices for black pearls down and market demand declined. Pearl exports from French Polynesia between 2000 and 2005 declined by more than 20 percent and their value declined by approximately 40 percent. Total production in 2005 was in the range of 8–9 tonnes and currently represents approximately 20 percent of total pearl market value (Table 1). Government regulatory measures now maintain a minimum standard for pearls exported from French Polynesia.

Recent years have seen a decline in the number of pearl farms in French Polynesia to 516 in 2006. They vary from small (approximately <5 ha. in area) to large (>40 ha in area). Most farms are situated in the Tuamotu and Gambier archipelagos. Pearl culture is French Polynesia's second largest economic resource after tourism and the first in terms of exports. The industry generates employment for thousands of families spread over 30 islands in French Polynesia and is an essential part of the social and economic life of the country.

Cook Islands

Cook Islanders generated income from the collection and sale of *P. margaritifera* MOP until the early 1970s (Strack, 2006). Round pearl culture from *P. margaritifera*, using the technique developed in French Polynesia, began in 1972 and in 1991 the Cook Islands Pearl Farmer's Association offered 30 000 pearls for sale at its first auction. The industry peaked in 2000 with export revenue of US$18 million, accounting for

20 percent of the country's gross domestic product. However, poor farming practices, particularly overstocking, meant that the oysters were susceptible to disease. The industry was virtually decimated by a disease outbreak towards the end of 2000 when a rise in water temperature resulting from limited flushing of the Manihiki lagoon, combined with a mass spawning of oysters, triggered a rapid rise in the levels of pathogenic bacteria (Heffernan, 2006). To help ensure the long-term sustainability of the Cook Islands pearl industry and avoid further problems with disease, on-going monitoring of water quality and a greater understanding of the bathymetry and hydrodynamics in Manihiki lagoon have been critical in developing a Pearl Farming Management Plan for Manihiki (Heffernan, 2006).

There were 205 pearl farms in the Cook Islands in 2003 with an estimated 1 million cultured adult oysters. However, as a result of increasing pearl production in French Polynesia, low international pearl prices and the continuing impacts of the year 2000 disease outbreak, pearl export revenue from the Cook Islands declined to about US$2 million in 2005. Currently, 78 percent of the Cook Islands black pearl farms are within the lagoon of Manihiki Atoll where 90 farms nucleate approximately 900 000 pearl oysters annually to produce approximately 300 000 saleable pearls. The remaining 20 percent of pearl culture occurs on Penrhyn Atoll where pearl culture began in 1994. Pearl production in the Cook Islands amounts to approximately 5 percent of world production of black South Sea cultured pearls.

Other countries

Cultured pearl production from *P. margaritifera* has received considerable research attention in other parts of the Pacific and some has resulted in commercial production. In 2000, a pearl farm was established in the island of Vanua Levu in Fiji. The farm is situated in a deep bay on a high island, and subject to nutrient-rich upwelling – a situation that differs greatly from that of pearl farms in the oligotrophic atoll lagoons of eastern Polynesia. Approximately 80 percent of the farmed oysters are obtained from spat collectors. Local communities are engaged in spat collection, which provides the much-needed income to communities close to the farm. The first auction of "Fiji pearls" in Japan in 2007 offered 30 000 pearls (Anon., 2007).

Cultured round pearls from *P. margaritifera* have been produced from a number of research and pilot projects in other Pacific nations including Solomon Islands, Kiribati and Micronesia (Fassler, 2002; Ito, Jackson and Singeo, 2004; Southgate, 2004). *P. margaritifera* has also been used for trial mabé pearl production in Tanzania in a project to determine the potential of small-scale pearl production to generate income for coastal communities in support of marine conservation efforts (Southgate *et al.*, 2006).

Cultured "black" pearls produced from *Pinctada margaritifera* in Kiribati, central Pacific. The pearls are held in a *P. margaritifera* shell.

COURTESY OF BEERO TIOTI

Akoya pearl oyster, *Pinctada fucata*

There is considerable taxonomic confusion about the Akoya pearl oyster which at this stage is probably best considered as an unresolved species complex encompassing *Pinctada fucata*, *P. imbricata*, *P. martensii* and *P. radiata*. Members of this complex have a wide distribution from the Mediterranean Sea, through the Red Sea and Indian Ocean, including the Persian Gulf, into the Pacific Ocean and throughout southeast Asia and northern Australia. It also occurs in the Caribbean Sea.

COURTESY OF AIMIN WANG

Akoya pearls being sorted in a factory in Guangdong Province, China.

Japan

The technique for culturing round pearls from pearl oysters was developed in Japan using the Akoya pearl oyster. Regular mass production of cultured pearls using this method has occurred in Japan since 1916. By 1926, there were 33 pearl farms in Japan and by 1938, this number had increased to 360, which produced more than 10 million pearls. Harvests of cultured pearls in Japan increased rapidly from the 1950s. In 1952, production was almost 10 tonnes; this increased to 52 tonnes in 1960 and reached a peak of 230 tonnes in 1966 produced from 4 700 farms (Strack, 2006).

Pollution of pearl farming sites became an increasing problem and in 1976, only 2 000 pearl farms remained. This number had declined further to approximately 1 000 farms producing about 35 tonnes of pearls by 1977. In the 1980s, production could not meet demand for high quality pearls and large quantities of low quality pearls flooded the market. By this time, there was also strong competition to the Akoya pearl market from China's increasing production of freshwater pearls. Following greater emphasis on larger and better quality pearls in the early 1990s, which saw prices increase, in 1996 an epidemic claimed vast numbers of pearl oysters in Japan and was a catastrophe for the industry. It is estimated that the epidemic caused the loss of approximately 75 percent of the oysters in Japanese pearl farms. By 1999, annual pearl production had declined to < 20 tonnes with a value of approximately US$130 million, compared to an annual value of US$550–600 million in the early 1990s. Annual production levels have since remained at about 20–25 tonnes.

Mie Prefecture today produces about 33 percent of the total Akoya pearl harvest in Japan (Strack, 2006), with Ehime and Kochi Prefectures also contributing significantly to the total. Kyushu Island has produced slightly greater volumes of pearls than Mie and Ehime Prefectures since 1996, with about 40 perecent of total production coming from Nagasaki Prefecture (Strack, 2006). Constraints affecting Akoya pearl production in Japan include: (1) the impacts of parasites such as *Polydora* spp., boring sponges and trematodes (e.g. Mizumoto, 1975); (2) periodic abnormal blooming of toxic dinoflagellate algae or "red tide" (e.g. Honjo, 1994); (3) seasonal changes in seawater temperature and reduced food availability (e.g. Tomaru *et al*., 2002); and (4) mass mortalities associated with pollution, over-crowding and viral infection (e.g. Miyazaki *et al*., 1999). Pearl farm management practices that reduce the risk of mass mortalities of oysters have been recommended to pearl farmers, and genetic programs to breed resistant strains of oysters have been initiated (Uchimura *et al*., 2005).

China

Marine pearl oyster cultivation began in China in 1961 and pearl production increased rapidly during the 1980s when private farms became established (O'Connor and Wang, 2001). Annual Akoya pearl production was estimated to be greater than 20 tonnes at the start of the new millennium (Wang *et al*., 2007). The major culture areas are in the southern provinces of Guangxi, Guangdong and Hainan with Guangxi Province producing about 8-9 tonnes of pearls annually. There are over 1 000 pearl farms along the coast of Leizhou in Guangdong Province which, together with farms in

Xuwen, harvest approximately 9-10 tonnes of pearls annually; Akoya pearl production from Hainan Province is less than one tonne (A. Wang, pers. comm., 2007).

China produced 5-6 tonnes of marketable cultured marine pearls in 1993 and this stimulated Japanese investment in Chinese pearl farms and pearl factories. Pearl processing is done either in Japan or in Japanese-supported pearl factories in China. The majority of the higher quality Chinese Akoya pearls are exported to Japan. Additionally, MOP from pearl shells is used in handicrafts and as an ingredient in cosmetics, while oyster meat is sold at local markets.

Pearl farm workers clean and sort nets used for pearl oyster culture on a floating pontoon in Li'an Bay, Hainan Island, China.

India and other countries

India began Akoya pearl culture research at the Central Marine Fisheries Research Institute (CMFRI) at Tuticorin in 1972 and the first experimental round pearl production occurred in 1973. Although a number of farms have been established, particularly along the southeastern coast, commercial pearl farming has not become established on a large scale (Upare, 2001). Akoya pearls from India generally have a diameter of less than 5-6 mm (Mohamed *et al.*, 2006; Kripa *et al.*, 2007).

Halong Bay in the Gulf of Tonking in Viet Nam has been famous for its natural pearls for many centuries (Strack, 2006). Since 1990, more than twenty companies have established Akoya pearl farms in Viet Nam and production exceeded 1 000 kg in 2001.

Akoya pearl culture has also been investigated on the Atlantic coast of South America (Urban, 2000; Lodeiros *et al.*, 2002), in Australia (O'Connor *et al.*, 2003), Korea (Choi and Chang, 2003) and in the Arabian Gulf (Behzadi, Parivak and Roustaian, 1997). However, information on commercial production of cultured pearls from these regions is not yet available.

Young women clean pearl oysters and culture equipment from boats in Li'an Bay, Hainan Island, China.

Winged pearl oysters, *Pteria* spp.

The common name "winged pearl oyster" relates to the elongated hinge of *Pteria* spp. There are numerous species of *Pteria* but only two, *Pteria penguin* and *Pteria sterna*, are used for commercial scale pearl culture. *Pteria penguin* is cultured throughout Southeast Asia, in Australia and in some Pacific island nations (Beer and Southgate, 2000) and *P. sterna* is commercially cultured in the Gulf of California, Mexico (Kiefert *et al.*, 2004; Ruiz-Rubio *et al.*, 2006). *Pteria* spp. are generally used for mabé pearl (also called half pearl or blister pearl) culture and less commonly for round pearl culture. It is generally acknowledged that this is more difficult to achieve with *Pteria* spp. than

Shell of *Pteria penguin* with mabé pearls. The pearls will be drilled from the shell for processing.

Pinctada spp. as a result of morphological differences between genera. Only in recent years has successful production of round pearls from *Pteria* spp. been reported (Farell *et al*., 1998; Yu and Wang, 2004).

Pteria penguin

Pteria penguin is the most widespread cultured winged pearl oyster. It is readily collected using spat collectors although hatchery production has been described (Beer, 1999; Yu and Wang, 2004).

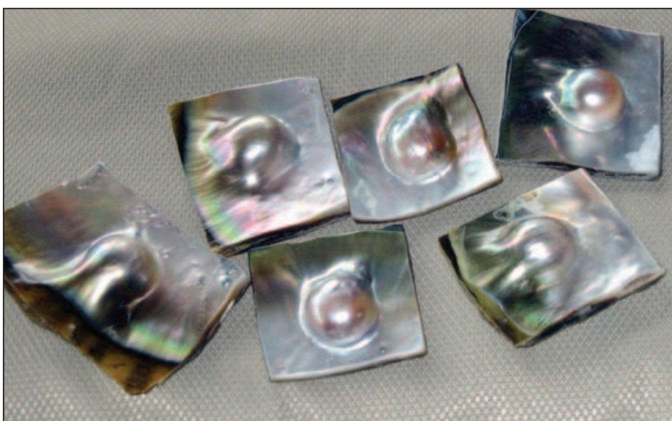
In the 1950s, Japanese companies began using *P. penguin* (called "mabé gai" in Japanese) on the Ryukyu Islands for production of mabé pearls. There are currently three or four companies in Ryukyu producing approximately 200 000 pearls per annum (Hisada and Fukuhara, 1999) from hatchery produced oysters.

Pteria penguin is widely distributed along the southern coast of China where it is used for hatchery-based pearl culture (Yu and Wang, 2004). Three companies have been established at Hainan Island and Leizhou Peninsula for cultivation of mabé pearls from *P. penguin* (Yu and Wang, 2004) and round pearls have also been produced from this species at Hainan Island.

The two major pearl farms at Phuket Island in Thailand were reported to hold 30 000 *P. penguin* for mabé production in addition to a number of smaller family farms that also produce mabé from *P. penguin* (Bussarawit, 1995). *Pteria penguin* collected as natural spat are used for production of mabé in Vava'u islands, Tonga (Finau, 2005).

Pteria sterna

There has been regular production of pearls, both mabé and round pearls, from *P. sterna* in Mexico since 1993. Development of a seeding technique for round pearl production from *P. sterna* was a breakthrough for the cultured pearl industry in Mexico (Nava *et al*., 2000) and research has also been carried out to determine factors that influence the quality of mabé pearls from this species (Ruiz-Rubio *et al*., 2006). The mabé are in the range of 12-15 mm and round pearls are generally sized between 6.5-8.5 mm, but may reach up to 14 mm. Current production is approximately 4 000 round pearls and 8 000 mabé annually.


Mabé pearls produced from *Pinctada magraritifera* in Tanzania, east Africa.

Natural spatfall of *P. sterna* can supply the oysters currently required by commercial pearl farms in Mexico. However, hatchery production of this species has been described (McAnally-Salas and Valenzuela-Espinoza, 1990; Araya-Nuñez, Ganning and Bueckle-Ramirez, 1995) as well as factors influencing nursery culture (e.g. Monteforte and Garcia-Gasca, 1994).

Other Pteria species

Pteria colymbus has recently been the subject of research in Venezuela and Colombia (Marquez *et al*., 2000;

Lodeiros *et al*., 1999), where it could be used to produce cultured round pearls of a size similar to Akoya.

Handicrafts made from lower grade pearls and pearl shell offer opportunities for income generation in coastal and island communities. The photograph shows participants in a pearl handicraft training workshop held in Kiribati, central Pacific.

COURTESY OF ANTOINE TEITELBAUM

Summary

The global cultured pearl industry is diverse in its methods, technological levels and products. In French Polynesia, oysters can be easily collected using spat collectors. This provided the opportunity for pearl culture to expand through the establishment of small- scale or family-based farms. An individual, or family, can enter the industry at a number of levels. They may simply collect spat for sale to a larger pearl farm, grow pearl oysters for their MOP, or produce mabé or round pearls. Furthermore, the pearl industry provides opportunity for the involvement of women and provides the raw materials for local handicraft manufacture, which may include lower grade pearls or pearl shell. In general, the pearling industry provides significant socio-economic benefits for coastal communities where it occurs (Tisdell and Poirine, 2000).

In contrast to family-based ventures, the dominant companies within the industry are large, wealthy and highly mechanized, and many have active research programmes. Hatchery cultivation of pearl oysters offers opportunity for selective breeding and stock enhancement, yet this area of research has been slowly embraced by the pearling industry compared to other aquaculture industries. Indeed, the two largest cultured pearl industries, in Australia and French Polynesia, are based on oysters collected from the wild. Furthermore, we still have limited understanding of the respective influences of genetics and environment on pearl quality. The next step in the evolution of the cultured pearl industry will probably be based on development of appropriate selective breeding programmes and improved knowledge of the factors influencing pearl quality.

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PART 2

PEARL OYSTER HEALTH MANAGEMENT

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2.1 Introduction

2.2 General

2.3 Disease diagnostic protocols

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2.1 Introduction

The pearl production industry has evolved significantly since its first development in Japan at the turn of the century. Expansion volume and species cultured for pearl production, principally throughout the Asia-Pacific region, has increased attention to health management, since pearl production relies entirely upon the health of the oyster. The pearl is a product of a strong immune response to soft-tissue irritation. The stronger the immune system, the better the pearl quality. However, in order to produce cultured pearls, the mother-of-pearl oyster (MOPs) receives regular handling, including tissue surgery to introduce the irritant ("nucleus") for the cultured pearl. Although pearl oyster aquaculture has not faced the types of disease epizootics which have impacted edible molluscs elsewhere in the world, the ongoing development of the industry necessitates movements of oysters, equipment and people that warrants increased attention to the risk of disease introduction and spread, and awareness of health management measures that can reduce or prevent such risks.

2.1.1 Purpose, approach and target audience

The purpose of this manual is to provide technical guidance in managing the health of pearl oysters, based on a review of the literature of South Sea pearl oysters. It is, however, hoped that the procedures outlined in this manual will be equally useful for health management of other pearl oyster species.

The first section deals with general information related to husbandry and handling, hatchery production; the second concerns introduction and transfers and risk assessment; the third provides detailed protocols for disease diagnostics; the fourth deals with disease zoning; the fifth deals with disease outbreak scenarios; the sixth describes the development of national strategies on aquatic animal health; and the last section provides useful references.

This manual is intended for people at national and state agencies or institutes and private sector individuals involved in pearl oyster health management both at farm and hatchery production levels.

2.2 GENERAL

All commercially important species belong to the bivalve Family Pteriidae (Gray, 1847), a sister Family to the true oysters, the Ostreidae (Rafinesque, 1815). All species discussed in this manual fall in the genera *Pinctada* (Röding, 1798), the pearly oysters; or *Pteria* (Scopoli, 1777), the winged oysters.

2.2.1 Husbandry and handling

Introduction

Tropical and sub-tropical sub-tidal bivalve species, such as *Pinctada* and *Pteria*, do not adapt as readily as inter-tidal or temperate species to rapid changes in temperature, salinity, turbidity and water pressure. Thus, rapid environmental changes can induce significant physiological stress. Such stress can reduce resistance to disease and infection by opportunistic pathogens (Snieszko, 1974), thus, this factor is a key consideration for all the husbandry and handling techniques recommended below for pearl oyster health management.

Collection

Movement of adult pearl oysters from deep-water sources, for transfer to holding tanks/nets/cages in shallow water, should take into account changes in water pressure

and temperature, where possible. Any extreme environmental changes should be followed by a period of convalescence with minimal/no handling, prior to further transportation (Dybdhal and Pass, 1985; Pass, Dybdahl and Mannion, 1987; Dybdahl, Harders and Nicholson, 1990). The period required will vary depending on the degree of environmental change, pearl oyster size, species, and level of shell fouling (epibionts). Where such information is not known, it is recommended that sub-samples of oysters from different species or size-groups be held in hanging baskets at the collection site for varying periods prior to transfer to the farm site. This will provide collection site-specific information required to determine the optimum convalescence period needed to reduce mortalities. Convalesence periods may range from 24 hours to 1week.

Spat collection also requires care, although depth and temperature considerations are less important as larval oysters tend to frequent the upper water column prior to settlement (Monteforte, Kappelamn-Pina and Lopez-Espinosa, 1995). Once collected in spat collector bags, the first health management measure is to minimise unwanted hitch-hikers, such as spat of other bivalve species, predators and fouling organisms. This is necessary to reduce food competition and/or asphyxiation. Such stresses during early development may compromise the quality of the shell and oyster health later in life. Removal of spat from the collector bags also requires care. Air exposure gives good detachment results but is particularly stressful to this stage of development of sub-tidal species and was found to have inferior post-detachment results compared with trials using hypersaline water (40–45 ppt) or sub-ambient salinities (25–30 ppt) (Taylor, Rose and Southgate, 1997a). Re-attachment and survival was found to be 100 percent 24 hours post-detachment using saline treatments.

Handling

Handling for monitoring, sorting, defouling or transfer purposes should be minimised as much as possible and undertaken under shaded conditions or where the pearl oysters can easily be immersed or kept wet with ambient seawater. Transportation requires specialized equipment to ensure adequate water exchange, maintenance of ambient water temperatures and to avoid overcrowding and particulate contamination (Pass, Dybdahl and Mannion, 1987; Dybdahl, Harders and Nicholson, 1990; Joll, 1994; Norton, 1994). No handling is recommended during convalescence periods or during seasons when water temperatures favour proliferation of infectious microbes or toxic algae. Handling stress, in addition to defence against opportunistic infections, is likely to accelerate pearl oyster health problems.

Defouling

Fouling organisms, also known as epibionts, affect pearl oysters, and other bivalves they use as substrate, in a number of different ways. Encrusting coralline colonies and sponges can spread over the hinge or shell margins inhibiting normal opening and closure for feeding and respiration. Heavy fouling may also increase the amount of mechanical energy required for shell opening. Widman and Rhodes (1991) noted a possible correlation between broken ligaments and barnacle colonization of the shells of bay scallops, *Argopecten irradians* during a growth study. Shell edge encroachment can stimulate mantle retraction and this, in turn, can cause permanent shell deformities ("double-back" Taylor, Southgate and Rose, 1997). Excessive colonization also significantly increases the weight of suspended cages or lines, to the extent that the line may sink in the water column or the oysters get stripped-off. If suspended over an unfavourable bottom, this can further reduce the oysters chances of survival. Other direct impacts of fouling can be competition for particulate nutrients (Lesser *et al.*, 1992), e.g. with filter-feeding organisms such as spionid polychaetes, barnacles, sponges and corals; and mechnical blockage of water circulation through holding cage mesh (Parsons and Dadswell, 1992). Interestingly, Lodeiros and Himmelman (1996) found

that growth of the tropical scallop, *Euvola ziczac*, was more severely inhibited by fouling of the pearl nets than by fouling directly on the shells, although, heavily fouled shells demonstrated higher mortalities than those with little surface colonisation. Thus, pearl oysters grown on long-lines, which are known to be heavily fouled, may not require as much cleaning as pearl shells held in suspension cages.

Fouling is usually controlled by manual removal (machete, blunt chisel, high pressure water hose) with frequency of cleaning varying with the nature of the fouling community, grow-out technology, holding depths and season of proliferation. Inevitably this increases the amount of handling required (see Section 2.2.1) and care is required to minimize the subsequent stress on the oyster. Ideally, methods which minimize removal from the water will reduce the stress of handling, e.g. underwater defouling by divers or cleaning of cages or individual oysters in tanks with flow-through seawater. Unfortunately, this stripping activity also means that the fouling organisms remain immersed and this increases their chances of survival, proliferation and re-attachment. This can be circumvented by moving the cages to a remote defouling station for either immersion or demersion cleaning. Interestingly, *P. maxima* appears tolerant of defouling, with maximum growth being demonstrated in oysters cleaned most frequently (every 2 or 4 weeks) (Taylor, Southgate and Rose, 1997). This is in contrast to other species, where defouling is correlated to reduced growth rates (Parsons and Dadswell, 1992).

As with other suspension-grown bivalves, holding depth may affect the degree and rate of fouling, with reduced fouling at greater depths (MacDonald and Bourne, 1989; Côté *et al.*, 1993; Claereboudt *et al.*, 1994; Lodeiros and Himmelman, 1996). Studies of growth of the winged oyster *P. penguin*, found mortalities of uncleaned oysters decreasing with increasing depth from 40 percent at the surface, to 33.3 percent at 4 m to 6.7 percent at 8 m in 10 m deep water (Smitasiri, Kajiwiwat and Tantichodok,1994). Chlorophyll *a* concentrations did not appear to vary with depth. In deeper water grow-out sites, however, the effect of surface fouling and cleaning has to be weighed against the decrease in food availability with increasing depth (MacDonald and Bourne, 1989; Côté *et al.*, 1993; Claereboudt *et al.*, 1994; Lodeiros and Himmelman, 1996).

The use of antifouling paint (Lee, 1992) or antifouling wax (Dybdahl, Harders and Nicholson, 1990) has proven useful in reducing the concentration and rate of colonization of both holding cages and shell by fouling organisms. However, the composition of any anti-fouling agent must be carefully assessed, since many are designed to combat mollusc settlement and may be toxic to the pearl oyster (especially larval and seed stages). The effect of any chemical on the ecology and water quality of the grow-out site also needs careful assessment. A rich fouling community usually indicates a healthy aquatic environment. An advantage of non-toxic anti-fouling agents, however, is the reduced amount of handling required.

Surgery

The most obvious disease concern with respect to surgery is the opening of the soft tissue which forms the first physical defence against tissue infection. In addition, the nucleus stimulates a defence response that is energetically costly to the pearl oyster. If not in optimum health, this tissue trauma and defence response may weaken the oyster to the extent that it may cease feeding and die. The haemocyte-mediated response to the artificial nucleus can also divert defence resources away from other irritants or infections, rendering the oyster more susceptible to opportunistic infections. Thus, a post-surgery convalescence period is recommended (as with post-collection and post-transportation).

Physiological stress induced by prying open the shell, holding in open air, etc., can be reduced by using relaxants (Norton, 1994). In addition, pre-operation treatments, such as varying seawater flow or feed to inhibit or stimulate gonad development, increase

physiological stress. Although high survival rates (mean of 75.3 percent) are reported in *P. martensii* (Deng *et al.*, 1995), special care is required to prevent exposure to additional stresses. Much lower survival rates and nucleus-retention are reported in *P. nigra*, depending on the development stage of the gonad. The highest survival (62 percent) and nucleus retention (61.5 percent) occurred as the gonads were enlarging (Meng *et al.*, 1994). This period coincides with the use of energy resources for gametogenesis, a process which is curtailed in conditions of energy deficit (MacDonald and Thompson, 1986; MacDonald, Thompson and Bayne, 1987; MacDonald, Thompson and Bourne, 1991; Thompson and MacDonald, 1990). This means that energy is available to repair tissue damage due to surgery as well as additional physiological challenges. Post-spawning oysters ("shrinking" and "transparent" stages) would have the least energy reserves and the resting stage of gametogenesis usually coincides with somatic growth, which may or may not reflect energy availability for use in tissue defence.

The different types of graft tissue used may also play a role in pearl oyster health and pearl formation (Tun, 1994; Wada, 1996; Wada and Komaru, 1996). Autograft methodology, using tissues from the individual being seeded, is the least likely to provoke an extreme foreign body response and is, thus, least energetically costly. Homografts, using tissues from other individuals of the same species, are likely to invoke a greater tissue response, but may be required for smaller pearl oyster species, which have less tissue available for the autograft technique. Heterografts, using tissue from other mollusc species, invoke the greatest tissue response. Tissue from incompatible species result in a massive haemocyte infiltration response, abscess formation, tissue rupture and "rejection" of the nucleus. This response is especially costly for the pearl oyster and may render it more susceptible to additional physiological challenges (disease, environmental changes, etc.).

Intervals between seeding with artificial nuceli and mabé (half-shell pearls) production, should take into account the fitness of the pearl oyster (e.g. assessed by demonstration of somatic/shell growth) and optimum energy surfeit periods, to enhance the success of repeat surgery.

2.2.2 Hatchery production

Introduction

With decreasing wild sources of pearl oysters and increasing interest in development of stocks of consistent, superior quality, more pearl oyster producers are using hatchery-production of seed for grow out (Fisheries Western Australia, 1997). Advantages of hatchery production are reduced pressure on wild populations of pearl oysters, ability to select individuals that have optimal characteristics for pearl productivity, and reduced need for transfer of oysters from remote sites with the associated risk of introduction of new pests or diseases. Disadvantages associated with hatchery production are an increased need to handle the early, more delicate, developmental stages and the need for specialized expertise and technology for spawning and successful rearing of the larvae to metamorphosis and grow-out size. Hatchery production is also equally, if not more, susceptible to opportunistic disease problems than wild populations, but careful management and biosecurity measures can reduce this susceptibility.

Broodstock

There are no reports of health problems in pearl oysters held and spawned as broodstock, although the same problems associated with gonad manipulation for surgical implantation procedures could be expected to apply to spawning manipulations for spat production (see Section 2.2.1). Of particular significance to pearl oyster broodstock development is the need to monitor the gonadal development to determine the optimum time to induce spawning. Opening of the oyster through mechanical wedging can damage the adductor muscle and mantle margins (Mills,

Tlili and Norton, 1997), as well as the hinge. The anaesthetic, propylene phenoxetol, appears to circumvent the need to physically pry the shell open and has been reported to cause minimal mortalities, even when used on a large scale (Mills, Tlili and Norton, 1997). These authors note, however, that optimal results are only obtained for oysters which are relaxed prior to immersion in the anaesthetic solution and which have been cleaned of fouling organisms at least 24 hr prior to anaesthesia. Handling stress reduces the gape achieved using anaesthesia and fouling organisms reduce the anaesthetic concentration. Recovery is most rapid in oysters given the minimum exposure required to provide an adequate gape for examination purposes.

In other bivalve species, repeat spawnings and prolonged holding within hatcheries have frequently been associated with outbreaks of disease. Typically, the infectious agents involved are present in the bivalves in the open-water environment, but in closed-circulation facilities, can proliferate to abnormally pathogenic levels of infection (Whyte, Cawthorn and McGladdery, 1994a, b). Control of such infections usually involves modifying husbandry practices to reduce physiological stress and prevent a build up of potential pathogens on tank surfaces and in pipelines (Elston, 1984). Chemotherapeutants can be applied, but the expense of repeated applications against ubiquitous marine organisms, the risk of development of drug-resistant pathogens, and potential adverse environmental effects (see papers on Chemotherapy in Shariff, Subasinghe and Arthur, 1992), usually make alternative strategies more attractive. Examples include moving animals to disinfected tanks, reducing stocking densities, food concentrations, and temperatures, and increasing monitoring and removal of mortalities.

Although infectious agents usually build-up within the holding system, another source of potential contamination is the algal food supply. Most hatcheries are supplied by filtered seawater, in order to minimize contamination and clogging of the system by macroplankters. This means that cultured algae is necessary to provide or supplement the food required for the animals under production. Careful control of the microbial load within the algal supply and delivery system is necessary to prevent the build up of opportunistic pathogens from this source (Elston, 1984).

Another health risk associated with hatcheries is the potential for introduction of infectious agents into the hatchery system via the gut and mantle contents of the broodstock. Although these infectious agents may not affect the adult oysters, the larval offspring may be susceptible to infection. Minimizing the period of exposure of the spawning adults to their spawning products is usually effective in reducing contamination of the larvae (Elston, 1984).

Seedstock

Successful seedstock production is a challenge for any hatchery operation, since young bivalves are extremely vulnerable to energy deficits induced by competition for nutrients, toxic by-products from contaminants and microbes, as well as rapid changes in environmental conditions (temperature, salinity, pH). The concentrated somatic growth effort at this stage of development leaves little energy “buffer” for other energy demands and requires special care in provision of adequate and uncontaminated food (Krishnan and Alagarswami, 1993a, b). If anything upsets the balance between feed and growth, mortality rates occur much more rapidly than in juveniles and adult stages. Seedstock mortalities can reach 100 percent within 12-24 hr, leaving little time for remedial action. Such heavy mortalities, along with undigested food particles, produce a nutrient base for proliferation of saprobionts and secondary infectious organisms. Monitoring is, thus, of paramount importance at this stage of hatchery production (Elston, 1984). This can take the form of direct observation of the larvae themselves (velar activity), or monitoring of tank sediment/flow-through effluent particulate matter for undigested algae (indicating reduced feeding).

Hatchery techniques, aimed at minimizing the stress of handling on younger developmental stages of pearl oysters, are being developed (Rose and Baker, 1994). A monitoring study using histological cassettes to hold individual spat reduced direct contact of the fragile and convoluted edge of the developing shell and was found to have a negligible effect on larval growth (Mills, 1997). Food consumption by the larvae in cassettes was less than by free larvae, however, conversion efficiency was greater – possibly due to less handling stress. Comparisons with growth rates from parallel studies showed that *P. maxima* appear to be slower growing than *P. margaritifera* and *P. fucata* and that spat held in farm-based nurseries grow faster than those maintained in the hatchery.

Stocking density is also important for the health of the early developmental stages of bivalve molluscs. Southgate and Beer (1997) studied the effect of stocking density on black-lip pearl oysters, *P. margaritifera* grown on plastic mesh trays and in pearl nets over a 19 week growth trial. Larger spat (>10 mm) were placed in plastic mesh trays (55 x 30 x 10 cm) and, interestingly, showed greatest growth at the highest density (100 spat/tray). Spat measuring 5-10 mm were placed 7 mm mesh pearl nets and showed greatest growth at the lowest stocking densities (20 and 50 per net), which is more consistent with results from studies with *P. maxima* (Taylor *et al.*, 1997). The reason for the difference in density effect between size-groups of black-lipped pearl oysters is unclear. It is known that pearl oyster spat are gregarious and clump together (Crossland 1957; Gervis and Sims 1992; Taylor *et al.*, 1997), which can result in smothering of the internal individuals and shell deformities. The initial high growth rates observed by Southgate and Beer (1997) may reflect a growth spurt following transfer from the hatchery to open-water, and precede a slowing down when the clumps begin to fill the available space in the trays. Stocking densities which induce clumping are well documented as causing shell deformities (Taylor *et al.*, 1997) and require more handling to break the clumps apart which, in itself, may cause shell damage. This would be expected to reduce the health, quality and survival potential for the oysters as they grow-out to commercial size.

Open-water spat holding techniques with good water exchange and nutrient provision may encourage a healthy start to spat growth (Taylor *et al.*, 1997; Mills, 1997), although Rose and Baker (1994) found lower mortalities in spat reared for 5 months post-settlement in downweller facilities in a hatchery (1-2 percent mortality) compared to those in open-water plastic cages (9-12 percent). The open environment is subject to wave action and predation which, affect spat growth and health. Although suspension culture reduces losses to benthic predators (such as, crabs, starfish, gastropods), Southgate and Beer (1997) noted the presence of the fish *Paramonacanthus japonicus* ("leatherjacket") in the pearl nets and trays used in their experiment. These fish graze on the soft margins and growth processes of the shell and may also attack mantle margins. Fish, as well as other predators, have also been reported from pearl producing areas in the Red Sea, Solomon Islands and Western Australia (Crossland, 1957; Sims, 1994; Friedman and Bell, 1996). Since spat are more vulnerable to predation than older pearl oysters, regular monitoring is necessary to prevent predators from building up, or getting trapped inside, pearl nets and cages. The health risks imposed by open-water challenges need to be assessed against the capital cost and expertise required for long-term holding within a hatchery.

Where open-water culture is used for spat grow-out, suspension culture plays a significant role in enhancing successful growth of *P. maxima* spat (Taylor, Rose and Southgate, 1997b). Surface waters contain a greater biomass and diversity of planktonic and particulate nutrients than deeper or bottom waters (Taylor, Rose and Southgate, 1997b).

Selective breeding

With the production of seed, the opportunity to improve the genetics of cultured stocks by selection of favoured traits, such as shell colour, productivity and disease-resistance,

presents itself (Wada, 1987). Ideally, animals selected for breeding should have demonstrable traits for survival at the sites for intended growth. Oysters from remote sites should be bred in facilities which keep them separate from local-local crosses, to minimise the chance of contamination of local stocks - both genetically or with potential pathogens (see Section 2.2.3). This also preserves the gene pool of the natural population for back-crosses if required to reverse the development of unwanted traits or reverse in-breeding (Wada, 1993; Wada and Komaru, 1994). Dauphin and Cuif (1995) describe a change in the colour of the black pearls from *P. margaritifera* which they attribute to increased mixing of different (genetically distinct) populations of black-lip pearl oysters. Selection for white shell in the Japanese pearl oyster *P. fucata martensii* was found to have an adverse effect on growth and survival, when compared with brown-white shell hybrids (Wada and Komaru, 1994). However, the inferior growth performance of the white shelled oysters was, subsequently, found to be useful in enhancing white pearl production when used as the donor tissue for implantation in hybrid recipient oysters (Wada and Komaru, 1996).

Although inbreeding problems usually take many generations to develop when starting from a "wild" broodstock, care should be taken to use a large number of parent stocks, the crosses from which should be carefully separated and followed. This is necessary to trace both good and bad traits which may develop. Many selective breeding programs - especially for fast growth - produce unexpected "side-effects", which may or may not have an adverse effect on health. Knowing the genetic line of each generation will help with the management of any such problems, should they arise.

Triploidy – Diploidy

Triploids are usually produced to ensure sterility and enhance somatic growth. In addition, diversion of energy reserves from gametogenesis may also enhance reserves available for tissue defence. There is some evidence that triploid American oysters (*Crassostrea virginica*) are faster growers and demonstrate more resistance to infectious agents than diploid individuals (Matthiessen and Davis, 1992), however, other oyster species show negligible differences in resistance (Nell *et al.*, 1994). Reversion to diploidy and mosaic triploids (Allen *et al.*, 1996) may complicate the interpretation of disease resistance and triploidy correlations. This may also result in some individuals which can reproduce, although the resultant embryos may not be viable (He, Lin and Jiang, 1996). Triploids should, therefore, be used in areas where accidental liberation can be avoided and any escapees easily controlled.

Although there is little information available on pearl oyster triploids and disease resistance, there is evidence that growth and pearl production is enhanced in triploid *P. martensii* (Jiang *et al.*, 1993). This indicates that triploid sterility may enhance soft-tissue defence mechanisms, at least in this pearl oyster species. It should be noted, however, that triploidy itself may enhance mortality (since triploidy is not a natural state). Lin, He and Jiang (1996) found significantly higher mortalities in triploids during the straight-hinge to juvenile stage of development, compared with diploids. Interestingly, embryos and adults showed no difference in rates of mortality.

2.2.3 Introductions and transfers

Introduction

Disease risks associated with uncontrolled introductions and transfers are well-recognized (Sindermann, 1986, 1991; Brock, 1992; DeVoe, 1992; ICES, 1995; OIE, 2006, 2007), especially with the increase over the last 20-30 years of hatcherybased seed production, remote setting and use of nonindigenous species for aquaculture. Disease risk assessment for any introduction or transfer of aquatic organisms requires an accurate knowledge of the health status of both the shellfish being moved and the shellfish in the receiving waters.

Drawing upon the primary literature and other information that is often not readily available (i.e., research laboratory reports, government technical reports and personal communications with colleagues), a comprehensive and worldwide synopsis on shellfish diseases of commercially important molluscs, echinoderms and crustaceans (Bower, McGladdery and Price, 1994; Bower and McGladdery 1997) and an Asia disease diagnostic guide for important pathogens of finfish, mollusc and crustaceans (Bondad-Reantaso *et al.*, 2001) were developed. A significant gap in the knowledge contained in these documents, however, is data for south sea pearl oyster infectious agents. This makes assessment of the disease risks associated with the movement of these molluscs particularly difficult. Historic movements of pearl oysters have, for the most part, been local in nature, however, increased pressure to supply seed and adult pearl oysters as local populations dwindle, is focusing attention on more remote sources and even international transfers (Benzie, 1994; Fassler, 1994; Sims and Sarver, 1994, 1996).

Local movements

Movements of pearl oysters within a region probably present minimal health risk concerns, especially if the stocks used have been moved traditionally from the source site to grow-out site, with no disease problems. The main precaution required for this established practice is that the source stock does not change its health status by becoming mixed with stocks from other areas or become depleted, resulting in collection of sub-optimal oysters.

Movements between remote areas within a country represent a greater health risk, since one population or stock may be susceptible to infection by an organism which is benign in the other stock. It is also harder logistically to monitor the health status of remote oyster populations, in which case a health check of the source stock, before transfer to the farm site, as a minimum precaution is highly recommended.

International movements

No international movements of live aquatic organisms should be undertaken without a detailed evaluation of the health status of the stock being introduced. The reasons given in Section 2.2.3 also apply to international transfers but, in addition, compatibility of the source habitat with the import habitat may also need to be evaluated. If these differ significantly (temperature, salinity, turbidity, fouling organisms, etc.) the source stock may be subject to severe physiological stress with the transfer, making them more susceptible to health problems following introduction. Although there is debate over the emphasis given such risks (Sims and Sarver, 1996), these questions can be answered by precautionary introductions of trial numbers of animals maintained in quarantine with an ambient water supply (e.g. Wang, 1994). Guidelines for quarantine assessments are given by ICES (1995) and OIE (2006). Additional concerns, such as the introduction/spread of toxic algal cysts can also be addressed by quarantine introductions (Dijkema, 1995). The standard protocol for minimising the risk of adverse effects from international aquatic animal transfers is introduction of a broodstock into quarantine for spawning. Once spawned and the spat generation have reached a size where the chance of survival is stable, the broodstock should all (100 percent) be examined for infectious agents of disease concern to the importing waters. If the broodstock are free of such agents, the spat generation can be checked. If they are also free of infectious organisms of concern, they can be released from quarantine.

Disease risk assessment

An assessment requires a detailed knowledge of what is present in both the source stock and the stocks present in the receiving site. In addition to disease, genetic and ecological impacts associated with movement of live aquatic animals should also be

assessed (ICES, 1995). The ICES (1995) Code of Practice is particularly useful for pearl oyster movements, since it aims at identification of unknown risks, unlike the OIE Code (2007) which concentrates on known disease agents. Currently, no pearl oyster diseases are listed in the OIE Code.

Ideally, a health profile (see Section 2.3) should be available for the source stock, however, such information may be rare for pearl oysters. Thus, one or more samples of the source stock should be examined for all pests and diseases before any movements to the pearl culture farm take place. Once the profile is obtained, the pearl farmer can determine whether there are any infectious agents or pests present which do not occur at the farm site. If so, the farmer is faced with the choice of finding another, more compatible, source or taking the risk of exposing the farm to potentially harmful additions. The methodologies used for health examinations are outlined in Sections 2.2 and 2.3.

2.3 DISEASE DIAGNOSTIC PROTOCOLS

2.3.1 Field collection of samples

Background information

Shellfish Health Questionnaire (Annex 2.1)

Field Data Sheet (Annex 2.2), note the following:

- hinge-lip length
- wet weight
- surface appearance (shell and soft-tissues)
- any damage to soft-tissues during opening of the shell.

Number of specimens collected (see Annex 2.3).Check the number required with the pathology laboratory and ensure each specimen is intact, i.e., no empty or mud-filled shells.

Shell surface, note the following:

- the presence of fouling organisms (barnacles, slipper limpets, sponges, polychaete worms, bivalve settlement, etc)
- shell deformities (shape, holes in the surface)
- obvious shell-fragility
- any abnormal colouration/smell
- any shell breakage or repair.

Inner shell, following removal of the soft-tissues note these observations:

- the presence of fouling organisms on the inner surface
- shell deformities (shape, holes in the surface, mud or water blisters)
- obvious shell-fragility
- abnormal colouration/smell
- pearls attached to the inner surface (cultured or wild).

Soft-tissues, note the following

- presence of abscess-like lesions, pustules or other tissue discolouration
- oedema (water blisters)
- overall transparency or wateriness
- any abnormal smell
- pearls (cultured or wild).

If fixing the tissue section on site, note the following:

- any worms or other organisms (small crabs, copepods, sponges) in the gut, mantle cavity or on the gills. Estimate numbers present (e.g. <50, >100, >1 000, etc.) and record.
- avoid fixation of tissues that contain pearls or sand or grit, where possible.

Sample size and condition

Samples collected due to abnormal mortalities should consist of live individuals wherever possible, along with samples of unaffected shellfish, as available. Freshly dead shellfish may be collected if the soft tissues are intact and show no obvious signs of necrosis. Where *in vitro* culture is being used as the primary diagnostic tool for specific parasites (such as *Perkinsus* sp.), moribund shellfish with tissues in early stages of necrosis, may still be used.

It is important to emphasize that many disease agents of shellfish have carrier states which are impossible to detect using routine diagnostic techniques. Where there is the possibility of such a disease agent, sampling should be designed to maximize the opportunity for detecting the pathogen. This may involve:

- repeat sampling over one or more seasons
- transportation of samples to a diagnostic facility for live-holding and temperature stressing prior to analysis
- duplicate sampling for use of multiple detection techniques
- selection of appropriate specimen size/age.

The number of specimens collected for a disease outbreak may vary according to the extent of the problem (different genetic lines, sites, ages, etc.) and performance of tests to be applied. Samples for disease-screening of healthy populations, however, are usually based on a sampling regime designed to provide 95 percent confidence of detection of a single infected individual in a given population size at 5 percent prevalence of infection (see Annex 2.3, shaded column) (Ossiander and Wedermeyer, 1973).

Sample preservation

Fixatives should be prepared and used with adequate ventilation to reduce inhalation of noxious gases.

1G4F (1 percent glutaraldehyde/4 percent formaldehyde)

1G4F is a fixative which can be used for light and electron microscopy examinations. Tissue samples should not exceed 2-3 mm in thickness. Following fixation, the tissue should be rinsed well in seawater before embedding or post-fixation for electron microscopy. Tissues can be stored in 1G4F at room temperature until ready for embedding or post-fixation and will tolerate long-term storage (months) in this fixative.

If glutaraldehyde is not readily available, or larger tissue samples have to be preserved, Davidson's fixative or 10 percent formalin (below) should be used. These fixatives are not suitable for electron microscopy or direct long-term storage. Both fixatives should be changed, either to 70 percent alcohol (ethanol or isopropanol) or fresh 10 percent formalin for long-term storage.

Stock solution:	formalin (37–40 percent formaldehyde solution)	5 gal
	Na_2HPO_4 (disodium phosphate)	284 g
	phenol red (pH indicator)	0.5 g
	NaOH (sodium hydroxide)	1.2 g
Working solution:	37–40 percent buffered formalin stock	120 ml
	50 percent glutaraldehyde	20 ml
	tap water	360 ml
	filtered natural or artificial seawater	500 ml

The working solution should be prepared immediately prior to use. Seawater will cause flocculence and /or precipitate but this does not adversely affect fixation.

Carson's fixative
Similar to 1G4F except for the substitution of paraformaldehyde for formaldehyde.

Davidson's fixative
Tissue up to 10 mm in thickness can be fixed. Prior to embedding, tissues can be transferred either to 50 percent ethanol for 2 hrs (minimum) and then to 70 percent ethanol for an additional 2 hours (minimum), or directly to 70 percent isopropanol. Best results are obtained if the fixative is made up in the following order of ingredients.

Stock solution:	glycerin	400 ml
	formalin (37-40 percent formaldehyde)	800 ml
	95 percent ethanol (or 99 percent isopropanol)	1200 ml
	filtered natural or artificial seawater	1200 ml
Working Solution:	dilute 9 parts stock with 1 part glacial acetic acid	

10 percent formalin
Tissue up to 10 mm in thickness can be fixed using this solution. Wash in a buffered solution of ambient salinity for 30 min to 4 hrs prior to paraffin embedding.

Working Solution:	formalin (37-40 percent formaldehyde)	10 ml
	filtered natural or artificial seawater	90 ml

Note: The same flocculence and precipitate may occur as noted for the working solution of 1G4F. This does not adversely affect fixation of the tissue.

Label requirements

Storage jars
- Collection date (+ date of fixation, if different);
- Geographic location (as exact as possible);
- Scientific name of the shellfish or initials of genus and species;
- Number of specimens in each jar and number of jars in the sample;
- Name of fixative;
- Name and telephone number of sampler.

Tissue cassettes
- Date of collection (day: month: year);
- Initials of genus and species (as for the storage jars);
- Specimen No.;
- Code for geographic origin of the sample.

Specimen No. and Code for geographic origin should cross-reference to information on the questionnaire (Annex 2.1) and field data forms (Annex 2.2).

Shipping box or cooler
- The complete address of pathology laboratory doing the diagnostic testing;
- The complete address of the sender;
- An inventory of the contents (e.g., 10 jars containing American oyster tissue samples in 1G4F fixative, 2 data sheets, 1 completed questionnaire form).

Mailing information

Contact the laboratory <u>before</u> collecting the sample

- to ensure the lab is ready to process the samples and that they are scheduled for examination;
- to verify mailing address, contact names and telephone numbers.
- to ensure that someone will be available to accept and properly store the parcel if arriving after normal working hours.
- to check number of specimens required by the pathology laboratory and determine whether or not live shellfish are necessary for tissue culture, bacteriology or other live specimen processing.

Ensure that fixative solutions and shipping containers are labelled for compliance with any chemical transportation requirements

All air shipments must meet International Air Transportation Association (IATA) regulations. Details can be obtained from the courier or airline company.

Shipping instructions for live shellfish
Pack shellfish in seawater soaked burlap, newspaper or paper towels with gelatine cold packs (ice packs are not allowed by airlines and freshwater leaks can affect the shellfish tissues) in a sealed watertight container. Label:

"LIVE SPECIMENS, REFRIGERATE <u>BUT DO NOT FREEZE</u>"

If being shipped by air also indicate:

"HOLD AT AIRPORT AND CALL FOR PICK-UP"

For all shipments:

- clearly indicate the name and telephone number of the contact person responsible for picking up the package at the airport or receiving it at the lab.
- ship **early in the week** to avoid arrival during the weekend with possible loss of samples due to improper storage.
- inform the contact person as soon as the shipment has been sent and, where appropriate, give them the name of the carrier and waybill number.

2.3.2 Gross external observations

- All gross external clinical signs or abnormalities, which may indicate a disease problem should be noted (new growth, damage, fouling, hinge-ligament rupture). Note: Pea crabs (*Pinnotheres* spp.) are commonly found in the mantle cavity of *P. maxima*. Although fairly large, they do not appear to harm the pearl oyster (Fisheries Western Australia, 1997).
- If external shell material is to be tested, it should be collected following processing of the soft tissues which may be damaged by shell sampling.
- If organisms in the soft tissues require culture (e.g. bacteriology), samples should be removed prior to tissue collection for histology.
- Larval suspensions should be examined under a dissecting or light microscope, before being shipped to the diagnostic laboratory. Any abnormalities should be noted and submitted with the sample (e.g., reduced velar activity, bacterial "swarms", fouling by stalked ciliates).

2.3.3 Gross internal observations

Soft-tissue surfaces (abscess, oedema, mantle retraction, pearls)

Features which should be noted in the soft-tissues to ensure accurate interpretation of subsequent histology material include:

- abscess lesions – yellow/green spots in the mantle;
- water blisters or systemic oedema;
- mud blisters – formed due to perforation of the shell by boring sponges (*Cliona* spp.) or polychaetes (*Polydora* spp.) and susbequent invasion of the tunnels by mud or other irritants which come into contact with the soft-tissues;
- mantle retraction – acute retraction will show new shell growth and no inner surface fouling;
- chronic retraction may be associated with no new shell growth and fouling of the inner periphery of the shell;
- pearls – within the tissues or attached to the inner surface of the shell;
- gill deformities – convoluted edges, cysts, filament fusion;
- mantle-dwellers – nematodes; flatworms; pea crabs, etc.

Smears and tissue squashes

- blood samples can be taken either by heart imprint or by haemolymph suspensions in seawater. These can be air-dried, fixed and stained by a number of different commercial rapid stain kits or techniques. Blood samples should be examined for systemic infections by bacteria and protistans, or for neoplastic changes in cell morphology.
- gut contents can be smeared to check for internal parasites (protistans, helminths, copepods) as well as toxic algae. These can be examined fresh or fixed and stained.
- abscess lesion contents can be examined by preparing a smear or tissue squash, fixing and staining. Routine histological stains can be used, or Gram stain kits, depending on the suspected aetiology.

2.3.4 Laboratory protocols

If the shellfish are delivered live to the diagnostic laboratory, then the information given for field collections and gross observations apply to the first step of laboratory examination (Sections 2.2.1 and 2.2.2).

Biosecurity

All samples received for diagnostic examination should be treated as if they are positive for infection. Thus, the diagnostic laboratory is responsible for disposing of sample waste and materials in a manner which will ensure that nothing infectious is spread from the laboratory to the surrounding enviornment. All materials, including transport containers and water, shellfish remains, parasites, microbial cultures, contaminated equipment and instruments should be autoclaved, incinerated, or chemically decontaminated.

Storage of materials and clothing used for diagnostic examinations should be kept separate from all other laboratory areas and activities. All tissues and shells that remain after tissue samples have been collected should be labelled "biohazard" during the disinfection and disposal procedures. Laboratory effluent or liquid waste should also be disinfected before release (i.e. no direct flushing of chemicals or liquid waste into the municipal waste collection system).

All samples received for diagnostic testing should be clearly labelled on receipt by the laboratory and be tracked throughout the examination process (from necropsy to diagnostic report).

Light microscopy

In general, bivalve molluscs are screened for diseases using histology, with individual specimens fixed separately. Duplicate tissue samples are stored in fixative to ensure tissue availability for additional sectioning, staining, electron microscopy, or molecular testing if required. Additional samples may be required if a disease agent is detected which requires live culture for specific identification.

Fixatives

The same fixatives described under Section 2.2.1 for field preservation of samples are recommended for use in the laboratory. Frozen tissues are unsuitable for fixation and light microscopy.

Tissue collection

Fouling organisms should be cleaned from the shell to prevent contamination of the soft tissues. Soft tissues should be examined for discolouration and deformity and any abnormal tissues preserved for histological examination, or collected for culture if bacteria or fungi are suspected.

Transverse body sections should contain samples of as many tissues as possible. Large specimens will require several sections to be cut from different areas of the body. This should be performed as quickly as possible and at cool temperatures to minimise processing-induced histology changes.

- Spat (< 20 mm in length) can be preserved whole in individual histology cassettes. This may require placing spat 1-3 mm in length inside a biopsy bag (or other commercially available cassette liner) to prevent the specimens slipping though the holes in the cassette. Once fixed and infiltrated with paraffin, the spat can be scraped out of the bag and embedded in a cluster in the paraffin block. Multiple specimens on a single slide provide sections through most planes of the body.
- Juvenile pearl oysters (< 20-40 mm hinge to lip length) can have a single cross section removed and placed within a single cassette. This is usually through the digestive gland behind the hinge, out towards the gills and mantle margin. The optimum orientation will include sections of the cardiac cavity, gonad, digestive gland, gills and mantle.
- Adult pearl oysters (> 40 mm) require multiple tissue sections to be removed for histological examination. Depending on the size of the oyster, these may fit within one or more cassettes, or each need an individual cassette.

Stressed pearl oysters can produce large quantities of mucous which adversely affect tissue collection and preservation. Tissues removed from oysters in this condition should be placed in the histology cassette and then rinsed quickly (seconds) in a dish of ambient temperature, clean, seawater to remove the surface coating of mucous before fixation.

Oysters in spawning condition also pose a problem for fresh tissue sampling and fixation. Fragile gonadal tissue and gamete release are difficult to keep intact for paraffin mounting on the microscope slide. Such oysters should either be removed from their shells or kept attached to the half-shell (cleaned of superficial fouling organisms) and placed in 10% buffered formalin or Davidson's fixative for 30 mins to 1 hour (depending on size) to allow fixation of the surface tissues. Following this treatment, tissue sections should be removed, as described above and fixed using the same fixative solution.

To achieve good preservation of tissues fixed within the shell, oysters should be anaesthetized prior to fixation. This can be achieved by adding propylene phenoxetol (1-3 ml/l) (Mills, Tlili and Norton, 1997) or magnesium chloride (14 gm/l) (Culloty and Mulcahy, 1992) to seawater containing actively swimming larvae or juveniles with their

valves agape[1]. Food may be provided for the oysters prior to adding the anaesthetic to encourage them to open their shells. Sufficient exposure to the anaesthetic is achieved when they do not close their shells on being disturbed (several minutes to several hours depending on species, size and water temperature). After fixation, the shells should be decalcified prior to embedding.

Decalcification

Pearl oysters fixed within the shell (juvenile or spat), or which have tissues which contain calcified material, should be decalcified prior to being embedded.

- Fix tissue in fixative of choice.
- Rinse well in running water.
- Decalcify, using any commercially-available chelating agent, e.g. 5 percent trichloroacetic acid (TCA). If EDTA is used (10 g of ethylenediaminetetraa cetic acid (99.5 percent powder) in 100 ml distilled water), use the following procedure:
  - Place tissue in a solution of EDTA.
  - Change solution every 2-3 days to ensure optimum decalcification.
  - Retain tissues in EDTA solution until decalcification is complete (tissue no longer producing gas bubbles and shells are rubbery to the touch).
  - Specimens may be left in EDTA solution for up to 14 days without affecting the staining qualities of subsequent histological sections.
  - Rinse well in running water (30 min–1 hour, depending on the size of tissue).
  - Store in 70 percent ethanol until ready to process.

Tissue storage

Duplicate tissue sections from all specimens should be kept in storage, either on-site, or at the diagnostic laboratory. The duration of storage depends on the purpose of the health examination, but should exceed the period required to do the initial diagnosis. Tissues kept in fixative should be stored away from points of combustion – 1G4F and 70 percent ethanol are combustible and should be stored with due caution. Tissues stored for electron microscopy should also be treated as toxic. No tissues or their fixatives should have access to open water or drainage points into open water, due to their toxicity. Consult the manufacturer's *Material Safety Data Sheets* for safe disposal of all chemicals used for fixatives and fixed tissues.

Paraffin blocks should be stored in a relatively cool place to prevent meltdown. Ideally, temperatures should not exceed 20 °C for paraffin block storage and the duration of storage depends on the purpose of the examination, as for tissues in wet storage.

Electron microscopy (EM)

Many intracellular and microscopic lesions cannot be identified directly using light microscopy, therefore, the increased magnification and resolution of electron microscopy is required. This is specialised technique, using toxic chemicals and highly sensitive equipment. Electron microscopes are not available in all diagnostic laboratories and analysis using this technique may, therefore, take longer than standard light microscopy. Electron microscopy can also be use as a confirmatory back-up to light microscope observations.

[1] N.B. These anaethetics have been used for adult pearl oysters and other oyster species - optimal anaethesia should not be fatal, which could potentially affect the histopathology. Conduct trials on larval juvenile recovery to determine optimum concentrations for the pearl oyster size, water temperatures, etc.

Fixation for transmission electron microscopy (TEM)

Note: all fixatives are toxic and should be handled in a fume cupboard/hood. The post-fixatives used for TEM tissue preparation are particularly dangerous and should only be used by laboratory personnel who have received training in their use.

- The standard electron microscope fixative is 2 percent gluteraldehyde in ambient seawater. 1G4F may be used if 2 percent gluteraldehyde is unavailable. Formalin and ethanol fixatives are unsuitable, and frozen tissues cannot be fixed for electron microscopy.
- Post-fix tissue in 4 percent osmium tetroxide and embed in a resin matrix suitable for ultramicrotome sectioning.
- Stain with lead citrate and uranyl acetate or an equivalent EM stain.

Negative stain

Tissues showing lesions, abscesses, granulomas, etc., but no obvious causative agent, can be processed for direct EM using negative-stain preparation[2].

Fresh tissues are ground down to a homogenous suspension in a buffer which is isosmotic with ambient seawater and supplemented with 2 percent gluteraldehyde. Some fragile viruses may be destroyed by this process, but if present in concentrations high enough to cause tissue pathology, virions should still be distinguishable.

- Drops from the tissue suspension are placed on clean Parafilm® and a carbon-coated 400 mesh copper grid is placed coated side down on top of the drop for 10 min.
- The grid is rinsed through two drops of phosphotungstic acid (PPTA) before being placed on a fresh drop of PPTA for a further 10 min.
- The grid is then air-dried and ready for examination.

Bacteriology

For specimens which require bacterial examination, external tissues should be disinfected prior to collecting samples. Such disinfection should be noted for correlation to subsequent histology sections, which may show evidence of surface tissue irritation by the disinfection procedure. Examples of procedures used for bacterial infections in bivalves are given below:

Smear preparations

Bacterial abscess lesions

Successful isolation of any single etiologic agent from such lesions has not been achieved to date. Gram-positive bacteria (*Micrococcus* sp.) and Gram-negative species (*Vibrio, Pseudomonas* and *Aeromonas*) have been cultured from abscess lesions in bivalves from Canada and the USA.

- Surface-sterilize tissues by wiping with 70 percent ethanol.
- Make a smear of abscess contents, air dry and stain with a commercial Gram stain kit, as per manufacturer's instructions.
- If bacteria are indicated by the Gram stain, attempt to culture the bacteria.

Non-fastidious marine culture media, such as marine agar (MA) or trypticase soy agar (TSA) and brain heart infusion agar (BHIA) have been used to culture bacteria from these abscesses, however, care is required to avoid contamination by surface or secondary bacteria.

[2] Note that the examination for viral particles in negative-stain preparations should be done by personnel with experience or training, because artifacts can easily be mistaken for viral particles, especially in bivalve molluscs.

<u>Nocardiosis of oysters</u>
Although not reported from pearl oysters, this bacterium is found in other oyster species and can be cultured, although cultures are not required for identification. Because infections involve Gram-positive bacteria – an unusual feature for the majority of marine bacteria – infections can be confirmed by routine Gram stains on histological sections or smears of suspected tissues to show the Gram-positive branching bacterial colonies.

Media culture

<u>Hinge ligament disease of juvenile bivalve molluscs</u>
Cytophaga sp., the causative agent of hinge ligament disease of juvenile bivalve molluscs, belongs to a group of bacteria characterized by the following features:

- gliding motility resulting in colonies having a rhizoidal appearance on agar culture plate surfaces
- no flagellar appendages;
- long and variable cell lengths ranging from 2.5 microns to several hundred microns;
- flexible cell walls of typical Gram-negative structure; and
- ability to metabolize biomacromolecules.

Cytophaga sp. is isolated from infected hinges of most bivalves and grown on seawater *Cytophaga* agar, with a low nutrient concentration:

50 percent agar-tryptone	0.5 g	60 percent (enriched) agar -tryptone	2.0 g
yeast extract	0.5 g	yeast extract	0.5 g
beef extract	0.2 g	sodium acetate	0.2 g
sodium acetate	0.2 g	artificial seawater	600 ml
artificial seawater	500 ml	distilled water	400 ml
distilled water	500 ml	agar	18.0 g
agar	11.0 g		

Boil to dissolve solutes. Adjust pH to 7.2. Autoclave. Pour into Petri plates.

Procedure

- Remove hinge ligaments from up to ten spat per sample, keeping the dorsal surface uppermost to prevent contamination with dissection products, use a pointed scalpel to sever the adductor muscle.
- Remove the soft tissues, separate the valves, and excise the hinge ligament into chilled sterile saline (1.5 percent w/v NaCl).
- Place ligaments in a chilled tissue grinder with 1.0 mL of ambient saline and homogenize.
- Ten-fold dilutions of homogenate may be prepared.
- Dispense 0.1 mL portions onto 50 percent *Cytophaga* agar[3] and spread with a glass rod.
- Incubate agar plates at 15 to 20 °C for 5-10 days.

Examination

- Examine agar surface for rhizoidal colonies.
- For further identification, rhizoidal colonies can be removed from agar surface, suspended in saline and subcultured onto enriched cytophaga medium or commercially-available marine agar (Difco).

[3] Note: *Cytophaga* bacteria are slow-growing and are quicky overwhelmed by other bacteria. Thus, initial isolation is made on the low nutrient medium, which reduces growth of other bacteria, and promotes gliding motility, enhancing recognition by behaviour and colony morphology.

Vibrio tapetis (Brown-ring disease) of Manila clams, *Tapes philippinarum*
Vibrio tapetis can be cultured on commercially-available marine agar or thiosulphate citrate bile salts sucrose agar (TCBS) supplemented to contain 2-3 percent NaCl.

Culture media:
- commercially-available marine agar (Difco)
- thiosulphate citrate bile salts sucrose agar (TCBS) (Difco) supplemented with 1 percent NaCl

Confirmatory tests:
- O/129® vibriostatic compound sensitivity disks (Oxoid), 10 and 150 mg
- Pathotec® cytochrome oxidase test strips
- Gram stain kit
- oxidative-fermentative medium, consisting of:

phenol red broth medium	16.0 g
glucose	10.0 g
yeast extract	3.0 g
sodium chloride	25.0 g
agar	3.0 g
distilled water	1 000 ml

Dissolve ingredients, adjust pH to 7.6, add 3 g of agar and boil. Dispense 10 ml into each test tube and autoclave. To conduct the oxidation-fermentation test, inoculate medium by making a stab in the medium using an inoculating loop or wire probe coated with bacteria from the colony. In a positive test, the lower pH of the acid formed from glucose fermentation changes the medium colour from orange to yellow.

Procedure
- Scrape material from the suspected site of infection using a sterile loop and smear onto marine agar or TCBS agar
  or
- Excise the suspected site aseptically and homogenize the tissue in about 1 mL of sterile seawater
  or
- If the animals are too small to be dissected, take a sample of packed larvae with a 1 ml syringe. Let larvae settle to the delivery end of the syringe and dispense 0.5 ml of larvae into 2 ml of sterile seawater or 1.5 percent (w/v) NaCl solution. Grind the sample using a tissue grinder. Allow the large particulate matter (such as large pieces of shell) to settle for 10 min. Using a sterile loop, smear a sample of the suspended material onto prepared Petri plates containing marine agar or TCBS agar.

Examination
- Incubate Petri plates at 10 to 20 °C. Check daily.
- If using TCBS media look for yellow colonies or a colour change in the medium from green to yellow within 48 hr.
- Characterize colonies which resemble the colony morphology and physiological characteristics described for *Vibrio* spp. in Bergey's Manual of Systematic Bacteriology (Holt and Krieg, 1984) (i.e. Gram-negative, cytochrome oxidase positive, motile, possessing the ability to ferment glucose (oxidative-fermentation test), and sensitivity to O/129® (Oxoid).

Note that *Vibrio* spp. are ubiquitous in the marine environment and most of the species associated with shellfish are thought to be facultative pathogens and, thus,

are not reportable disease agents. The only known mechanism for identifying *Vibrio tapetis* is to use a suspected isolate challenge to healthy Manila clams by injection into the pallial cavity. Development of clinical signs of the disease occurs four weeks after injection.

Immunodiagnostics and nucleic acid probes

To date, there are no commercially available immunodiagnostic tools for any known disease agents of pearl oysters. Tools are under development for other shellfish, however, and may become available for rapid screening of microbial agents, such as *Vibrio harveyi* in the near future (Bachère *et al*., 1995).

Record-keeping

An essential component of accurate disease diagnosis and efficient analysis of case submissions is meticulous record-keeping.

Field collection records

The information outlined in the Shellfish Health Questionnaire (Annex 2.1) and Field Data Sheet (Annex 2.2) should be kept at pearl farm to ensure relevant information is readily available if a disease problem arises. Having data on conditions when the pearl oysters were healthy is an invaluable reference for comparing with field conditions associated with a disease situation.

Case-tracking

Diagnostic submissions should be traceable from when they leave the farm to when they arrive at the laboratory via registered couriers. On arrival at the laboratory, they should be logged-in immediately to prevent accidental misplacement and ensure rapid processing for preservation. An example of a laboratory case-log is shown in Annex 2.4.

Specimen data-sheets

Specimen data sheets are spreadsheets designed to record all observations from individual oysters, including:

Specimen No.	Haemocyte infiltration*	Viruses	Shell Sponges
Length	Diapedesis*	Intracellular Bacteria	Shell Polychaetes
Weight	Oedema	Extracellular Bacteria	Surface Fouling
Sex	Abscesses*	Surface Protistans	Shell Deformities
Maturity*	Hyperplasia	Internal Protistans	Abnormal Odour
Food content*	Metaplasia*	Intracellular Protistans	
	Neoplasia	Metacercariae	
	Ceroid*	Sporocysts/Rediae	
	Concretions*	Cestodes	
	Adipogranular*	Nematodes	
	Necrosis*	Surface Turbellarians	
		Internal Turbellarians	
		Copepods	
		Decapods	
		Fungi	

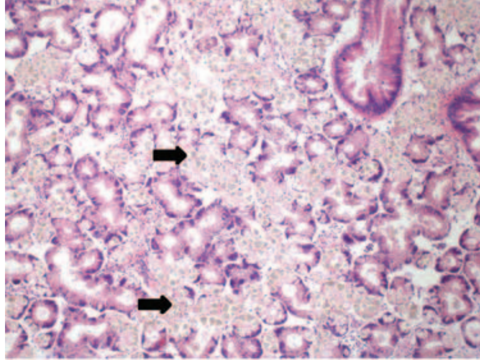
Most observations consist of counts per tissue section. The factors marked with an asterisk are scored by qualitative scale (Annex 2.5).

Diagnostic report filing

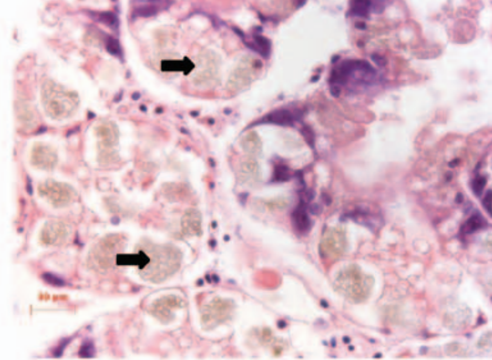
Diagnostic reports are sent directly to the client who submitted the samples for diagnosis. If the health check was for an introduction or transfer request, the diagnostic

PLATE 2.1.1

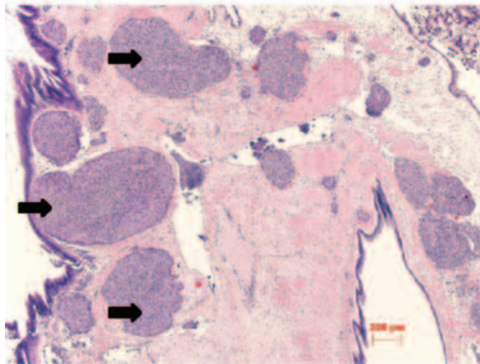
Examples of histopathological observations (concretions, abcess-like lesions, granuloma, neoplasia) on molluscan species



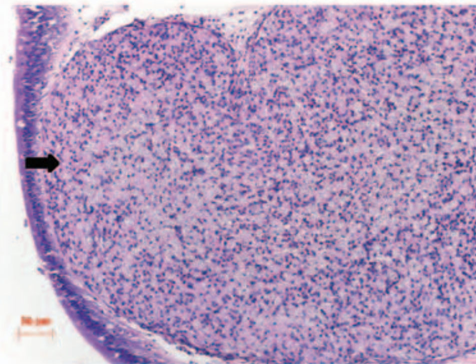
Low magnification of concretions (arrows) in the digestive gland of a scallop (*Placopecten magellanicus*)



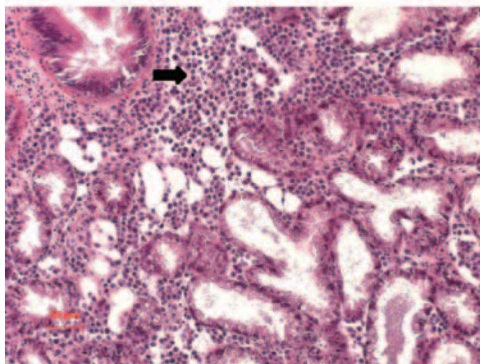
High magnification



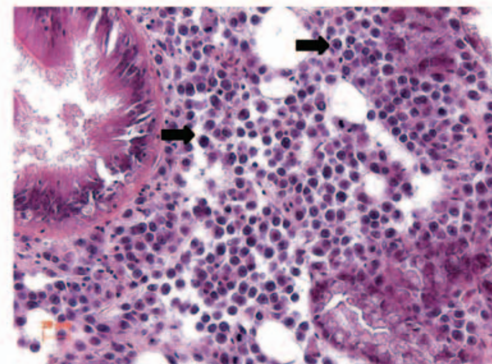
Low magnification of abcess-like lesion (arrows) in the mantle of a scallop (*Placopecten magellanicus*)



High magnification of a granuloma (arrows)



High magnification of disseminated neoplasia (neoplasia) in the connective tissue of the digestive gland of a mussel (*Mytilus trossulus)*

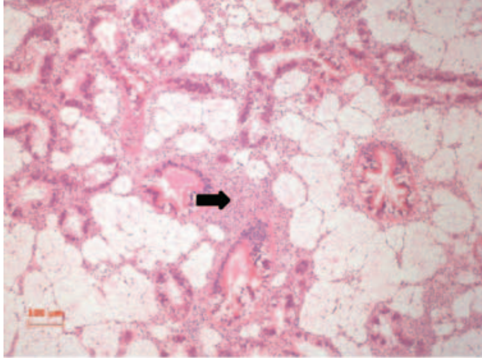


High magnification

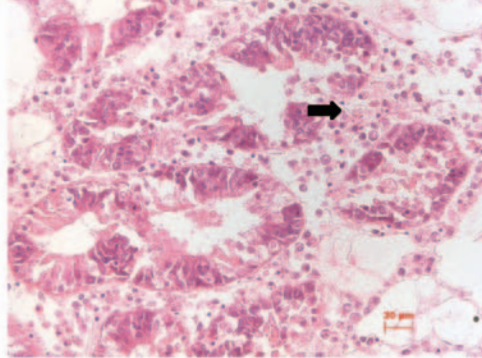
COURTESY OF F.C.J. BERTHE

PLATE 2.1.2

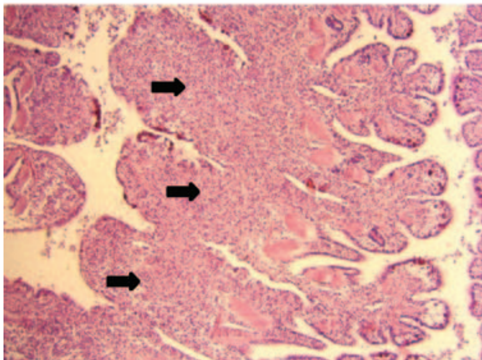
Examples of histopathological observations (haemocytic infiltration, congestion) on molluscan species



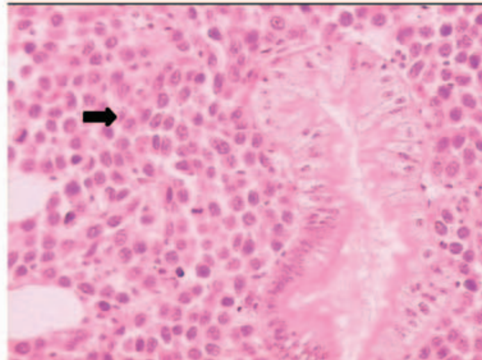
Low magnification of haemocytic infiltration (arrows) of the digestive gland connective tissue of a flat oyster (*Ostrea edulis*)



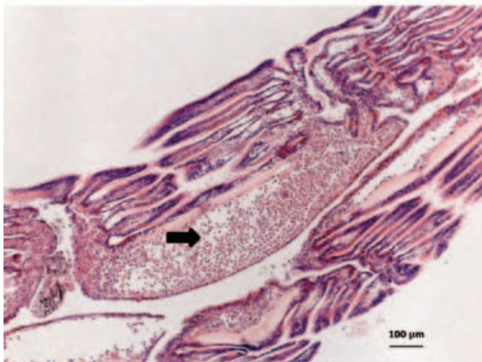
High magnification



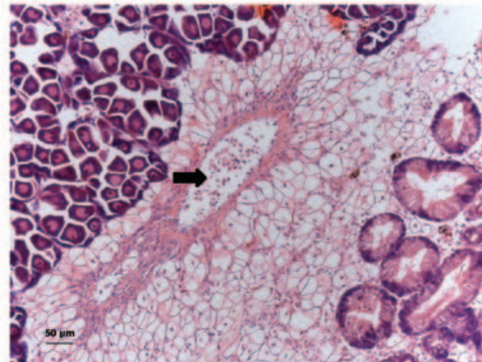
High magnification showing infiltration (arrows) of the gill filaments



Haemocyte infiltration associated with disseminated neoplasia (arrows) in the connective tissue of the digestive gland of a mussel (*Mytilus galloprovincialis*)



Congestion (arrow) of a vessel in the gill of an Eastern oyster (*Crassostrea virginica*)



High magnification of the connective tissue between digestive gland and gonad (arrow)

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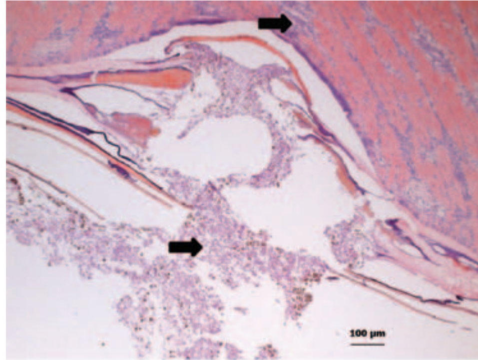
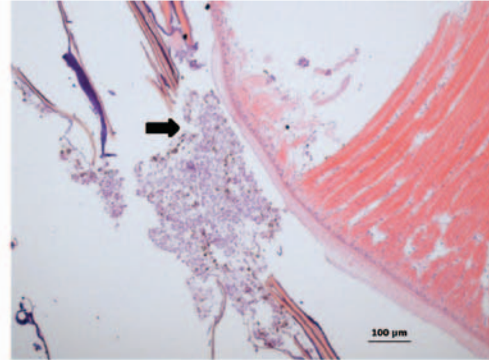
PLATE 2.1.3

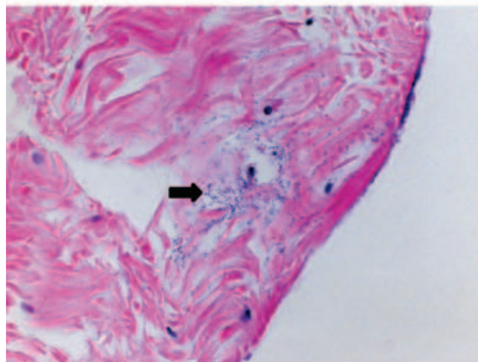
Examples of histopathological observations (Cliona lesion, haemocytic infiltration, *Vibrio* sp. infection, oedema-type lesion, starving oyster) on molluscan species



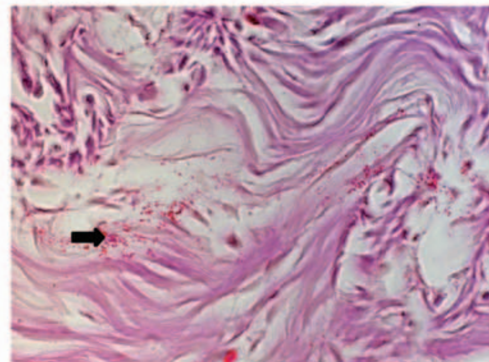
a) Low magnification of *Cliona* sp. lesion of the shell. Note haemocytic infiltration (arrows) of the adjacent muscular tissue (Eastern oyster, *Crassostrea virginica*)



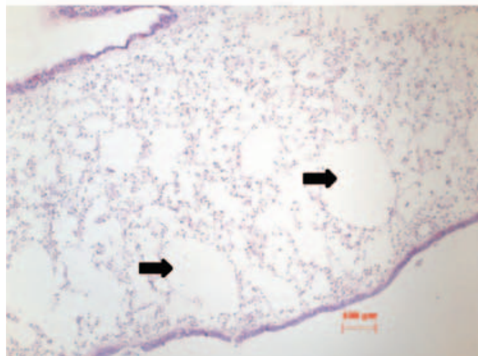
b) High magnification shows the breach of shell integrity (arrow)



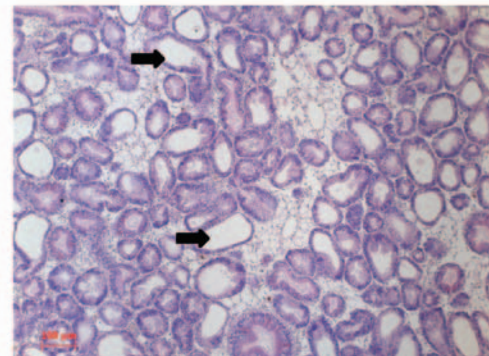
c) *Vibrio* sp. infection (arrow) of the foot of an abalone (*Haliotis asinina*)



d) Gram stain of *Vibrio* sp. infection (arrow)



e) Low magnification of oedema type lesion (arrows) in the mantle of Eastern oyster (*Crassostrea virginica*)



f) Low magnification of the digestive gland of a starving oyster (*Crassostrea virginica*) (arrows)

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laboratory should check with the client whether they want the health report sent directly to the transfer licensing authorities or the client will send a copy with their request. Copies of the diagnostic report should be kept on file as well as with the specimen data sheets.

2.4 HEALTH ZONATION

Introduction

The concept behind zonation is to establish a reference health profile for a geographic area or facility which can be used as the basis for monitoring any changes in the health status of the culture stock and/or to assess the risk of disease transfers. This method of health management is recommended by the OIE (2006) and is applied on a regional basis for shellfish aquaculture in Canada, parts of the United States of America and the European Union. Stock from facilities or areas with identical health profiles present negligible health risks compared with those with different health profiles. Land-based facilities can be given a health status based on isolation from surrounding facilities. Since this is harder to assume with open-water, health zonation can be conducted on a geographic/hydrgraphic basis. With pearl oyster farms, the facility-based concept may apply more readily than with other mollusc culture systems, since there is little direct exchange between farms and the number of farms in any given area is usually limited. Where neighbouring farms undertake stock exchange (seedstock, broodstock, etc.), ,the zonation approach may be more applicable.

Facility-based health status

In order to establish an accurate health profile, a facility (hatchery, culture production) should have at least four (4) consecutive health checks over a period of 18-36 months and at periods most likely to detect disease agents (e.g. post-transportation, post-spawning, post-surgery). Fewer health checks may be required for land-based facilities with a sterile water supply. Separate samples should be examined for different year-classes, species and stocks from different sources (unless they have been mixed). Numbers of oysters which need to be examined will vary with the number being held at the facility but should comply as closely as possible with 95 percent confidence of detecting an infectious organism at 2 percent prevalence, i.e. a maximum sample size of 150 animals (see Annex 2.3). Once the consecutive checks have been completed, a health status report can be compiled for the facility site. This can be used for requests to transfer stocks to other facilities or to assess risks from importations from other sites or facilities (based upon their health status records, if available).

Geographic area

The same protocol for establishing a health profile can be applied to a geographic area. This is usually applied to discrete bays or areas with a well-established exchange of oysters, e.g. collection of local source stock or seedstock from a local hatchery, or hydrographic water exchange. Four consecutive health checks are recommended and samples of wild stock should consist of 150 oysters (unless stocks are limited or considered to be endangered). Where several facilities fall within a single geographic zone, the growers may decide to provide broodstock from one farm, seedstock from another, etc. The results from each can then be pooled to provide the health status for the zone.

Health status maintenance

Following establishment of a health status profile for a facility or geographic zone, this can be maintained by annual samples of stocks.

2.5 DISEASE OUTBREAK INVESTIGATION PROCEDURE

Introduction

A systematic procedure to help identify the cause or causes of disease events and prevent the further transmission of the pathogen is required to control an ongoing and to prevent future disease events. Any disease outbreak investigation will strongly benefit from using an epidemiological approach which assesses the pattern of infection within the affected population (Lilley *et al*., 1998).

Lilley *et al.* (1998) outlined the nine basic steps to a disease outbreak investigation developed for investigating outbreak of epizootic ulcerative syndrome (EUS) of fresh and brackishwater finfish in Asia. This approach is also applicable to pearl oyster health disease investigations. The nine steps include: (1) establishing a diagnosis, (2) establishing a "case definition", (3) confirming that an outbreak is actually occurring, (4) characterising the outbreak in terms on time, affected/unaffected species, and place, (5) analysing the data, (6) developing a working hypothesis, (7) making intensive follow-up with different laboratory testing and further epidemiological analysis, (8) establishing control and prevention measures and (9) reporting. These nine steps may not necessarily be included in every investigation, nor will an investigation always follow the same sequence; they may be undertaken simultaneously.

One of the most important criteria to establish, as quickly as possible, is whether or not a disease outbreak is due to an infectious agent or due to stress and/or secondary opportunistic infection. This can be achieved by sending samples of affected and unaffected oysters to a diagnostic laboratory as soon as abnormal mortalities are detected. Since some tests may take weeks, some interim control measures will be required pending diagnostic results.

A careful record of the pattern of mortalities may assist in determining the nature of the problem (although not the cause). Generally-speaking, environmental stress-related mortalities may affect animals throughout the stock. Stocking density effects will tend to cause mortalities at the centre of the cultured population, with peripheral lines/cages showing relatively little effect. Toxins or pollutants may cause the same pattern of mortality or manifest themselves as acute mortalities throughout the stock. Infectious disease agents will show a progressive pattern of mortality spreading between neighbouring lines or nets.

Diagnostic submission

As soon as abnormal rates of mortality are detected, samples of moribund (not dead) and unaffected (if available) oysters should either be preserved on site (see Section 2.2.1) or submitted live to a diagnostic laboratory with molluscan shellfish health expertise. If there is evidence of a microbial (bacterial or fungal) infection, fresh rather than preserved samples should be sent to the laboratory. Phone/Fax the laboratory in advance to let them know the nature of the problem, determine the number of samples they need and ensure that they are prepared for the diagnostic case. The laboratory may need to prepare specialized media for tissue culture and fixatives.

Quarantine (isolation)

Quarantine is a holding method which prevents the escape or release of shellfish and any of their disease agents. Different periods of quarantine may be required depending on the disease. Quarantine is also applied to shellfish being introduced to a site from an area where there is a disease agent of concern or no health history information (see Section 2.2.1). Quarantine, by definition, is therefore difficult to manage in open-water.

Isolation is used to prevent infection by organisms from the surrounding environment. This may occur within a site where there is a disease outbreak and unaffected stocks are placed under isolation (while the diseased stock fall within quarantine). Isolation can be conducted under two different conditions:

- *Land-based isolation* prevents access to the shellfish by other shellfish or associated disease agents from surrounding waters. Although shellfish may not be introduced to an isolation facility or site, they can be released into the surrounding waters.
- *Geographic isolation* is the spatial separation of shellfish from neighbouring shellfish beds or sites.

Disinfection

Disinfection involves the application of chemical treatments at sufficient concentrations, and for sufficient periods of time, to kill or inactivate harmful organisms. Since the inherent toxicity of disinfectants negates safe use in open-water, or flow-through systems, disinfection can only be applied with reasonable control within hatcheries, tanks or land-based holding facilities. Disinfectants must be neutralised before release into the surrounding environment, especially seawater treatments, which produce residual oxidants which are particularly toxic to bivalves (OIE, 2007).

If a disease agent is detected, disinfection of tanks, nets and equipment is recommended. Stock infected by an opportunistic pathogen may be destroyed, or transferred following surface disinfection to clean holding tanks at lower stocking densities, water temperatures, and with new food sources.

Where stocking densities are high, shellfish should be rotated between disinfected tanks as frequently as practical. Each new batch of shellfish introduced to a facility should be placed in pre-disinfected tanks. Because the presence of organic matter will reduce the disinfection capacity of most disinfectants, filtering influent water is recommended. In addition, all surfaces should be thoroughly cleaned prior to disinfection. The detergent used for this purpose should be compatible with the disinfectant and both should be compatible with the surface being treated. All waste from washing should be disinfected before disposal.Regular air- or heat-drying of pipelines (daily), tanks and other equipment (e.g. algal culture carboys), in addition to disinfection of their surfaces, is also recommended. All chemical treatments should be undertaken in a manner that prevents their release into the surrounding environment.

Treatments

Chlorine is usually applied as sodium hypochlorite (Chlorox®, household bleach, etc.). Fill all pipelines with 50 ppm chlorine (= 50 mg/l). Allow an exposure time of at least 30 minutes before flushing with clean seawater. This solution is effective against most microbial agents as well as labyrinthulid protozoans. Chlorinated seawater should be neutralised prior to release from the holding facility.

Iodophors are applied as alkaline solutions (Wescodyne®, Betadine®), at an iodine (I_2) concentration of 200-250 ppm, for a contact time of at least 10 minutes, however, they are not effective against certain protistans (Bower, 1989) and may need to be supplemented by air- or heat-drying of tank surfaces and pipelines.

Testing treatments

Following a disease outbreak, or the initial set-up of a disinfection system and prior to introducing fresh shellfish stock or flushing effluent from the facility, routine bacteriological culture of disinfected surfaces and water for ubiquitous bacteria (such as *Vibrio* spp. and *Pseudomonas* spp.) should be performed. If present, this indicates that the disinfection procedure used was inadequate.

Residual monitoring

Residual or free chlorine (or other halogen) ions are indicative of the degree of binding to/ oxidation of organic compounds or organisms in the water being treated. If no residual oxidizing ions remain after a set period of treatment, it is assumed that:

- inadequate disinfectant was administered; and
- viable pathogens may have survived the treatment.

The baseline for most disinfection facilities is a free residual chlorine (or equivalent oxidizing ion) level of 5 ppm, following water treatment for a minimum of 10 min. However, the baseline for effective disinfection varies significantly between facilities, depending on water salinity, temperature, turbidity, volume, organic content, pH, flow-rate, etc. Establishing the concentration and contact time for the type of disinfectant chosen requires pre-testing prior to release of the water into the surrounding environment.

Pre-testing procedure

Treat water with varying concentrations of an appropriate disinfectant (chlorine, iodophor, peroxide, ozone or ultra violet) and graph residual oxidant concentrations after 10, 15, 30, 45 and 60 sec and after 5, 10, 15, 30 and 45 min:

- If concentrations decrease to < 5 ppm within 10 min of water treatment, the concentration or type of disinfectant is ineffective.
- If concentrations are > 5 ppm after 10 min but continue to decrease after the 30 min interval, disinfection is continuing and the starting concentration should be increased or use another type of disinfection because viable pathogens may still be present after 30 min.
- The disinfectant concentration which results in a point where residual oxidant concentrations begin to stabilize at >5 ppm (and can be increased with additional disinfectant) represents the concentration required for disinfection.

Sterile waste disposal

Both chlorine and ozone produce long-lived residual oxidant compounds in seawater. Seawater at 35 ppt salinity contains 60 ppm bromide ion which produces hypo-bromite in the presence of ozone. Disinfected artificial seawater, at the same salinity, produces bromine and hypobromous acid. Since these are toxic to larval oysters, treated seawater should be passed through an activated charcoal filter before being released or used for live mollusc larvae.

Reducing agents such as sodium thiosulphate or aeration may also be used for halogen neutralisation, but these do not remove toxic chloramines and are not recommended for seawater facilities.

A log of neutralisation of disinfection procedures and monitoring results is highly recommended for ensuring that neutralisation is adequate to prevent negative environmental impacts.

Chemotherapeutants

To date, there are no chemotherapeutants which are recommended for use in open-water or flow-through mollusc farms. Although effective antibiotics are available, their prophylactic use or use against a build up of opportunistic microbes is not recommended due to the risk and consequences of antibiotic resistance.

Personnel

Only authorised personnel should be allowed contact with shellfish that are subject to disease investigation. Entry-exit points should be made secure against unauthorized access.

Log-keeping

Log-books should be kept during a disease investigation to record:

- entry-exit times of authorized personnel
- shellfish mortalities (date, time, method of disposal)
- disinfection administration

- residual levels and neutralisation
- samples sent to laboratories for disease diagnosis.

Investigation

Mortalities with no immediately apparent cause may take years to investigate, depending on the epidemiology of infection. This means that disease control measures must be balanced in terms of cost and practicality with the continued operation of the farm. Disease control measures and research plans are most effective where the farm management and personnel actively participate. The latter have the day-to-day contact with the shellfish which provides the information essential for accurate interpretation of disease observations and experimental results.

2.6 NATIONAL STRATEGIES ON AQUATIC ANIMAL HEALTH

In recent years, countries are developing and implementing "national strategies" on aquatic animal health management (AFFA, 1999; Olivier, 2004; Kanchanakhan and Chinabut, 2004; Amos, 2004). In Asia, the development of the "national strategy" was an outcome of an FAO-supported regional technical cooperation project on "health management for the responsible movement of live aquatic animals" (Bondad-Reantaso, 2004). The "National Strategy on Aquatic Animal Health" contains the actions plans of the government at the short-, medium, and long-term to implement the Asia Regional Technical Guidelines for the Responsible Movement of Live Aquatic Animals (FAO/NACA, 2000). The essential components (or elements) of the "national strategy on aquatic animal health" include the following: (i) policy, legislation and enforcement, (ii) pathogen list and information system, (iii) diagnostics, (iv) health certification and quarantine, (v) risk analysis for aquatic animal movement, (vi) surveillance, monitoring and reporting, (vii) zoning, (viii) response and contingency plans to disease emergencies, (ix) research, (x) institutional structure, (xii) human resources development and (xiii) regional and international cooperation.

There are varying processes involved in the development of the "national strategy". In some countries, the take-off of national strategy development was immediate; for some others, it went through a slow process for various reasons. Although not aimed specifically at diseases of pearl oysters, the development of such strategies is a valuable framework for enhancing basic health management and biosecurity education and awareness for aquatic animal health at both a national and regional levels. Experience in Asia provided some essential considerations for ensuring success in developing and implementing a "national strategy". These include: (i) strong national coordination, (ii) a good driver for the process (through the Competent Authority, a commission, a committee, a task force, or a focal person) with clear terms of reference, (iii) needs assessment and prioritization exercise, (iv) active stakeholder consultation, (v) approval from highest authority, (vi) an implementation strategy, (vii) a monitoring and review plan, (viii) development of proposal for addressing the different component (elements) of the national strategy and (ix) resource and funding allocation.

Development and implementation of national strategies on aquatic animal health within the broader aquaculture development and biosecurity framework should be continuously pursued. The national strategy is comprehensive enough, and using the concept of "*phased implementation based on national needs*", it could provide a good and strong entry point for capacity building for many countries regardless of aquatic animal health infrastructure or economic development.

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ANNEX 2.1

Shellfish Health Questionnaire

Name and address of client ____

____

Contact telephone number ____________ Fax____________ E-mail ______________

Reason for submission:
Stock transfer (indicate from where to where) _________________________________

Abnormal mortalities (give approx. percentage of losses) _________________________
Other ____

Growing conditions:
Growing Method ____

Water temperature _____________ Salinity _____________ Turbidity __________

Other environmental observations (e.g. predators, high rainfall, wave action, pollution, etc.) ____

____

____

General observations on the stock being submitted:
Appearance (gaping, shell damage, fouling, etc.)_______________________________

____

____

____

Behaviour (feeding, growth, maturation, movements, etc.)_______________________

____

____

Recent handling history of the stock or neighbouring stocks_____________________

____

____

Comments

____

____

____

____

ANNEX 2.2

Field Data Sheet

Species ______________________________

Location ______________________________

Date ______________________________

Name & phone number of sampler ______________________________

Specimen No.	Length (mm)	Weight (g)	Boring sponge	Boring worms	Surface gill-worms (Turbs)	Clam-worm	Fungus	Oedema	Abscess	Shell blisters, bumps, repairs, etc.	Soft-tissue/ gill deform-aties, etc.	Foul odour. liquefaction, etc.	Notes Page ___ of ___
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													

ANNEX 2.3

Sample sizes needed to detect at least one infected host in a population of a given size, at a given prevalence of infection, with 95 percent confidence (Ossiander and Wedermeyer, 1973)

	Prevalence (%)						
Population size	0.5	1.0	2.0	3.0	4.0	5.0	10.0
50	46	46	46	37	37	29	20
100	93	93	76	61	50	43	23
250	192	156	110	75	62	49	25
500	314	223	127	88	67	54	26
1000	448	256	136	92	69	55	27
2500	512	279	142	95	71	56	27
5000	562	288	145	96	71	57	27
10000	579	292	146	96	72	29	27
100000	594	296	147	97	72	57	27
1000000	596	297	147	97	72	57	27
>1000000	600	300	150	100	75	60	30

ANNEX 2.4

Example of a laboratory case-log

Case No.	Date (out of water)	Location	species	No.	Date fixed	Date infiltrated/ embedded	Date sectioned	Date stained	Culture	Slides read (date + person)	Report sent (date + person)	Remarks	Computer file No.

ANNEX 2.5

Histology qualitative scales

Gonad maturity

0 – no gametes (resting or immature)
1 – developing gametes attached to gonoduct epithelium (ripening)
2 – gametes filling gonoducts (mature)
3 – evidence of loss of mature gametes (spawning)
4 – residual mature gametes (post-spawning)
5 – gamete resorbtion by haemocyte infiltration (recovery)
6 – prespawning resporbtion (spawning failure)

Gut content

0 – none
1 – small amounts of food in one or other intestinal look
2 – food in both intestinal loops or one intestinal loop plus partial stomach
3 – food in both intestinal loops and stomach

Infiltration

0 – no haemocytes present in the connective tissue (rare)
1 – haemocytes 1–2 cells deep around the intestine and stomach epithelia
2 – thick layer of haemocytes around the stomach and intestine, +/– small numbers of focal aggregations in the connective tissue
3 – systemic infiltration of haemocytes throughout the connective tissue

Diapedesis

0 – none
1 – minor diapedesis across the odd duct or intestine wall
2 – diapedesis across several epithelial borders
3 – extensive diapedesis across stoamch, intestine and duct epithelia

Metaplasia

0 – none
1 – < half the tubules with flattened epithelia
2 – > half the tubules with flattened epithelia
3 – almost all tubules with flattened epithelia
4 – chronically flattened epithelia

Adipogranular storage tissue

0 – none
1 – small deposits in mantle only
2 – large amounts throughout the mantle
3 – mantle and peripheral digestive gland connective tissue
4 – mantle and throughout the diegstive gland
5 – throughout the connective tissue of the whole tissue section.

Ceroid (connective tissue)/concretions (digestive tubule epithelia)

0 – none
1 – minor accumualtion in connective tissue/digestive tubules
2 – moderate accumulation
3 – heavy accumulation

Necrosis

0 – none
1 – focal (limited)
2 – focal (moderate)
3 – systemic
4 – systemic + saprobionts

PART 3

EXPERIENCES IN DEALING WITH PEARL OYSTER MORTALITIES

3.1 Review of pearl oyster mortalities and disease problems
J. Brian Jones

3.2 The Cook Islands experience: pearl oyster health investigations
Ben Diggles, P. Mike Hine and Jeremy Carson

3.3 The Australian experience: pearl oyster mortalities and disease problems
J. Brian Jones

3.4 The Japanese experience: pearl oyster mortalities and constraints
Katsuhido Wada

3.5 The French Polynesia experience
Franck C.J. Berthe and Jean Prou

3.6 Pearl oyster health: experiences from the Philippines, China, the Persian Gulf and the Red Sea
Melba G. Bondad-Reantaso, Sharon E. McGladdery, Daisy Ladra and Wang Chongming

3.1 Review of pearl oyster mortalities and disease problems

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ABSTRACT

The aquaculture of pearl oysters is an expanding multimillion dollar industry in the tropical marine environment of many countries, including Australia, French Polynesia, the Middle East, China, Southeast Asia and Japan. Despite the size and extent of the industry there is remarkably little known about the diseases and parasites of the genus *Pinctada*. There is a growing awareness among the industry that, as with other molluscs under cultivation, disease can be an important constraint and that translocation of shellfish poses a serious risk. This paper will review the known diseases caused by pathogenic agents as well as those with a non-infectious aetiology. Management techniques, which can be used to minimize the impact of disease, will also be discussed.

INTRODUCTION

The aquaculture of pearl oysters of the genus *Pinctada* is an expanding industry in the tropical or sub-tropical marine environment of many countries including the Middle East, China, Japan, South-East Asia including Australia and French Polynesia. Contemporary farming practice requires a supply of oysters to replenish oysters harvested for their shell, meat and pearls. These activities, in turn, require introductions of seed stocks from hatcheries and the wild-harvest sector and movements of oysters around the coast. The movement and introduction of marine molluscs to new areas has a long history of initiating major disease impacts on aquaculture industries (Grizel *et al.*, 1986). Recent severe mortalities in Japan, Australia and French Polynesia suggest that *Pinctada* spp. are no exception (Chagot *et al.*, 1993; Miyazaki *et al.*, 1999; Hirano, Sugishita and Mobin, 2005). This paper reviews the major diseases of *Pinctada* spp. and management practices that reduce their effect on the industry.

INFECTIOUS DISEASES AND PARASITES

As with other mollusc species, techniques for the routine culture of virus, fungi and protozoans affecting pearl oysters are not generally available and both fungi and protozoa, with the exception of *Perkinsus* spp. are still routinely identified by

histopathology. Though molecular techniques such as Polymerase Chain Reaction (PCR) are useful for identifying specific pathogens, histology is still preferable as it is capable of detecting multiple infections and other physiological problems such as starvation or loss of condition following spawning. In pearl oysters, the most common response to an insult is an inflammatory reaction. Cellular elements that participate in the inflammatory response and wound repair include agranular amoebocytes, basophilic semi-granulocytes and eosinophilic granulocytes. A typical response of aggregation, phagocytosis, hyperplasia and encapsulation has been described (Feng, 1988; Suzuki, 1992). The haemocyte accumulations, infiltrations and granuloma formations seen in the absence of aetiological agents therefore represent a spectrum of past or existing inflammatory response to a variety of antigens, including soluble antigens, not visible histologically.

Viruses

Pass, Perkins and Dybdahl (1988) described intranuclear viral inclusion bodies present in the digestive gland epithelium of oysters (*Pinctada maxima*) from Australia. These inclusion bodies have been demonstrated to consist of large, icosahedral, enveloped virus particles (Humphrey *et al.*, 1998).

Norton, Shepherd and Prior (1993a) reported that papova-like viral inclusions occurred commonly in the epithelium of the palp in both wild caught and farmed oysters (*P. maxima*) in Queensland, Australia. Mild to moderate digestive gland hyperplasia and degeneration are associated with heavy infections suggesting that the causative agents may potentially be pathogenic (Humphrey *et al.*, 1998) and as a result movement of live infected oysters between Queensland Northern Territory and Western Australia is currently prohibited.

Suzuki, Kamakura and Kusuda (1998) and Kitamura *et al.* (2000) reported the presence of a birnavirus in the haemocytes and digestive gland tubules of *Pinctada fucata martensii* in Japan. The pathological significance of the birnavirus is uncertain. It should be noted that pearl oysters, as with other oyster species, probably sequester a wide variety of viruses from the environment - not all of which will be pathogenic to oysters and some, such as hepatitis and Norwalk virus, would be pathogenic to humans.

In 1995, there was a mass mortality of *Pinctada margaritifera* in pearl farms in several Atolls of the Gambier Archipelago, French Polynesia (Comps, Herbaut and Fougerous, 1999; 2001). On-going research has identified a 40 nm virus associated with necrosis of the adductor muscle, the histopathology of which Comps, Herbaut and Fougerous (2001) suggested was similar to that described in Akoya oysters by Miyazaki *et al.* (1999). Severe mortalities of *P. maxima* in Exmouth Gulf, Western Australia during 2006 are also believed to have a viral aetiology, though no infectious agent has yet been identified.

"Akoya virus"

Mass mortalities of Japanese oyster (*P. fucata martensii*) were first noticed in Yusu Bay and Uchiumi Bay, in Ehime Prefecture, Japan during 1994 and have subsequently occurred annually both at these sites and other bays in western Japan. Mortalities include juvenile, adult and seeded oysters. In 1996 and 1997, losses were reported to be 50 percent of all oysters in production in western Japan (Miyazaki *et al.*, 1998; 1999) though local losses of about 80 percent have been recorded (Tomaru, Kawabata and Nakano, 2001). The mortalities have been the subject of intensive study within Japan including a workshop in September 1999 to overview research on the Japanese pearl oyster mortalities (Muroga, Inui and Matsusato, 1999).

Gross pathology associated with the syndrome includes poor growth of shell on valves, sluggish closing of valves, atrophy and a distinctive yellowish to red-brown colour of adductor muscle and watery appearance due to lack of nutrient storage

(Miyazaki *et al.*, 1998). The histopathology includes necrosis, haemocyte infiltration, atrophy, swelling and vacuolization of adductor and foot muscle fibers. Digestive diverticulae with large lumen and greatly reduced vacuolation and lysosome activity by epithelial cells are consistent with starvation (Shinomiya *et al.*, 1997; Miyazaki *et al.*, 1998). Kurokawa *et al.* (1999) showed that the mortalities were associated with an infectious agent causing the distinctive colour change as well as the histopathological changes to the digestive gland. Miyazaki *et al.* (1999) independently described a small (25–33 nm) virus (Akoya virus) which affected all smooth muscle fibers, not just adductor muscle, affecting feeding, respiration and cardiac function. Miyazaki *et al.* (2000) showed that the effects of the virus could be reduced by injections of a recombinant feline interferon-w.

The Japanese mortalities were associated with illegal imports of Chinese pearl oysters (Wada, 1997) and subsequently large numbers of hybrid akoya/Chinese pearl oysters have been produced in Japan because the hybrid is resistant to the disease, although producing pearls of low quality (Miyazaki *et al.*, 2000). The disease may kill other molluscs, including *Chlamys nobilis* and *Crassostrea gigas* (Miyazaki *et al.*, 1998).

Tomaru *et al.* (2001) suggested, based on sampling in Uchiumi and Yusu Bays, that there was a relationship between water temperature and mortality in autumn (September to November) and that these mortalities were not due to toxic algae. They hypothesized that, when *Nitzschia* spp. (which is inedible to oysters) dominated the culture area, the health of the oyster deteriorated due to food limitation and high water temperature and that subsequently, the infective agent affected the digestive glands causing death by starvation. This hypothesis may explain the results obtained by Hirano, Kanai and Yoshikoshi (2002) who demonstrated, using contact infection trials with diseased oysters in pocket-type cages in the sea, that infection (as expressed by mortality) was not transmitted. Further analysis of these mass mortality events by Hirano, Sugishita and Mobin (2005) concluded that the disease is not due to viral, bacterial, fungal or parasitic organisms but might be due to organic pollution effects from neighbouring fish farms, a view challenged by Nagai *et al.* (2000) who again emphasized the infective nature of the disease and its dependence on high water temperature.

Rickettsiales

Polymorphic rickettsiales have been described from *P. maxima* from the South China Sea (Wu and Pan, 1999) and from *P. margaritifera* in the Pacific (Comps, Fougerouse and Buestel, 1998). These appear, by light microscopy, to be identical to the rickettsiales also seen in Western Australia in *P. maxima*. Rickettsiales have also been reported from *P. maxima* in Queensland and the Northern Territory in Australia (Humphrey *et al*., 1998). Worldwide, rickettsiales are recognized as asymptomatic infections in a range of molluscs including *Crassostrea gigas*, *C. virginica* and *Mya arenaria* and have been associated with mass mortalities of the scallop *Placopecten magellanicus* (Sparks, 1985).

Bacteria

Bacterial culture under tropical conditions, where water temperatures are around 29 °C to 33 °C, frequently results in the isolation of large numbers of commensal or contaminant organisms, which cannot be readily distinguished from potential pathogens. However, a number of studies have identified bacteria, particularly species of the genus *Vibrio*, as pathogens of oysters, often associated with stress induced by poor management practices or environmental perturbations. Bacterial infections usually incite haemocytic inflammatory lesions (Pass, Dybdahl and Mannion, 1987; Suzuki, 1995). The formation of conspicuous deposits of brown-black conchiolin on the inner nacreous surface of the shell of *P. maxima,* described by Perkins (1996),

was observed in 12–14 percent of shell by Humphrey *et al.* (1998) and has also been reported in *P. margaratifera* in French Polynesia (Marin and Dauphin, 1991; Cuif and Dauphin, 1996). Though common in many bivalves and attributed to bacterial irritation (Paillard, Maes and Oubella, 1994), the aetiology is still uncertain.

Haplosporidium

An unnamed species of *Haplosporidium* was found in a batch of *P. maxima* spat from the Carnarvon hatchery, Western Australia, in 1993 (Hine and Thorne, 1998). The same haplosporidian was subsequently found in *P. maxima* spat from a marine farm site at King Sound in the Kimberley region of Western Australia in 1995 and again in spat at a marine farm site in Broome in 2000 (Jones and Creeper, 2006). The 1995 incident involved spat that had been free of infection on leaving the hatchery and 6 weeks later were found to have a prevalence of infection of 4.6 percent. On re-testing 15 days later, the prevalence had increased to just over 10 percent, at which point the spat were destroyed. Electron microscope examination confirmed that the parasite was the same parasite as that reported by Hine and Thorne (1998). The relationship between this haplosporidian and one occurring in *Saccostrea cucculata* in the same area is currently under investigation (Bearham *et al.*, 2007).

Perkinsus

Perkinsus sp. is routinely diagnosed by thioglycollate culture rather than histology. It has been commonly isolated by culture from tropical Australian molluscs, including *Pinctada* spp. in the absence of any pathology (Goggin and Lester, 1987, 1995). *Perkinsus*-like protozoa were described by Norton *et al*. (1993b) in focal granulomatous lesions in adult *P. maxima* from a population undergoing high mortality in Torres Strait, Australia, though a causative relationship could not be established. Park *et. al*. (2001) were unable to demonstrate the presence of *Perkinsus* in *P. fucata martensii* from Korea either by thioglycollate culture or histology.

Other agents

A number of infectious organisms do not appear to cause significant tissue damage or inflammatory response from their host. These include turbellarians and Ancistracomid-like ciliates. While turbellarians caused no apparent damage or host response in the study by Humphrey *et al*. (1998), northern hemisphere studies on the turbellarian *Urastoma cyprinae* have shown that it is attracted to oyster gill mucous (Brun, Boghen and Allard, 1999) and causes biochemical changes to gill mucous of infected *Crassostrea virginica* (Brun, Ross and Boghen, 2000). Therefore, absence of histopathological change does not mean that there is no effect on pearl oysters. In 2001, an intracellular rhynchodid-like ciliate was found in oysters (*P. maxima*) from the Exmouth Gulf and Montibello Islands in Western Australia. The ciliate appears similar to those described from mussels (*Mytilus* spp.) in Europe (McGladdery and Bower 2002; Jones and Creeper, 2006). Sampling has shown that the ciliate occurs in high prevalence and abundance in juvenile oysters, disappearing from oysters over about 90 mm diameter.

Gregarines occur in *P. maxima* in Australia and appear not to cause significant damage (Humphrey *et al.*, 1998). However, an unidentified intracellular gregarine parasite, described from the gut of *P. margaritifera* from atolls in the Tuamotu Archipelago, French Polynesia, from the Fiji Islands and from the Red Sea (Gulf of Aqaba), causes local or complete destruction of rectal epithelial cells and may be associated with mortalities in French Polynesia (Chagot *et al.*, 1993).

Metacercariae of a bucephalid trematode occur in the gonad of *P. radiata* in the Persian Gulf (Khamdan, 1998). Third and fourth stage larvae of the ascariid nematode has been reported to occur in the adductor muscle, digestive gland and gonads of

Pinctada spp. The adult nematode occurs in loggerhead turtles, *Caretta caretta* (Berry and Cannon, 1981).

Larval cestodes of the family Lecanicaphalidae which include the genera *Tylocephalum* and *Polypocephalus* are commonly associated with discrete focal or multifocal granuloma in interstitial tissues. Larval *Tylocephalum* sp. have been reported in most bivalves examined in northern Australia (Wolf, 1976, 1977; Hine and Thorne, 2000). Larval *Tylocephalum* sp. are not considered host specific and may occur at high prevalence and intensity in oysters where they may reduce the condition of their host (Sindermann, 1990).

The copepod *Anthessius pinctadae* was described from *P. mararitifera* from the Torres Strait (Humes, 1973). The copepod also occurs in *P. maxima* throughout northern Australia (Humphrey *et al.*, 1998). In moderate numbers, this copepod causes erosion of the oesophageal epithelium and entrapment in the digestive gland resulted in encapsulation and a haemocytic response. Thus, under certain conditions, the parasite may be potentially pathogenic or predispose the oyster to infection.

Fouling organisms

Boring molluscs *Lithophaga* spp. are common in Australia and produce large holes of 1-2 cm sometimes disrupting or breaching the nacreous layer. Polychaete worms invading the shell nacre are also common, resulting in "mud blisters" (Humphrey *et al.*, 1998). Boring sponges, family Clionidae, including the bright red coloured *Cliona* sp., are a major problem for the industry in Australia, leading to severe erosion of the shell matrix and premature removal of shells from pearl production (Vblayudhan, 1983; Taylor, Southgate and Rose, 1997). Treatment and prevention involves regular scrubbing of shell, either manually or by high-pressure hose. There has also been some success with freshwater baths for 30–60 min, while trials are underway testing anti-fouling paints.

Commensal animals

Pea crabs and shrimps are common, occurring in up to 85 percent and 72 percent of Australian *P. maxima* oyster populations respectively (Humphrey *et al.*, 1998). Apart from local oedema in the mantle and depressions in both the mantle and gill tissues caused by the pea crabs, no pathology is associated with these commensal organisms (Dix, 1973).

DISEASES WITH NON-INFECTIOUS AETIOLOGY

Temperature

There is little published information on the histopathology associated with temperature, but it has a marked effect on the oysters. Tomaru *et al.* (2002) showed that growth in height, length and thickness of the shells of *P. fucata martensii* was limited by water temperatures less that 20 °C. Pouvreau and Prasil (2001) showed that temperatures over 30 °C had a negative impact on growth of *P. margaratifera*. Likewise, Mills (2002) showed that survival of *P. maxima* spat was greatest between 23 °C and 32 °C, with 35 °C resulting in high mortalities.

Toxic algae

During a red tide event in Ago Bay, Japan in 1992, the maximum cell density of *Heterocapsa circularisquama* reached over 85 000 cells/ml and there was a concurrent mass mortality of pearl oysters. Subsequent trials (Nagai *et al.*, 1996; Nagai *et al.*, 2000) showed that mortality of two-month-old pearl oysters (*P. fucata*) was closely correlated with the cell density of *H. circularisquama* and that, on exposure, oysters rapidly contracted their mantles and closed their shell valves. Negri *et al.* (2003) showed that *Trichodesmium* blooms also caused poor condition in pearl oysters.

Coral spawning

Mortalities caused by low oxygen levels associated with coral spawning are not uncommon, though not reported in the literature.

Starvation

Degenerative changes, not associated with causative agents, are relatively common and include oedema, increased pigmentation in macrophages in interstitial tissues and kidney and mineralisation. Oedema is believed by Humphrey *et al.* (1998) to be a degenerative response in a physiologically compromised oyster and is also seen commonly in oysters removed from the water column and held at high ambient air temperatures. The significance of increased brown pigmentation in cells (so-called brown cells) is unclear but is believed to be associated with prior cellular breakdown and detoxification (Zaroogian and Yevich, 1994) though it should be noted that brown pigmentation of the heart and epithelium of the mantle tissue is normal. Lamellar mineralization is associated with pearl formation, however, mineralization may occur without nacre formation (Comps, Herbaut and Fougerouse, 2000).

Miscellaneous

Tearing of the adductor muscle through the practice of wedging open oysters for seeding operations also results in a recognizable wound healing response in the affected muscle (Norton, 2000). A non-specific inflammatory haemocytic infiltration in the adductor muscle is typical of this change (Humphrey *et al*., 1998).

Slightly refractile ovoid brown pigmented "protistan parasites" have been described in cytoplasm of digestive gland epithelium of *P. maxima* in Australia (Wolf and Sprague, 1978) and in *P. margaritifera* from the Red Sea (Nasr, 1982). These bodies occur commonly in both healthy and diseased oysters. They are "residual bodies", storage or secretory products which are ultimately released from the cell (Pass and Perkins, 1985) and are not of pathological significance.

Though neoplasia are rare, neurofibromatous tumours in Australian *P. maxima* were recorded by Humphrey *et al*. (1998). Two polypous mesenchymal tumours in *P. margaritifera* have also been described from Australia (Dix, 1972).

MANAGEMENT OPTIONS

There are two factors that together make pearl farming less susceptible to disease-induced mass mortalities than other molluscs. The first is the panel based culture system, which apart from providing a degree of predator protection, makes monitoring of individual shells possible. The second is the requirement to lift and clean biofouling off the panels every four weeks or so, which means that shell is regularly inspected for mortality.

The majority of the internationally notifiable diseases of molluscs have been spread by human activities, usually associated with aquaculture. Movement controls to prevent the indiscriminate movement of shell between areas and between countries is thus the first step in disease management. Within Australia, regional differences in distribution of several agents including Papovavirus-like inclusion bodies were identified by Humphrey *et al.* (1998) and form the basis for regional control measures including batch testing of animals for disease status prior to authorizing movements. In Western Australia, the use of quarantine sites and the provision of a mandatory five nautical mile exclusion zone for pearl oyster farming activities around farms serves, in part, as a barrier to prevent the spread of disease from farm to farm.

CONCLUSIONS

Pearl oysters are relatively free of serious pathogens – so far. However, there has been little study of causes of mortalities of pearl oysters in regional areas where farming is

now spreading. For that reason, both government agencies and industry members need to be careful about the source of their stocks and the movement of oysters between regions if the disasters that have befallen other shellfish industries are to be avoided.

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3.2 The Cook Islands experience: pearl oyster health investigations

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ABSTRACT

A survey of the health of black-lip pearl oysters (*Pinctada margaritifera*) and other bivalves in Manihiki Lagoon, Rakahanga Lagoon and Penrhyn Atoll, Cook Islands, was conducted in May 1998. That study revealed the presence of a few trivial infections (e.g. gregarines, ciliates, trematode metacercariae) and three potentially pathogenic groups, boring clionid sponges, fungal infection of drill holes caused by an *Ostracoblabe implexa*-like fungus and a species of *Perkinsus* which occurred at low intensities in kuku (*Arca ventricosa*). The *Perkinsus* sp. was most probably *P. olseni* which has been previously reported from the region, however it appeared that pearl oysters were less susceptible than many other hosts and therefore perkinsosis may not be a great threat to pearl farming in the Cook Islands.

Subsequent to the original survey, a disease outbreak was reported in black-lip pearl oysters cultured in Manihiki Lagoon in November 2000. The disease was characterized by retraction of the mantle and deposition of a broad, brown conchiolin deposit on the nacre inside the pallial line. Overall, 64.7 percent of oysters examined showed signs of the disease at all sites examined within the lagoon, with the prevalence of the lesions ranging from 3.3 percent in wild oysters from Site 3, to 100 percent in cultured oysters from Site 2. There was no evidence of widespread infection of any viral, protozoan or metazoan pathogens in the diseased pearl oysters examined. A variety of bacteria were isolated from the hemolymph and adductor muscle of the oysters sampled for bacteriology, most notably *Vibrio harveyi.* However, there was little evidence to suggest one pathogenic strain of bacteria was present in diseased oysters and there was no significant correlation between the severity

of conchiolin deposits and the severity of bacterial infections. A follow-up survey of all three sites in December 2003 showed that a high percentage of the oysters in Manihiki Lagoon exhibited shell scarring as the brown lesions were overlaid with new nacre, while oysters sampled from Penrhyn Atoll were also affected with similar gross signs to those observed in Manihiki Lagoon in November 2000. These data suggest that the November 2000 disease outbreak in Manihiki Lagoon was an unprecedented event. It appears that the disease outbreak in November 2000 was associated with infection of pearl oysters by opportunistic vibrios following a transient reduction in lagoon water quality due to a long period of unusually calm, dry weather. The situation may have been exacerbated by high oyster stocking densities and occurred at a time of the year when oysters may have been further stressed after spawning. Management of the problem may, therefore, be based on controlling the stocking density of oysters in lagoons and modifying husbandry practices to reduce stress on oysters when they are spawning. Ongoing monitoring of key water quality parameters in the lagoons would also be useful so that the epidemiology of disease outbreaks which may occur in the future might be better understood.

INTRODUCTION

Culture of black-lip pearl oysters (*Pinctada margaritifera*) in the Cook Islands (Sims, 1994) has increased dramatically in recent years and production of black pearls now rivals tourism as the major source of foreign exchange (Rowntree, 1993). Because of their importance to the Cook Islands economy, a survey of the health of *P. margaritifera* and other bivalves was done in May 1998, funded by the Asian Development Bank (Hine, 1998). The survey was carried out to determine the health status of farmed and wild pearl oysters in Manihiki lagoon. Oysters were also examined from Rakahanga, a nearby island, Penrhyn Atoll, another site of black pearl culture and Aitutaki. *Perkinsus olseni*, an internationally notifiable organism, infects pearl oysters (Norton *et al.*, 1993; Hine and Thorne, 2000), but it occurs more commonly in members of the families Tridacnidae (Goggin, 1996), Arcidae and Isognomonidae (Goggin and Lester, 1987; Hine and Thorne, 2000). Therefore members of those families were also sampled to determine whether *Perkinsus* occurs in the lagoon. Subsequent to that survey, a disease outbreak was reported in black lip pearl oysters cultured in Manihiki Lagoon in November 2000 (Diggles and Hine, 2001). This paper presents some of the results of investigations conducted by the National Institute of Water and Atmospheric Research (NIWA) to determine the identity of the agents and environmental conditions associated with diseases of black-lip pearl oysters in the Cook Islands.

PEARL OYSTER HEALTH INVESTIGATIONS

Materials and Methods

In the May 1998 health survey, 547 pearl oysters were sampled from 11 sites within Manihiki lagoon. At one of these sites, 40 wild virgin shells and 20 drilled and hung virgin shells were sampled. At another site, a sample was collected of 50 oysters fouling the farm site. Also, adult pearl oysters were sampled from the nearby island of Rakahanga (n = 58) and from Penrhyn Atoll (n = 49) and spat from Penrhyn Atoll (n = 16) and Aitutaki (n = 12). In addition, 42 pipis (*Pinctada maculata*), 77 razor shells (*Isognomon isognomum, Isognomon perua*), 50 kuku (*Arca ventricosa*) and 50 paua (*Tridacna maxima*) from Manihiki were sampled. The shells of all animals sampled were measured and examined externally and internally for pests. A standard section was cut through the digestive gland, gonad, mantle, gills, adductor muscle, heart, kidney and foot and fixed in 10 percent formalin made up with seawater for histology. The remaining tissues of 50 *P. margaritifera,* 21 *I. isognomum,* 25 *A. ventricosa* and 3 *P. maculata*, were incubated in Ray's fluid thioglycollate medium (RFTM) (Ray, 1966; Bushek and Allen, 1996) for the detection of *Perkinsus.*

During the disease outbreak in November 2000, black lip pearl oysters (n = 300) and water were sampled from 10 sites within Manahiki lagoon for histopathology, bacteriology and toxicology. Examination began by noting the presence of grossly evident infections with metazoan parasites and fouling organisms (mudworms, fungi, boring sponges, etc.). The dorso-ventral measurement (DVM) of each oyster was recorded to the nearest mm before the oyster was opened by severing the adductor muscle. The nacre of each shell valve was examined for abnormal conchiolin deposits and the severity of conchiolin lesions was assessed using a six-grade qualitative scoring system (Table 3.2.1).

The first 10 oysters examined from each site were subjected to bacteriological analysis prior to being processed for histopathology and RFTM incubation. Samples of 0.1 ml of hemolymph were obtained from the pericardium with a sterile 1 ml syringe and 25 G hypodermic needle and inoculated onto one half of plates containing thiosulphate citrate bile salt sucrose agar (TCBS) and for oysters from sites 1 to 6, Tryptone Soya Agar (TSA) with 2 percent NaCl added (TSA+2). The other half of each plate was streaked with a flamed wire loop which had swabbed the surface of the adductor muscle which had been cut with a sterile scalpel to provide an uncontaminated surface. Sub-samples of 0.1 ml of seawater samples obtained for plankton analysis from the top and the bottom of the water column from each site were also examined for waterborne bacteria in a similar manner. The bacteriology plates were incubated at room temperature (30 °C) and examined for bacteria after 18 hrs and again after 36 hrs. Samples of different colony types observed were sub-cultured to ensure purity before being stored in long term preservation medium (Beuchat, 1974) for up to 2 weeks prior to identification.

Biochemical characterization of selected isolates was undertaken by subjecting them to 52 phenotypic tests using methods described by Baumann, Baumann and Mandel (1971), Furniss, Lee and Donovan (1978) and West and Colwell (1984). Phenotypic data were compared to a probabilistic data matrix for *Vibrio* species (Bryant and Smith, 1986) using a regularly updated version of the Bacterial Identifier program (Bryant and Smith, 1991). An acceptable identification was reached when the identification score equaled or exceeded 0.98.

The first 10 oysters examined from each site were also examined for the presence of *Perkinsus* sp. by placing excised samples of mantle, digestive gland, gills, foot and kidney into RFTM. Tissues were incubated in RFTM in the dark between 7 and 14 days then removed, blotted to remove excess RFTM and placed in dilute Lugol's iodine before examination under a dissecting microscope at 40x magnification for stained enlarged hypnospores (Ray, 1966; Bushek and Allen, 1996).

All 30 oysters from each site were sampled for histopathology. An oblique transverse section, approximately 5-7 mm thick, was cut from each oyster using a scalpel. The section was oriented to include mantle, gonad, digestive gland, gills, foot and sometimes kidney. The tissues were fixed in 10 percent formalin in filtered seawater for at least 96 hrs before being embedded in paraffin on the cut surface. One section 6 µm thick was cut and stained with hematoxylin and eosin (H&E) using standard histological techniques before being examined under a compound microscope.

TABLE 3.2.1
The qualitative scoring system used to grade the severity of abnormal conchiolin deposits in oysters from Manihiki Lagoon

Grade	Abnormal conchiolin lesion severity
0	apparently healthy, no lesions evident
1	1 or 2 focal lesions
2	<25% of shell valve perimeter affected
3	25 to 50% of shell valve perimeter affected
4	50 to 75% of shell valve perimeter affected
5	>75% of shell valve perimeter affected

Samples of pearl oysters were also collected from two sites in Manihiki Lagoon and a control site in Penrhyn Lagoon for toxicological analysis. The two samples from Manihiki lagoon included a sample of relatively healthy wild oysters (n = 4, lesion grade 0 to 1) and a sample of diseased cultured oysters (n = 5, lesion grade 3 to 5). The samples from Penrhyn Lagoon consisted of two sub-samples of 6 oysters without lesions. One sample consisted of 6 wild oysters and the other of 6 cultured oysters from the same locality. All samples were analysed for selected heavy metals (zinc, copper, arsenic, mercury chromium, cadmium, nickel and lead) using nitric/hydrochloric acid digestion followed by inductively coupled plasma mass spectrometry (ICP-MS) determination methods and total hydrocarbons by accelerated solvent extraction (ASE) or sonication extraction followed by gas chromatography/flame ionization detection (GC-FID) quantification.

A follow-up survey conducted in December 2003 saw a total of 654 pearl oysters sampled for pathological and microbiological analysis from 12 sites within Manihiki Lagoon (n = 357 oysters), 6 sites from Rakahanga Lagoon (n = 120 oysters) and 6 sites from Penrhyn Atoll (n = 177 oysters). The histological and microbiological methods used to examine oysters in the 2003 survey were identical to those used during the original disease outbreak in November 2000.

RESULTS

May 1998

Gross observations: *Pinctada margaritifera*

Three types of shell infection could be macroscopically identified; these are boring sponges, mudworm tunnels and fungal-like infestations derived from holes drilled in the shell for suspension-hanging.

Boring sponges appeared as orange inclusions, 1.0-5.0 mm across, underlying the nacre. Two types were apparent. One comprised of equally spaced inclusions of 1.0-3.0 mm (but usually 1.6-2.1 mm) diameter. Inhalent-exhalent holes 0.7-1.1 mm in diameter could be seen on the outer surface of infected valves. The second type of boring sponge was only observed in two wild oysters from one site. It appeared as orange inclusions of unequal size, 3.5-5.0 mm in diameter, angular to ovoid in shape, that were widely distributed throughout the shell, including nacre underlying the adductor muscle attachment. Holes on the outer surface of the shell were 1.0-1.4 mm in diameter, but erosion of the shell between holes were <5 mm in diameter. These infestations appeared severe enough to cause mortalities, either by causing detachment of the adductor muscle, or disintegration of the shell.

Mudworm tunnels were only seen in 5 oysters. They appeared as 1-3 mm wide dark brown to black straight tunnels extending up to 14 mm from the edge of the nacre toward the centre of the shell. The corresponding outer surface of the shell had a shallow indentation into the surface between the layers of shell. These infestations appeared to be trivial and unlikely to cause any adverse effect on oyster health.

Infections associated with holes drilled in oyster shell for suspension-hanging occurred at the site of the drilled hole, but progressed as a greenish brown or yellow discolouration of the inner shell extending toward the centre of the shell. The overlying nacre was thin and can easily be broken by slight pressure. The infections appeared to develop in oysters with drill holes made too far from the edge of the shell, causing damage to the underlying soft-tissues of the oyster (presumably the mantle).

Histopathology: *Pinctada margaritifera*

In 18-68 percent of *P. margaritifera* from different sites in Manihiki lagoon, a gregarine-like apicomplexan occurred between the epithelial cells, or underlying the basement membrane of the posterior gut. Occasionally low density infections occurred in connective tissue throughout the oyster. Gregarines in the connective tissues were

usually larger than those located between epithelial cells. Although there was no host inflammatory reaction to connective tissue infections, gregarines in the gut epithelium were often partially engulfed by brown cells. Prevalence was highest in wild shell (63-68 percent). Gregarines were less prevalent (10 percent) in Rakahanga oysters and absent from Penrhyn oysters and spat transferred from Penrhyn to Aitutaki.

A few ciliates with a distinctive pellicle occurred in the gut of 3–14 percent of pearl oysters from Manihiki. These ciliates were never numerous and there was no apparent tissue damage associated with their presence. Rakahanga oysters had similar levels of ciliate infection (3 percent). Infection levels were much higher in Penrhyn adults (39 percent) and Aitutaki spat derived from Penrhyn (42 percent), but were absent from Penrhyn spat. Although quantification was not possible, infected Penrhyn oysters appeared to have elevated levels of degeneration of the digestive diverticula epithelium.

Trematode metacercariae were encysted in the connective tissue of the mantle of five oysters. Infections were light and none of the encysted helminths caused a host cellular response.

Examinations for the presence of Perkinsiid protozoan parasites using RFTM incubation gave negative results.

Histopathology: *Pinctada maculata*

The epithelium and underlying connective tissue of the posterior gut was infected with gregarine-like apicomplexans that were morphologically indistinguishable from those at the same site in *P. margaritifera.* The prevalence (29 percent) was also similar, but the intensity of infection was higher, with many gregarines crowding the gut epithelium. Despite this, there was no evidence of a cellular response by the host, suggesting that these parasites do not cause disease. Ciliates resembling those in *P. margaritifera* were also present. An un-identified thick-walled protozoan cyst, 22 µm in diameter, occurred in the connective tissue of one pipi. It contained a few basophilic refractile reniform spore-like bodies 4 x 8 µm in diameter. RFTM incubation tests gave negative results.

Histopathology: *Isognomon* spp. and *T. maxima*

No infections or abnormalities were observed and incubation of tissue samples in RTFM gave negative results.

Histopathology: *A. ventricosa*

Gregarines, indistinguishable from those seen in other hosts, occurred in the digestive epithelium of the posterior gut of one kuku. Coccidian sporozoites infected the connective tissues of the digestive gland of four *A. ventricosa.* There appeared to be eight sporozoites per sporocyst and two sporocysts per oocyst, tentatively placing this organism within the eimeriine genus *Dorisiella.* Ciliates were common in the gut and digestive diverticulae and, in one animal, infected the adductor muscle, causing dissociation of the muscle fibres.

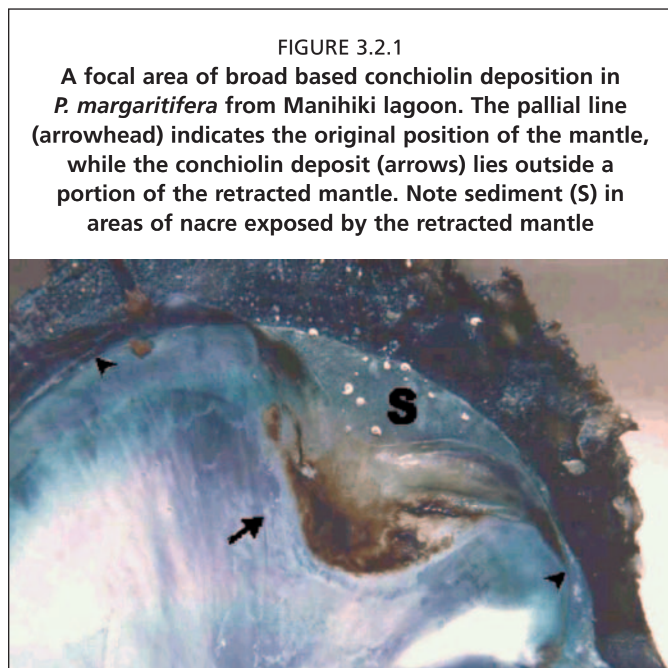
Groups of *Perkinsus* schizonts, 12–18 µm across, comprising individual schizonts 4-5 µm long, occurred in the connective tissue between the digestive diverticula and the gonad follicles of three (6 percent) and in the heart of one (2 percent) of the kuku examined. There was no apparent cellular response to these light infections. RFTM incubation gave two positives, being two of the four kuku in which *Perkinsus* was detected by histology.

November 2000

Gross observations

A total of 194 of the 300 oysters examined (64.7 percent) in November 2000 exhibited varying degrees of a broad, brown conchiolin deposit on the nacre inside the pallial

FIGURE 3.2.1
A focal area of broad based conchiolin deposition in *P. margaritifera* from Manihiki lagoon. The pallial line (arrowhead) indicates the original position of the mantle, while the conchiolin deposit (arrows) lies outside a portion of the retracted mantle. Note sediment (S) in areas of nacre exposed by the retracted mantle



line (Figure 3.2.1). The inner edge of the abnormal conchiolin deposits approximated the general position of the outer surface of the retracted mantle. Abnormal conchiolin deposits were found in oysters from all sites examined. Prevalence of the lesions ranged from 3.3 percent in wild oysters from site 3, to 100 percent in cultured oysters from site 2 (Table 3.2.2). The mean severity of the lesions ranged from 1 in wild oysters at site 3, to 3.3 in cultured oysters at site 9 (Table 3.2.3).

Microbiology

A variety of bacteria were isolated from the hemolymph and adductor muscle of the oysters sampled for bacteriology. Most oysters had mixed bacterial infections and there was little evidence to suggest one pathogenic strain of bacteria was present in diseased oysters. Bacteria were not always isolated from oysters exhibiting abnormal conchiolin deposits, while

TABLE 3.2.2
Prevalence and mean score of abnormal conchiolin deposit lesions on nacre inside shell valves

Site examined	Lesion prevalence	Mean lesion score	Range	Standard deviation
Site 1	93.3 %	2.9	0 – 5	1.44
Site 2	100 %	2.7	1 – 5	1.32
Site 3	3.3 %	1	0 – 1	–
Site 4	60 %	1.2	0 – 3	0.51
Site 5	73.3 %	1.7	0 – 4	1.08
Site 6	53.3 %	2.1	0 – 4	1.31
Site 7	70 %	2	0 – 5	1.3
Site 8	70 %	2.9	0 – 5	1.6
Site 9	56.7 %	3.1	0 – 5	1.5
Site 10	66.7 %	2	0 – 5	1.38
All sites	64.7%	2.33	0 – 5	1.42

TABLE 3.2.3
Comparison of the mean severity of abnormal conchiolin deposits and the mean severity of bacterial infections of the adductor muscle and hemolymph as detected by plate culture using the following grading system: 0 = no bacteria, 1 = 1 to 10 colonies isolated, 2 = 10 to 100 colonies isolated, 3 = > 100 colonies isolated. A total of 10 oysters and 2 water samples (0.1 ml) were examined by bacteriology from each site. Nd = not done

Site	Prevalence of conchiolin lesions	Mean lesion intensity	Prevalence of bacterial infections by plate culture	Mean bacterial infection severity (TCBS)	Mean bacterial infection severity (TSA+2)	Mean severity of bacteria in water, Top	Bottom
1	80%	1.3	90%	1.1	1.4	1	1
2	100%	2.4	90%	1.5	1.6	1.5	1.5
3	0%	–	30%	1	1.3	0	1.5
4	40%	1	50%	1.5	1.5	1	1.5
5	90%	2.1	70%	2.3	2	1.5	1
6	80%	2.5	40%	1	3	0	1
7	50%	1.8	0%	–	Nd	1	0
8	60%	1.8	10%	1	Nd	1	1
9	80%	3.3	0%	–	Nd	1	2
10	80%	1.3	50%	2.2	Nd	0	1

TABLE 3.2.4
Bacteriology results for selected isolates obtained from pearl oysters and water during field sampling

Isolate number	Sample source	Identification
1	Site 1, bottom water, yellow on TCBS	*Vibrio* sp. not identifiable
2	Site 1, oyster D hemolymph, yellow on TCBS	Vibrio tubiashii
3	Site 2, oyster A adductor muscle, yellow on TCBS	*Vibrio harveyi*
4	Site 2, oyster F, hemolymph, yellow on TCBS	*Vibrio harveyi*
5	Site 5, oyster E adductor muscle, yellow on TCBS	*Vibrio pelagius biovar II*
6	Site 1, oyster F adductor muscle, green on TCBS	*Vibrio harveyi*
7	Site 2, oyster D adductor muscle, green on TCBS	*Vibrio harveyi*
8	Site 5, oyster H small green colonies on TCBS	*Staphylococcus-Micrococcus*-like sp.
9	Site 2, bottom water, cream on TSA+2	*Vibrio* sp. not identifiable
10	Site 2, bottom water, clear on TSA+2	Not recovered on subculture
11	Site 2, oyster F, hemolymph, clear on TSA+2	*Vibrio mediterranei*
12	Site 1, oyster C, hemolymph, cream on TSA+2	*Vibrio tubiashii*
13	Site 1, bottom water, spreading on TSA+2	*Vibrio* sp. not identifiable
14	Site 2, bottom water, spreading on TSA+2	*Vibrio harveyi*
15a	Site 2, oyster F, hemolymph, spreading on TSA+2	*Vibrio* sp. not identifiable
15b	Site 2, oyster F, hemolymph, spreading on TSA+2	*Vibrio* sp. not identifiable
16	Site 1, oyster P, from brown stain on nacre	*Vibrio* sp. not identifiable
17	Site 4, oyster F, adductor muscle, very small colonies	*Acinetobacter*-like

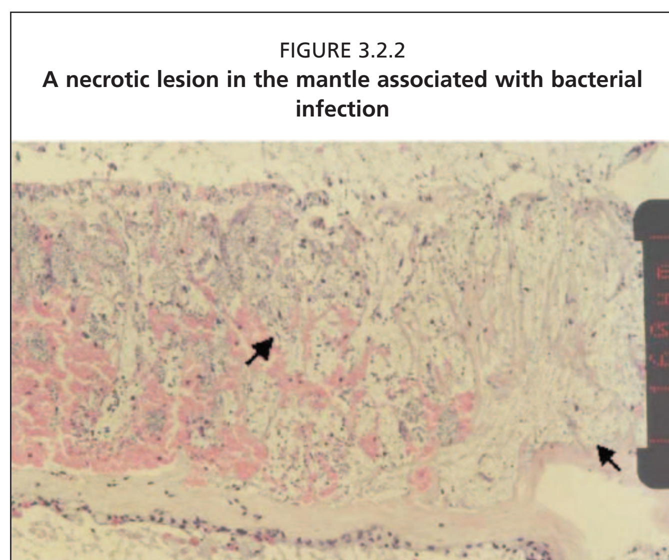
some oysters without obvious conchiolin deposits had bacterial infections (Table 3.2.3). There was no significant correlation between the severity of conchiolin deposits and the severity of bacterial infections. Only light to moderate numbers of bacteria were isolated from water samples, with more bacteria being isolated from bottom samples. The identifications for 17 representative isolates of bacteria obtained from pearl oysters and from water collected during field sampling are summarised in Table 3.2.4. Isolates of *Vibrio harveyi* predominated in samples from adductor muscle, hemolymph and water. All isolates of *V. harveyi* examined had different biochemical phenotypes. Undescribed species of *Vibrio* were also common in water, hemolymph and from the surface of conchiolin deposits. Less common isolates included *V. tubiashii* from hemolymph, *V. mediterranei* from hemolymph and *V. pelagius* biovar II from adductor muscle.

Histopathology

Most bacteria occurred in the mantle epithelium and were associated with necrosis and sloughing of epithelial and sub-epithelial cells (Figure 3.2.2). Other sites of infection included the gonad and digestive gland tubule epithelium. Oysters with higher conchiolin scores (2 or more) tended to have depleted or resorbing gonads (Figure 3.2.3). Infections with gregarine protozoans were noted principally in the epithelium of the mid and hind gut (Figure 3.2.4), but also in the sub-epithelial connective tissue surrounding the gut. There was no significant correlation between the intensity of gregarine infection and conchiolin lesion severity.

Other notable pathological lesions in the oysters examined by histopathology included breakdown, sloughing and focal necrosis of the digestive gland epithelium

FIGURE 3.2.2
A necrotic lesion in the mantle associated with bacterial infection



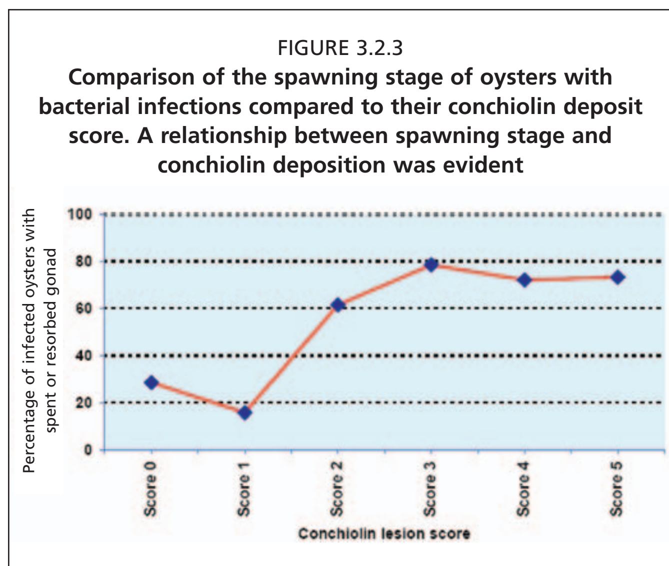


FIGURE 3.2.3
Comparison of the spawning stage of oysters with bacterial infections compared to their conchiolin deposit score. A relationship between spawning stage and conchiolin deposition was evident

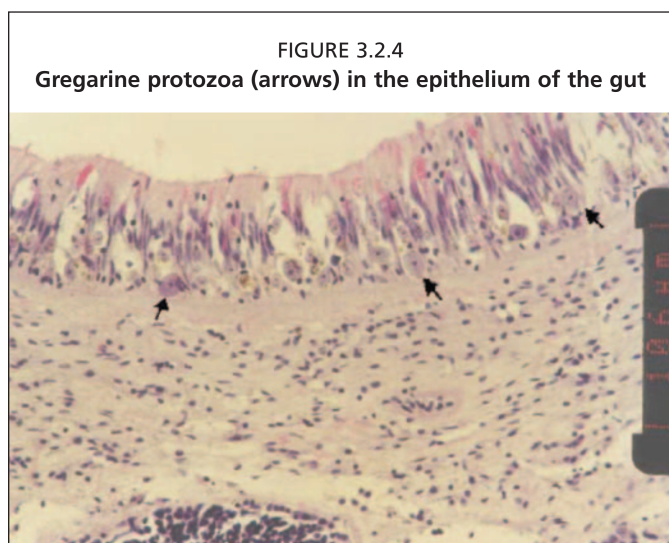


FIGURE 3.2.4
Gregarine protozoa (arrows) in the epithelium of the gut

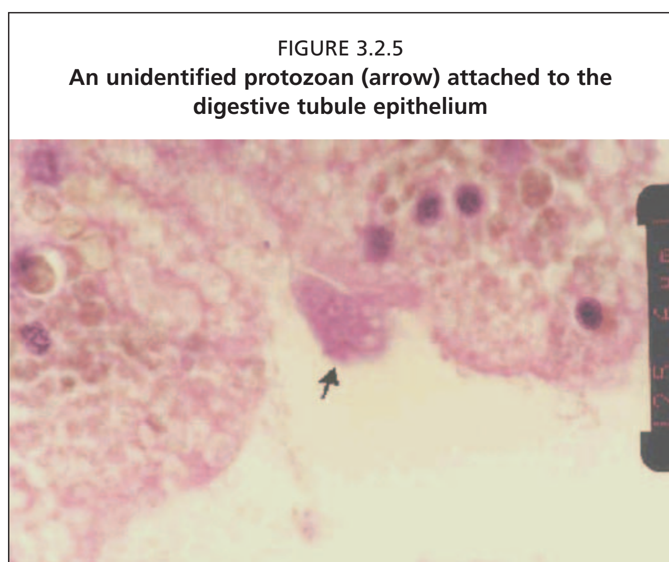


FIGURE 3.2.5
An unidentified protozoan (arrow) attached to the digestive tubule epithelium

(32 percent of oysters), atrophy of digestive gland tubules (5 percent of oysters), and abnormal kidney pathology (necrosis or hyperplasia of the kidney epithelium, 2.7 percent of oysters). Parasitic infections also occurred at low prevalence, including trematodes in the foot and mantle (4 percent of oysters), copepods in the gut and digestive tubules (6.6 percent of oysters) and an unidentified protozoan found attached to the digestive tubule epithelium with a stalk-like process in two oysters (0.7 percent prevalence, Figure 3.2.5). Groups of heavily basophilic prokaryote-like organisms were also present in the periphery of the mantle of all oysters examined (Figure 3.2.6). These may have been harmless symbiotic organisms as their presence appeared to bear no relationship with oyster health.

Thioglycollate incubation

None of the 100 oysters examined were positive for *Perkinsus* by incubation of tissue samples in RTFM.

Plankton analysis

None of the plankton species found in the water samples were known to be toxic, hence the possibility of a bloom of a toxic plankton species appeared highly unlikely.

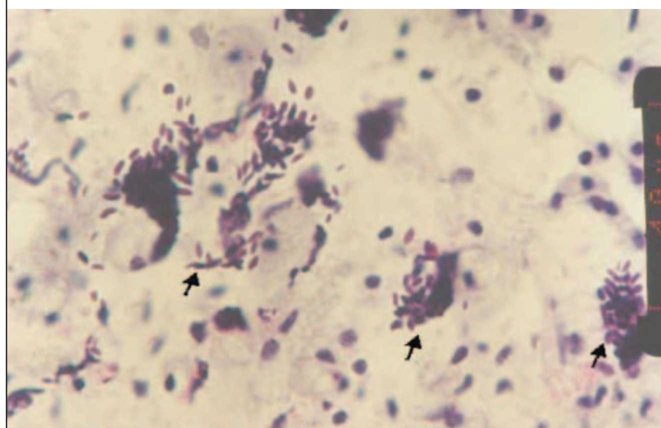
Toxicology

Analysis of samples of oysters taken from Manihiki Lagoon showed that lead levels were elevated in cultured oysters with grade 3–5 conchiolin lesions compared to wild oysters with grade 0 and 1 lesion (Table 3.2.5). The levels of lead in the affected oysters were higher than levels of concern recommended by the Food and Drug Administration (FDA) of the United States of America (Table 3.2.5). Healthy oysters from Penrhyn lagoon had very low levels of lead compared to the oysters from Manihiki lagoon, but had elevated arsenic, though these were below levels of concern listed by the FDA. Levels of zinc were elevated in oysters from Manihiki lagoon, while hydrocarbons were higher in oysters from Penrhyn.

December 2003

A significant proportion of both wild and cultured oysters from Manihiki and Penrhyn lagoons displayed brown coloured conchiolin deposits in the nacre of one or both shell valves (Diggles and Maas, 2004). The oysters from Penrhyn were the worst affected, while this lesion was absent from oysters sampled from Rakahanga Lagoon. The prevalence and intensity of this lesion in oysters from Manihiki Lagoon was much lower than recorded in November 2000, but its persistence suggests that the oysters in Manihiki remained affected by environmental stress in at least some parts of the lagoon. The emergence of the conchiolin lesions in oysters from Penrhyn lagoon may be related to stress from poor water quality due to low levels of dissolved oxygen recorded by water quality monitoring buoys in that lagoon.

FIGURE 3.2.6
Groups of deeply basophilic prokaryote-like organisms (arrows) in the mantle



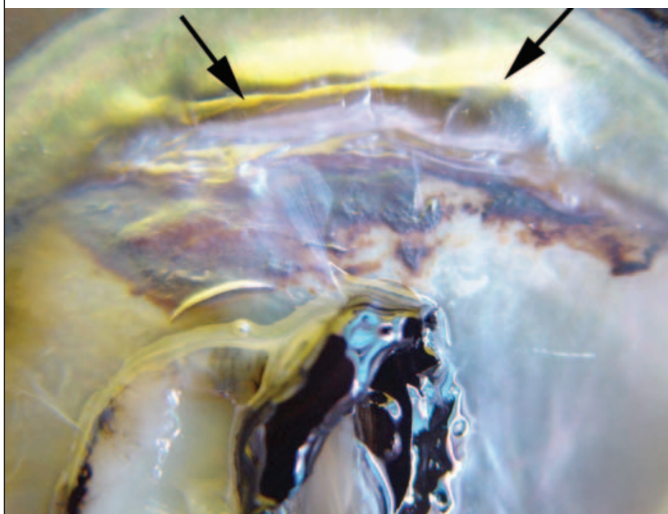
Signs of recovery from the previous outbreak of bacterial disease in November 2000 were observed in both wild and cultured oysters from Manihiki Lagoon. In these oysters the conchiolin lesions remained visible but had been overlaid by newly deposited nacre (Figure 3.2.7). This permanent scarring of the nacre of the shell was also associated with development of a prominent check around the periphery of the shell (Figure 3.2.8), indicating that shell growth ceased for a significant period of time subsequent to November 2000. Recovering oysters were not observed in any of the earlier samples of oysters from Manihiki Lagoon from May 1998 or November 2000, suggesting that the November 2000 disease episode was unprecedented and had significant long term effects on the health of surviving oysters in Manihiki Lagoon.

TABLE 3.2.5
Comparison of toxicology tests for oysters from Manihiki and Penrhyn lagoons. Results are for 4 to 6 pooled oysters from each site. Notable differences between lagoons are in bold

Sample name	Manihiki Lagoon, cultured oysters	Manihiki Lagoon, wild oysters	Penrhyn Lagoon, cultured oysters	Penrhyn Lagoon, wild oysters	USA FDA levels of concern
Range of conchiolin lesion severity	Grade 3 to 5	Grade 0 to 1	Grade 0, healthy	Grade 0, healthy	
Zinc (mg/kg)	52	80.3	13.6	6.35	n/a
Copper (mg/kg)	0.33	0.53	0.38	0.48	n/a
Arsenic (mg/kg)	7.27	5.79	13.1	10.5	86
Cadmium (mg/kg)	1.02	0.85	1.63	1.61	3.7
Mercury (mg/kg)	<0.005	0.008	0.004	0.004	1
Chromium (mg/kg)	0.13	0.16	0.08	0.11	13
Nickel (mg/kg)	0.1	0.19	0.1	0.16	80
Lead (mg/kg)	2.7	0.08	0.007	0.009	1.7
Hydrocarbons					
C7-C9 (mg/kg)	<10	<10	<4	<4	-
C10-C14 (mg/kg)	<20	<20	<8	<7	-
C15-C36 (mg/kg)	50	100	160	183	-
TOTAL	<80	100	160	180	-

FIGURE 3.2.7
This photo of an oyster taken from Manihiki lagoon in December 2003, around 3 years after the November 2000 disease event shows conspicuous regrowth of nacre over conchiolin deposits (arrows) laid down during the disease event

FIGURE 3.2.8
Regrowth of nacre over conchiolin deposits immediately proximal to a prominent check in shell growth (arrows) in an oyster taken from Manihiki lagoon 3 years after the November 2000 disease event



DISCUSSION

The study of May 1998 revealed the presence of a few trivial infections (e.g. gregarines, ciliates, trematode metacercariae) and three potentially pathogenic groups, boring sponges, the fungus from infected drill holes and *Perkinsus.* The identity of the orange sponges that bore into Manihiki and Rakahanga oyster shell is currently unclear, as is the taxonomy of clionid sponges. The descriptions of many species differ from the descriptions of the same species by other authors. The more common orange sponge resembles *Cliona celata,* but the description by Thomas (1979) is too vague to be of use and *C. celata* has been reported from cold temperate waters (Baxter, 1984). The other, less common, boring sponge resembles *Cliona vastifica,* which infects *P. margaritifera* in French Polynesia (Mao Che *et al.*, 1996), but *C. vastifica* ranges from dark green to yellowish grey in colour and the dimensions of the sponge differ from those given by Thomas (1979). *Cliona vastifica* and *C. celata* are the most widespread clionid infestations in pearl shells (Thomas, 1979; Mao Che *et al.*, 1996).

The organism associated with the infection of drilled holes appeared hyphae-like and most closely resembles the fungus *Ostracoblabe implexa* from *P. margaritifera* in French Polynesia (Mao Che *et al.*, 1996). *Ostracoblabe implexa* is primarily a fungal pathogen in shells of bivalves in temperate waters (Li *et al.*, 1983), however the site and growth of the organism reported here appears to be the same as for *O implexa.* The burrowing fungus appears to gain entrance through drill holes and obtains its nourishment from the breakdown of the proteinaceous shell matrix. However, when it reaches the inner shell surface it sets up an irritation that changes the cellular structure of the mantle, resulting in deposition of conchiolin-rich nacre over the fungus. This results in the thin nacre, lacking refringence and lustre that overlays the infection and which can be readily broken by light pressure. Fortunately it appears that infection is rare, except through holes drilled in the shell for suspension hanging and improvements in technique will overcome the problem.

Perkinsus is potentially the most serious pathogen. The species in Manihiki lagoon is probably *P. olseni*, as this species is known to occur around northern New Zealand (Hine and Diggles, 2002), Australia (Lester and Davis, 1981; Hine and Thorne, 2000),

Korea (Choi and Park, 1997; Park, Choi and Choi, 1999; Lee *et al.*, 2001) and Japan (Hamaguchi *et al.*, 1998). This suggests that it is an Indo-Pacific species and that it is likely therefore that the Manihiki *Perkinsus* is *P. olseni.* It has been shown that another *Perkinsus, P. atlanticus*, which occurs in clams in Spain, is actually *P. olseni* (Robledo *et al.,* 2000), that was probably introduced into Spain in Manila clams (*Ruditapes decussatus*) from Southeast Asia.

Perkinsus olseni was originally described in association with mass mortalities among abalone (*Haliotis* spp.) on the coast of South Australia (Lester and Davis, 1981). It was subsequently shown that it infects many families and species of molluscs (Goggin and Lester, 1987; Hine and Thorne, 2000) and that isolates from one species can infect many other species (Goggin *et al.*, 1989). Any one isolate has developmental stages of sizes that vary from host to host and therefore size does not distinguish species. In the great majority of infections, the parasite occurs as spherical clusters of schizonts without eliciting a host response (Hine and Thorne, 2000), as was observed here. When *P. olseni* causes disease, the other histozoic developmental stages (merozoites which develop to meronts and then to schizonts) are present. What triggers the parasite to change from a benign parasite into a proliferating pathogen is unknown. It does appear, however, that pearl oysters are less susceptible than many other hosts and therefore perkinsosis may not be a great threat to pearl farming.

The study in November 2000 suggested that the outbreak of disease associated with brown conchiolin deposits on the nacre had a bacterial aetiology. There was no evidence of widespread infection of any viral, protozoan or metazoan pathogens in the diseased pearl oysters examined. The presence of the gregarine protozoans in the gut epithelium was not correlated with the severity of the conchiolin lesions. The 1998 survey found that these protozoans also occur in apparently healthy pearl oysters, as also found by Humphrey *et al.* (1998). We consider the association of gregarines with diseased *P. margaritifera* both here and previously in French Polynesia (Chagot *et al.*, 1993) as likely to be incidental and they are considered unlikely to cause disease under normal circumstances (Humphrey *et al.*, 1998). Their presence in *P. maculata* and *A. ventricosa* indicate that both other pteriid species and other bivalve families are infected by these apparently benign parasites. The basophilic prokaryote infections of the mantle seen in the November 2000 survey do not appear to have previously been reported, although similar inclusions have been reported from the digestive epithelia of *P. margaritifera* from French Polynesia (Comps *et al.*, 1998).

The predominance of the bacterium *V. harveyi* and other vibrios in cultures taken from affected oysters, together with the occurrence of pathological lesions consistent with bacterial infection in many affected oysters, indicates that infection by *V. harveyi* and other opportunistic vibrios, was associated with this disease syndrome. There was no evidence to suggest that the disease syndrome was primarily caused by a single pathogenic strain of *V. harveyi*. This is a fundamental difference between the disease syndrome of *P. margaritifera* in Manihiki Lagoon and that of Brown Ring Disease (BRD). BRD is also characterised by abnormal brown conchiolin deposits adhering between the pallial line and the edge of the shell (Paillard and Maes, 1994). However, BRD is caused only by strains of a pathogenic bacterium, *Vibrio tapetis* (formerly known as *Vibrio* P1) (Borrego *et al.*, 1992; Paillard, Maes and Oubella, 1994; Novoa *et al.*, 1998) and the disease can be reproduced by experimental exposure to these strains.

The anomalous conchiolin deposition in BRD occurs as a definite thin brown ring on the nacre around the perimeter of the shell (Paillard and Maes, 1994). In contrast, most of the oysters sampled from Manihiki Lagoon displayed a broad conchiolin deposition resulting from retraction of the mantle, with the deposits lying outside the edge of the mantle. This condition was virtually identical to that described for *Pinctada maxima* from Western Australia associated with the presence of *Vibrio harveyi* (see Dybdahl and Pass, 1985; Pass, Dybdahl and Mannion, 1987; Perkins, 1996).

Bacterial diseases in aquaculture are often associated with opportunistic bacteria which invade hosts which are stressed due to unfavorable conditions such as overcrowding, abnormally high or low water temperatures and/or poor water quality. Pass, Dybdahl and Mannion (1987) found that *V. harveyi* infection in *P. maxima* was associated with poor water quality conditions, low water temperatures and overcrowding during transport of oysters to lease sites. Figueras *et al.* (1996) found the highest prevalence of BRD in storage areas where clams were kept at high population densities for at least 1 month. In Manihiki Lagoon, one potential stressor which may have been related to the onset of mortalities was the high stocking density. Prior to the disease outbreak the number of oysters cultured in Manihiki Lagoon was reportedly at an all time high (B. Ponia, MMR, pers. comm.).

There is also evidence to suggest that a decline in water quality preceded the disease outbreak. Water exchange in the semi-enclosed Manihiki lagoon is poor, at around one water exchange every 2 months (Anderson, 1998). A long period of unusually calm, dry weather was reported prior to the disease outbreak. At the time oysters were first sampled in late November 2000, water temperatures in Manihiki Lagoon were normal (29 °C), but dissolved oxygen (DO) levels were low, ranging between 1 and 3 mg/l (S. Sharma and G. Frost, SOPAC, pers. comm.). However, during the one week period in which field sampling was conducted, the mean DO of the lagoon increased to over 6 mg/l, probably due to high wind conditions which would have helped with aeration and water exchange in the lagoon (S. Sharma and G. Frost, SOPAC, pers. comm.). These water quality data, albeit limited, suggest that the disease outbreak followed a transient period of poor water quality in Manihiki lagoon.

High stocking densities reduce the food supply available to each oyster (Anderson, 1998). Starvation may have been indicated in some of the diseased oysters by pathologies such as atrophy of digestive gland tubules. Reduced food supply can also be an important stressor in bivalve molluscs (Tomaru *et al.*, 2001) and can cause mortality (Numaguchi, 1995). Spawning is another potential stressor which may have predisposed oysters to disease. We found that oysters with bacteria present in sections and with high lesion scores (2 or more) tended to have depleted or resorbing gonads.

Anecdotal evidence supplied by oyster farmers suggest the performance of pearl oysters in Manihiki Lagoon in the years following the November 2000 disease outbreak has been reduced compared to pre-outbreak times. Certainly many of the oysters which survived were still showing signs from the disease outbreak in a follow up survey of oyster health done 3 years later in December 2003 (Diggles and Maas, 2004). These signs included permanent scarring of the nacre of the shell and development of a prominent check around the periphery of the shell, indicating that shell growth ceased for a significant period of time subsequent to November 2000. These signs of disease had never been previously seen by pearl oyster farmers throughout the production history of the lagoon, indicating that the November 2000 disease outbreak in Manihiki Lagoon was unprecedented and had significant long term deleterious effects on oyster health in that lagoon.

In conclusion, our data suggest that an unprecedented disease outbreak in *P. margaritifera* in Manihiki lagoon in November 2000 was associated with vibriosis caused by *V. harveyi* and other opportunistic vibrios. The disease outbreak appeared to follow a transient reduction in lagoon water quality associated with a period of calm weather and was probably exacerbated by high oyster stocking densities. Furthermore, the mortalities occurred at a time of the year when oysters may have been further stressed after spawning. Management of the disease could, therefore, be based on controlling the stocking density of oysters in the lagoon, and modifying husbandry practices to reduce stress on oysters when they are spawning. Ongoing monitoring of key water quality parameters such as water temperature, DO, chlorophyll α, and both sediment oxygen demand (SOD) (Anderson, 1998) and biological oxygen demand

(BOD) may also be useful so that the epidemiology of disease outbreaks which may occur in the future might be better understood.

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3.3 The Australian experience: pearl oyster mortalities and disease problems

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ABSTRACT

Australian aboriginals were trading pearls long before Europeans "discovered" pearls in Australian waters in 1812. By the end of the nineteenth century, fishing for "mother of pearl" shell and the incidental pearls was well established. The industry was heavily affected by the decline in value of mother of pearl after World War II (WWII). Artificial seeding of pearls (*Pinctada maxima*) began in Australia in 1956, under agreement with the Japanese and production grew until major mortalities in the 1970s affected industry growth. The mortalities were traced to inadequate management practices resulting in *Vibrio harveyi* infections and, following improvements to shell handling techniques, mortalities were eliminated. The *Pinctada maxima* industry in Western Australia and Northern Territory is the most valuable in Australia and is heavily regulated. Smaller industries exist in Australia farming non-maxima pearl oysters. Though a range of parasites have been identified in pearl oysters, none have caused serious problems for the industry, however, mortalities in Exmouth Gulf in 2006 with an unknown aetiology have forced changes to industry protocols.

HISTORY OF THE INDUSTRY

A well-established trade in pearls and mother-of-pearl (MOP) shells existed among the aboriginal people of Australia long before pearls were "discovered" by Europeans in Queensland waters in 1812. Though initial shell harvests were in the Torres Strait - Thursday Island area of Queensland, an industry soon developed in Western Australia, first at Shark Bay in the 1850s for pearls from *Pinctada albina albina* and soon after, in Nicol Bay, for pearls from *P. maxima*. Initially aboriginal and Malay "free" divers were used but, with the increasing demand for MOP shells and with the arrival of hard-hat compressed air diving suits in the 1880s, pearling moved north to the town of Cossack (now abandoned) and later to the port of Broome. By 1910, Broome was producing 75 percent of the world's output of pearl shell which involved more than 400 luggers

and 3 500 people. However, the collapse in market price for MOP after World War I (WW1) was equally dramatic. By 1939, the number of luggers had reduced to 73 and after WWII, only 15 boats resumed pearling (Malone, Hancock and Jeffries, 1988; O'Sullivan, 1995).

In 1956, Pearls Proprietary Ltd. introduced pearl culture at Kuri Bay (see Plate 1) in association with Japanese and other overseas investors and under agreement with the Japanese Government. By 1973, Kuri Bay was said to produce around 60 percent of the world's finest round pearls and by 1987 there were 11 companies farming pearls in the Broome region (Malone, Hancock and Jeffries, 1988). In 2001, there were five pearl hatcheries and 16 companies operating farms in Western Australia from North East Cape to the Northern Territory border though recent rationalization in the industry saw the number of hatcheries reduced to three by 2006. The annual quota that can be seeded in Western Australia consists of 572 000 wild stock shell and 350 000 hatchery produced pearl oysters. At present, leases in Western Australia cover 150 square nautical miles and that area is predicted to increase to 280 square nautical miles by 2010. Availability of suitable sites has been identified as a critical constraint to industry expansion. Annual production is now in excess of AUS$190 million with production of pearls from *P. maxima* being supplemented with "black" pearls farmed in *Pinctada margaritifera* in the Shark Bay area.

The Northern Territory has a history of over exploitation of natural pearl oyster beds, which began in Darwin Harbour, when beds were depleted early last century. The western grounds, near Bathurst Island, were discovered around 1929-1930 and were fished out by 1939. The eastern grounds, off Boucat Bay, were discovered in 1936 and by the 1950s were no longer commercially viable. Presently, pearl culture operations in the Northern Territory are centered on Bynoe Harbour, Coburg Peninsula, and the English Company Islands. Pearl cultivation also occurs in Darwin and Bynoe Harbours. An annual quota of 420 000 *P. maxima* oysters can be seeded that is split up between 7 pearl licensees. There are currently two hatcheries (J. Humphrey, Darwin DPIE, pers. comm.)

In Queensland, the pearling beds collapsed from overfishing in the 1930s. In 1999-2000, there were approximately 20 licensed areas for pearl cultivation in Queensland, but not all were in use and about 16 000 shells were seeded in that reporting period. In 2003-2004, the value of the pearling industry was reported as AU$338 000 and there has been little growth since then. There is some minor production from mollusc hatcheries. The main species farmed is *P. maxima*, with some production from *P. margaritifera*, *P. radiata* and *Pteria penguin* (T. Hawkesford, Queensland DPIE, pers. comm.; Lobegeiger, 2001).

In New South Wales, a small *P. imbricata* farm has met with considerable public and political opposition, however, the experience gained has been used to develop other *P. imbricata* farms in Queensland and Western Australia.

DISEASE ISSUES

Unexplained mortalities of oysters on lease sites in the Kuri Bay region commenced in the 1970s and occurred subsequently in leases in the Broome area and at Darwin. Deaths of more than 50 percent of the oysters following transfer from the "80 Mile Beach" fishing ground to the farms were recorded, but not among wild stocks or oysters harvested then "dumped" near the grounds. The stock situation became so serious in the early 1980s that the Western Australian Government was forced to impose a moratorium, until December 1987, on the number of companies licensed to fish for and farm shell. Results published by Dybdahl and Pass (1985) and Pass, Dybdahl and Mannion (1987) attributed the losses of the oysters to the conditions during transport that allowed build up of the pathogenic bacterium *Vibrio harveyi*. Their report made recommendations to modify management practices. These

modifications were successful in reducing the mortalities but the problem also made the companies acutely aware of disease issues and the potential for transfer of disease between areas. Pass, Perkins and Dybdahl (1988) described a virus from the digestive gland of *P. maxima*. The inclusion bodies were visible in apparently healthy oysters and no pathological significance could be ascribed to them (see Plate 2). In 1993, a papovavirus-like infection and a *Perkinsus*-like infection (Norton, Shepherd and Prior, 1993) were described from *P. maxima* collected from the Torres Strait region. In 1994, industry approved funding by the Fisheries Research Development Corporation (FRDC)of a survey to determine the disease status of the oyster industry across all three affected states. The survey was undertaken from 1995 to 1998 (Humphrey *et al.*, 1998) (Table 1). Shell disease was independently described from *P. maxima* in 1996 (Perkins, 1996) and a haplosporidian, found in 1993 from the same oyster species was finally described in 1998 (Hine and Thorne, 1998). The emergence of an intracellular ciliate in the digestive gland of oysters in the Exmouth Gulf region was reported by Jones and Creeper (2006). A severe mortality with an infectious aetiology occurred in *Pinctada maxima*, but not other *Pinctada* species growing in Exmouth

Gulf in 2006. The cause is still under investigation. The pathogens and diseases of *Pinctada maxima* in Australia are listed in Table 3.3.1.

TABLE 3.3.1

Parasites and diseases previously recorded in pearl oysters Pinctada maxima in northern Australia. QLD = Queensland, NT= Northern Territory, WA= Western Australia

Aetiological agent	Disease/Pathology	Geographic location	Reference
Viruses			
Papova-like virus	Epithelial hypertrophy of palp	QLD	Norton *et al.*, 1993b; Humphrey *et al.*, 1998
Intranuclear viral inclusions	None	WA, NT, QLD	Pass *et al.*, 1988; Humphrey *et al.*, 1998
Bacteria			
Rickettsiales : Large form	None	WA, NT, QLD	Humphrey *et al.*, 1998
Rickettsiales : Small form	None	WA	Humphrey *et al.*, 1998
V. alginolyticus		WA	Humphrey *et al.*, 1998
V. anguillarum		WA	Humphrey *et al.*, 1998
V. harveyi	Mortalities	WA	Dybdahl and Pass, 1985; Pass *et al.*, 1987
V. mediterranei		WA	Humphrey *et al.*, 1998
V. parahaemolyticus		WA	Humphrey *et al.*, 1998
V. pelagius		WA	Humphrey *et al.*, 1998
Vibrio sp.	Mortalities	WA	Dybdahl and Pass, 1985
V. splendidus II		WA	Humphrey *et al.*, 1998
Corynebacterium sp.		WA	Humphrey *et al.*, 1998
Erwinia hebicola		WA	Humphrey *et al.*, 1998
Photobacterium sp.		WA	Humphrey *et al.*, 1998
Pseudomonas putrefaciens		WA	Humphrey *et al.*, 1998
Protozoa			
Intracellular cilliate	None	WA	Jones and Creeper, 2006
Gregarines	None		
Haplosporidium sp.	None	WA	Hine and Thorne, 1998
Perkinsus sp.	Mortalities	QLD	Norton *et al.*, 1993a
Apicomplexan			Hine and Thorne, 2000
Cryptosporidia-like		NT	Humphrey *et al.*, 1998
Metazoa; Crustacea			
Conchodytes maculatus	None		Bruce, 1989; Chace and Bruce, 1993
Pinnotheres villosus			Dix, 1973
Anthessius pinctadae		NT, WA	Humphrey *et al.*, 1998
Metazoa: Platyhelminthes			
Larval lecanicephalid cestodes		WA, NT, QLD	Humphrey *et al.*, 1998

WESTERN AUSTRALIAN PEARL OYSTER MANAGEMENT

The most highly regulated pearling industry in Australia is that in Western Australia. The regulatory regime was heavily influenced by the disease problems encountered in the 1980s. Management of the translocation of pearl oysters in Western Australia is based on the concept of creating a closed population of known disease status that can then be tested with a high degree of confidence. The coastline has been divided into zones for stock management purposes so the movement of oysters between zones is subject to their being tested for disease. Hatcheries are licenced and are subject to annual inspection and disease testing of production batches. Imports of live shell from out-of-State are generally prohibited. A decision was taken by the industry in 1998 to increase the test sample size from 150 to 300 oysters to further improve the probability of detecting pathogens.

For a disease problem to develop, the causative agent must already be present around the area or must be introduced, most likely by human agency. Management regimes and freedom from disease certification minimize the risk of introduction by human agency, but pathogens cannot be detected and excluded with absolute certainty. Therefore, in a disease or mortality event on a pearl farm in Western Australia, the farm would be quarantined. This is one reason why, in addition to the zones, there is currently a five nautical mile buffer zone around each farm. It is generally not possible to site another farm within this zone without agreement between the farm owners but, in any event, adjacent farms cannot be closer than 2 nautical miles.

In a disease emergency, there would be a buffer zone created around the quarantined farm of at least 1–2 miles (depending on the organism and its likely rate of spread). Farms within the buffer zone would also be quarantined. Thus, the closer the farms, the greater the chance of disease spread and the more expensive (in terms of impacts and losses) would be the problem of containing a disease should it occur. Pearl farms, unlike marine fish cages, cannot be "towed away" if trouble strikes.

Disinfection protocols existed for vessels involved in shell movement between farms and for equipment used by visiting seeding technicians prior to the 2006 mortality event. Epidemiological investigations following the mortalities suggested that the involvement of divers and their gear in the spread of the pathogen had been overlooked. These are also now subject to disinfection protocols when moving between farms.

The following are some of the major requirements for *P. maxima* pearl hatcheries in Western Australia, or for producing spat for Western Australia: (a) high level

PLATE 3.3.1
Pearl oyster locations in Australia

Scenic shot of Kuri Bay, the original lease site for Paspaley pearls and occupied continuously by them since they pioneered pearl seeding in the 1950s.

Pearling lugger waiting for high tide while at the Broome jetty in the 1990s.

of filtration of incoming water to remove other larvae and most bacteria; (b) use of axenic algal cultures where possible to reduce the build-up of bacteria in the larval tanks; (c) testing of all outgoing spat batches and (d) filtration of all effluent water from the hatchery to prevent disease spread from a hatchery to the environment.

Filtration of hatchery inflow water and bacterial control through hygiene is not unique to the pearling industry. Anyone who has seen the results of a barnacle settlement or a sabellid tubeworm settlement in the pipes will appreciate the economic value of filtration. The problem, however, is not confined to bacteria free in the water column. Bacteria on biofilms can produce exotoxins, including proteinases and ciliostatic toxins that are pathogenic to larvae (Tubiash, 1975; Nottage and Birkbeck, 1987; Nottage, Sinclair and Birkbeck, 1989; Riquelme *et al.*, 1996).

The control of bacterial growth in mollusc hatcheries is often associated with the routine use of antibiotics (Jeffries, 1982; Prieur, 1990; Moore *et al.*, 1993; Riquelme *et al.*, 1996). This practice is discouraged in Western Australia, in part because no drugs are registered for such use and also because of the disease resistance problems which can occur (Kerry *et al.*, 1994; Riquelme *et al.*, 1996; pers. obs.).

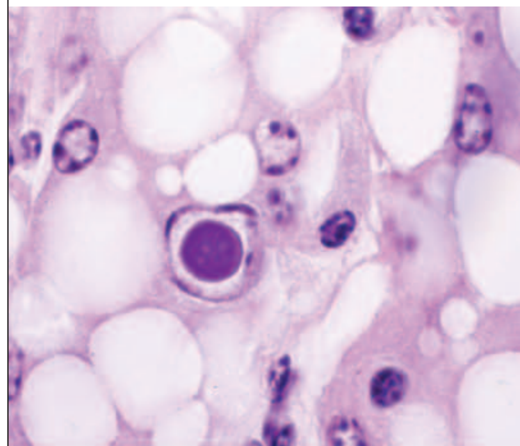
Sorgeloos (1995), in discussing hatcheries generally, commented that the reduction of bacterial loads, particularly in the feed and culture water, should be considered to be important and that strict hygiene measures should be taken including regular disinfection and dry-out of the complete culture circuit (including piping) between production cycles. This is common practice in pearl hatcheries.

The principle behind the requirement that effluent be filtered is recognition that hatcheries can be a source of infection for the surrounding wild fishery. In particular, it would be possible to breed a virulent strain of pathogen, possibly drug resistant, which would be released in large numbers into the environment (McVicar, 1997). Studies in British Columbia (Canada) have associated an increased risk of *Aeromonas salmonicida* in sea cages which are within 10 km of infected cages and, even where release of pathogens can not be clearly demonstrated, public perception of a "disease risk" from a hatchery may be enough to force closure.

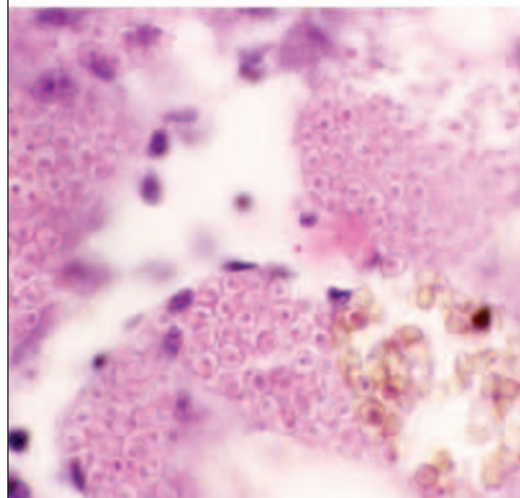
CONCLUSION

A number of disease issues have arisen in the industry during the last 5 years, including vibriosis, *Haplosporidium* sp., a rickettsia in the digestive

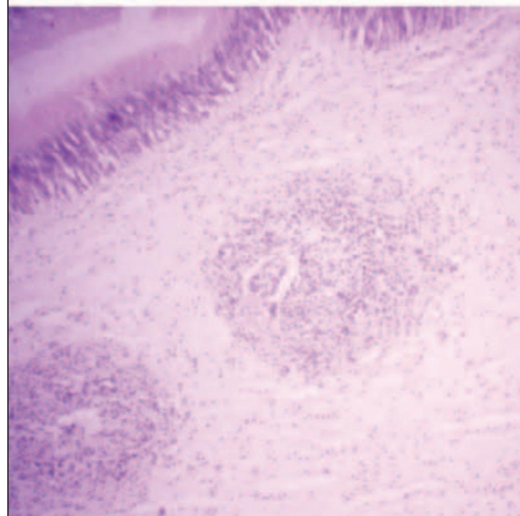
PLATE 3.3.2
Diseases of pearl oysters in Australia



Small intranuclear virus-inclusion in digestive gland of *Pinctada maxima* (H & E, x1000).



Haplosporidian spores in digestive gland of *Pinctada maxima* (H & E, x1000).



Larval cestodes associated with discrete focal granuloma in interstitial tissues of *Pinctada maxima* (H & E, x 200).

gland and an intracellular ciliate. These incidents have demonstrated the value of the management regimes in place and the close links that have developed between the industry and the government fish health laboratories. These links proved to be of great value in generating early reporting of mortalities and in cooperation between companies and government to contain and mitigate the impact of the mortality event in 2006.

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3.4 The Japanese experience: pearl oyster mortalities and constraints

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ABSTRACT

This paper provides a historical overview of pearl fishery in Japan and discusses the different disease organisms encountered by the pearl farming sector. These include parasites and pathogens, fouling organisms, predators and red tide. Mass mortalities of Akoya pearl oysters experienced in the 1950s, 1970s and 1990s are discussed. The paper was concluded with an emphasis on the importance of evaluating the susceptibility or tolerance to disease when pearl oyster seed are translocated as this is fundamental to preventing potential mass mortalities that may be caused by infectious pathogens. which need to be understood by These concepts are fundamental to the prevention of mass mortality caused by infectious pathogens and must be understood by farmers, scientists and policy makers.

HISTORY OF FISHERY OF PEARLS IN JAPAN

The earliest record of a pearl fishery appeared in India in 400 BC. Since then, fishermen have collected wild oysters from the sea and harvested the natural pearls throughout the entire distribution of the species in the Mediterranean Sea, the Red Sea, the Persian Gulf, Sri Lanka, the Middle East, Atlantic, South and North Pacific Oceans including Japan and China (Wada, 1991). The natural resources of oysters decreased partly due to over-fishing in most of beds and partly due to the low productivity of natural pearls from wild animals. For example, Ogushi (1938) reported that only natural pearls weighing 9 rin (1 rin = about 3.75 mg which is international unit of pearls for trading) could be harvested from visceral parts of about 200 Japanese (Akoya) pearl oysters *Pinctada fucata martensii*. It is interesting to note that Japan is located at the most northern latitude of the natural distribution of pearl oysters in the world and that the culture technique has been developed in this country by using the northern subspecies of tropical species (*Pinctada fucata*).

The Japanese government issued regulations to control over-fishing of natural pearls and conducted transplantation and culture experiments before World War II. Culture of pearls started at the end of 19th century. In 1893, Kokiti Mikimoto did the first experiments in which he succeeded in producing semispherical pearls. Tatsuhei Mise and Toukichi Nishikawa, were also famous figures in the history of methods for producing spherical pearls. These three pioneers are often cited as inventors of

the main techniques for the insertion operation for producing spherical pearls, which were mostly established by about 1916. The introduction of hanging methods for rearing the animals has increased the production of cultured pearls. The Japanese pearl industry contributed to many techniques of operation for insertion of nucleus and graft, wintering, cleaning the shell, collecting and rearing natural spat, juveniles and adult oysters, and hatchery production of seed (Ikenoue, 1992).

The number of cultured pearls produced was carefully recorded during the initial stage of the industry (1926-1945). There were, for example, 669 in 1926, 7 749 in 1935 and 10 883 in 1938. Estimated maximum weight of annual production was 2 500 to 3 000 kan (1 kan = 3.75 kg) during this period. Production was about 50 kan when culture restarted in 1946 after World War II and increased to 1 000 kan in 1950. There was a remarkable increase of production during the period from 1955 (6 543 kan) to 1966 (39 522 kan). The rapid expansion of the production depended primarily on the demand of world market as well as Japanese economy and also on the development of new techniques and expansion of culture sites throughout the distribution of the pearl oyster population in Japan. However, a long economic slump in 1975 resulted in a production decrease to 1 298 kan. Recovery from reduced production after a long slump of about ten years occurred and production was around 17 000 kan every year from 1985 to 1995. Due to mass mortality which became acute in 1996 and 1997, the annual production recently decreased to less than half of the value produced during each year of the last decade. The causes of increased mortalities are under investigation.

PARASITES AND PATHOGENS

Parasites of *P. fucata martensii* have been known since the early stages of development of the Japanese pearl industry. Damage to the pearl industry by parasites has been of particular significance since 1952. The rapid increase in damage over the whole pearl oyster-producing area seems to have been caused by the transplantation of the pearl oyster and complicated by the lack of biological data on the parasites. Mudworms, sponges and a trematode are the main parasites affecting production of pearls.

Mudworms of the genus *Polydora* produce a mud tube and blister in bivalve shells. *Polydora ciliata* (Johnson) is recognized as the most important parasite (Mizumoto, 1975). Since 1960, damage to the industry by mudworms has been occurring in the whole pearl-culturing area. Oysters are fatigued, resulting in heavy mortality. In 1970, more than 50 percent of pearl oyster cultivated were infected by mudworm in the main farming areas (i.e. Mie, Wakayama, Oita, Kumamoto, Miyazaki and Kagoshima prefectures) in the central and southern waters of Japan. Spawning of the mudworm occurs in May, June and October in Ago Bay, Mie Prefecture. Larvae settle on the shell and mud tubes and blisters are formed in summer and winter. To exterminate mud worm, the industry use brine treatment in the larval setting season. The pearl oysters are dipped into sea water for about five min, followed by a 15 min freshwater treatment, then in 22 percent brine for about 20 min and then exposure air in the shade for 15 min. Biological control using natural enemies of mud worm is also being explored (Funakoshi, 1964; Mizumoto, 1975).

The extent of the damage caused by the trematode *Bucephalus varicus* in the pearl industry was recognized around 1960 and the life history of this parasite has been studied and reported by Sakaguchi (1968). Pearl oysters infected by worms cannot be used in any pearl production, either as mother shell or as mantle piece donor because they only produce poor pearls with spotted or thin pearl layers. The infection has spread over the whole pearl-culturing areas and a rate of infection as high as 40 percent was reported in some farms in Japan. The pearl oyster is the first intermediate host of the parasite. Larval trematode stages found in pearl oysters are young sporocytes. The sporocytes mature in spring when the water temperature rises. The germ-balls

in mature sporocytes develop into cercariae, which are motile and are released from the oyster tissues through summer and autumn. The second intermediate hosts are small fish, such as *Atherina bleekeri, Spratelloides japonicus* and *Engraulis japonica*, which are abundant in the waters near the pearl farms. The *B. varicus* cercariae invade the muscular tissue of the fish and encyst as metacercariae. Fish species, such as *Caranx sexfasciatus*, *C. equula* and *C. ignobilis* are the final hosts, which ingest fish infected with the metacercariae. Once ingested, the metacercariae emerge from their cysts and mature into adults in the digestive system of the fish. Another trematode species *Proctoeces ostrea* has also been found in the internal organs of pearl oysters and is common throughout the pearl farms, but damage to the industry is not severe (Sakaguchi, Kamakura and Kusuda, 1970). A proposed practical method to control these parasites is to avoid culture of pearl oysters in September when these trematodes undertake their peak period of transmission. It is impossible to remove the host fish from the coastal areas (Sakaguchi, 1968; Mizumoto, 1975).

There are fewer reports of microbial pathogens (bacteria and virus) causing mortalities of Japanese pearl oysters than those in the tropical species. Kotake and Miyawaki (1954, 1955) isolated two strains of unidentified bacteria from Japanese pearl oysters during an episode of mortality. Miyazaki *et al*. (1999) reported isolating an unidentified virus as a cause of the mass mortality that has emerged recently. These results were not confirmed, either as to the identity of the pathogen or its pathogenicity to pearl oysters (Nakajima, 1999; Hirano, Kanai and Yoshikoshi, 2002). Suzuki, Kamakura and Kusuda (1998), Suzuki (1999) and Kitamura, Jung and Suzuki (2000) also isolated a birnavirus from Japanese pearl oyster.

FOULING ORGANISMS

Many species of invertebrates and seaweeds inhibit growth and injure pearl oysters by attaching to the shell, particularly at the hinge, often interfering with the opening of the shell, which may cause mortality. Fouling organisms include barnacles, sponges, worms, edible oysters, and seaweeds. The dominant species of barnacles in the Japanese pearl farms are *Balanaus variegatus tesselatus* and *B. amphitrite.* The polychaete worms *Hydroides norvegica* and *Dexiospira forminosus,* as well as the bryozoan, *Dakaria subovoidea*, are also fouling organisms in Japan. Periodic physical cleaning of the shell or dipping the oysters in fresh water are the practical defense measures taken against these organisms. Shell cleaning is carried out during the period from April to November. Freshwater treatment is effective particularly for removing larvae or young stages of fouling animals on the shell.

PREDATORS

The predators of cultured pearl oysters include the eel, *Anguilla japonica*, the black porgy, *Sparus* sp. and globe fish, *Sphoreoides* sp., as well as the octopus, *Octopus* sp. They especially attack the pearl oysters just after the nucleus operation.

RED TIDE

One of the important constraints has been the effect of red tide on marine cultured organisms. *Gymnodinium mikimotoi* is a well-known dinoflagellate red tide which was named after the late Kokichi Mikimoto, one of the inventors of pearl culture in Mie Prefecture, where they found this species. Since then many records have described the damage to the pearl oyster by this dinoflagellate in Japan. The other red tide species reported are *Heterocapsa circularsquama*, *Gonyalaux* sp. and *Cocurodium* sp. Abnormal blooms of these toxic dinoflagellates have been suspected as the cause of mass mortalities in pearl farms in localized areas. Most of the Japanese pearl farms are located in semi-closed coastal estuaries or small bays, sites that favour abnormal blooming of red tides in Japan.

A large-scale bloom of *Heterocapsa circularsquama* occurred in Ago Bay in 1992 causing a new type of red tide mass mortality that had not seen before with red tides of *Gymnodinium mikimotoi* or other species (Matsuyama *et al*., 1995). They reported that in the summer of 1992 in Ago Bay the maximum cell density of *Heterocapsa* sp. was 87 420 cells/ml and it caused mass mortality of pearl oysters, although mortalities of cultured and feral finfish were not observed. That was the first report of this species of red tide causing mass mortality of pearl oysters in Japan. Many studies have been reported in Japan on the factors that stimulate phytoplankton blooms, physiological damage and mortality of pearl oyster, prediction of blooms on the basis of the field observation and experimental exposure of animals to red tide dinoflagellates (e.g. Honjo, 1994; Nagai *et al*., 1996; Iwata *et al*., 1997). Nagai *et al*. (1996) used laboratory experiments to determine the mechanism by which *H. circularsquama* caused mortality of juvenile pearl oyster. The lethal dose 50 (LD_{50}) was approximately 20 000 cells/ml after 24 hr of exposure to *H. circularsquama*. They suspected that the cause of death was the direct action of cells. Immediately after exposure to algal cells, animals rapidly contracted their mantles, closed their shell valves, then contracted gills and heart beat became irregular until it stopped. Transporting the pearl nets that hold pearl oysters from the bloom area to a non-blooming site is a usual procedure to avoid mortalities in the case of red tides in Japan.

MASS MORTALITY

In pearl farms located at the end of semi-closed estuaries or bays, mass mortalities occurred in the summer season in Mie Prefecture between the 1950s and 1970s. Based on the analysis of seawater data, high temperature was considered to be the primary cause of the mortalities, which ranged from 4 to 70 percent. At the time of summer mortalities, water temperatures rose over 29 °C, the oxygen content was low and hydrogen sulphate concentration was high, particularly near the bottom, all of which are suspected to have contributed to the mass mortalities. Physiological condition of the pearl oysters is variable and is dependent on the phytoplankton availability in pearl farms before and during periods of high water temperature. Mortalities are strongly influenced by physiological condition. These experiences motivated the farmers and scientists to recognise the importance of monitoring the condition of seawater in pearl farms. Co-occurrence of high water temperatures and low levels of planktonic food are sometimes the main cause of mass mortalities. Food availability and temperature are important environmental factors for physiological conditioning of bivalves in culture. Elucidation of the influences of food deprivation on the mortality of pearl oysters was studied in laboratory rearing experiments (Numaguchi, 1995a, b). Two-year-old pearl oysters were held in tanks of seawater filtered through a series of filters (10, 5, 1 µm) for 115 days during June to September at the natural temperature of 23 °C to 29 °C. The mortality of unfed oysters increased remarkably when the condition index (dry meat weight/dry shell weight) dropped below 4 (initial value 13.7), dry meat losses increased more than 70 percent and the weight of the crystalline style decreased below 10 mg (initial weight: 30 mg). A level of 28 °C is a critical temperature indicator for some physiological effects in pearl oysters based on experiments on filtering rate, food intake and oxygen consumption (Uemoto, 1968; Numaguchi, 1995a, b). Mass morality observed in western Japan from summer to autumn may, therefore, be caused by these factors in association with the effect of oceanic seawater which is warmer and includes less food plankton than coastal seawater.

Since 1994, mass mortalities have been occurring which are causing significant economic losses to the pearl culture industry in western Japan (Sorimachi, 2000). This disease occurs from summer to autumn and the affected oysters exhibit a yellowish-red coloration of the adductor muscle. Histopathological changes commonly appear in the loose connective tissue of the mantle and adductor muscle in the affected pearl

oyster. Various factors such as toxic dinoflagellate blooms, infectious diseases caused by a filterable virus-like agent and environmental factors were suspected as causes of the mortality. To clarify the cause of this epizootic, experimental transplantation of the mantle piece of the affected oyster into healthy oysters, cohabitation of affected and healthy oysters and inoculation of filtrate of affected oyster hemolymph into healthy oysters were performed. After 2 to 3 months, healthy oysters displayed the signs similar to those of spontaneously affected oysters and mortality occurred. These results indicated that the mass mortality of the cultured pearl oysters is caused by an infectious filterable agent. However, the agent of the disease has not been conclusively identified. Mortality decreased when water temperature declined in October-November indicating the disease is highly dependent on water temperature. Low water temperatures in the previous winter appeared to suppress the occurrence of the disease during the following growing season (Kurokawa *et al*., 1999; Miyazaki *et al*., 1999; Muroga, Inui and Matsumoto, 1999; Morizane, 1999; Takami, 1999; Yoshikoshi, 1999; Nakajima, 1999; Sorimachi, 2000; Maeno *et al*., 2001; Morizane *et al*., 2001; Uchimura *et al*., 2001; Tomaru, Kawabata and Nakano, 2001). Management practices have been recommended for the pearl farmers to reduce mortalities. In some regions, prohibition on transplanting live pearl oyster shells from the area where the infection was suspected has been proposed. Imports of live oysters from out of Japan are generally prohibited.

Funakoshi (1999) discussed the history and current problems caused by mass mortality to pearl culture in Japan. He commented that after the 1970s many pearl farmers have tended to produce fast-growing and large-size mother-of-pearl (MOP) shell for oyster production. These appear more resistant to mass mortalities and can produce larger pearls. This has resulted in most MOP shells being produced from natural or artificial (hatchery) setting spat at the southwestern region of Japan. The new infectious disease emerged in part of these regions around 1995 and then spread over to pearl farms in other regions after 1997. It was suspected that the unknown pathogen was introduced by the transplantation of seeds or MOP. Also there have been introductions of live shells from out of country for genetic selection or crossing, and this is suspected, without evidence, as the cause of invasion of the pathogen.

CONCLUSION

When live shells are introduced from other prefectures or areas, or when pearl oyster seed are introduced, it would be desirable to carefully evaluate their susceptibility or tolerance to disease. These concepts are fundamental to the prevention of mass mortality caused by infectious pathogens and must be understood by farmers, scientists and policy makers. Routine monitoring of the physiological condition of cultured animals and oceanographic aspects of water quality by farmers, cooperatives or local governments at every culture site is also necessary.

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3.5 The French Polynesian experience

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ABSTRACT

French Polynesia is currently the world's largest producer of cultured black pearls with exports worth US$150 millions annually. The activity has become of prime socio-economic importance the archipelagos with strong support and control of the government. In the mid-1980s, mass mortality of the black-lipped pearl oyster, *Pinctada margaritifera*, has occurred in several atolls and challenged the development of pearl farming activity. Consequent studies and surveys have described several pathological conditions. None of those is considered to pose significant threat to the industry. However, they are monitored by a surveillance program in order to prevent and control emerging diseases. The government of French Polynesia has developed efforts to maintain high quality of the pearls and sustain demand of the markets. This paper reviews a successful model of development based on proactive policy and cooperation among stakeholders.

INTRODUCTION

The pearl industry in French Polynesia is based on the culture of the black-lip pearl oyster, *Pinctada margaritifera cumingii*. Annual exports of pearls have increased over the past two decades. They reached more than 8 000 kg/year in 2005 after a peak over 11 000 in 2002. Although annual turnovers have significantly decreased since 2000 (from a record value of US$252 millions to a rather stable US$150 million figure since 2005), French Polynesia is the world's largest producer of cultured black pearls (Anon., 2007). The socio-economic importance of this activity could hardly be overemphasized as it has become a major source of livelihoods. It also contributes to maintain island communities in remote and isolated locations of the archipelagos. Approximately 1 000 farms are currently under operation in 34 islands and atolls located from Gambier Islands to northern Tuamotu. The activity generates direct and indirect employment

for about 7 000 people. While pearl farming in French Polynesia meets an undeniable success, the industry has not evenly grown. In the mid-1980s, mass mortality of oysters has occurred in several atolls and challenged the development of pearl farming activity. The government of French Polynesia has introduced stringent quality control system in order to maintain high quality of the pearls and sustain market demand. Here we review this experience and lessons learnt from the past.

OUTBREAKS OF MASS MORTALITY

In 1985, the pearl oyster industry has faced series of mass mortality outbreaks in several islands of the Tuamotu Archipelago. During these outbreaks, recorded mortality rates were over 60 percent in some locations based on enumeration of tagged individuals in wild populations (Cabral, 1990). The mortality persisted up to 1986 and also affected cultivated stocks (Intés, 1994). Mortality of pearl oysters was accompanied by shell disorders, mantle lesions and necrosis of the adductor muscle (Grizel *et al.*, 1986). These signs led to the description of complex syndrome currently known as "syndrome 85" (Comps, Herbaut and Fougerouse, 2000). While no abnormal mortality was reported, these signs have been recurrently observed although at low prevalence in some islands until 1996-1997.

Despite many investigations and attempts to give the 1985 outbreaks a cause, no infectious aetiology was demonstrated. The presence of a gregarine in affected pearl oysters was reported (Chagot *et al.*, 1993). Gregarines, when present in low numbers, are usually regarded harmless to pearl oysters and the role of this parasite was not clearly assessed later (Fougerouse *et al.*, 1994; Comps *et al.*, 2001). It is accepted that a deleterious combination of biotic and a-biotic environmental factors, along with stress caused by culture handling, crowding and grafting, contributed to cause mass mortality of pearl oysters (Grizel, 1986; Cabral, 1990; Dauphin and Cuif, 1990; Cuif and Dauphin, 1996; Dauphin and Denis, 1987).

Between 1982 and 1983, six hurricanes wiped out shallow bottom beds in certain lagoons. Takapoto lagoon revealed oligotrophic conditions characterized by nitrogen and phosphorus deficits and low concentrations in bacteria and phytoplankton. Although the pearl oysters contributed to the suspended particulate matter used by micro-organisms, this appeared to have a negative balance against the rate of oyster grazing and indicated a limited carrying capacity for the lagoon (Vacelet, Arnoux and Thomassin, 1996). Such conditions may have also exacerbated recovery of the pearl oyster lagoons which were restocked, post-typhoon.

It is likely that such poor conditions associated with local high densities of oysters on culture rafts also may have exacerbated detrimental effect of pathogens. This paper reviews the pathogens reported from *P. margaritifera* in French Polynesia.

MAIN PATHOGENS RECORDED IN FRENCH POLYNESIA

There is very little known about diseases of pearl oysters in French Polynesia before any development of the industry. Initial studies focused on parasites having potential to induce pearl formation (Seurat, 1906; Dubois, 1907) and it was only during the 1985 mass mortality that light has been shed on pathogens of pearl oysters in French Polynesia.

Shell disorder, or disease, is part of the "syndrome 85" (Grizel *et al.*, 1986; Comps *et al.*, 2001). This disorder is characterised by brown organic matter deposits on the inner shell associated with inflammation in the mantle displaying yellowish coloration and swelling. Bio-mineralization disorders had previously been interpreted as consequences of mechanical trauma or chemical stress (Grizel, 1986; Dauphin and Cuif, 1990; Fougerouse *et al.*, 1994). Although the disorder has persistently been recorded by farmers in numerous areas from 1996 to 1997, the prevalence was low in comparison with the prevalence observed in 1985 (Comps *et al.*, 2001).

Microstructure and composition of the affected shells have shown malformations in the nacreous layer associated with biochemical abnormalities occurring during the biomineralisation process (Marin and Dauphin, 1991, 1992; Cuif and Dauphin, 1996). Electron-dense particles were observed in the granulomatous tissue contiguous to similar organic deposits and interpreted as possible virus-like particles (Combs *et al.*, 2001). This condition has also been compared with brown ring disease of clams, *Tapes philippinarum* and *T. decussatus*, caused by *Vibrio tapetis* (Paillard, Maes and Oubella, 1994; Borrego *et al.*, 1996) and a contagious aetiology was suggested (Cabral, 1994 cited in Cuif and Dauphin, 1996), although evidence of a pathogen has yet to be found (Cuif and Dauphin, 1996). In the course of brown ring disease of clam, infection with *V. tapetis* provokes disorganization of the periostracal lamina and a brown periostracum deposit in the inner surface of the shell (Paillard and Maes, 1990, 1995) very similar to those reported in *P. margaritifera* (Comps *et al.*, 2001). According to the authors, this might also result from physiological changes caused by the environmental, culture handling and grafting conditions considering that such organic deposits in the shell of marine bivalves are a frequent sign of reaction stimulated by wounds, parasites or debris (Aldermann and Gareth-Jones, 1971; Perkins, 1996).

Abscess-like lesions were reported in the adductor muscle of weak oysters displaying also abnormal mucus secretion. These lesions are characterised in histology by focal necrosis of the muscular tissue and haemocytic infiltration (Comps *et al.*, 2001). The study shows that prevalence of these symptoms vary; with highest prevalence being observed in Raiatea, Manihi and Takapoto lagoons. Virus-like particles – the very nature and role of which is still unknown – were reported from similar granulomatous tissues (Comps, Herbaut and Fougerouse, 1999). Similar lesions of necrosis, atrophy, swelling and vacuolization of muscle fibres were associated with virus particles in *P. fucata martensii* during a mass mortality which occurred in western regions of Japan in 1996 and 1997 (Miyazaki *et al.*, 1999). The size of the particles is apparently slightly different with virus-like particles in *P. margaritifera* of 40 nm while akoya-virus particles measure 33 nm (Comps *et al.*, 2001). A papova-type viral infection is also reported from the gold-lip pearl oyster *P. maxima* associated with nucleus hypertrophy of the epithelial cells of the labial palps (Norton, Shepherd and Prior, 1993).

Abscesses in the pearl bag are reported in the course of grafting and certain cases were possibly associated with the presence of intracellular procaryotes (Comps *et al.*, 2001). The secretory epithelium of the pearl sac may be highly damaged with accumulation of haemocytes and cellular debris surrounded by strong inflammatory reaction. Micro-organisms may be introduced in the pearl bag with mantle tissue introduction during the course of grafting and become a cause of abscess formation. In response to mechanical wound or to the accidental introduction of a foreign body during grafting, local abnormal secretion of periostracum by the epithelium of the pearl sac sometimes induces formation of whitish paraspherical bodies (Comps, Herbaut and Fougerouse, 2000).

Micro-colonies of bacteria were found in the epithelial cells of the digestive tubules (Comps, Fougerouse and Buestel, 1998; Comps *et al.*, 2001). The bacteria exhibit characteristics of members of Rickettsiales. All of these were reported during routine examinations and were not associated with significant disorder of pearl oysters, except in certain instances where fibrous layer surrounds some of these micro-colonies of bacteria.

The gregarine described by Chagot *et al.* (1993) is commonly reported from several lagoons (Gambier, Raiatea and Manihi) in the digestive tract of *P. margaritifera* with no particular impact on the host (Fougerouse *et al.*, 1994; Comps *et al.*, 2001).

Although scarcely observed, stages of a cestode, possibly related to the genus *Tylocephalum*, were recently reported from Raiatea and Gambier islands (Combs *et al.*,

2001). Such observations confirm previously reported occurrence of helminths from the same islands (Seurat, 1906).

Shell damages in the black-lip pearl of French Polynesia may be caused by various boring organisms (Mao Che *et al.*, 1996). These include cyanobacteria, green algae, the marine phycomycete fungus *Ostracoblabe implexa* and clionid sponges, *Cliona margaritifera* and *C. vastifica*. Apparently, *Ostracoblabe implexa* would be more damaging to the nacreous layers of the shell compared to clionid sponges limited to outer prismatic region of the shell (Cuif and Dauphin, 1996, Comps, Herbaut and Fougerouse, 2000). However, clionid sponges have been shown to produce perforation throughout the shell three layers and even induce haemocytic response in adjacent muscular tissue of edible oysters (Groman and Berthe, unpublished data).

HEALTH MANAGEMENT

These different studies have shown a reasonably good health condition of *P. margaritifera* in French Polynesia despite persistent manifestation of the syndrome 85. The most serious concern probably comes from described virus-like particles and intracellular prokaryotes. The mass mortality outbreaks encountered by the pearl industry in Japan strongly contributed to reinforce this concern (Miyazaki *et al.*, 1999).

Transfers of pathogens via movements of live molluscs are generally recognized as a major cause of disease outbreaks and epizootics. In French Polynesia, hatchery production is still in development and despite the economic importance of this industry, the pearl oyster culture is strongly dependent on natural spat collection. This collection occurs in atolls where natural stocks are abundant and where spat production is significant. Closed lagoons, such as Takapoto or Hikueru, were traditionally exploited for this specific purpose. Collected spat are then spread to non-collecting atolls where there is usually limited or no natural stock. The spatio-temporal variability in production of wild spat as well as lack of regulation regarding transfers of spat from collection to farming areas are underlying causes of numerous transfers between islands (Cabral, Mizuno and Tauru, 1985; Prou, Benett and Tiapari, 1999).

The exponential development of the pearl oyster industry has been accompanied by an increasing number of farms and farmed atolls. A direct consequence of this has been a rise in the frequency and volume of oyster transfers from collecting to non-collecting atolls. Animal exchanges increase mixing of oysters populations from the different atolls among Polynesian archipelagos (Society Islands, Marquesas Islands and Tuamotu–Gambier) as underlined by the significant genetic homogenization trend between populations previously distinct (Arnaud-Haond, 2003). The oyster transfers also generate anemone dissemination and this is becoming an important ecological problem in some islands with economic consequences as a result of the increased frequency needed for cleaning livestock and rearing structures (LeMoullac *et al.*, 2003). The great demand for spat also had, as a consequence, high densities of reared pearl oysters in the collecting atolls, such as, for example, Takaroa.

In order to prevent and control spreading of any emerging disease in the French Polynesian pearl farming sector, surveys were undertaken and a basic surveillance programme was proposed for implementation (Combs *et al.*, 2001; Thébault, 1999). This program was initiated in 2003 as a network based on five islands of the French Polynesian archipelagos. The programme is based on monitoring of pearl oyster farms and producing areas by routine collection of samples and investigation of abnormal mortality outbreaks. The strong collaboration between actors of the scientific community and delegates of pearl oyster administration in atolls (through meetings, coordination, training courses) enabled increased awareness of farmers for the benefit of the industry.

CONCLUSION

The pearl farming in French Polynesia has been extremely successful. Involvement of the government and cooperation of research and private sectors are two key components of this model. French Polynesia has developed a strong proactive policy in support of the pearl industry with efforts to develop and implement a national strategy for pearl oyster health management. This policy is still under review with new management measures being considered. Among those, quotas of pearl farms are discussed to reduce the risk of diseases of pearl oysters and avoid problems of poor quality of the pearls.

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3.6 Pearl oyster health: experiences from the Philippines, China, the Persian Gulf and the Red Sea

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ABSTRACT

Available information, based on field and literature survey, on pearl oyster health in the Philippines, China, the Persian Gulf and the Red Sea, are compiled in this paper. While the case studies presented here are limited, the collected information, nevertheless, represent the currently available knowledge. There are some similarities in the abnormalities experienced by the countries particularly shell damage due to heavy fouling, clionid sponge and *Polydora* infection as well as presence of unidentified parasites, inclusion bodies and rickettsia-like organisms. Some farm management practices are discussed.

THE PHILIPPINE EXPERIENCE

The Philippine Pearl Oyster Industry is based primarily on the gold- or silver-lip pearl oyster, *Pinctada maxima*, along with the winged pearl oyster, *Pteria penguin*, for round, half-round and three-quarter shells and other shell products (Ladra, 1994;

Ladra, 1997). Pearl farming used to be a minor mariculture activity in the country has now grown to be the eighth dollar earner for the fishery sector.

There are at least 30 registered marine pearl farms in the Philippines, with areas covering 1–242 hectares/licensee and occupying a water area if more than 2 000 ha (BFAR, unpublished data). The farms are located in the provinces of Masbate, Sulu, Zamboanga, Tawi-tawi, Guimaras, Davao, Palawan, Quezon, Pangasinan and Samar. The best culture sites can be found in the southern part of the country in Suu, Tawi-tawi and Basilan.

The industry is involved in collection, production and trade. In Bulacan Province, for example, more than 2 000 workers are involved in the jewelry trade; in Cebu Province, there are about 49 mother-of-pearl (MOP) costume jewelry operators and shell-craft processors. Revenue generated from pearl oyster products has increased tremendously from US$9 million in 1991 to US$12 million in 1994 (Table 3.6.1) and to US$15 billion in 2005 (Table 3.6.2).

During the early 1990s, the Pearl Oyster Industry experienced growing mortality losses, as well as shell deformity problems, to the extent that some formerly productive sites have become unworkable.

With the support of the South Sea Pearl Industries of the Philippines[1], the BFAR and collaborating agencies[2], took an initiative to undertake a preliminary assessment of the pearl oyster health problems was conducted in 1996. The assessment involved farm visits, interview with pearl farmers and examination of pearl oyster specimens (gross examination of shell abnormalities and histological examination).

The case-histories presented by the various farms visited under the Pearl Oyster Health Initiative revealed patterns of disease and mortality which matched several of the scenarios of pearl oyster health problems in other countries. Notably, many farmers were reporting high losses shortly after arrival of wild MOP shell stock at the farm site. Plates 1-3 show some characteristic abnormalities observed from Philippine pearl oysters.

Wild oysters collected from deep water (> 60 feet) and raised directly to the surface undergo pressure changes that are assumed to have some impact on physiological functions. In addition, rapid changes in ambient temperature result from transfer from deep to surface waters. Problems with heavy fouling (e.g. encrusting and sessile invertebrates) of oysters collected from shallower waters are also common. These fouling organisms can overgrow the complete shell, including the lips and hinge, impeding feeding and growth. Cleaning of heavily fouled oysters requires removal from the holding system and air exposure which, if not conducted under shaded/ conditions can also be physiologically taxing. Transportation to off-site areas for de-

TABLE 3.6.1
Revenue from pearl oyster products (in US$, 1991–1994)

Commodity	1994	1993	1992	1991
Raw shells	359 030	422 506	3 014 745	898 456
Jewelry	3 601 226	1 775 983	1 656 835	1 997 930
Shell blanks	2 970 000	1 788 258	1 250 497	1 103 037
Processed shells	172 429	176 914	731 954	95 060
Pearls	5 585 906	3 278 042	1 473 726	3 780 000
Shell button	–	2 929 646	9 453 210	9 103 837
Total	**12 689 590**	**10 371 349**	**9 453 210**	**9 103 837**

[1] "Coron Development Corporation, Guian South Sea Pearl Farm, Hikari South Sea Pearl Corporation, Sommaco South Sea Pearl Corporation, Sea Queen, Tawi-Tawi Pearl Farm.

[2] Philippine Council for Aquatic and Marine Resources Development (PCAMRD), Technology Application and Promotions Institute (TAPI) both of the Department of Science and Technology of the Philippines; Canada's Department of Fisheries and Oceans (DFO-Canada), Thailand's Aquatic Animal Health Research Institute (AAHRI) and Canadian Executive Service Organization (CESO).

TABLE 3.6.2
Philippine export data (cultured pearl and mother-of-pearl and products) for 2005

Product	Exporting country	Weight (kg)	FOB value (Philippine pesos)	FOB value (US$)
Cultured pearl Unworked	Hong Kong	100	19 211 428	371 320
	United States of America	27	9 471 247	183 060
	Switzerland	13	12 727 597	246 000
	Japan	8	9 833 828	190 069
	Total	**148**	**51 240 100**	**990 450**
Cultured pearl worked	Hong Kong	744	255 731 281	4 865 482
	Japan	332	127 437 447	2 463 121
	Australia	275	159 615 969	3 055 070
	United States of America	119	110 051 239	2 127 079
	Switzerland	34	29 751 172	575 033
	Others	53	28 514 732	551 135
	Total	**1 557**	**707 101 840**	**13 666 920**
Shell buttons	China PR	36 000	1 955 704	37 800
	Japan	6 886	5 975 589	115 458
	Hong Kong	674	615 685	11 900
	Korea	525	336 298	6 500
	Germany	350	783 679	15 147
	Others	261	903 365	17 499
	Total	**44 096**	**10 570 320**	**204 304**
Mother of pearl unworked	Hong Kong	316 731	8 114 619	156 840
	China	54 619	6 818 370	131 876
	Korea	52 524	8 105 513	156 664
	Japan	19 300	2 672 973	52 050
	Italy	10 100	827 811	16 000
	Others	39 507	843 622	15 919
	Total		**27 382 908**	**529 259**
Mother of pearl worked	Hong Kong	15 010	1 823 254	35 240
	Thailand	13 200	134 623	2 602
	Germany	2 836	3 404 891	65 810
	United States of America	2 604	1 936 302	37 425
	Spain	1 453	591 988	11 442
	Others	3 060	4 110 549	79 449
	Total	38 163	12 001 607	231 068
	Grand total			**15 622 601**

fouling would be the ideal situation for farm management, but involve more exposure of the pearl oysters to air/shallow holding as well as time and transportation expense.

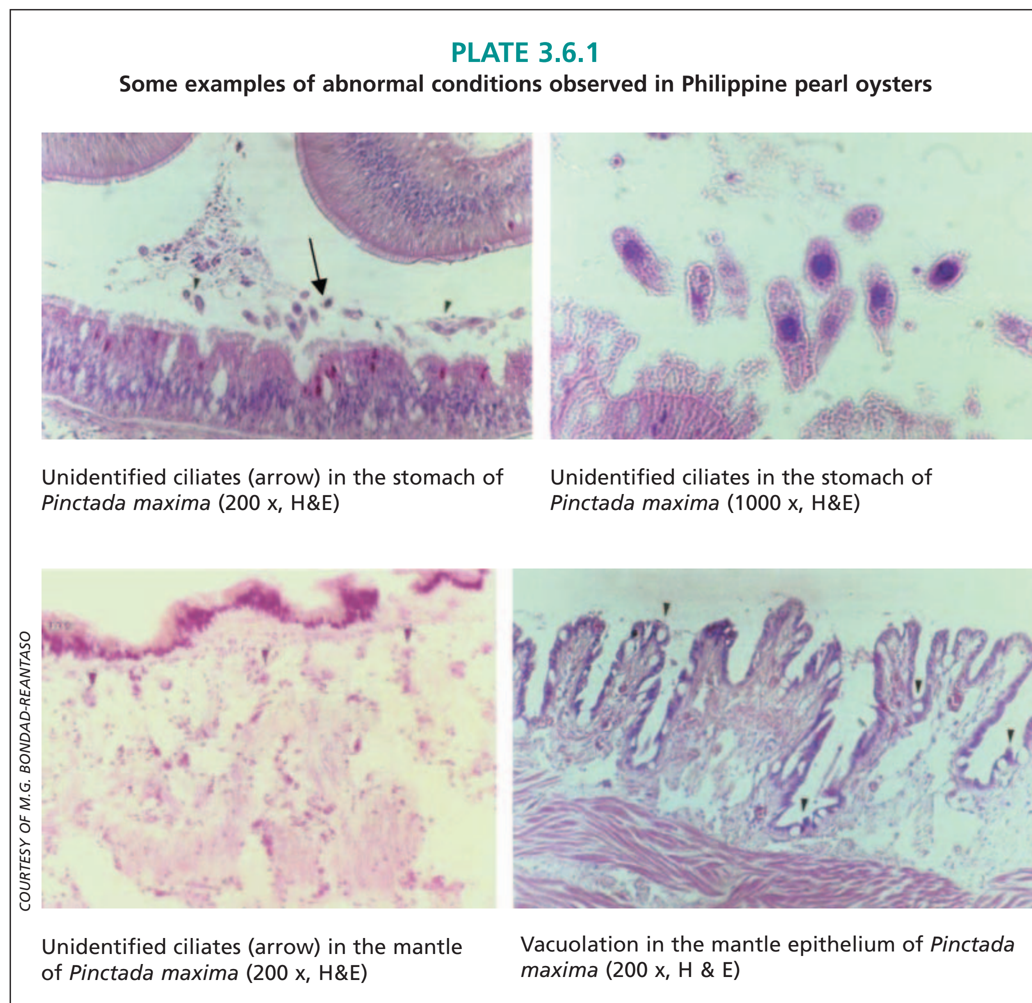
The problem of heavy fouling experienced in the Philippines and its effect on shell quality and survival is a situation similar to that described from the Persian Gulf as well as elsewhere. This problem shows no host-specificity and applies to both *P. maxima* and *P. penguin*.

Biomineralization problems similar to those described from French Polynesia were also reported at certain Philippine pearl farms, both in *P. maxima* and *P. penguin*, however, the extreme examples demonstrated by *P. margaritifera* were not observed.

In addition to the above information, collected through farmer interviews, gross examination of shells provided the following observations:

a) heavy fouling: mollusc encrustment as well as multi-taxa fouling, particularly in *P. penguin*;
b) shell damage due to clionid sponge;
c) blisters at the adductor muscle attachment of *P. penguin*;
d) *Polydora*-related tunnel damage to inner shell;
e) mantle recession in *P. maxima* and
f) inner shell discolouration.

PLATE 3.6.1
Some examples of abnormal conditions observed in Philippine pearl oysters



Unidentified ciliates (arrow) in the stomach of *Pinctada maxima* (200 x, H&E)

Unidentified ciliates in the stomach of *Pinctada maxima* (1000 x, H&E)

Unidentified ciliates (arrow) in the mantle of *Pinctada maxima* (200 x, H&E)

Vacuolation in the mantle epithelium of *Pinctada maxima* (200 x, H & E)

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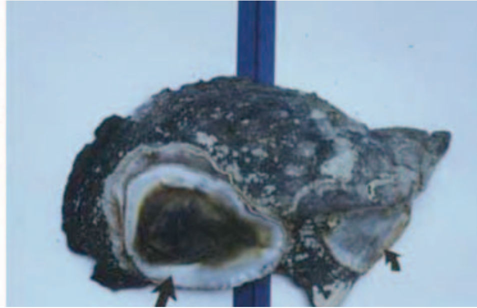
Preliminary analysis of histological sections collected showed the presence of inclusion bodies in digestive tubule epithelia; rickettsia-like organisms (RLO) in the kidney, digestive tubules, and gills of *P. maxima*; parasitic ciliates in the mantle, stomach and intestine; vacuolisation of the mantle epithelium; and extreme metaplasia of the digestive tubules indicative of starvation. The significance of the infectious organisms and the various histopathologies observed on pearl oyster health was not pursued, due to very low prevalence. However, these observations provide a useful base-line reference for ongoing monitoring of pearl oyster health in the Philippines.

With respect to the mortalities reported by some of the farms, it is likely that no single factor is responsible. There was no evidence of mortality patterns indicative of spread of an infectious agent, as most farmers reported patchy mortalities throughout their holding systems and with negligible correlation to size/age. Thus, it is possible that the mortalities are due to the cumulative effects of pressure and temperature changes, heavy fouling and stress due to defouling, transportation method and transfer to open waters. A further assessment and long term studies on the health situation of cultured pearl oysters in the Philippines is necessary.

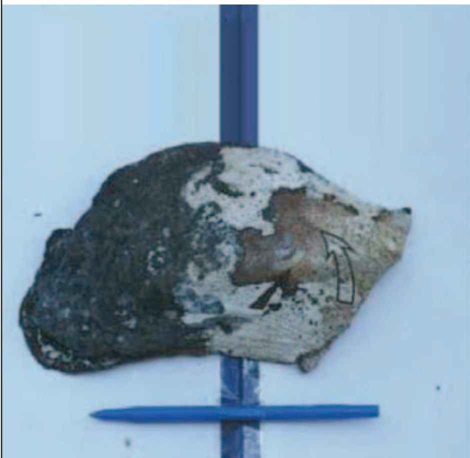
PLATE 3.6.2

Some examples of abnormal conditions observed in Philippine pearl oysters

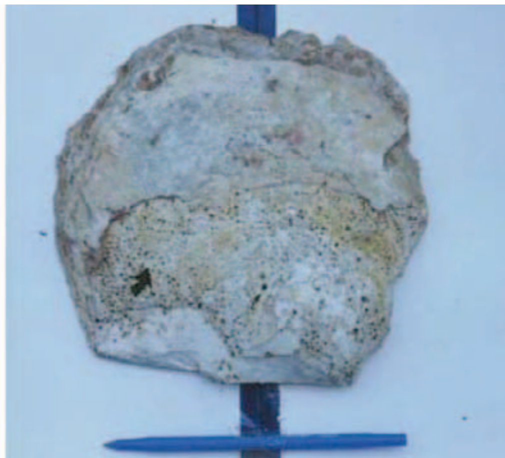
Multi-taxa fouling on *Pteria penguin*



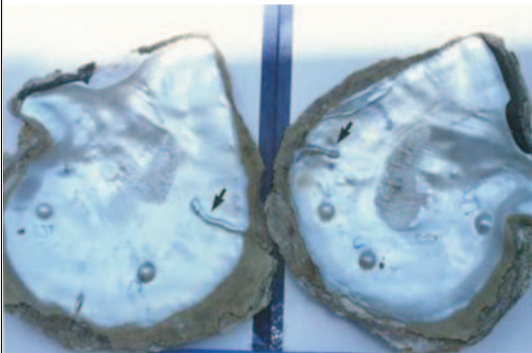
Mollusc encrustment on *Pteria penguin*



Boring sponge on *Pteria penguin*



Boring sponge on *Pinctada maxima*



Mud tunnels caused by *Polydora* sp. on *Pinctada maxima*

Mud tunnels caused by *Polydora* sp. on *Pteria penguin*

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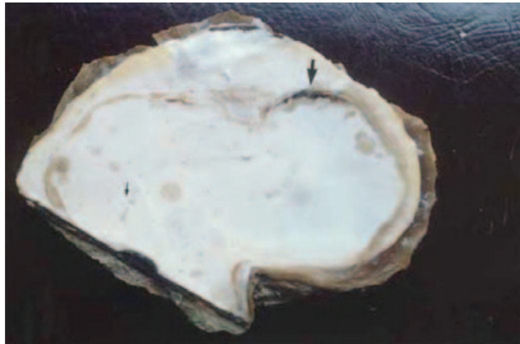
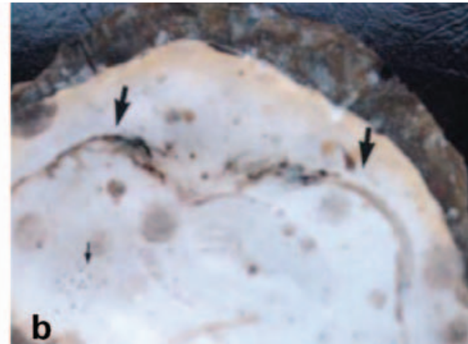
PLATE 3.6.3

Some examples of abnormal conditions observed in Philippine pearl oysters



Shell of *Pinctada maxima* showing erosion of inner surfaces (arrows) probably related to chronic mantle retraction; thin arrows show complete penetration by boring sponge.



Shell of *Pinctada maxima* showing erosion of inner surfaces (arrows) probably related to chronic mantle retraction; thin arrows show complete penetration by boring sponge.

Dense multi-taxa fouling in *Pteria penguin*

Extensive shell damage due to clionid boring sponge in *Pteria penguin*

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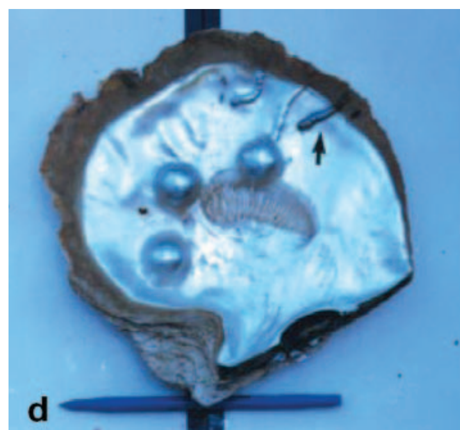
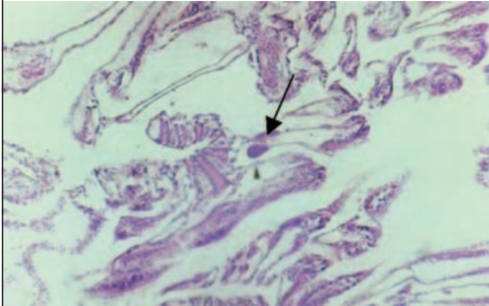


Dense multi-taxa fouling in *Pteria penguin*

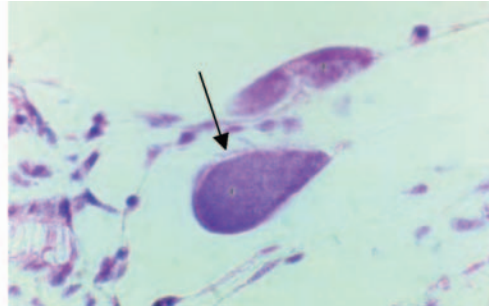
Extensive shell damage due to clionid boring sponge in *Pteria penguin*

PLATE 3.6.4

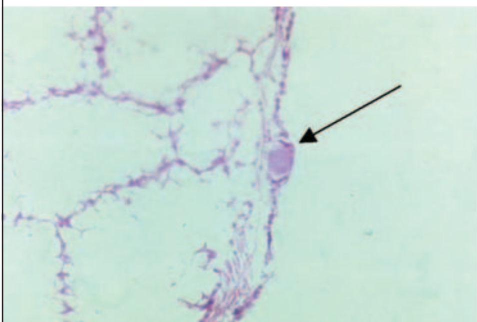
Some examples of abnormal conditions observed in Philippine pearl oysters



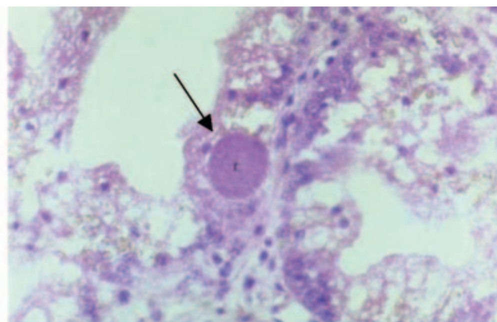
Rickettsia-like organisms (RLO, arrow) in the gills of *Pinctada maxima* (200 x, H & E)



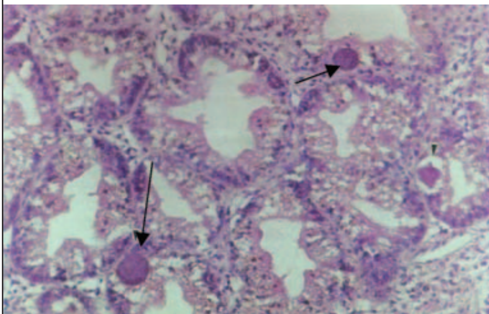
Rickettsia-like organisms (RLO) in the gills of *Pinctada maxima* (1000 x, H & E)



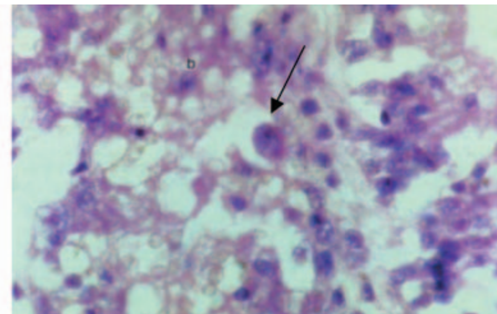
Rickettsia-like organisms (RLO, arrow) in the kidney of *Pinctada maxima* (200 x, H & E)



Rickettsia-like organisms (RLO) in the digestive tubules of *Pinctada maxima* (1000 x, H & E)



Inclusion bodies in the digestive tubule epithelia of *Pinctada maxima* (arrows, 800 x, H & E)



Inclusion bodies in the gills of *Pinctada maxima* (arrows, 800 x, H & E)

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THE CHINESE EXPERIENCE

Pearl oyster culture in China began 45 years ago. In 1958, five pearl farms (*Pinctada fucata*) harvested round pearls through insertion of a mantle piece into the body of wild oysters (Jin, 1996). The following year, after successful breeding of *P. fucata*, many pearl farms were established (Chen, 1995; Jin, 1996; Li, 1999). In 1978, the first group of nucleus pearls from cultured *P. maxima* were harvested and the yield increased year by year as techniques improved (Xie *et al.*, 1985; Xie, 1995).

The provinces of Guangdong, Guangxi and Hainan are the main location of the farms producing marine pearls in China, with *P. fucata* and *P. maxima* as the main cultured species. Culture techniques involve floating rafts with suspended cages (Xie *et al*,. 1985; Xie, 1995). At present, the yield is more than 34 000 kgs compared to a low of 15 kg in early years (see Table 3.6.1).

In recent years, *P. fucata* became smaller than before, due to inbreeding since 1966 (Jin, 1996). This is one of the main problems limiting cultured pearl development and many researches have worked on options to restore the quantity of pearl oyster, especially polyploid reared oysters (He and Jiang, 2002; Chang and Wang, 2002).

The second problem which limited cultured pearl development was disease. The most serious disease is the "black shell disease" which can be found everywhere (Jin, 1996; Xie, 1995). It is a destructive disease caused by *Polydora* sp. Saturated salt-water soaking was often used to cure this disease (Xie, 1995). Aside from *Polydora* infections, other agents such as the sea mussels *Lithophaga malaccana* and *Botula silicula,* the clam, *Gastrochaena cunieformis* and piddock, *Zirfaea minor*, may also cause shell-perforation (Xie *et al.*, 1985). Parasites such as cestodes, trematodes and nematodes were also commonly found on the gills, mantle, foot, gonad and digestive gland of the pearl oysters (Xie, 1995). Some predators, such as Anguilliformes, Sparidae, Tetrodonidae, Scylla, Octopodidae, Asteroidae and Cymatiidae finfish were also harmful to pearl oysters. Rickettsia-like organisms (RLOs) are the main microbial pathogens reported and which have been associated with heavy mortalities to both *P. fucata* and *P. maxima* (Wu and Pan, 1997; Wu and Pan 1999a, b, c).

TABLE 3.6.1
Marine pearl production in China[1] (in kg)

Location	1990	1991	1992	1993
Guangdong	2 934	3 709	6 170	8 155
Guangxi	1 497	1 558	2 857	4 375
Hainan	60	68	300	15
Total	**4 500**	**5 336**	**9 327**	**12 545**
Location	**1994**	**1995**	**1996**	**1997**
Guangdong	12 737	16 382	14 055	12 393
Guangxi	5 963	10 831	11 277	11 393
Hainan	15	19	21	280
Total	**18 715**	**27 232**	**25 353**	**24 278**
Location	**1998**	**1999**	**2000**	**2001**
Guangdong	19 594	23 734	26 091	21 883
Guangxi	8 654	8 836	11 249	7 125
Hainan	250	510	1 280	1 215
Total	**31 498**	**33 080**	**38 620**	**30 223**
Location	**2002**	**2003**	**2004**	**2005**
Guangdong	23 042	20 133	20 890	22 845
Guangxi	11 065	9 191	8 500	11 025
Hainan	200	350	280	
Total	**34 307**	**29 674**	**29 670**	**33 870**
Location	**2006**			
Guangdong	24 634			
Guangxi	9 500			
Hainan	353			
Total	**34 487**			

[1] Internal material, Fishery Department of Ministry of Agriculture, China

THE PERSIAN GULF EXPERIENCE

In the Persian Gulf, three species of pearl oyster (*Pinctada margaritifera*, *P. fucata* and *P. radiata*) are reported to be severely affected by fouling organisms (Doroudi, 1993a, 1994, 1996). Most destruction of the shell is caused by clionid sponges (*Cliona vastifica*, *C. margaritifera* and *C. carpenteri*) and shell-boring mussels (*Lithophaga hanlyana* and *L. malaccana*). Cultured pearl oysters are more severely affected than wild oysters and mortalities were attributed to the shell destruction (Doroudi, 1994). Levels of mortality, however, were not reported. Other fouling organisms found included barnacles, oyster spat and tube-dwelling polychaete worms, however, these were not linked to poor pearl oyster performance and mortality. On natural beds, the principal fouling organisms found were sponges, encrusting algae and ascidians (Doroudi, 1996). An experimental evaluation of cleaning frequency and effect of fouling on growth of *P. radiata* revealed no significant difference between approximately three, six and 13 weekly intervals in cleaning between January and April 1993 (Doroudi, 1993b). The reason for this may have been the short experimental period.

Similar problems have also been reported with the clionid sponges *C. margaritifera* and *C. lobata* and other fouling organisms in raft-culture of pearl oysters along the southwest coast of India (Alagarswami and Chellam, 1976; Thomas, Ramadoss and Vincent, 1993).

THE RED SEA EXPERIENCE

Mortalities of black-lip pearl oysters (*P. margaritifera*) in the Dongonab Bay of the Sudanese Red Sea were tentatively linked to a spherical parasite (Nasr, 1982) similar to one described from *P. maxima* in Australia (Wolf and Sprague, 1978). These spheres have since been identified as sequestered autophagous inclusions (Perkins, 1996), so it is unlikely that they were the cause of the Dongonab Bay mortalities – more likely an effect. No mortalities have been reported from this area since the original description (Nasr, 1982).

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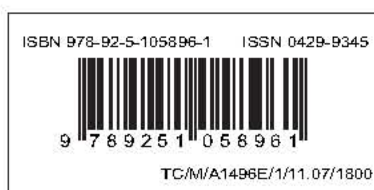
The pearl oyster industry is a growing multibillion dollar sector of molluscan aquaculture. The end product of pearl farming, the pearl, is unique to this sector. Pearl production is entirely based upon health. The pearl itself is a product of the oyster's immune defences as a response to soft-tissue irritation. Today, most disease problems are caused by opportunistic pathogens taking advantage of oysters weakened by the stress of handling, including pearl surgery and sub-optimal growing conditions. Further development of the industry will inevitably lead to increased risk of disease introduction, spread or emergence. Against such an unwanted future, health management is the critical line of defence. This publication provides guidance on the management of pearl oyster health and reviews pearl oyster mortalities and disease problems that will be useful for designing programmes aimed at reducing the risks from diseases. Part 1 consists of pearl oyster health – the current interest in it and an overview of the cultured marine pearl industry. Part 2 examines pearl oyster health management and consists of seven sections, namely: (a) introduction; (b) general information on husbandry and handling, hatchery production, introductions and transfers; (c) disease diagnostic protocols dealing with field collections of samples, gross external examination, gross internal examination and laboratory protocols; (d) health zonation; (e) disease outbreak protocols; (f) national strategies on aquatic animal health; and (g) references. Certain countries in the pearl oyster producing regions have acquired a great deal of experience in health management of cultured species. Experiences from Australia, the Cook Islands, Japan, French Polynesia, the Philippines, China, the Persian Gulf and the Red Sea are included in Part 3 which also contains a general review of pearl oyster mortalities and disease problems.



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