European Commission

Community Research

Proceedings

EUROHAB
Science Initiative

European Initiative on Harmful Algal Blooms

Harmful algal blooms in European marine and brackish waters

ENERGY, ENVIRONMENT
AND SUSTAINABLE DEVELOPMENT

EUR 18592 EN
Research in enclosed seas — 5

EUROPEAN COMMISSION

Edith CRESSON, Member of the Commission
responsible for research, innovation, education, training and youth

DG XII/D-3 — Marine sciences and technology

Contact: Ms E. Lipiatou
Address: European Commission, rue de la Loi 200 (SDME 7/84),
B-1049 Brussels — Tel. (32-2) 29-66286, Fax (32-2) 29-63024

European Commission
Directorate General
Science, Research and Development

EUROHAB
SCIENCE INITIATIVE

Harmful Algal Blooms in European Marine and Brackish Waters

Report of an international workshop organised jointly by the MAST Programme of the European Commission, DG XII, NUTEK (Swedish National Board for Industrial and Technical Development) and the University of Kalmar, Department of Marine Sciences.

Kalmar, Sweden, November 5-7, 1998

Edited by
E. Granéli, G. A. Codd, B. Dale, E. Lipiatou,
S.Y. Maestrini, H. Rosenthal

Research in enclosed seas series – 5

EUR 18592

Acknowledgements

Cover photos:
Upper left: Bloom of the toxic dinoflagellate *Dinophysis* spp in a Norwegian Fjord. Photo courtesy of Ø. Paulsen.
Lower left: A high biomass bloom of the prymnesiophyte *Phaeocystis pouchetii* deposited on a Belgian beach. Photo courtesy of V. Rousseau.
Right: A high biomass bloom of the toxic cyanobacteria *Nodularia spumigena* in the Baltic Sea. Photo courtesy of U. Larsson.

Monique de Bie is thanked for the hard work she put into the shaping of this document.

The editorial work done by Linda Medlin is also greatly appreciated.

The European Commission, NUTEK and Kalmar University are acknowledged for their support, without which the Workshop would never have been accomplished

A great deal of additional information on the European Union is available on the Internet. It can be accessed through the Europa server (http://europa.eu.int).

Cataloguing data can be found at the end of this publication.

Luxembourg: Office for Official Publications of the European Communities, 1999

ISBN 92-828-6612-2

Printed in Belgium

PRINTED ON WHITE CHLORINE-FREE PAPER

TABLE OF CONTENTS

HARMFUL ALGAL BLOOMS IN EUROPEAN MARINE AND BRACKISH WATERS

Microalgae in marine and brackish waters of Europe regularly cause «harmful effects», considered from the human perspective, in that they threaten public health and cause economic damage to fisheries and tourism. These episodes encompass a broad range of phenomena collectively referred to as «harmful algal blooms» (HAB). They include discoloration of waters by mass occurrences of microalgae (true algal blooms that may or may not be «harmful»), toxin-producing species (toxic «blooms» that may be harmful even in low cell concentrations), and several organisms, which are protists but normally referred to as algae. A broad classification of HAB distinguishes three groups of organisms: 1) the toxin producers, which even with low biomass can contaminate seafood, causing sickness and death in humans eating the seafood, or sickness and death in the shellfish and fin-fish themselves; 2) the high-biomass toxin producers (cyanobacteria), which can have similar harmful effects; and 3) the high-biomass bloom species, which can cause either anoxia that indiscriminately kills off marine life, or unpleasant foam or gelatinous masses that are a nuisance for tourists who may develop allergic skin reactions after bathing.

BENEFITS TO SOCIETY

Harmful Algal Blooms (HAB) affect the marine ecosystems in several ways leading to economical losses and/or health problems for humans. They also cause structural changes in the natural food webs that may be indirectly linked to human well being.

Research directly addressing the causes of HAB development in different parts of Europe will provide a basic knowledge that will indicate counter measures necessary to return marine and coastal waters affected by human impact back to more pristine conditions. The suite of countermeasures may well be very different in different parts of Europe. For example, water and airborne inorganic/organic nutrients can play a very different role in different marine waters.

A decrease in both the number of HAB and their intensity will reduce the harmful effects on fisheries and aquaculture, and reduce disturbance of marine ecosystems with the less known effects on the parts of the food web not directly exploited by humans. However, these effects should not be disregarded, since the structure of marine food webs are very intricate and disturbances on levels other than fish or shellfish may as well in the end also affect human interests.

A change in the water quality of marine waters leading to re-establishment of more pristine food webs would also mean that shellfish and fish harvested or caught for human consumption would be safer concerning the content of algal toxins. This would both improve the quality of food for humans and free some of the resources otherwise used for monitoring and surveillance of algal toxins in food products. Fish kills occurring in aquaculture could also be minimised (e.g. reducing insurance costs to the producers), which would lead to a more stable economy for fish and shellfish farmers with fewer hazardous events that today can mean substantial economical losses in aquaculture industry.

A decrease in the frequency of HAB causing large biomasses floating ashore would also be of importance for recreation and tourism in many European countries. Clean beaches with clear water as opposed to large masses of floating algal scum and gelatinous substances will prevent significant economical losses for the tourist industry such as those observed in the Mediterranean, the North Sea coasts and in the Baltic.

A general increase in the objective knowledge on the causes/occurrences of HAB will also be beneficial for the public awareness of the associated problems. This should help to moderate the overreactions due to often poorly informed media information that regularly occurs today.

Goals of EUROHAB (European Initiative on HAB research)

In order to consider the possibilities of reducing the occurrence and impact of harmful algal blooms in Europe, there is an urgent need to understand the mechanisms by which they are harmful.

The goals of EUROHAB are:

- To define the unique characteristics of some HAB species that enable them to outcompete and dominate the plankton communities.
- To distinguish between natural causes versus anthropogenic impact (overenrichment of nutrients and metals by eutrophication, overfishing, global warming) on the bloom formation and toxicity of harmful algae (HA).
- To understand the interactions between HAB and the food web which determine the outcome of HAB.
- To develop databases in order to establish the extent to which HAB are increasing in frequency and extent.

SUMMARY

Harmful algal blooms and nutrients

Although we know that some harmful algal species have bloomed over centuries, and others even thousands of years before, both scientists and the public are concerned that the size and frequency of these blooms have increased. Such evidence is found e.g., in the Baltic Sea and the northern Adriatic. In addition to increased algal biomass production, there is an increasing number of reported exceptional blooms (often toxic) of dinoflagellates, flagellates and cyanobacteria (blue green algae), during the past 15-20 years for most European marine waters. Toxic algal blooms have caused problems in European marine waters. They may be catastrophic to the ecosystem, causing mass mortality of fish and even (for cyanobacteria) seabirds or mammals. The *Chrysochromulina polylepis* bloom in the Kattegat-Skagerrak, in 1988, covering marine waters belonging to Denmark, Sweden and Norway, damaged the entire marine biota.
Blooms of toxic phytoplankton may also break the food chain, by not being grazed by zooplankton.

From the human point of view, toxic blooms are undesirable because they (i) poison commercially important shellfish and humans eating the shellfish, (ii) cause large scale fish kills (e.g., in aquaculture), and (iii) foul waters in recreational areas (inflicting economic losses on the tourist industry).

Some of the microalgae involved in these blooms are widely occurring species throughout most European countries, whereas others are only known to occur in a specific sea. For example, Diarrhoeic Shellfish Poisoning (DSP), is an illness that causes diarrhoea and vomiting in humans who have ingested mussels that have been feeding on a microalgae that occurs in most European waters. In contrast, the species causing multi-million dollar losses to the tourist industry over the past decade have more restricted distributions (e.g., those producing enormous accumulations of mucous in the northern Adriatic, and those producing skin irritating toxins in the bathing areas of the Baltic).

One of the key questions still to be answered is how to distinguish natural causes versus anthropogenic impact on the occurrence of HAB. Several anthropogenic forces might be considered to be involved in the formation of HAB. Among them are: eutrophication; disruption of the marine food chains by overfishing; increase in the mobilisation of essential or inhibiting trace metals to microalgal growth, and intensive aquaculture in certain sensitive marine areas.
Eutrophication of marine and enclosed brackish waters is a problem of major concern not only in Europe but also throughout the world. Human activities have significantly increased the input of nitrogen and phosphorus to estuarine and marine waters over the last 50 years. The nitrogen load to the North Sea has increased by a factor of 2.5 from 1950 to 1980 whereas phosphorus seems not to have increased.
Traditionally, scientists and water quality managers have focused on terrestrial, anthropogenically generated sources, such as agricultural runoff, wastewater and groundwater discharge, as dominant nutrient inputs. However, recent studies suggest that we need to include atmospheric deposition in the budget of anthropogenic nutrient inputs. In Europe, whereas marine nutrient loading is increasing, national and international authorities have targeted agricultural, municipal and industrial wastes for reduction and improved treatment. In contrast, atmospheric emissions, specifically rainwater containing N compounds, are not controlled and have increased greatly over the past 4 decades. It has recently been estimated that for the north-western European shelf, atmospheric deposition of N may exceed estuarine exports.

In the Baltic Sea region, 80% of atmospheric deposition originates from the British Isles, and Western and Southern Europe (France, Benelux, Germany). Atmospheric and terrestrial nutrient inputs in the Mediterranean appear to have increased about 3-fold in the 1960-1983 period.

For some parts of Europe, lowering of the pH in soils due to acid precipitation disrupts terrestrial and fresh water ecosystems. Acidification is not a problem in seawaters due to their strong buffering capacity. However, acidification in soils increases the mobility and leaching of essential or inhibiting trace metals to algal growth. The input of metals such as iron, copper, aluminium, manganese, cobalt to marine waters has increased several fold in the last decades. Some of these metals have been connected to HAB occurring in different European seas.

The increase in precipitated atmospheric nitrogen deposition, directly on the sea surface and secondarily by river runoff, added to the relatively constant phosphorus load is a very important factor for the increase of algal blooms seen in European marine waters.

Another important aspect to consider is that evidence is accumulating that shows the production of toxins by algae is not only related to genetic inheritance but is also affected by external factors, such as nutrient concentrations. Many of the most toxic species, e.g., the species producing Paralytic Shellfish Poisoning (PSP), sometimes deadly to humans, increase their toxin content several fold when grown in media with low phosphorus and high nitrogen concentrations. Thus even if eutrophication might not be involved in the increase of HAB, the blooms might have become more toxic due to the higher input of nitrogen to European marine waters.

Why is there a need for co-ordinated research on HAB in Europe?

Many countries share European marine waters, and there are no physical barriers dividing the waters among them. Even if certain countries are not sharing the same sea, currents are continuously transporting water masses from one country to the next. Thus, a HAB population in one country might be triggered by nutrients originated in another country. Nutrients are also transported by winds. Another process possibly triggering HAB is the transport of merchandise by shipping. The amount of ballast water in large modern ships potentially contains seed populations of harmful algae enough to initiate a bloom in another marine area when discharged in exchange for cargo.
EUROHAB is formulated to generate the required research to manage better the effects of toxic/harmful marine microalgae in the marine and brackish waters of the EU.

During the past years, The European Commission has funded a number of initiatives on harmful algae, ranging from specific workshops to different projects included in programmes of the Commission. These projects usually have addressed specific problems related to a particular species or to a specific geographic location. For example, NUTOX, DOMTOX, Project on Ballast waters and COMWEB (MAST III Programme) focused on monitoring and control of harmful toxic algal blooms, EHUX and MEICE, focused on algal physiology, EULIT and CLEAN, on controlling factors of harmful blooms dynamics, NIRO on the modelling of algal bloom formation, as well as BASIC, PHAEOCYSTIS and EROS 21 (ENVIRONMENT&CLIMATE Programme). In each maritime country of the European Union, considerable efforts are devoted to harmful algal bloom monitoring and related research. Some European countries also have developed national plans. However, the somewhat limited scale of these observations allows very few general conclusions to be drawn.

European scientists participate actively in various international groups and fora where research on harmful algae is discussed and ideas exchanged. With the establishment of an increasing number of national (EU and non-EU), regional, and international initiatives, it becomes increasingly important to gather and focus the many national efforts in Europe.

The programme GEOHAB has been recently initiated by IOC, SCOR and ICES with very similar aims to those of EUROHAB but on the global scale.

Since HAB have been identified as a major problem by the Coastal-GOOS, the Health of the Oceans (HOTO), and the Living Marine Resources (LMR) panels, and have been associated with a switch in the links between nutrients, primary production, zooplankton and fisheries which are the topic of

GLOBEC, there is a need to collate and disseminate data and information on HAB from these programmes

This need for European co-ordination especially applies to HAB, since research on this subject does not yet allow a theoretical generalisation. It is therefore hazardous and often unjustified to apply results obtained in a given area and a given species to another situation.

To our knowledge, no reports on the trends of Harmful Algal Events are available at the European scale. This information would be a highly valuable contribution towards determining management options of the problem. The first step towards collecting and archiving a database of harmful events has already been accomplished (e.g. of decadal events, see Fig. 1). EUROHAB provides the framework for the next step, through a co-ordinated action of the different EU countries that would facilitate trend analysis.

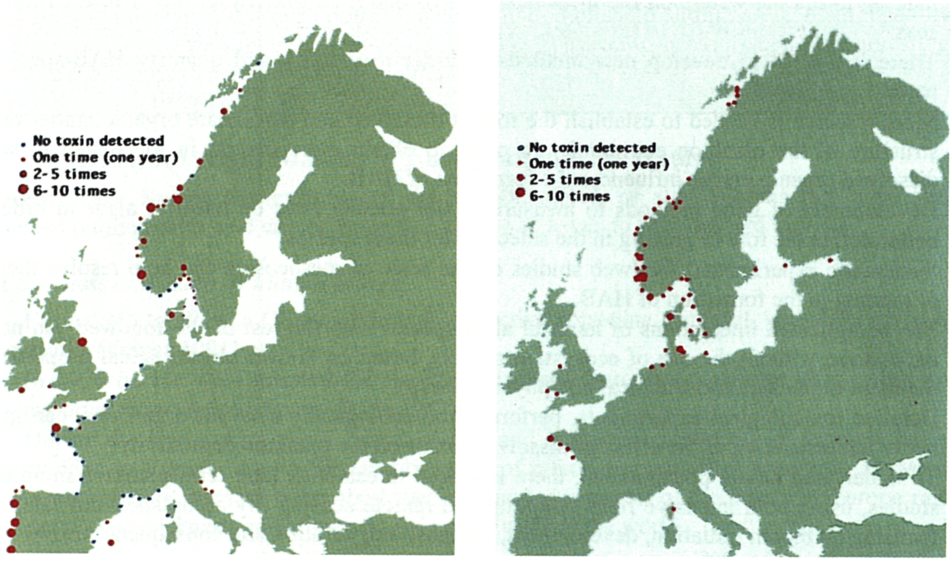


Fig. 1. Locations on different European coasts where shellfish was found with high levels of PSP or DSP toxins (left panel) and occurrences of animal and plant mortalities (right panel), both between 1987 and 1996. (Courtesy of ICES-IOC WGHABD and IFREMER, C. Belin)

If we are to understand the basic scenarios leading to harmful algal events, there is an urgent need to compare situations in different European countries. This would allow for more rational planning for research based on the important processes already known and the identified gaps. Strategies aiming at better management of harmful algae problems can most efficiently be developed through collaboration between national teams and comparison of their results. This can only be achieved through a generic action spanning many years.

All the factors stated above point to the advantage of an integrated European research effort to document the reasons for the different HAB occurring in our marine waters, as a basis for designing responsible strategies for better management of marine resources. A strong, integrated research programme would speed up this process. Participating in the EUROHAB initiative will be of major significance for European countries. It will also stimulate a major advancement in the management and mitigation of the effects caused by harmful microalgae.

Gaps in knowledge

Although a great deal is known about the ecology and physiology of harmful algae and of harmful algal blooms, major gaps still exist in our understanding of the factors controlling the population dynamics of harmful algae. Why and how harmful algae blooms develop is not understood at present.

Research requirements

The ultimate objective is to predict and if possible mitigate the development of HAB. The following areas of research need to be addressed:

- Small and large-scale laboratory experiments, enclosure and field studies of HA are needed to gain knowledge of the growth characteristics and toxin production.
- To determine the importance of inorganic nutrients (such as nitrogen, phosphorus and metals) loading to marine waters on the influence of HAB and their toxicity in the different European seas.
- There is a-need to develop new methods in order to identify and quantify HAB-species in natural communities.
- More research is needed to establish the role of dissolved and particulate organic matter on the structure of the plankton community in order to obtain more insight in the extent to which dissolved organic matter influences the formation of HAB.
- Development of good methods to measure *in situ* grazing rates on harmful algae in order to better define the role of grazing in the selection for these species.
- Systematic experimental foodweb studies on the scale of mesocosms can help resolve the role of grazing in the formation of HAB.
- The complicated interactions of harmful algal species with the rest of the foodweb can not be understood without the use of ecosystem models, based on known physiological properties of the main organisms and chemical and physical forces.
- Detailed toxicological experiments, performed on ecologically relevant organisms, can give a better understanding of the effect of dissolved toxins on the plankton community.
- To understand bloom phenomenon, there is a need to establish long term plankton monitoring studies, using both intensive field sampling and remote sensing (by aeroplanes and satellites), focusing on bloom initiation, development, composition, duration and consequences.
- Studies of microfossils archived in bottom sediments can provide information on blooms in relation to environmental changes (e.g., due to climate, eutrophication, etc.) before the present day.
- There is a need to identify sites of introduction of HAB-species from ballast water from larger ships and to evaluate the dimension of the problem for Europe.
- Characterisation of newly discovered toxins and development of detection and analytical methods are needed, in order to achieve a better knowledge of the different types of toxins. Identification of potential new toxin producers is also needed.
- Determination of the enzymatic processes involved in the metabolism and elimination of HAB toxins from shellfish.
- More research is needed to understand the overall importance of stratification, low turbulence, surface winds and irradiance for the initiation and the development of blooms for the occurrence of many HAB.
- There is a need to improve the knowledge on HAB species physiology in order to understand their competitive ability in relation to other species.
- There is a need of reference culture collections throughout Europe, with access for European scientists, in order to perform comparative studies between regions.
- There is a need to investigate the possible strategies to mitigate HAB. Possible strategies involve biological control (bacteria, viruses, zooplankton grazing), or flocculants (clay minerals) that will remove harmful algal cells from the water column.

1. INTRODUCTION

WHAT ARE HARMFUL ALGAL BLOOMS?

Microscopic, single celled algae are the main component of the phytoplankton in the sea, responsible for most of the primary production that sustains the marine food web. However, a few of the thousands of species found in European waters are known for their far-reaching damage to the coastal economy of many countries. These noxious events are named " harmful algal blooms " (HAB).

Some of these algae form massive slime growths along coastal areas, as in the Baltic and Adriatic Seas. Their nighttime respiration can deplete oxygen supplies so that fish suffocate, and their unsightly appearance causes substantial losses to tourism.

Other harmful algae produce potent toxins. Some of these toxins are lethal to finfish; they have already killed millions of finfish outright. Accumulated toxins can seriously affect shellfish health, because they filter the algae from the water. Acute and chronic illness in human can occur through the consumption of shellfish that are contaminated by the toxins. Hence, blooms of some toxic species can lead to bans of shellfish harvesting and major loss to commercial fishing industries.

1.1 Harmful Algal Blooms in European marine waters

In recent years, Europe has experienced the harmful effects of algal blooms that have seriously threatened public health, and caused enormous economic losses to fisheries and tourism.

1.1.1 Sickness and death of human beings

- From 1946 to 1963 in Obidos, Portugal, collective poisoning occurred, including seven human deaths (Franca 1991).
- In 1976 people were hospitalised for PSP in several European countries after eating mussels exported from Galicia, Spain (Fraga 1987).
- During the past twenty years, human deaths and large-scale outbreaks of sickness have been avoided in Europe, due to the establishment of effective monitoring programs. However, the threat remains, because toxic algal species found along most of the coasts of Europe regularly cause acute sickness, sometimes fatal, in other parts of the world.

1.1.2 Economic losses to fisheries

- In 1988, a massive bloom of *Chrysochromulina polylepis* in Scandinavian waters caused heavy mortalities of farmed fish and total collapse of the natural community (Rosenberg et al. 1988, Underdal et al. 1989).
- Periodic banning of shellfish from the markets has caused heavy economical losses for the shellfish industry. In 1995, for instance, several mussel raft areas were closed to harvesting in Ria de Pontevedra, Galicia, for more than 40 weeks due to persistent DSP and ASP outbreaks (Report from the Galician Monitoring Center, 1996), while along the French Atlantic coast, 1000 tons of mussels died together with a large variety of wild life due to a bloom of *Gymnodinium mikimotoi* (Arzul et al. 1995).

1.1.3 Economic losses to tourism

- In the Adriatic Sea, in 1989, algal blooms caused massive amounts of unsightly mucilage to float on the sea near beaches (Penna et al. 1993) resulting in heavy reductions in tourism for years, with correspondingly severe economic losses to the region.
- Similarly, in the Mallorca Islands, blooms discolouring the sea caused a major impact on the local tourist industry (Delgado et al. 1997).

- It is important to note that even HAB restricted to rather small areas are damaging for countries, which attract tourism by recognition of their pristine waters.

1.2 Are harmful algal blooms increasing?

HAB are an ancient phenomenon; there is a scientific record of such events from Venezia in 1729 (Fonda Umani et al. 1989), and several records from early European settlers in North America (Dale and Yentsch 1978). However, the striking increase in reports of HAB during the past decades has caused increasing scientific and public concern. Determining the extent to which the frequency and intensity of harmful blooms is increasing is one of the main questions to be answered by research in this field.

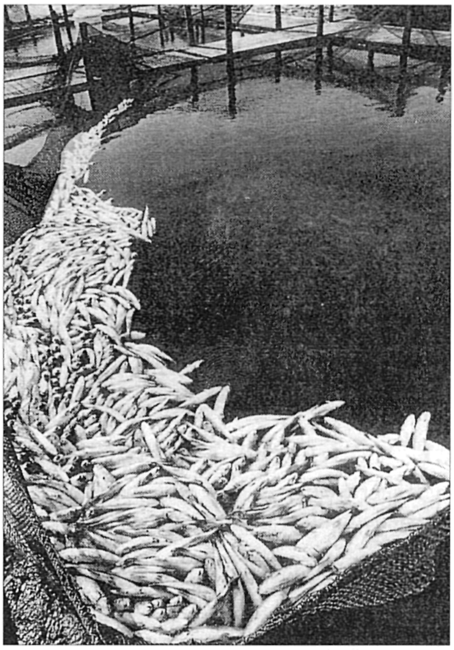


Fig. 2. Fish cage farm struck by a sudden bloom of the toxic algae *Chrysochromulina polylepis*. Toxic algal bloom shave caused fish kills in various countries. About 35 salmonid farms were hit by a large scale toxic algal bloom in Scandinavia. Over a few days Swedish and Norwegian farmers suffered economic losses of about 7 million dollars. Some 130 Norwegian farms, with a commercial value of more than 85 US dollars had to be relocated to fjords not affected by the bloom. Photo: O. Tollesby, AMBIO, 1989: 18(4). (Authorised publication by courtesy of Elisabeth Kessler, Editor-in-chief)

Evidence for increase in frequency, duration and extent of some HAB:

- Satellite imagery has shown the extension of the cyanobacterial bloom in the Baltic Sea from 1982 to 1993 (Kahru et al. 1994).
- Examples of discoloured waters affecting tourism appear to be a recent phenomenon.

Other factors contribute to the observed increase in reported HAB:

- The greatly increased capability for detection, especially of monitoring systems, which record the presence in marine waters of potentially-harmful species and/or the presence of toxins in commercial shellfish to a far greater extent than before (Anonymous 1998).
- The changes in cultural habits. Shellfish are presently eaten in summer when they are more likely to be contaminated, where previously they were not.
- Aquaculture. Altogether, the demand for farmed sea food has greatly increased; farmed sea food represents at present 23% the total captures of fisheries (Rana 1997), and this contribution is expected to increase greatly (Pedini and Shehadeh 1997). Whereas wild fish are often able to swim away to avoid harmful blooms, caged fish are particularly vulnerable.

Fig. 3. Mucilage in the Northern Adriatic Sea after the algal bloom in 1989. Surface accumulation (left panel) and a diver and a piece of mucilage (right panel). Photograph provided by courtesy of Dr. Attilio Rinaldi.

TYPES OF HARMFUL ALGAL BLOOMS IN EUROPE

Europe experiences a wide range of HAB producing varied serious effects.

- Algal toxins accumulated in shellfish have caused diarrhoea, amnesia and paralyses in humans in many countries (Egmond et al. 1993), even when the microalgae were present at low cell concentrations in seawater (Fig. 1 A)).
- Algae produced water-soluble toxins have caused mass mortalities of fish in several regions (Fig. 1 B) (Dahl and Tangen 1990, McMahon and Silke 1998).
- Cyanobacteria that can produce toxins and accumulate high biomass are a permanent threat in the Baltic Sea (Sivonen et al. 1989).
- A few non-toxic algae have (i) discoloured seawater in many marine waters, or produced (ii) enormous amounts of unpleasant foam (North Sea; Lancelot et al. 1987) which accumulates along beaches, or (iii) ugly mucilage (Adriatic Sea) floating near the beaches.

1.3 The role of human activities

Human activities can contribute to the increase of HAB by:

- Introduction of alien species, via (i) deballasting of large boats (Hayes 1998, Hallegraeff 1998) or (ii) transportation of living shellfish (Honjo et al. 1998).
- Heavy selective fishing, which decreases grazing upon herbivores (Botsford et al. 1997, Pauly et al. 1998), with subsequent alleviated losses for phytoplankton (Verity and Smetacek 1996); in summer, when potentially toxic species represent a significant fraction of the community, lack of grazing might contribute to the built up of a bloom.
- Changes in the amount and composition of the nutrient reservoir due to various types of loading (sewage discharge, transfer of air pollution, agricultural runoff). For instance, changes in N:P:Si ratios will favour nonsiliceous species, including most harmful species (Schöllhorn and Granéli 1996, Sandén and Hakanson 1996).

- Reduction of the natural hydrodynamic regime by building dams on rivers, walls in harbours, or artificial harbours, which provide refuges for phytoplankton growth and sediment for accumulation of cysts as seedbeds. e.g.,, in France, a dam built on a river where once tides provided a bi-daily flushing soon generated a retention zone where noxious events have occurred since (Merceron 1987).

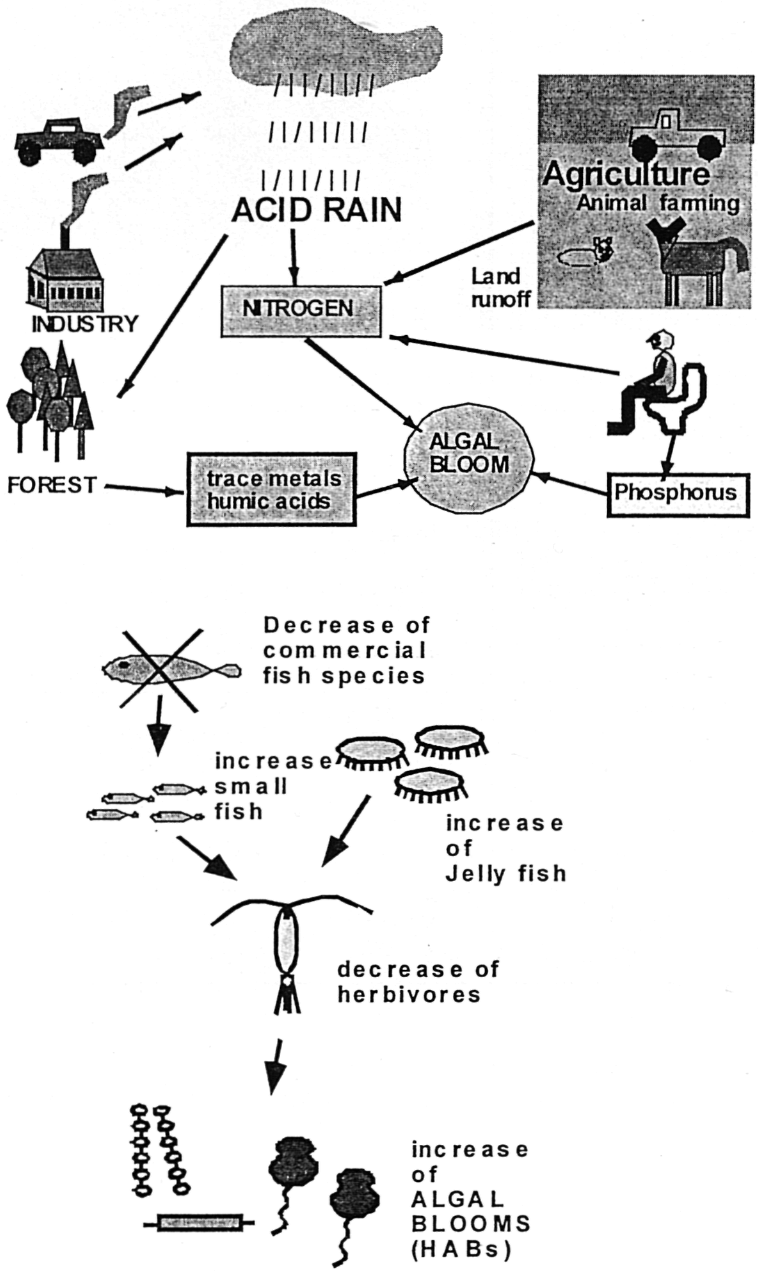


Fig. 4. Schematic view of two kinds of anthropogenic influences on HAB formation: effect of nutrients (upper panel) and overexploitation of commercial fisheries (lower panel).

1.4 Research needs for Europe

Experience in Europe to date shows critical gaps in HAB research.

- Accurate identification in real time is needed for species responsible for observed HAB e.g., it took days before *Chrysochromulina polylepis* was identified as the responsible organism for the 1988 bloom in Scandinavian waters (Kaas et al. 1991).
- Identification of some still inadequately identified toxins. For instance, the toxic cyanobacteria *Microcystis aeruginosa* produces a range of lipids compounds which inhibit fish gills; none has been hitherto characterised (Bury et al. 1998).
- Adequate time series measurements must cover all stages of bloom development (e.g., previously, measurements of environmental factors have been concentrated during or after a bloom, missing vital information regarding early (= potentially causative) stages of the bloom). A limited number of critical stations should be established for long-term monitoring to establish trends.
- The effects of the HAB should be understood for the whole ecosystem; this can be achieved only by using different approaches and further integration of data in models.
- Understanding the fate of toxins (detoxification).
- Scientists from all European countries should establish a culture collection of HAB species.

1.5 Responsible management of marine resources

The overall goal of such research is to provide a sound scientific basis for utilising marine resources, such as fisheries and tourism, with minimum risk of damage to public health and the natural environment. Hence, some practical questions should form the framework for European research on HAB.

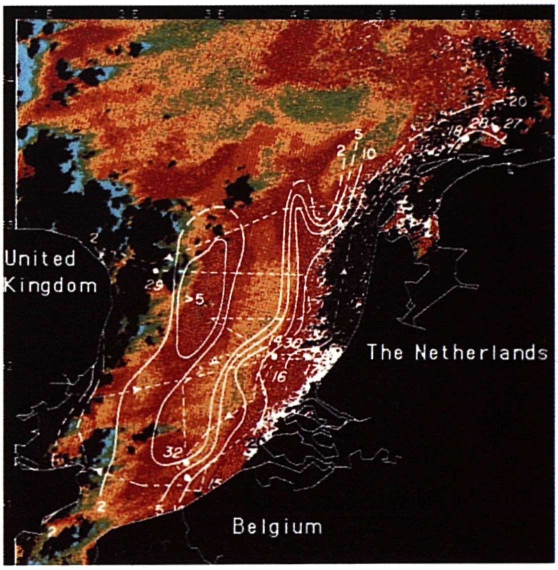


Fig. 5. Satellite image of the Dutch coastal area, showing a calculated chlorophyll a in a *Phaeocystis*-dominated bloom (colour), overlaid with chlorophyll a measured (isolines and values) from a research cruise during May 1986. Photo courtesy of Remote Sensing group at CCMS Plymouth Marine Laboratory. (see also Holligan et al. 1989)

1.5.1 Can we give adequate warning of HAB?

Previous research has provided few simple models allowing to give a range of probability for the appearance of harmful events. For instance, the toxic dinoflagellate *Dinophysis fortii* was shown to appear in the Inland Sea of Seto, in Japan, as soon as the temperature of water exceeded 8°C (Ozaka

1985). In the Galician rías, after reversals of the wind circulation and subsequent downwelling events, *Gymnodinium catenatum* populations from shelf waters are advected to the coast where they congregate. Satellite pictures show the warm surface waters approaching the coast, and the cold water band disappearing (Fraga et al. 1988). The Galician Monitoring Centre uses these patterns and the satellite pictures as a predicting tool: sampling is done in offshore warm water by means of a rosette coupled to a helicopter. When *G. catenatum* populations are present in surface waters, it is assumed that a few days later they will be "pushed" into the rías, and thence a sudden PSP outbreak will probably take place (Pazos and Maneiro 1998). Using all available data in order to provide such tools would help users of the coastal zone.

1.5.2 Can we prevent HAB?

At present the answer is clearly " no ", because bodies of water are too large, and we lack basic understanding of the causes of HAB. However, in a limited number of situations, attempts are possible. For instance, increasing turbulence by e.g., destruction of installations or enlargement of channel linking a pond to the open sea can greatly diminish the risk of HAB appearance in some low-turbulence waters. In the long term, any decision aiming to restore more pristine environments would help to diminish some HAB risks. Reduction of loading of nutrients from terrestrial origin is the most obvious; in the Black Sea, for instance, reduction of agricultural activities of bordering land has led to a significant decrease of HAB occurrence (Lancelot et al. 1998). However, this solution would not apply in the many other cases where harmful events are not related to high biomass of the responsible species. For them, prevention is unclear at the moment.

1.5.3 Can we alleviate the consequences of an HAB already progressing?

Some attempts have been made in Japan and Korea: spreading of clay in order to flocculate the toxin-producing species (Honjo 1994), spreading of surfactant to protect farmed fish against algal toxins (Ono et al. 1998). However, such attempts to eliminate the harmful population should be viewed with great caution, because ultimately the entire natural community could be damaged.

2. ALGAE PRODUCING TOXINS ACCUMULATING IN THE FOOD WEB

2.1 Introduction

Several marine phytoplankton species are known to be potent toxin producers (Fig. 6, Table 1). In order for these toxins to have harmful effects, these species do not need to be present in concentrations sufficient to discolour the water. Toxins may be passed through, accumulated and concentrated in the food chain, thus affecting predators (including man). Of particular concern in this regard is the accumulation of toxic species by shellfish which can cause human health problems, leading to the closure of shellfish-fisheries at certain times of the year in all coastal European countries (Fig. 7). Additionally the potential effect of toxin transfer through marine food chains is a concern, especially where they may impact on commercially important larval fish.

The questions that are prominent in the minds of managers and scientists are:

- Why are there seasonal, interannual and geographic variations of toxic events?
- Can toxin production, acquisition by marine organisms and persistence be understood and predicted?
- Can shellfish be made safe for human consumption once contaminated with microalgal toxins?

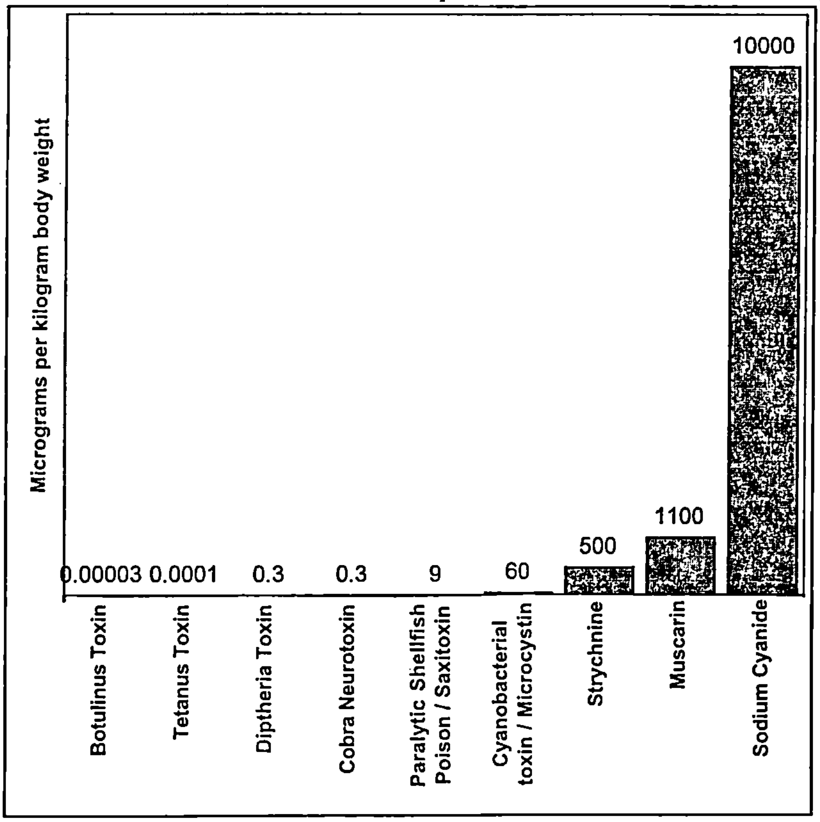


Fig. 6. Comparison of dose necessary to kill humans by algal produced toxins (PSP and cyanobacterial toxins) and other known poisons. (Redrawn from Error! Reference source not **found.seagrant/ issues/PSP/PSP.pdf)**

2.2 Organisms

2.2.1 Current Knowledge

There are some 20 known phytoplankton species producing toxins that accumulate in the food web. Organisms of this nature, with their effects, are listed in Table 1. The table shows those toxic species known to occur in Europe and also those species that are known to be toxic in other parts of the world and have been recorded as occurring in Europe (Lassus 1988).

In characterising HAB and managing their consequences and routine monitoring for harmful species, there is a need for precise identification of organisms. In this regard it is important that within the community a pool of taxonomic expertise is maintained. It is obviously important to be able to distinguish potentially toxic organisms in the phytoplankton assemblage. However, there is a further level of difficulty imposed by the need to distinguish closely related species with different toxin production capabilities. For example, in Table 1 the *Pseudo-nitzschia* group contains 5 toxic species and there are other morphologically similar *Pseudo-nitzschia* species that are non-toxic. These various species require cumbersome preparation before they can be distinguished on microscopes. This is a time consuming process and cannot be undertaken on a routine basis. Molecular probes are being developed for *Pseudo-nitzschia* (Fig. 8), however; they are not yet robust enough for routine monitoring work. The inability to distinguish potentially toxic from non-toxic species routinely is an impediment to investigation of HAB; it is also a problem for those undertaking monitoring.

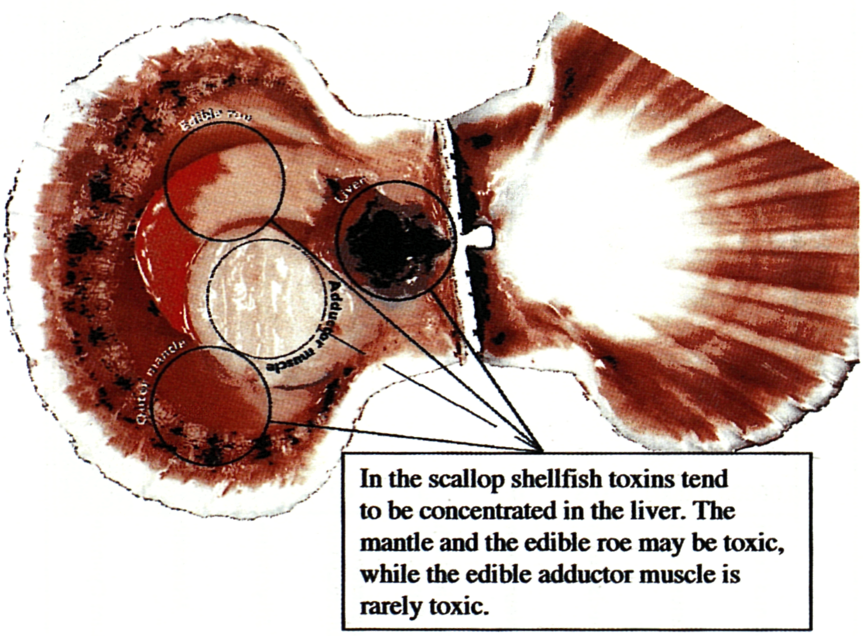


Fig. 7. Dissected scallop showing the edible parts where algal toxins accumulate. Photo courtesy of Tom McKinnes, Fisheries Research Services, Aberdeen, UK.

Most of these toxic species produce a suite of compounds of varying toxicity at different stages of their cell cycle and species of the same genus can produce a different range of compounds and hence exhibit varying toxicity characteristics and hazards. The situation is made more complex by the apparent lack of some HAB strains within the same species to produce toxin. Toxin content is our primary concern with regard to these particular HAB species due to their potential to accumulate in shellfish and cause illness in human consumers of contaminated food products. Again our inability to distinguish between toxic and non-toxic cells reduces the effectiveness of our research into HAB and our monitoring programmes.

Marine phytoplankton have a variety of life histories and of particular note in these toxic organisms is the fact that many of them have a resting stage as part of their life cycle (Fig. 9). Resting stages fall to the sediments, and it is here that many species reside for much of the year. The possession of a resting stage is not a prerequisite for HAB formation but their prevalence in harmful species suggests that it may offer some competitive advantage. These resting stages are important in providing the inoculum for blooms and can also be a mechanism for removing cells from the population. Their role in bloom initiation and decay means that a thorough understanding of life cycles is critical to our understanding of the dynamics of HAB.

Syndrome	Type of toxin	Species	Symptoms
Paralytic Shellfish Poisoning (PSP)	Saxitoxin, Neosaxitoxin and other derivatives	*Alexandrium tamarense* *Alexandrium catenella* *Alexandrium minutum*	Headache, dizziness, nausea tingling paralysis
Diarrhoeic Shellfish Poisoning (DSP)	Okadaic acid, Dinophysis toxins	*Gymnodinium catenatum* *Dinophysis acuminata* *Dinophysis acuta Dinophysis caudata Dinophysis mitra* *Dinophysis norvegica* *Dinophysis rotundata* *Dinophysis tripos* *Dinophysis sacculum* *Prorocentrum delicatissima* *Prorocentrum lima* *Prorocentrum tepsium sp. indet* *Prorocentrum seriata*	Diarrhoea, nausea, vomiting, abdominal pain; hepatotoxic cardiotoxic, chronic exposure may promote tumour formation in the digestive system
Amnesic Shellfish Poisoning (ASP)	Domoic acid	*Pseudo nitzschia multiseries* *Pseudo nitzschia pseudodelicatissima Pseudo nitzschia australis Pseudo nitzschia seriata*	Nausea, vomiting, diarrhoea, abdominal cramps, dizziness, hallucinations, confusion, shorterm memory loss seizures
Neurotoxic Shellfish Poisoning (NSP)	Brevetoxins	*Gymnodinium breve*	Chills, headache, diarrhoea, vomiting and abdominal pain, muscular, aches, dizziness, anxiety, sweating and peripheral tingling
Cyanobac-terial toxins	Nodularins, Microcystin, Saxitoxin, Neosaxitoxin	*Nodularia spumigena* *Microcystis spp. Anabaena spp* *Aphanizomenon flos-aquae* New species	Weakness, recumbence, pallor, vomiting, vomiting, diarrhoea. Death occurs due to pooling of blood in the liver and respiratory arrest

Table 1. Different harmful algal species, their toxins and its effects.

HAB species display different behavioural strategies for survival, growth and bloom formation. Motility is one of several behavioural traits. The flagellate species (for example *Alexandrium* spp., *Dinophysis* spp.) have a range of swimming abilities, which they may exploit in a variety of ways in bloom formation. For example, some species (*Alexandrium* spp.) migrate through the water column on a daily basis, exploiting surface sunlight during the day and deep nutrients by night. Yet other species (*Dinophysis* spp.) use their swimming behaviour to maintain their position in a narrow layer of the water column. The ability of a particular species to interact in these ways with physical features, such as pycnoclines, fronts or internal waves may be of prime importance for the competition of HAB species with other algae. The formation of chains by bloom species seems likely to be another

behavioural trait, conferring the dual advantage of increased swimming capacity and a decreased likelihood of grazing (Fig. 10 B). These behavioural strategies are only known from a small number of species, they remain to be elucidated for the majority of HAB species.
HAB species can also have nutritional strategies, which enable them to out-compete other microalgae. Some autotrophic species (*Alexandrium catenella*) appear to use dissolved organic nutrients while others (*Dinophysis* spp.) rely on mixotrophy to supplement their carbon requirements. These phenomena are only known for a few species but their potential importance in bloom dynamics mean that they need to be investigated for all HAB species.

2.2.2 Gaps in knowledge

Over the last 20 years toxins have been newly discovered in several species, for example Yessotoxin in *Protoceratium reticulatum* (Yasumoto and Satake 1998). It is probable that further species exist with such capabilities. It is important that monitoring agencies remain aware of this possibility and that the research community is able to respond with appropriate investigations (for example taxonomy and toxin profiling of the species concerned).
Some information is available on the mechanisms of toxin production of toxic HAB species held in the laboratory, however, our knowledge and understanding of field populations is poor. In addition, with current methodology, it can be difficult to determine full toxin profiles (e.g., of *Dinophysis* and *Pseudo-nitzschia* species). Furthermore, there are no standards for some toxins (e.g., Yessotoxin) available in Europe.

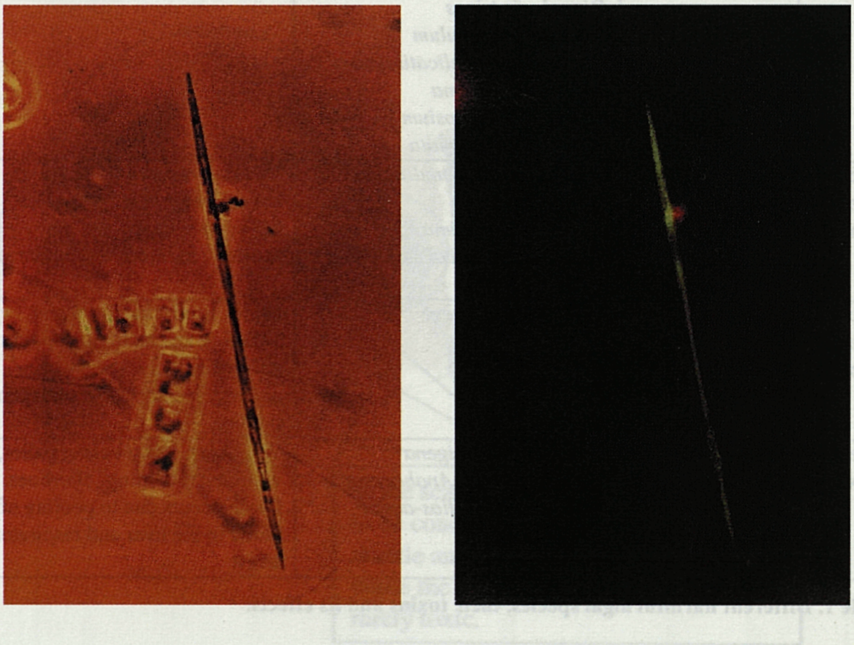


Fig. 8. A ribosomal RNA-targeted oligonucleotide specific for *Pseudo nitzschia pungens* f. *multiseries*, applied to a natural sample. A transmitted light micrograph is shown at the left panel. A chain of needle-like diatoms that could be either P. pungens f. pungens (non-toxic) or *P. pumgens* f. *multiseries* (toxic) is seen, in addition to other non-toxic plankton. The two forms of *P.pungens* are indistinguishable using transmitted light microscopy. However, when the same frame is viewed using epifluorescence microscopy (right panel) the fluorescein labelled rRNA probe (green) is visible, identifying the *Pseudo-nitzschia* chain as a *P.pungens* f. *multiseries*-the toxic variety linked to amnetic shellfish poisoning. Photo provided by D. Anderson (courtesy of C. Scholin and K.Buck)

To facilitate environmental studies into the mechanism of toxin production and accumulation in shellfish the biosynthetic pathways of toxin production; the physiological regulation of toxin accumulation in cells and the genes involved need to be elucidated. Investigation into the latter is complex as HAB associated with toxin production generally have an associated bacterial microflora and may have virus infection, the role of which has not been determined.

The life cycles of a few toxic species have been documented (e.g., *Alexandrium tamarense, Gymnodinium catenatum*) but the distinction of the various life-cycle stages is difficult and time consuming by conventional means. In laboratory culture the manipulation of a species through these stages is usually achieved by nutrient deprivation. However, it is not clear what internal cell processes are truly responsible for triggering these transitions. There have been very few studies of these processes in the environment because of the difficulty in distinguishing the life-cycle stages. Further, the factors causing life-cycle changes in the environment are not clear. In the field, resting stage formation in some species has been noted even during apparent nutrient sufficiency.

It has been documented for *Alexandrium tamarense* and *Gymnodinium catenatum* that the resting stage (cyst) is toxic. Toxin containing cysts may be an important transport mechanism of toxins to the sediments and to benthic marine organisms. However, cysts have very robust cell walls and the efficiency of transfer of the toxins from cysts to benthic organisms is not known. It is possible that some benthic organisms will simply pass cysts through their digestive systems and expel them in their faeces.

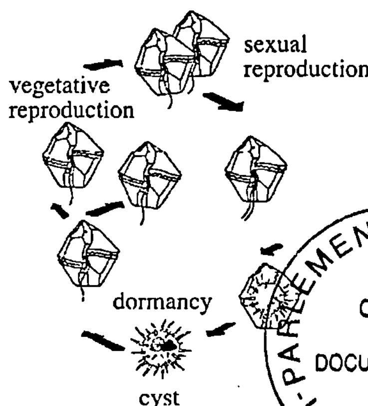


Fig. 9. Life cycle of the dinoflagellate *Lingulodinium polyedrum*.

The physiological requirements and tolerances of species for environmental variables, such as temperature, salinity, turbulence, light and nutrients can determine species distribution and responses to changing environments. Relatively few HAB species have been characterised with regard to these variables. All HAB species must be characterised in this respect if we are to understand and predict their distribution and occurrence in natural waters.

To carry out much of this research we require cultures of HAB species. There has been great progress in this area but it is clear that many of these species are fastidious and difficult to maintain in the laboratory. Whereas many of the autotrophic HAB species can be maintained in culture, others (notably the *Dinophysis* species) have not been successfully maintained under laboratory conditions. This highlights our lack of knowledge of the maintenance requirements of these organisms. It is obviously necessary in order to complete a number of goals in this research (taxonomy, life cycle research, physiological requirements) that culture collections are improved, expanded and made widely available.

2.2.3 Research needs and justification

To improve the efficiency and effectiveness of monitoring programmes and to ensure they remain up to date and to improve our understanding of the variability of toxin production and to allow us to predict toxin production, research programmes are required to address:

- The development of rapid and cost effective means of identifying potentially harmful species and their toxicity.
- The investigation of newly discovered toxic species. Including the taxonomy of the species concerned (allowing accurate identification for monitoring) and the isolation and characterisation of unknown toxins and production of toxin standards for method and inter-calibration

- The elucidation of the biosynthetic pathways of toxin production and the definition of factors which influence toxin production and changes in toxin profiles at a cellular level emphasising intraspecies differences and nutrient assimilation.

> The determination of the genes involved in toxin production and regulation taking into account potential sources from organisms such as bacteria and viruses.
> Understanding the mechanisms of toxin production and definition of genes involved may also lead to the development of molecular tools which will allow investigations of toxin production and accumulation in shellfish during HAB events. An additional benefit of such research could be better techniques (more specific, cheaper, faster, easy to handle) for use in shellfish monitoring programmes and in research for example in mixed cultures to investigate the toxin content of single cells.

- The establishment of the toxin content of resting stages of all toxic species and the investigation of the passage of toxins from resting stages to benthic marine organisms

In order to understand and better forecast bloom dynamics, our understanding of the life cycles of toxic species and their physiological requirements needs to be improved. Research should focus on:

- The collection, isolation and maintenance of cultures of HAB species
- The development of robust, simple, functional probes to follow physiological changes inside cells in order to determine the factors triggering life-cycle transformations.
- The development of tools to rapidly identify life-cycle stages and the investigation of life cycles of toxic species in the laboratory and in the field
- The investigation of the behaviour of HAB in natural waters or at least in more *in-situ* like cultures.

The sediment stock of cysts provides a record of species that have occurred in the area. One issue that is of concern is whether toxic events are increasing in frequency. In suitable sites (with little bioturbation) long term changes in the populations of toxic species could be investigated. This has been done for *Gymnodinium catenatum* (Dale et al. 1993). By sampling strategic sites throughout Europe a baseline could be established in order to determine trends in event occurrence in future years. Research should address:

- The investigation of changes in cyst-forming species in recent sediments as indicators of change in bloom frequency.
- The establishment of a European baseline of strategic sites for the monitoring of cyst stocks in order to follow potential future change of bloom frequency.

2.3 Community interactions

2.3.1 Current knowledge

The growth of HAB species takes place within the pelagic community composed of a large number of other organisms interacting in many ways. The success of the HAB species will not only depend on growth, but also on the results of these interactions with other organisms in the plankton community, such as competition for resources, chemical conditioning of the water by previous organisms, predation or diseases. In order for the HAB species to be successful, its growth rate must exceed the sum of all of the loss processes. Many HAB species do not have especially high growth rates compared to other phytoplankton species. Their success seems instead to be based on survival strategies, such as swimming behaviour, resting stage production, release of allelopathic substances and grazing resistance.

2.3.2 Gaps in knowledge

Factors affecting phytoplankton growth are light and nutrients, for which there is competition among members of the community. For a few toxic species the nutrient uptake rates and general requirements are known under laboratory conditions, however, there is very little information on the performance of toxic species under natural conditions in the presence of other species.

It has been noted that some phytoplankton species produce compounds that are detrimental to their competitors (allelopathy), again this potential is only known for a few of these species and has not been investigated for most.

There are a variety of mechanisms for reducing the numbers of toxic cells, of which grazing is probably the most important in the early part of bloom dynamics. We have some evidence for toxic species that they have adaptations to avoid being grazed. For example *Alexandrium* species may be bioluminescent, which leads to a reduction in grazing rates. We also know that some species (*Alexandrium* spp., *Dinophysis* spp.) are poor food for predators, either because of their toxin content or because of their poor nutritional qualities. However, for many of these species we are not certain even what organisms are capable of feeding on them so much remains to be investigated in this area.

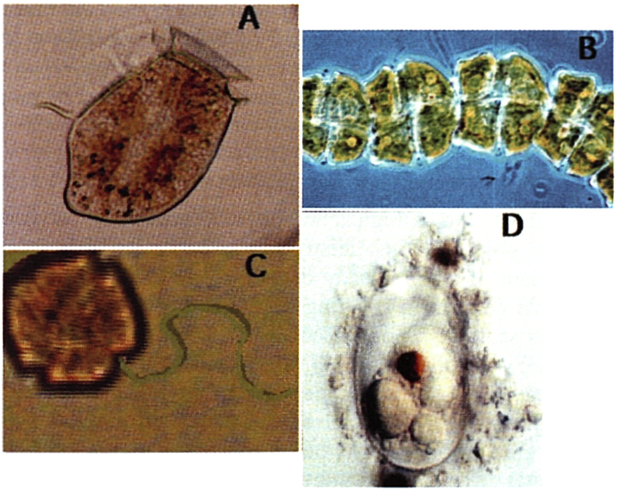


Fig. 10. A cell of the DSP producing dinoflagellate *Dinophysis acuta* (70 μm length) (A) and two PSP toxin producing species: *Gymnodinium catenatum* (cell diameter: 40 μm) (B) and *Alexandrium tamarense* (diameter: 30 μm) (C), and (D): *A. tamarense* cyst (length: 60 μm). Photos: (A, C) Christina Esplund-Linquist and Edna Granéli, (B) courtesy of M. Teresa Moita, (D) courtesy of Yasuwo Fukuyo.

Changes in higher trophic levels, such as over exploitation of piscivorous (fish eating) fishes, will lead to an increase of zooplankton-eating fish and consequently a higher predation pressure on the zooplankton, which are consuming the phytoplankton. If this results in a significant reduction or the zooplankton stock, the grazing pressure on phytoplankton will be relaxed. Thus, changes in higher trophic levels can lead to a release of phytoplankton (among them HAB species), from zooplankton grazing.

In the later stages of a phytoplankton bloom, two phenomena have been noted that can be important in reducing species numbers, often in a dramatic fashion. These are parasites and bacterial or viral infections. Like other phytoplankton, HAB species can have species-specific parasites or viruses that can be an important factor responsible for the termination of HAB. There is very little information available on these phenomena.

Most of the field studies on HAB species have focused on the toxic species and very little information has been gathered on the other components of the plankton community at the same time. The consequence is that there is hardly any information available on how the HAB species interact with the rest of the community under natural conditions.

2.3.3 Research needs and justification

To better understand and forecast bloom dynamics, research needs to focus on:

- Field and mesocosm studies including all components of the plankton community (from viruses and bacteria to zooplankton and fish) in order to place HAB in their community context
- Experimental work into specific interactions between HAB species and their predators, parasites, viruses and phytoplankton competitors in order to understand the most important factors involved in the respective HAB species success in forming blooms and the processes behind the termination of blooms.

2.4 Physical and chemical conditions leading to HAB formation.

2.4.1 Current knowledge

The characteristics of the organisms and their interactions within the community are fundamental to determining whether or not they grow. However, all of these processes are set in the context of a wider, hydrodynamic picture. Microalgae (whether toxic or not) are governed by the water body in which they are contained. The nature and history of that water body and its movements are a crucial key to unlocking the puzzles of HAB formation.

2.4.2 Gaps in knowledge

There is a broad understanding of the biological and physical processes that govern HAB. However, the interaction of the biology, physics and chemistry as it affects microalgae occurs over a wide range of scales. From the movement of a water body containing a HAB from offshore to inshore to the microturbulence that prevents cells from dividing successfully. Our understanding of the detailed interactions of these various scales is poor, especially with regard to the selection of a particular species (possibly a toxic one) over others. Much of our understanding has been derived from field sampling programmes that have employed bulk measures of phytoplankton (for example chlorophyll) and their activity (for example oxygen). It is clear that these will never tell the whole story and in particular can never address the question of why one species instead of another, which is fundamental to our understanding of HAB. To answer this question and generate realistic data to assist in modelling, field programmes and mesocosm experiments need to be able to undertake detailed sampling over large and small scales. At present this generates numbers of samples which are unrealistic for analysis by conventional means.

2.4.3 Research needs and justification

To improve our understanding of HAB and to allow development of realistic models to allow forecasting of such events, research programmes are required to address:

- The development of continuous methods for identification of planktonic organisms
- The definition of small scale processes and their effects on different HAB species
- The initiation, growth and termination of HAB over wide scales

2.5 Aquaculture, fisheries and human health.

2.5.1 Risk Assessment

2.5.1.1 Current Knowledge

Toxic groups such as ASP, PSP and DSP accumulate in a range of shellfish and adversely effect a variety of organisms including humans. Examples of the latter include food poisoning outbreaks in a number of European countries. Organisms as diverse as copepods, birds and whales may also be affected. Therefore these toxins influence public safety, fisheries, wild life and aquaculture. The effects of toxic HAB on aquaculture and fisheries is primarily economic in terms of availability of stocks, the feasibility of marketing the produce and in maintaining the local economy. Risk assessment, which leads to risk management, can lead to improved protection and utilisation of resources by both industry and regulators. An example of risk assessment in terms of consequences to human health include assessing the following; the likelihood of human exposure, routes of exposure, extent and frequency of exposure, chronic and acute effects, and avoidance of exposure.

2.5.1.2 Gaps in knowledge

In the field of HAB risk assessment has been carried out for determining safe levels of microcystins in drinking water but it has not been effectively applied to other HAB toxins. There may be sufficient information to carry out risk assessment for some toxic compounds, for example those involved in PSP, but this information has not been assessed and applied. Unfortunately there are several toxin groups where the information required doing a risk assessment is not available.

2.5.1.3 Research needs and justification

Several research issues need to be addressed:

Human Health.

A detailed review on toxicological data available for established toxins must be compiled in order to summarise knowledge on hazard characteristics, exposure levels, adverse health effects and to assess if current information is good enough to develop risk assessment programmes. Gaps in knowledge must be summarised and research programmes designed to provide the information required. Detailed investigation of the toxicology, including chronic and acute effects of new compounds and toxin groups where information is insufficient to allow risk assessment.

- Research programmes to support efforts to find the causative organisms of new toxins and assess their frequency of distribution within member states.
- Support for a social research programme to improve public information and avoid media misrepresentation.

Aquaculture.

- Investigations into the survival of different life stages of causative organisms of toxic HAB in shellfish in order to define criteria for stock transfer;
- Development of methodology to assess the risk of toxic HAB in the selection of new aquaculture sites this could include the development of cyst survey methods and applications in the context of estimating the potential risk of toxic species in a new site.
- Evaluation of the economic cost of harvesting closures due to HAB toxins in relation to industry value and national monitoring programmes.

Fisheries

- Develop risk assessment strategies and methodologies to ensure the safety of products from wild fisheries;
- Investigate programmes to determine if toxic HAB pose a threat to commercial fisheries by adversely effecting fish larvae.

Syndrome	Type of toxin	Abbreviation	Marine organisms affected
Paralytic Shellfish Poisoning (PSP)	Saxitoxin, Neosaxitoxin Gonyautoxin-1 bis 4 N-Sulfocarbamoy-ltoxins Decarbamoyl-toxins	STX NEO GTX 1 to 4 B1+2,C1 to C4 e.g., dcSTX, dcNEO	Mussels, clams, sea scallops, oysters, gastropods, lobsters crabs, herring, mackerel salmon, menhaden, sandlance whales, sea birds, squid, zooplankton and other benthic invertebrates like sponges
Diarrhoeic Shellfish Poisoning (DSP)	Okadaic acid, Dinophysis toxins	OA DTX-1 to 3	Mussels, mackerel, finfish
Amnesic Shellfish Poisoning (ASP)	Domoic acid	DA	Mussels, anchovies, sea birds, scallops
Neurotoxic Shellfish Poisoning (NSP)	Brevetoxins A Brevetoxins B	PbTx-1,7,10 PbTx-2,3,5,6,8,9	Scallops, clams, oysters, tunicates, sea turtles, dolphins
Cyanobacterial toxins ? so far unknown toxins	Neurotoxins: Anatoxin, Hepatoxins: Microcystins Nodularin		

Table 2. Toxins and affected marine organisms.

2.5.2 Quality control and quality assurance

2.5.2.1&2 Current Knowledge and gaps in knowledge

Quality control of products requires that they meet set standards, which in the case of HAB toxins are defined in Council directive 91/492. However, inconsistencies in these standards exist, particularly in the definition of compounds belonging to some of the toxin groups e.g., DSP. This makes the establishment of quality control criteria difficult. Related to quality control is the issue of quality assurance i.e., defining the quality of the data generated. This incorporates processes that range from sampling design to sample analysis. Quality assurance programmes are currently in operation throughout the EC for the measurement of trace metals in fish/sediment e.g., the QUASIEME programme. This was initially established using EU funding and led to the stage where participant laboratories regularly analyse contaminated samples, prepared by the central laboratory and their performance is evaluated. Similar systems have not been applied in relation to toxic HAB. However, the basis of such a programme for shellfish toxicity testing could be established as the result of a recent BCR programme. This programme produced shellfish material contaminated with two of the PSP toxins. Knowledge gained in this project could provide the basis of a research programme, which develops processes to allow the preparation of the range of material required to run such a programme as QUASIEME. However, such a programme would also have to incorporate initial research on the effects of differences in sampling criteria, frequency, storage and storage conditions of samples. Similar programmes for phytoplankton analysis would require initial research into similar criteria to that described for shellfish.

2.5.2.3 Research needs and justification

- Implement research to investigate effects of changing sampling criteria, frequency, storage and transport conditions on toxicity and phytoplankton measurements.
- Implement research to allow the development of a programme analogous to QUASIEME to establish and evaluate quality assurance in toxicity testing and the detection of toxic HAB species in both research and monitoring.
- Evaluate the toxicological of compounds and establish quality control criteria.

This research will assist in the provision of effective quality control and assurance must be addressed in order to provide the accuracy, precision of data generated between European countries and effective protection of public health, fisheries and aquaculture.

2.5.3 Fate and effect of toxins in marine organisms

2.5.3.1&2 Current knowledge and gaps in knowledge

HAB cells are food for herbivorous marine organisms. The endotoxins of the microalgae are ingested and accumulated in the grazers, sometimes affecting them negatively, but sometimes the grazers appear unaffected by the presence of toxins and will thereby act as a vector transferring toxins to other components of the food web.

In the first case, zooplankton behavioural changes and even lethal effects have been observed when copepods have been feeding on, for example, *Alexandrium* and *Dinophysis* species. The effects of HAB toxins on other grazing organisms, such as plankton-eating fish and shellfish larvae are, however, almost totally unknown. Not only are acute lethal effects on different organisms to be expected, but also sublethal effects, which might affect processes, such as reproductive success.

In the case of transferring of toxins, the most well known example (and probably the one with most economical impact) is the accumulation of toxins in molluscs (mussels and oysters) and the concomitant effects on humans who consume them. However, there is evidence that toxin transfer to humans can also occur via the ingestion of other marine animals such as fish, crabs and lobsters. Besides the lethal effects of the toxins on humans, lethal effects have also been observed on other mammals, such as whales, domestic animals and birds (Anderson 1994).

The extent of accumulation, metabolism and depuration of HAB toxins once ingested is known to various degrees depending on the accumulating species. For instance, little is known about accumulation of toxins in zooplankton and fish, but more is known about the accumulation of different toxins in shellfish.
Filter feeding bivalves and gastropods molluscs concentrate HAB toxins to different extents and for different periods depending on the species, which means that data from one species may not necessarily be extrapolated to another. The reason for this variability is largely unknown, however, the level of toxicity could depend on several factors: toxin content of the HAB species ingested, the proportion of toxic cells represented in the total food available for the animal, environmental conditions and intrinsic factors. One of the most studied toxin groups are the paralytic shellfish toxins where uptake and detoxification experiments have been undertaken and in some instances models of toxin kinetics proposed. However, these studies relate to a limited number of species and little is known about the enzymatic mechanisms involved in the metabolism and detoxification processes. Even less is known about the mechanisms for the other two main groups of toxins (ASP and DSP toxins). Understanding of the processes is essential in our quest to develop management systems for contaminated shellfish.

2.5.3.3 Research needs and justification

- Research on toxin accumulation, detoxification and biotransformation rates in exploited shellfish species.
- Development of commercial systems for the cleansing of shellfish once contaminated with toxin.
- Development of accurate, rapid and user friendly methods for toxin analysis in shellfish to be performed at aquaculture sites.
- Determination of the enzymatic processes involved in the metabolism and elimination of HAB toxins, particularly for those commercially important species where detoxification is extremely slow (for example scallops).

2.5 Benefits

Furthering our understanding of the seasonal, interannual and geographic variations in toxic events; the variability of toxin production, acquisition and persistence in organisms and their potential for detoxification will lead to:

- Improved health protection of shellfish consumers
- Rational planning of shellfish collection and marketing
- Scientifically based assessment for aquaculture site selection
- Innovation in traditional marketing practices (e.g., removing organs with unsafe levels of toxins, industrial processing and treatment of moderately toxic seafood to obtain toxin free products with good organoleptic properties.
- Modification of existing legislation on toxicity criteria of seafood products according to new results of toxicological studies
- Harmonisation and standardisation of ongoing monitoring programmes will lead to better exploitation of available data at a European scale
- Design of more cost-effective monitoring programmes applying more automated techniques for cell identification and toxin detection
- Sound and basic information to provide key points for consideration in future environmental impact evaluation of coastal modifications

3. FISH KILLING SPECIES

3.1 Introduction

Several HAB species are non-toxic to humans, but may harm natural ecosystems and kill farmed fish with serious economical consequences for the aquaculture industry. The species causing fish kills are not a uniform group, but include members from different algal classes. The dinoflagellates *Gymnodinium mikimotoi, G. corsicum* and *G. galatheanum*, members of the haptophyte genera *Chrysochromulina* and *Prymnesium* (Edvardsen and Paasche 1998), the raphidophyte *Chattonella* spp., *Fibrocapsa japonica* and *Heterosigma akashiwo, H. inlandica*, the silicoflagellate *Dictyocha speculum,* and finally the diatoms *Chaetoceros convolutus* and *C. calcitrans* all form fish killing algal blooms in European marine waters. *Gymnodinium mikimotoi* was first recorded in European waters in 1966 and has since then formed recurrent blooms from the Spanish Atlantic border in the south to western Norway in the north, causing death to about 3000 tons of farmed fish and about 900 tons of mussels (Moestrup 1994). The first recorded toxic bloom of a *Chrysochromulina* species occurred in 1988 (Dahl et al. 1988), when *C. polylepis* formed an extensive bloom in Skagerrak and Kattegat. The bloom caused death of a wide range of marine organisms in addition to 900 tons of farmed fish. In 1991 *Chrysochromulina leadbeateri* killed 600 tons of farmed salmonids in Lofoten in Northern Norway. *Chrysochromulina*-blooms with minor fish-kills have occurred in northern Europe as well. *Prymnesium* spp. has recurred yearly since 1989 in a fjord system in western Norway where it has killed about 1500 tons of farmed fish. The raphidophytes *Chattonella* spp. and *F. japonica* were recorded in Europe in 1991. The first fish kills due to this newly found species were recorded in 1998, where a large bloom of *Chatonella* aff. *verruculosa* caused extensive fish kills in the North Sea - Skagerrak area. *Heterosigma akashiwo* killed fish off the Brittany coast in 1994, and farmed fish off the south coast of Portugal in 1997.

Aim: To develop a better understanding of HAB responsible for fish kills in order to limit their impacts, and to seek commonalties within these species to establish possible functional groupings.

3.2 The organism

3.2.1 Current knowledge

3.2.1.1 Taxonomy

Is crucial to know the precise taxonomic identity of the causative organism of a harmful bloom in order to apply knowledge from previous similar blooms to the species at hand. The majority of the 50 *Chrysochromulina* species that are described occur in European waters (Green and Leadbeater 1994), and many more species await description. Blooms of *Chattonella* species have been recurrent in European waters since 1991. These species differ morphologically from those that regularly form toxic blooms in Japan. Most of the ichthyotoxic (fish-killing) species are difficult to preserve and to identify.

3.2.1.2 Life history

Knowledge on the life history for these fish-killing species is fragmentary. Sexual reproduction has been demonstrated in some dinoflagellates, raphidophytes and haptophytes, but appear to have been lost in other species within the same groups. Some species of *Chrysochromulina* and *Prymnesium* produce both haploid and diploid cells, suggesting that they have a sexual haplo-diploid life cycle (Edvardsen and Paasche 1998). *Chattonella marina* and *C. antiqua* from Japan can reproduce sexually and produce cysts that may seed the next bloom. The life cycle of the raphidophytes in Europe have, however, not been studied. The life history in the ichthyotoxic dinoflagellates (*G. mikitomoi*) is practically unknown. Information on the role of the alternate phases in the life cycle of these species is crucial in order to understand and predict the bloom development.

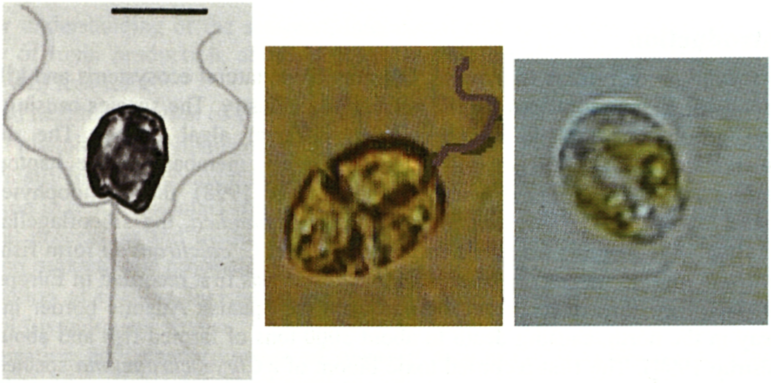


Fig. 11. Cells of fish killing microalgae: left panel: *Chrysochromulina polylepis* (diameter: 8µm), middle panel: *Gymnodinium mikimotoi* (diameter: 20 µm), and right panel: *Prymnesium parvum* (diameter: 8 µm). Photos Christina Esplund-Lindquist and Edna Granéli.

3.2.1.3 Behavioural strategies and physiology

Fish-killing species are photosynthetic. Growth rate in response to environmental factors such as salinity, temperature and light have been examined for some of these species. Many (e.g., *Chattonella* spp., *C. polylepis* and *P. parvum/patelliferum)* appear to be eurythermal and euryhaline, adapted to variable conditions, typical for coastal water. The growth requirements for trace metals have been studied for some of the species. The Raphidophytes appear to have a strong demand for iron, whereas the growth of *C. polylepis* is promoted by additions of selenium and cobalt, and the growth of *G. mikimotoi* is similarly promoted by additions of selenium. A number of fish killers, like *Chrysochromulina* spp., *Prymnesium* spp. and *Gymnodinium galatheanum* are able to feed on bacteria and other protists i.e. they are mixotrophic. This mode of nutrition may be an advantage forcompeting other phytoplankton species.

All the fish killing species are flagellates with the ability to swim and many species show phototactic behaviour. Vertical migration (e.g., *Chattonella* spp., *Gymnodinium mikimotoi,* tropism), attachment of cells to surfaces such as macroalgae (e.g., *Prymnesium*) are additional behaviour strategies that have been demonstrated in some species. These features may be of advantage in the competition for substrates e.g., in stratified waters with low nutrient concentrations above the pycnocline and high below.

3.2.1.4 Toxins and toxicity

Ichtyotoxic species produce toxins that can be exudated from the cell into the surrounding water. In some cases, toxins produced by organisms e.g., *Gymnodinium* spp., *Prymnesium* increase the cell membrane permeability and disturb ion balance in cells of a wide range of organisms (e.g., fish, other algae). These toxins are very labile and they do not appear to accumulate in the food chain. The chemical structure of toxins in *Prymnesium parvum*, and *Gymnodinium mikimotoi* have been examined to a limited extent (Yasumoto et al. 1990). The character of toxins in other species, such as in raphidophytes and *Chrysochromulina polylepis* is practically unknown.

3.2.1.5 Dynamics of toxin production

Growth conditions influence toxin production in many algae. The toxicity can be promoted by nutrient limitation (e.g., *Chrysochromulina* and *Prymnesium).* The high toxicity sometimes observed in nature has been difficult to reproduce in the laboratory. Unknown factors may influence toxin production of these species in nature.

3.2.2 Research requirements

- Rapid and accurate methods to identify and enumerate fish killing species in nature. Further information on ultrastructure, morphology and genetics of these species is needed in order to improve their identification and understand their phylogenetic affinities.
- Determination of the role played by alternate phases of the life cycle. To what extent do cysts or benthic stages occur? Which environmental factors are involved in the shift from one cell type to the other? What is the role of the different life cycle stages in the bloom formation of these algae?
- To what extent are growth requirements met by natural concentrations of nutrients and tracemetals such as iron, selenium and cobalt etc for fish killing species? To what extent can their behaviour strategy be to an advantage in the competition for nutrients?
- There is a need to identify the toxins in fish killing species, examine the biosynthetic pathways and their mode of action. What causes the variability of toxin cell content?

Type of toxin	Effects	Species	Symptoms
irritating substances (unknown)	physical damage of the gills, inhibiting oxygen uptake	*Chaetoceros concavicornis* *Ceratium fusus* *Gymnodinium mikitomoi* *(= G. nagakiense)* *Gyrodinium aureolum* *Noctiluca scintillans* *Chrysochromulina leadbeateri,* *C. polylepis* *Phaeocystis pouchetii* *Chatonella antiqua* *C. marina*	abrasion of the gills epidermis, clogging of the gills by excess mucous produced in response to irritating substances, stripping of the protective mucous layers
harmful chemicals, neurotoxins, hemolytic or blood agglutinating substances (unknown)	physiological damage to gills, major organs, intestine, respiratory or circulatory systems, interfere with osmoregulation.	*Chrysochromulina leadbeateri* *C. polylepis* *Prymnesium parvum* *Prymnesium patelliferum* *Chatonella antiqua* *C. marina*	malfunction of the brain and heart due to blood hypoxia, tissue and blood cell decay, fish death
unknown	acute toxicity	*Heterosigma carterae* *(= H. akashiwo)*	fish death
unknown	acute toxicity	*C. polylepis*	fish, phytoplankton, copepods, see-stars death

Table 3. Toxins involved in fish kills and their symptoms.

3.3 Community interactions

Blooms of fish killing species are a result of complex interactions between the organisms in planktonic community, such as bacteria, other algae, zooplankton (see Fig. 12).

3.3.1 Current knowledge

3.3.1.1 Competition for substrates

Fish killing alga species will compete with other algae and bacteria for inorganic and organic substrates. Work with multi-species cultures in the laboratory suggest that *Chrysochromulina polylepis* grows fast relative to others when the nitrogen sources is NH_4^+ or humic acids are under limiting conditions. Also *C. polylepis* thrives well under phosphorus limitation. To what extent these abilities are a general feature for fish killing species is not known. Apart from using dissolved nutrients, some of the fish killing species (*Chrysochromulina polylepis, Prymnesium* spp., *Gymnodinium galatheanum*) have been shown to feed on bacteria and other protists, i.e. they are mixotrophic. This may be the reason why *C. polylepis* grows well under P-limiting conditions. The role played by mixotrophy in bloom formation and persistence is unknown.

3.3.1.2 Allelopathy

Toxins produced by fish killing algae can affect their competitors like other algae and bacteria (termed allelopathy). This has been shown for the prymnesiophytes *Chrysochromulina polylepis* and *Prymnesium parvum* and for the dinoflagellate *Gymnodinium mikimotoi.* We know very little about the allelopathic effects of these algae. It is to be expected that allelopathic effects will depend upon the cell concentration and the cell toxin content of the toxic algae. The cell toxin content can be quite variable, depending on ratios of inorganic limiting nutrients; it depends also on the phase of growth (see above). Thus, fish killing algae will affect other algal species only under certain circumstances. There is a clear need to study these allelopathic interactions in much more detail in order to determine its potential role in bloom dynamics in nature.

3.3.2 Grazing

Potential predators of fish killing algal species include a variety of different protist groups (flagellates and ciliates) and metazooplankton. Fish killing species like *Gymnodinium mikimotoi* and *Chrysochromulina polylepis* have been shown to be a poor food for most predators (copepods and protistan grazers) when supplied as the only food source. At high cell concentrations, the predators mortality rate generally increase due to exudation of toxic substances. Experiments with mixed assemblages of fish killing algae and other non-toxic species have shown that the fish killing species actually can be a good food source for the zooplankton. The experiments also indicated that the predators could not avoid ingestion of toxic species. To what extent this is a general feature is unknown.

In one case a fish killing algal species has been shown to be a good food source for a specialised predator. That was in the case of the heliozoan *Heterophrys marina,* which is able to sustain growth when fed *Chrysochromulina polylepis* in monoculture in low algal concentrations (Tobiesen 1991). However, when supplied with dense suspensions of *C. polylepis,* this predator was killed by exudates excreted by the alga to the medium.

3.3.3 Infections

Viruses and pathogenic bacteria that lyse prymnesiophytes and raphidophytes have only been described recently. A number of studies have shown that the mortality of algae due to such pathogens is host-specific. In one case, the crash of a natural bloom of the raphidophyte *Heterosigma akashiwo* was reported to be due to a virus. Apart from virus also some algicidal bacteria have been found that lyse the raphidophytes *Chattonella antiqua* and *Heterosigma akashiwo* and the dinoflagellate *Gymnodium mikimotoi.* Two types of algacidal bacteria have been found. The first type excretes compounds into the water that kill the algae. The second type does not excrete such compounds into the water, but attacks the algal cells directly. Although parasitic protists are known to parasitise algae, no studies exist that have described parasites of fish killing species. Thus, identifying the various pathogens of fish killing species is a major challenge for future research. Also, we need to study the role of pathogens in the termination of fish killing blooms.

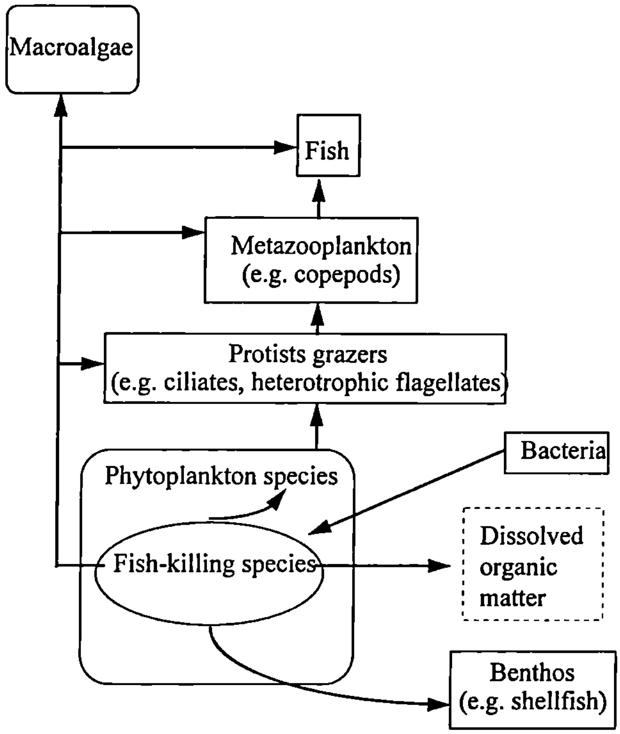


Fig. 12. Food web interactions including fish killing phytoplankton species.

3.3.4 Research requirements

- To what extent do external nutrient sources and their elemental composition favour blooms of fish killing species? Does mixotrophy play a role?
- What is the role played by toxins in determining in bloom dynamics?
- What role does specific grazers and pathogens play in the various stages of the blooms?
- Identify functional similarities among fish killing algae that will allow generalisations that can be used for modelling of bloom development.

3.4 Physical and chemical factors leading to the developments of HAB

An estimation of the effects of chemical and physical control variables on the development of a harmful event requires the integration of the knowledge on the species into realistic models of coastal circulation.

3.4.3 Current knowledge

3.4.3.1 Physics interact with biology at different scales.

At mesoscale, a population is transported, may be retained into confinement zones (small-scale eddies) and may grow at different rates depending on residency times of water. These processes are crucial in determining blooms events: they can be simulated through local hydrodynamical models, using real (and not averaged) meteorological time-series coupled with simple phytoplankton models.

Most of these HAB species have the ability to move vertically +/- 10 meters per day. They can either migrate according to a dial periodicity or, in stratified water columns, be confined into thin-layers embedded into the pycnocline (Gentien 1998). The causes leading to this situation are unknown. However, it appears that migration provides an advantage over other species, allowing uptake at night of nutrients in deeper water and use of the internal pools in the light at surface during the day.

Restriction of the population into pycnocline thin-layers may provide an advantage in confining dynamically the population, or in providing the best compromise for the species in terms of nutrition, environmental conditions and, possibly, growth factors. Understanding of the formation and maintenance of these thin-layers will provide the best compromise for defining growth of the population, such definitions being required for a correct formulation of a population dynamics model.
Algal cells are affected by direct mechanical strain on their membranes due to dissipation of small-scale turbulence in the viscous range and due to collisions with other particles. It has been demonstrated that some species can be arrested in their cell cycle due to turbulence with a negative effect on the growth rate. When two cells collide, they may remain together and present a higher sedimentation rate. This process leads to an increased mortality rate. These processes are very important when considering population dynamics because growth and mortality are the prominent processes to be considered in this context. However, it is not possible to extrapolate from the results obtained on few species to the entire range of fish-killing species.
Mixotrophy (i.e., the ability for a cell to meet its nutritional requirements using various substrates) has been recently documented for only few species (Jones et al. 1993). Simulations based solely on inorganic nutrients nutrition are invalid in the case of mixotrophic species.
In order to determine to what extent, substrate may control the population development of a given HAB species, it is necessary to know what are its nutritional preferences, which may be depend on the cell history and environment. Shifting from one to another substrate implies changes in biochemical pathways (e.g., enzymatic induction, requirement for another enzymatic cofactor, etc.) which need to be documented for each species of interest. The use of this recently acquired knowledge into simulations of population dynamics scenarios requires mathematical formulation of preferences and adaptations to different substrates. This is only possible through extensive experimental work.

3.4.4 Research requirements

- Develop experimental procedures for studying the effect of small-scale turbulence on HAB growth and mortality rates
- Determine factors leading to the establishment and maintenance of thin-layers
- Encourage the development of coupled physical-biological 3D-realistic model
- Quantify, formulate and parameterise the processes related to mixotrophic ability.

3.5 Research Benefits

The understanding of the control of HAB by forcing should allow:

- to develop an understanding of the population dynamics by simulation of essential processes (e.g., growth, mortality, behaviour, etc.),
- to establish an operational model of HAB by successive approaches of increasing complexity,
- to estimate the relative influence of various human activities on the development or on the occurrence of HAB.

4. High-Biomass HAB (HB-HAB)

4.1 Introduction: problems and occurrences in Europe

High-biomass HAB are massive nearly-monospecific algal blooms that, although non-toxic, induce adverse effects on the marine ecosystem: foam accumulations, anoxia with associated mass mortality of benthic macrofauna, reduced yields of harvestable marine resources, and loss of the recreational value of coastal regions (Smayda 1997). There is mounting evidence that at least some of these negative impacts are the visible manifestations of disruptions of the marine food web due to the eutrophication-induced unbalance between biomass production and consumption by the higher trophic levels. Non-silicified phytoplankton generally dominates these blooms, although some diatom-HB-HAB have also been reported. HB-HAB occur in nearshore coastal waters, and are induced by quantitative and qualitative changes in nutrients (N:P:Si but also the balance between organic nutrient and inorganic nutrient) driven by natural variability and/or anthropogenic activities. Some of HB-HAB like the well-known colonial haptophyte *Phaeocystis* sp. are recurrently blooming in the Barents Sea, Norwegian fjords, and in the continental coastal waters of the North Sea (Lancelot et al. 1987). In the latter eutrophicated area, *Phaeocystis* colonies are recurrently succeeding the spring diatoms and are sustained by nitrate remaining after the decline in diatoms. The most visible harmful effect of *Phaeocystis*-HAB is the deposition of thick layers of odorous foam on the beaches bordering the North Sea (Fig. 13). Other HB-HAB, like the mucilage-producing phytoplankton of the Adriatic Sea or the summer bloom events in the northwestern Black Sea are more episodic. Although the link between cultural eutrophication and HB-HAB has still to be assessed, records of bloom occurrence in the north-western Black Sea show a decrease of bloom events correlating with the reduced usage of fertilisers in agriculture caused by the economic pressure faced by many of the countries around the Black Sea since 1991. Visible positive signs of ecosystem recovery accompany this decrease in bloom events.

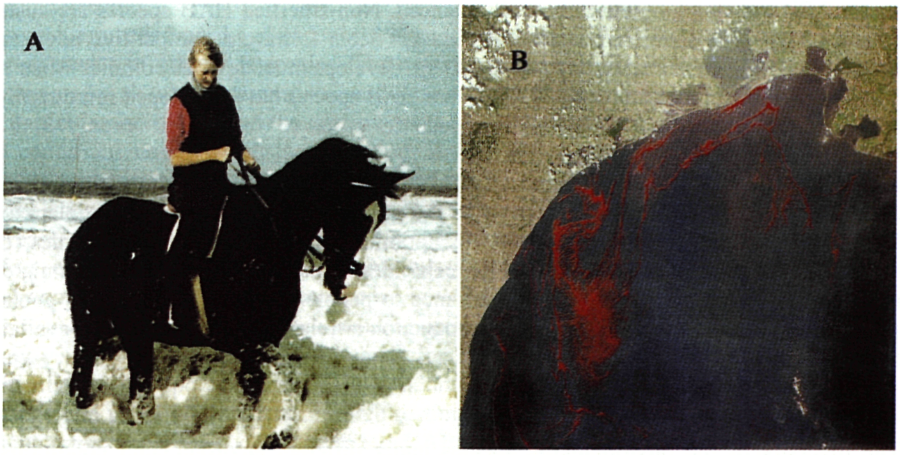


Fig. 13. Two examples of HB HAB. HB-HAB have a negative impact on recreational activities by producing mucilage or odorous foam in the water and on the beaches. *Phaeocystis* mucilages are also affecting aquaculture by clogging the gills of shellfish. Their impact on fisheries is mostly indirect by clogging the fishermen's nets. Accumulation of *Phaeocystis* mucus on a Netherlands beach in June 1986 (left panel) Photo: M.Veldhuis. AMBIO, 1987: 16(1). (Authorised publication by courtesy of Elisabeth Kessler, Editor-in-chief) And a colour enhanced satellite photograph of an algal bloom in the Adriatic in the summer of 1989 (right panel). Photo: NASA.

Considering the high variability of HB-HAB events and their nuisances, we need to develop a common integrated environmental methodology combining monitoring, process-level and modelling studies to assess, compare and predict HB-HAB bloom events within European marine waters in response to natural and anthropogenic forcing.

4.2 Causative organisms

4.2.1 Current knowledge

There are some recurrent well-documented HB-HAB species such as *Phaeocystis* sp. in northern European waters, and episodic HB-HAB species from a wide range of taxa. Most of the episodic HB-HAB species are dinoflagellates but also some diatom species and cyanobacteria might cause HB-HAB (Smayda 1997).

The taxonomic position of most of the HB-HAB species is known. There are considerable problems associated with the molecular diversity of different species. It is also unclear whether there is exchange of genetic information between different HB-HAB species occurring along the European coastline. The molecular diversity of some species is remarkably low between geographically isolated populations whereas for other species, a high genetic variability is established even within one population.

Probably the best-studied HB-HAB species in European waters is *Phaeocystis* (Lancelot et al. 1987); information from other species of interest is scarcer. Most of the HB-HAB species are permanent residents in marine waters occurring, however, at low abundance for most of the time with outbursts under specific conditions reaching high biomass within a short period of time. It is known that marine sediments are the seeding grounds for the water column and that a number of planktonic organisms are producing resting spores.

Little is known about the mechanisms responsible for the successful inception and development of HB-HAB. There is information available on the competition of some phytoplankton species in relation to the overall nutrient availability and the ratio between different nutrient species (Riegman et al. 1990). Diatoms, for instance, appear to be better competitors than non-siliceous phytoplankters if all nutrients (N:P:Si) are stoichiometrically well balanced. Non-silicified HAB species are sustained by nitrogen and phosphorus sources, but generally do not seem to have a competitive advantage over diatoms as long as silicate is available. *Phaeocystis* sp. develops mucilaginous colonies when nitrate is the dominant nitrogen source (Lancelot 1995). Other HAB species have developed mixotrophy, which combines the autotrophic and heterotrophic mode in order to fulfil their energy and nutrient requirements (Granéli et al. 1997).

One type of major harmful events linked to HB-HAB are those resulting from the production of mucilaginous polysaccharides, either directly like the polysaccharide-producing phytoplankton of the Adriatic Sea (Herndl 1992), or indirectly after disruption of ungrazed *Phaeocystis* colonies (Lancelot 1995). Both produce large amounts of polysaccharides, which subsequently accumulate in the environment with related nuisances (visible nuisance to people, clogging of fish gills, anoxia, etc.). Some observations suggest that the mucilage production is related to an unbalanced availability of nitrogen and phosphorus (Herndl 1992).

4.2.2 Gaps in knowledge

- The diversity of the phytoplankton species under different environmental conditions and in different areas sensitive to HB-HAB is still poorly understood. Especially the link in diversity between these sensitive areas and the adjacent areas needs to be investigated
- Generally, the molecular diversity within a given population of HB-HAB and between different, occasionally geographically more separated populations has not been thoroughly studied. The role of horizontal gene transfer is an important issue, however, because it determines the genetic plasticity of the species of interest.
- Only limited information is available on the role of resting cells in the formation of phytoplankton blooms and on the development of HB-HAB in particular. The role of the

sediment as a seeding reservoir for the water column deserves more attention. Only recently, the appropriate molecular tools became available to determine the resting spores on a species level. The abundance and survival conditions of these resting stages need to be known. The importance of ballast water from cargo ships for introducing new species, or increasing blooms needs to be determined. Generally, the life history of the HB-HAB species is only poorly known, with the possible exception of *Phaeocystis* sp.

- There is detailed enough information available from lab studies for only a rather limited number of potential HB-HAB species to allow characterisation of these species as opportunistic or overspecialised. Detailed studies are needed for a larger set of potential HB-HAB species to determine whether one of the above-mentioned strategies prevails.
- The mechanisms leading to the excessive formation of polysaccharides are not well understood despite progress made in recent years. It is essentially unknown whether rapid shifts from oligotrophic to eutrophic conditions or vice versa, or shifts in the nutrient ratios trigger the polysaccharide production and release by HB-HAB species.

4.2.3 Research needs and justification

- Long-term data on the population dynamics should be re-evaluated and information from different long-term data sets compiled in order to identify common trends between sites sensitive to HB-HAB. Monitoring programs should be continued and exchange of information between European countries re-enforced. Long-term data sets are essential for the identification of common trends in areas sensitive to HB-HAB. Efforts to establish and continue long-term data sets are therefore necessary.
- Cultures of HB-HAB should be established and provide the basis for studying the organisms of interest. The genetic variability within and among different populations should be determined. The establishment of a culture collection is the basis for concerted scientific research on HB-HAB. Organisms of interest should be sent to a central culture collection site. The determination of the genetic variability of individual cells within a given population and between different populations is essential if monitoring and control of HB-HAB species by molecular tools is to become feasible.
- Research is needed on the abundance and survival characteristics under different conditions of resting stages of HB-HAB species. The environmental conditions leading to the formation of resting stages should be determined, and their relation to the survival rate of these resting stages. Of particular interest is also the grazing pressure (or the absence of it) on the resting stages and the conditions under which they develop. Ballast water as a source for introduction of new species, and critical increase of existing seedbeds has to be evaluated. Modern cargo ships carry vastly greater amounts of ballast water that is more frequently exchanged. This means also increased transfer of potentially HB-HAB species into areas where either they were not present before, or they were present in lower amounts. Since this represents a potential hazard to coastal environments, research is urgently needed in this area.
- The role of coastal sediments as a seeding ground for marine plankton has been suggested for many years, and promising new methods for identifying some of the species, which produce particular resting spores, should help in addressing this important question. Knowing the life strategy and the conditions under which resting spores are formed and remain viable offers the potential to combat the occurrence of HB-HAB species at least in specific, highly affected areas.
- Detailed studies under defined lab conditions are needed to determine the competitive capabilities of HB-HAB species compared to their natural competitors. This approach provides essential information for predictive models.
- One key question is: What makes a HB-HAB species successful? We need to know for each HAB species of interest its nutrient stoichiometry and the mechanisms regulating photosynthesis and nutrient (ammonia, nitrate, phosphate, silicate) uptake rates. We also need to assess the occurrence of unique nutritional mixotrophic strategies combining autotrophic / osmotrophic / heterotrophy.

- Another key question is: What factors are triggering the polysaccharides production and are that a common property of HB-HAB species? We need to know for each mucilage-producing HB-HAB species of interest, the radiation and nutrient conditions, which may be involved in polysaccharide production. The production of polysaccharides by HB-HAB species is one of the main causes of nuisance in marine environments, and it has diverse effects on the functioning of marine ecosystems.

4.3 Community interactions

4.3.1 Current knowledge

HB-HAB are characterised by the dominance of mono- to oligospecific blooms of phytoplankton. Possible selective factors leading to such a development of HB-HAB might include the competition for inorganic nutrients during the onset of the blooms. The inorganic nutrient load received by European marine seas has considerably increased over the past decades (Hallegraeff 1993, Wulff et al. 1990). Efforts to reduce the inorganic nutrient load have been successful in certain regions. However, there are, long-term data sets available showing that the ratio of the major nutrients (nitrogen, phosphorus, silicate) has changed over the last 25 years, partly as a consequence of efficient reduction of the phosphorus input (Cadée and Hegeman 1993). Thus, in a number of areas there is a notable shift from a nitrogen- to a phosphorus-limited system, while in other regions, a decline in the N:P:Si ratio has been found. There is accumulating evidence that large areas of the European coast, particularly the Mediterranean Sea, are P-limited. Competition for nutrient species determines the development of specific populations.

Competition for organic nutrients could also be important. Generally, autotrophic organisms are considered to take up nutrients in inorganic forms. There is, however, considerable evidence that phytoplankton (including HAB species) express ectoenzymatic activity to cleave phosphate both from organic P and N-compounds. The utilisation of dissolved (DOM) and colloidal organic matter (COM) by phytoplankton has not yet received adequate attention (Carpenter et al. 1995). This nutritional strategy might be particularly important for HAB species under conditions of unbalanced nitrogen and phosphorus supply. A considerable number of these species are considered mixotrophic. It has been shown that mixotrophic flagellates take up colloidal and particulate organic matter.

Developments of HAB can be interpreted as a major disturbance of the traditional food web structure (Buskey et al. 1997). The biological control mechanisms of HB-HAB species are, at least temporarily offset. Possible mechanisms causing the inefficient grazing of non-toxic HAB species are:

- The bloom period is short enough to minimise grazing loss to mesozooplankton due to delayed response in mesozooplankton activity caused by their longer generation time. Especially in enclosed marine systems with a restricted exchange of water and under low turbulence conditions, nutrients derived from point-sources (locally restricted) can lead to sporadic blooms even under overall oligotrophic conditions (such as frequently observed in bays along the Adriatic coast) (Herndl 1992).
- HB-HAB species might be poorly edible for indigenous mesozooplankton because of inadequate size; poor food quality or inhibiting compounds stored intracellularly (Buskey et al. 1997). This unpalatibility can be uniformly active or selectively inhibit grazing by specific zooplankton species, which could lead to alterations in the grazing food web structure.
- Increase in the viscosity of the water (by the excessive release of polysaccharides) might affect the locomotion, sensing and rhapdorial feeding on phytoplankton by mesozoooplankton. This increase in viscosity is thought to impair the gas exchange across gill surfaces in fish. Although this has been suggested for some time already, there are only a few measurements on viscosity alterations of the water column due to intensive blooms.
- Production of infochemicals (allelopathy) might cause reduced or complete inhibition of mesozooplankton grazing. There are several compounds known from specific phytoplankton species such as acrylic acid release (in *Phaeocystis* sp.), or co-metabolites. One might assume that the production of such compounds might lead to selective advantages of the species producing them over other phytoplankton species.

- It is known for about a decade now that viruses are an important and dynamic component of marine food webs. Viruses are abundant in all coastal areas (but are also present offshore) reaching abundances of 10^9 ml^{-1}. As far as we know now, virus-host systems are species-specific.
- It might well be that the increased production of polysaccharides represents a protective shield for the HAB species.

A number of HB-HAB produces nuisance material in the form of polysaccharidic substances. This mucoid material is frequently remarkably resistant to bacterial degradation. It has been shown that under nutrient-limiting conditions phytoplankton release more than 50 % of the photosynthetically fixed carbon into the ambient water (Herndl 1992). These polysaccharide fibrils are coagulating under low-turbulence conditions forming marine snow and, in specific European coastal areas, mucilage (marine snow aggregates) is formed to which microorganisms adhere. Due to their buoyancy, these aggregates can remain suspended in the water column for a considerable period of time and eventually sediment to the bottom covering vast areas of the benthic biota, causing mortality especially among the filter- and suspension-feeding macrofauna community.

4.3.2 Gaps in knowledge

- Knowledge concerning possible large-scale alterations in the inorganic nutrient load of European marine waters is limited. Existing data sets should be more thoroughly investigated. In coastal areas with limited water exchange and under low-turbulence conditions, point sources of nutrients might be important and lead to the development of HB-HAB.
- The extent to which mixotrophy occurs in HB-HAB species is also poorly known. It might offer a selective advantage over exclusively autotrophic species especially under nutrient limited conditions. Therefore, more systematic investigations are required to determine the selective role of mixotrophy on the formation of HB- HAB. Moreover, distinction should be made between the uptake of COM and bacterioplankton. It might well be, that bacterivory is a more efficient strategy to combat N or P limitation than feeding on colloidal organic matter because of the higher N and P content of bacteria.
- The apparent lack of grazing pressure on HB-HAB by mesozooplankton is obvious although the exact causes are unknown for most cases and certainly diverse. Moreover, the general biochemical composition of the HB-HAB species and its relation to zooplankton grazing pressure and zooplankton development deserves more attention. Viscosity alterations in marine systems due to the production of polysaccharides by HB-HAB is essentially unknown as is the role of infochemicals as a means of communication between the potential prey and its predator.
- It is an intriguing question why the viruses are not more efficiently controlling the hosts, i.e. the mono- to oligospecific HB-HAB species. Given the high biomass reached in this type of HAB, one might assume that the contact rate between viruses and their host species is rather high which would make the control of these HAB by viruses efficient.
- The production of polysaccharides by HB-HAB and its accumulation in the water column shows large interannual variability. Despite considerable efforts to determine the coagulation and degradation of this material, a number of intriguing questions still remain to be answered.
- There is an urgent need for innovative approaches to determine the chemical characteristics of the material in the water column and its sedimented counterpart over the time of occurrence in order to elucidate the reasons for its refractory nature

4.3.3 Research needs and justification

- One of the most important unanswered questions is: Why do some specific phytoplankton species reach high biomass levels, thereby causing harmful effects to the system, while others do not?
- Research is needed to determine the long-term trends in the inorganic nutrient composition along the European coast and its possible role in the occurrence of HB-HAB. Particularly, the relation between the diatom vs. non-silicified bloom-species to N, P, and Si availability has to be investigated more intensively.

- The extent to which changes in the nutrient ratios (N:P:Si) affect the phytoplankton species composition in general and the development of HB-HAB in particular needs to be investigated.
- Mixotrophy as a strategy to overcome nutrient-limitation and outcompete potential competitor species has to be investigated. There, distinctions should be made between feeding on COM and particulate organic matter (most likely bacteria). Is COM taken up at similar efficiencies as bacteria and what is the threshold level for feeding on bacteria for different mixotrophic species?
- Inorganic nutrient ratios critically determine the composition of phytoplankton blooms including HB-HAB. The potential importance of mixotrophy in the development of at least some HB-HAB has received little attention thus far. Information on threshold levels of food concentration, and on the role of COM is urgently needed, since there is increasing evidence that a number of HB-HAB species are mixotrophic.
- The selectivity of zooplankton species feeding on HB-HAB needs to be investigated including determinations on developmental rates of zooplankton species fed with different HB-HAB species as compared to non-HB-HAB species.
- Viscosity alterations of seawater from HB-HAB should be compared with the viscosity under non-bloom conditions. The influence of viscosity on feeding by micro- and mesozooplankton should be investigated as this increase in viscosity might alter the food web structure.
- While there is knowledge available on the selectivity of zooplankton species feeding on phytoplankton virtually nothing is known on the viscosity alterations of seawater when HB-HAB are present. These viscosity alterations, however, might have a tremendous effect on the microbial food web as well as on the mesozooplankton.
- The release of infochemicals by HB-HAB species should be investigated since it might be an additional mechanism for HB-HAB species to avoid or reduce predation and thereby become numerically dominant over other indigenous phytoplankton species.
- Research should focus on the role of viruses in controlling HB-HAB. Particularly, attention should be paid to the protective mechanisms of HB-HAB species to inhibit or suppress viral infection or lysis.
- Only recently has the role of viruses in marine systems been addressed. While the general importance of viruses in the marine environment has been established to some extent their interaction with HB-HAB needs to be elucidated. Based on what we know thus far, one would suggest efficient control of this high biomass due to high encounter rates with viruses. Since this is obviously not the case counteracting mechanisms are obviously operating.
- Why do microorganisms colonising these aggregates not degrade the polysaccharidic material? Is P- and/or N limitation influencing bacterial degradation rates of this material? Obviously, these polysaccharide fibrils undergo abiotic modification rendering the material refractory for microbial degradation. Polysaccharidic material efficiently adsorbs metals and charged molecules, thereby influencing the hydration stage and consequently the degradability of the material. Detailed chemical analysis linked with microbiological studies is needed to improve our knowledge on the factors limiting degradation of this material.
- Polysaccharide aggregates and mucilage concentrate on boundary layers such as pycnoclines and eventually sediment to the bottom occasionally covering large areas. Microbial activity and the associated oxygen consumption of the sedimented matter covering the bottom cause the sediment underneath to become anoxic, leading ultimately to higher phosphate release out of the sediment which, in turn, stimulates production. These sedimentation events might affect also areas not directly under the influence of HAB due to lateral transport of mucilaginous material. Thus monitoring of the prevailing current patterns in coastal areas along with the dynamics of coastal HAB is required as well as information on the factors influencing the degradability of the material.

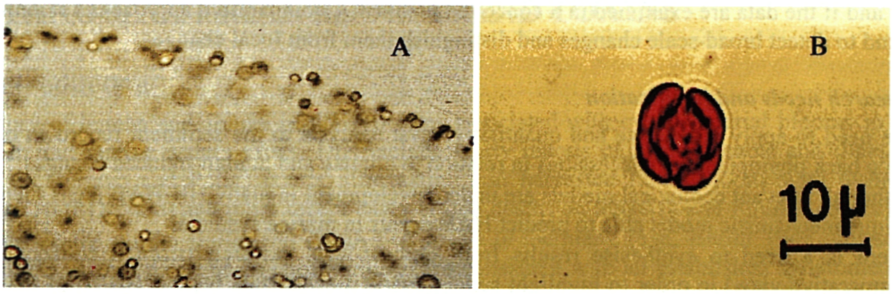


Fig. 14. The eurythermal and euryhaline genus *Phaeocystis* is one of the most widespread marine haptophytes, with most species sharing the ability to produce nearly monospecific blooms in many nutrient-rich environments. Its unusual heteromorphic life cycle, which alternates between gelatinous colonies and different types of free-living cells (vegetative non-motile, vegetative flagellate and microzoospores), sets it apart from other members of the class (photo (right panel) courtesy of Veronique Rousseau (left panel), AMBIO, (Authorised publication by courtesy of Elisabeth Kessler, Editor-in-chief)).

4.4 Prediction of HB-HAB.

4.4.1 Current knowledge

The frequency and extent of HB-HAB events are often correlated to the increasing human pressure on the marine environment. Human-induced changes are, however, occurring against a background of natural changes. Adequate prediction and management of HAB require an understanding of these two causes of ecosystem change. Our present inability to separate human-induced from natural changes results from the lack of adequate baselines and uncertainty as to whether observed changes are local, regional or on a broader scale. Adequate spatio-temporal monitoring in combination with paleoceangraphic studies and the development of coupled hydrodynamical-biological models on appropriate spatio-temporal and trophic resolution levels are necessary to resolve these deficiencies.

Because historical data on phytoplankton successions are often limited, paleoceanographic analysis of sediment records using adequate biomarkers is an appropriate tool to assess the historical impact of nutrient changes on the marine environment. For instance, opal measurements in sediment records have been of great value to reconstruct fluctuations in diatoms in Lake Ontario back to 1700 and relate them to human perturbations; and fossil dinoflagellate cysts have proved useful for tracing some phytoplankton changes resulting from cultural eutrophication (Dale et al. in press), industrial pollution (Sætre et al. 1997), and climatic change (Thorsen and Dale 1998).

Mechanistic models describing carbon, nitrogen, phosphate and silicon cycling are available or presently developed. These models should be applied to HB-HAB dominated ecosystem over seasons and years and related to the physical (meteorological) and nutrient forcing. These models constitute a useful research tool for analysing HB-HAB dominated-ecosystem properties, in particular their resistance to destabilisation. Such biological models have to be based on the best knowledge available on mechanisms driving HAB. When validated, these models are of great value to predict HB-HAB events and to determine the measures to be taken to counteract HB-HAB and/or their harmful consequences. Such high-trophic resolution ecosystem models have been already developed for some recurrent HB-HAB ecosystems as the *Phaeocystis*-dominated ecosystem of the coastal North Sea (Lancelot and Billen 1985) and could be implemented for other recurrent HB-HAB ecosystems.

Long-term individual monitoring programmes have a long tradition around the European coast. However, most of them are isolated, and there has been little comparison between data sets. Adequate monitoring programmes are of prime importance to monitor changes in HB-HAB and collect suitable data for model validation. The ability of long-term monitoring programmes to detect and characterise changes in the marine environment will be best realised if a consensus is reached on the data to be collected and if the data are examined in a concerted manner. A comparative approach is scientifically more sound to detect broad-scale changes and distinguish them from local changes.

4.4.2 Research needs and justification

- We need to apply qualitative and quantitative cyst analysis to the sediment record in order to trace back phytoplankton successions in relation to climate and human-induced changes.
- Although relevant ecological models are under development in many HB-HAB areas there is no unique mechanistic model able to predict HAB events in marine systems.
- There is a need to synthesise results from process-level research (from a conceptual and comparative view) in order to highlight some generic mechanisms behind HB-HAB that could lead to the formulation of a conceptual, predictive biological model of HB-HAB.
- There is an urgent need to implement a co-ordinated network of research on HB-HAB in key strategic marine areas of contrasting morphodynamics, climatic conditions and watershed characteristics. The monitoring programme should include traditional physico-chemical and biological data but should also be a platform for testing new in situ instrumentation, in situ taxonomic and functional probes as well as use information provided by remote sensing.

4.5 Research benefits

It is a recognised national and international task to secure sustainable use and development of the European marine and coastal zone for future generations. Coastal nations will have to face significant costs related to the management of coastal areas in the future and society will have to cover the costs in the long term. The need is for a comparative integrated research approach combining interactively existing historical time-series, newly designed monitoring, laboratory and field process-oriented studies and biological modelling. This will provide information on basic concepts leading to the development of HB-HAB, despite the variability of HB-HAB phenomena. Furthermore this knowledge is expected to provide tools to predict HB-HAB development as a function of natural and human-induced variability. These tools can be used, in turn, to provide guidelines for the establishment of an economically feasible and sustainable management practice for marine waters - a practice, which will cover both marine resources and environmental perspectives. This should have a positive economic and environmental impact for society through more cost-effective counter measures.

5. CYANOBACTERIAL BLOOMS AND TOXINS

5.1 Introduction

A history of human and animal health problems associated with cyanobacterial blooms in European brackish and marine waters exists. This includes early reports of human deaths and illnesses along Swedish, German and Polish coasts after eating fish harvested from lagoons with cyanobacterial blooms (Haff Disease) and more recent cases of dog deaths on Swedish and Finnish coasts and cattle and dog deaths on the Baltic and North Sea coasts of Germany (e.g., Berlin 1948, Gussmann and Bicks 1985, Nehring, 1993). Such poisonings are consistent with an established syndrome of human and animal illnesses and deaths after exposure to, and ingestion of, cyanobacterial cells and toxins from freshwater, estuarine and marine sources (Codd 1998; Sivonen 1998).

The cyanobacteria which can dominate such blooms in the Baltic Sea and in nutrient-enriched brackish waters elsewhere in Europe, e.g., Portugal and England, include genera and species with a high, but variable, capacity for toxin production, including *Nodularia spumigena, Aphanizomenon flos-aquae, Anabaena* spp. and, in places, *Microcystis aeruginosa*, which is more frequently found in freshwaters. The toxins of brackish and marine cyanobacteria include potent hepatotoxins, neurotoxins, skin-irritatory compounds and cytotoxins (Codd 1998, Sivonen 1998).

Marine and brackish water cyanobacterial blooms have additional adverse socio-economic effects in European environments, affecting the amenity value of coastal areas and causing economic losses to recreation, tourism and aquaculture (Lindholm 1998). This section presents information on hazards presented by cyanobacterial blooms in European marine and brackish water environments identifies gaps in knowledge on the causes and consequences of such blooms and the toxins produced, and identifies research needs required to manage these problems.

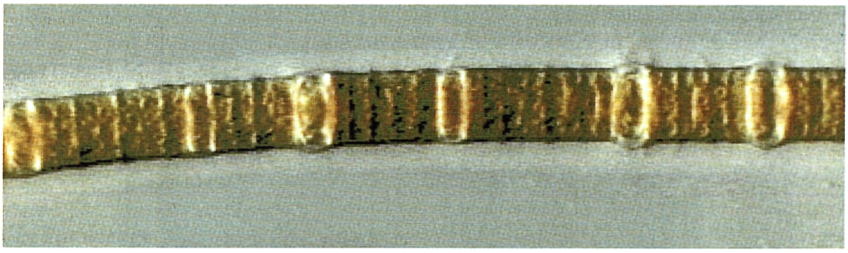


Fig. 15. A chain of the cyanophyte *Nodularia spumigena*, which forms blooms in the Baltic Sea during late summer (chain width: 10 µm). Photo Christina Esplund-Lindquist and Edna Granéli.

5.2 Cyanobacteria and their toxins

5.2.1 Current knowledge

In the open Baltic Sea, *Aphanizomenon* and *Nodularia* annually form mass populations in late July early August. In the coastal areas, freshwater species, such as *Microcystis, Anabaena, Gomphosphaeria* and *Oscillatoria/Planktothrix* can form regional blooms. Cyanobacterial blooms from the Baltic Sea have been tested for toxicity since 1985 and toxic blooms have been detected every summer. By strain isolation and studying the toxins from bloom samples, it was found that strains of *Nodularia spumigena* were responsible for the toxicity of the blooms and that the toxin involved was nodularin, a hepatotoxic cyclic pentapeptide. Non- toxic *Nodularia* isolates from the Baltic Sea also exist (Sivonen et al. 1989). However, they cannot be distinguished from the toxic isolates by morphological characters. Nodularin-containing blooms also occur in brackish water locations on the English coast. The few isolates of *Aphanizomenon* so far obtained from the Baltic Sea have been non-toxic, although toxic *Aphanizomenon* blooms occur elsewhere. Estuaries and associated brackish waters in e.g., Germany, Poland and Latvia have had blooms of *Anabaena* and *Microcystis* and estuaries in Portugal, *Microcystis aeruginosa*.

The taxonomy of the cyanobacteria is under revision. In the future, it will be increasingly based on molecular and genetic characters, in addition to the traditional structural and biological features. Several *Nodularia* species have been described from the Baltic Sea based on morphological characters. The taxonomic status of *Aphanizomenon* species is uncertain, but two species have been suggested to be present in the Baltic Sea. The only information on the genetic diversity of Baltic Sea cyanobacteria is a report of two genotypes of *Nodularia*, detected in the southern Baltic proper in the end of July 1996 (Hayes and Barker 1997).

Cyanobacteria are prokaryotic organisms capable of plant-type photosynthesis. *Nodularia*, *Aphanizomenon* and *Anabaena* are filamentous nitrogen-fixing genera. *Oscillatoria/Planktothrix* is a filamentous, non-nitrogen fixing assemblage. *Microcystis* and *Gomphosphaeria* are colonial, unicellular cyanobacterial genera. Specific features of these members of the plankton, which increase their ability to form blooms, include: the use of gas vacuoles to regulate buoyancy, the ability to store phosphorus and nitrogen and the capacity of some genera are to fix nitrogen from the air into biologically useful ammonia for growth. Some cyanobacteria develop resting spores (akinetes), which, in general, are induced by deprivation of phosphorus. *Aphanizomenon* and *Nodularia* are both nitrogen-fixing genera, and by obtaining all their demands of nitrogen and carbon from the air, phosphorous might be the limiting factor for these species. High temperatures and stable conditions appear to favour the growth of these cyanobacteria. The role of organic matter, from river runoff, in the regulation of the growth and production of toxins by bloom-forming cyanobacteria is under investigation.

Molecular genetic tools have been developed for a relatively small number of filamentous cyanobacteria (Thiel 1994), although none of these are bloom forming species. There is however no obvious reason to suspect that these tools cannot be applied to one or several bloom-forming cyanobacteria in marine and brackish water environments.

It is well established that cyanobacteria can produce potent toxins. Cyanobacteria are known to produce several types of toxins: hepatotoxins and neurotoxins. Like other Gram negative bacteria, they may also contain lipopolysaccharide cell layers, which may be pyrogenic (causing fever) and toxic.

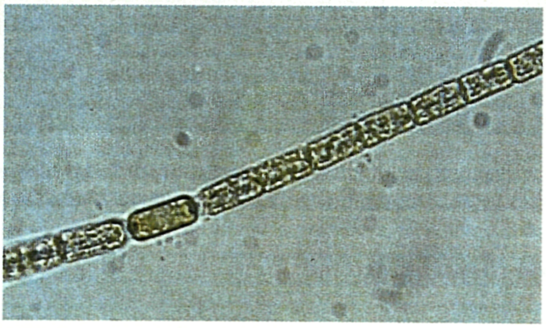


Fig. 16. Photo of a chain of the cyanophyte *Aphanizomenon* sp., isolated from the Baltic Sea (chain width: 5 μm). Photo courtesy of Christina Esplund-Lindquist and Edna Granéli.

Cyanobacterial hepatotoxins include cyclic peptides called microcystins (heptapeptides) and nodularin (pentapeptide). The peptides contain novel amino acids, D-amino acids and methylated amino-acids. Over 60 different microcystin variants have been characterised from cyanobacteria. Microcystins and nodularin inhibit important cell-regulating enzymes namely serine/threonine specific protein phosphatases. The hepatotoxins are readily transferred into liver cells via bile acid carriers, so that lethal doses in vertebrates cause death by haemorrhaging into the liver tissue. The toxins can also be taken up and accumulate in a range of aquatic food web organisms, including plankton-grazers, aquatic plants, molluscs and fish. Microcystins and nodularin, are also potent tumour promoters. The most frequently found cyanobacterial toxin in brackish water environments in Europe is nodularin, produced by *Nodularia* in the Baltic Sea. Laboratory studies have shown that highest amounts of hepatotoxins are produced under conditions that also favour growth (Sivonen 1996), and that the availability of phosphorous might be a limiting factor for the growth and toxin production of Baltic *Nodularia* (Lehtimäki et al. 1997). Microcystins produced by *Microcystis aeruginosa* and other cyanobacteria in freshwaters are occasionally found in estuaries and brackish waters in Europe.

Several cyanobacterial neurotoxins are known, including anatoxin-a, homoanatoxin-a, anatoxin-a(s) and saxitoxins. Around 20 different saxitoxin variants have been described from cyanobacteria. Cyanobacterial neurotoxins block neurotransmission and cause death in acute doses by respiratory paralysis. These toxins are commonly associated with freshwater blooms in Europe, and although not (yet) found in Baltic studies, their presence in other brackish and marine waters cannot be excluded, especially where such waters receive discharge from eutrophic rivers.

Several methods are available to detect and analyse cyanobacterial toxins. Bioassays include mouse bioassay, brine shrimp and other invertebrate tests, the use of freshly isolated mammalian and fish hepatocytes (Bell and Codd 1996). Mosquito larvae and plant seedlings have also been proposed, although these methods are of low sensitivity and specificity. Additional toxin detection procedures, with high sensitivity and specificity, are being developed. These are based on immunodiagnostic and enzyme-based principles and offer minimum detection levels below health guideline concentrations. Chemical methods are also being developed for the detection and analysis of cyanobacterial toxins, including separation by high-performance liquid chromatography, gas chromatography and capillary electrophoresis and identification by UV-spectrum, amino acid analysis, mass spectrometry and nuclear magnetic resonance The lack of quantitative analytical standards for cyanobacterial toxins (especially for nodularin and the numerous microcystin and saxitoxin variants) limits the efficient application of several biological and chemical analysis methods for the toxins in marine and estuarine environments.

> The correct identification of species that produce harmful cyanobacterial blooms is absolutely necessary in order to predict the development of the blooms. It is essential that new tools are developed for measurements of the growth and toxin production of bloom-forming cyanobacteria, in order to evaluate the effects of different environmental parameters in both the laboratory and in the field. More detailed information on the effects of light and nutrient composition on the physiology of bloom-forming cyanobacteria is also required.

5.2.2 Gaps in knowledge

- The taxonomy of toxic bloom-forming cyanobacteria is inadequate to provide understanding of the growth dynamics and relative abundance of cyanobacterial sub-groups in natural populations in marine and brackish waters. And there is no complete genetic characterisation.
- Genetic characterisation of marine and estuarine cyanobacteria, to support environmental research and water quality management, is lacking.
- The nutrient uptake kinetics for major nutrients, which support harmful marine cyanobacterial blooms (inorganic phosphorous and combined nitrogen), are not known.
- We do not know why cyanobacteria produce toxins.
- The genes and enzymes responsible for cyanobacterial toxin production and their regulation are not understood. (Toxic and non-toxic species cannot be distinguished by microscopy.)
- Analytical methods to measure the growth rates of filamentous cyanobacteria species in bloom samples are lacking.
- The effects of land-derived organic substances on coastal and marine bloom-forming cyanobacteria are not known.
- The over-wintering strategies of *Anabaena, Aphanizomenon* and *Nodularia* in the Baltic Sea are not known?
- The mechanisms that trigger akinete formation and germination are unknown.

- The existence of unrecognised populations of toxin-producing cyanobacteria, e.g., picoplanktonic and benthic forms, and toxins in estuarine and marine waters is an unknown factor.

5.2.3 Research needs and justification

Further research is needed to improve the taxonomy and to better understand the physiology of bloom-forming cyanobacteria in estuarine and marine environments The ultimate goal is to predict and mitigate the development of harmful cyanobacterial blooms. The following areas of research need to be addressed:

- A polyphasic taxonomic approach is needed to identify the species that produce harmful cyanobacterial blooms and toxins.
- Strain-specific markers should be developed, in order to identify potential toxin-producers in natural populations.
- Small- and large-scale laboratory experiments, enclosure and field studies of cyanobacteria are needed to provide knowledge of growth characteristics and toxin production.
- Genes and enzymes involved in cyanobacterial toxin production should be identified and are necessary for the understanding of the function and regulation of these toxins. This knowledge is also needed for designing molecular tools to detect toxin-producers and provide a possible rationale for variations in toxin production by blooms.
- Characterisation of emerging toxins and development of detection and analytical methods are also needed in order to achieve a better understanding of the different types of toxins, their health and biological significance, and abundance.
- Molecular genetic methods, including DNA transfer systems, should be developed for harmful bloom-forming cyanobacteria, in order to investigate the function and regulation of genes involved in growth, toxin production, and other cellular processes.
- The impact of dissolved organic matter, which is introduced into marine and brackish environments from land, on cyanobacterial growth and toxin production, must be further investigated in order to evaluate the risk of harmful blooms as a result of high nutrient loading from rivers.
- Studies on the occurrence and distribution of akinetes and vegetative cells on a long-term (year to year) basis, is needed to understand the survival strategies of cyanobacteria between bloom seasons.

5.3 Community interactions

5.3.1 Current knowledge

The ability of brackish water and marine cyanobacteria to compete for resources with other groups of phytoplankton is not well understood. Specific growth rates of harmful cyanobacteria are usually lower in comparison with other algal bloom-forming groups. However, certain specific physiological characteristics may provide cyanobacteria with adaptive advantages under some environmental conditions. The ability to fix atmospheric nitrogen allows some cyanobacteria to compete successfully when dissolved nitrogen is in limited supply, by using a nitrogen source that cannot be used by other phytoplankton. Additionally, the relatively high storage capacity of cyanobacteria for phosphorus and nitrogen can result in a competitive advantage under fluctuating nutrient concentrations. When inorganic nutrients are in short supply, many phytoplankton species are able to utilise nutrients (e.g., nitrogen or phosphorus) bound to organic compounds. Whether marine and estuarine bloom-forming cyanobacteria are able to utilise organic nutrients remains to be investigated. The role of dissolved organic matter in the outcome of competition for nutrients between cyanobacteria and other phytoplankton is unknown. Trace elements (especially iron) can limit the growth of some genera of marine cyanobacteria (Wilhelm 1995).

In general, cyanobacteria are known to be good competitors for light in comparison with other phytoplankton groups. In addition to photoadaptation and pigment content regulation, buoyancy regulation can be a specific advantage for cyanobacteria when light levels are sub-optimal (Walsby 1994). Furthermore, floating cyanobacterial layers can reduce the availability of light for other phytoplankton. The possibility that harmful cyanobacterial species directly inhibit the growth of other phytoplankton by means of bio-active compounds also exists. The algicidal properties of cyanobacterial toxins and other compounds have been recognised in freshwaters. So far, there is no clear evidence that this kind of interaction plays a determining role in bloom development in marine or brackish environments.

Although competition for resources may have an important role in structuring natural phytoplankton communities, the occurrence of cyanobacterial blooms might be additionally explained by relatively low loss rates compared to those of other phytoplankton. Decreased grazing losses could be caused by: lack of grazers that feed on cyanobacteria (possible cause: cascade effects from overfishing); avoidance of cyanobacteria by herbivores (due to e.g., poor palatability of cyanobacterial cells, possibly caused by their toxicity and/or size selection); and/or direct harmful effects on grazers by cyanobacterial toxins. Cyanobacterial toxin concentrations in aquatic environments can affect several trophic levels in brackish water food webs due to poor edibility and food quality of bloom species (Paerl 1996). Moreover, the high sensitivity to these toxins of certain groups of organisms (e.g., protozoa, rotifers) may lead to disturbances of processes including the microbial loop, with effects on other organisms e.g., crustaceans (Christoffersen 1996) and on nutrient regeneration in the water column. On the other hand, floating layers of cyanobacteria are usually associated with a large variety of micro-organisms, including micro and mesozooplankton. This suggests that possible negative effects may be species-specific. Microorganisms associated with cyanobacterial blooms, may even provide a mutualistic type of interaction. Products of cyanobacterial carbon and nitrogen fixation are partly excreted and may be used by heterotrophic bacteria, which may, in return, provide the cyanobacteria with certain growth factors. On the other hand, negative effects of freshwater cyanobacteria on heterotrophic bacteria have been observed. This may also occur in marine or brackish waters, when senescent cells release toxins to the environment. Because senescent cells remain trapped in the floating layer, released toxins may affect the functioning and structure of the associated microbial community. The high density of organisms within these communities makes them especially susceptible to viral, bacterial or fungal infection. The roles of microbial pathogens and parasites on the termination of cyanobacterial blooms in estuarine and marine waters are not well understood so far.

In cyanobacterial aggregates, different biologically mediated nutrient transformations (e.g., nitrogen fixation, ammonium and nitrate assimilation, ammonification, nitrification, denitrification and phosphorus remineralisation) may take place. However, the relative roles of nutrient recycling versus anthropogenic nutrient loading in marine cyanobacterial bloom development and maintenance are unknown.

Cyanobacterial toxins can be transferred to higher trophic levels in food chains and affect e.g., vertebrates, but little is known about their accumulation, transformation and possible detoxification mechanisms in estuarine and marine communities. Filter-feeding shellfish can ingest quantities of toxic cyanobacteria, e.g., *Nodularia,* sufficient to present acute toxicity to mammals. Fish can be affected by the toxins through the gastro-intestinal tract and the gills. Evidence indicates that freshwater fish have been killed by cyanobacterial toxins (Rodger et al., 1994) raising the possibility that cyanobacterial toxins may be transferred to humans if fish or shellfish, which have been contaminated through the food chain, are consumed. Objectionable tastes and odours, caused by cyanobacterial products, can be imparted to fish and crayfish in freshwaters. To what extent the palatability of fish from brackish or marine waters can be negatively influenced by cyanobacterial metabolites is not yet known.

There is no conclusive evidence at present that the success of cyanobacteria can be explained by their ability to produce toxins. From an evolutionary point of view, two possibilities are most obvious. First, toxins could selectively reduce grazing losses. Secondly, toxins may affect the activity of competitors either directly or indirectly through e.g., their metal-binding capacity in situations where phytoplankton growth is controlled by trace metal availability. However, neither of these possibilities is well established. It is possible that cyanobacterial toxins may have multiple, or even no, natural functions.

5.3.2 Gaps in knowledge

- What role does competition for resources (light, nutrients) play in the formation of harmful cyanobacterial blooms in marine and brackish waters?
- What is the significance of the ability to use dissolved organic matter as a nutrient source in the development of harmful cyanobacterial blooms in marine and brackish waters?
- How do harmful cyanobacteria affect phytoplankton-grazers? If there are negative effects of harmful cyanobacteria on grazers, does a reduced grazing loss help to explain the mass occurrences of harmful cyanobacteria?
- What are the effects of known cyanobacterial toxins and other bioactive compounds on the wider phytoplankton community?
- Can cyanobacterial toxins and other bioactive products provide a competitive advantage for cyanobacterial bloom-formers?
- What is the importance of nutrient transformation in cyanobacterial bloom formation and maintenance? Do cyanobacteria populations promote a net input of nitrogen into the marine system?
- Can cyanobacterial toxins be transferred to higher trophic levels in food webs and affect marine top carnivores, e.g., piscivorous fish, seals, piscivorous birds etc.?
- What roles do microbial pathogens and parasites of phytoplankton (viruses, bacteria, fungi, protozoa) exert during the initiation, persistence and decline of harmful cyanobacterial blooms?

5.3.3 Research needs and justification

- Systematic experimental growth studies (continuous and semi-continuous cultures, and competition experiments) should be carried out in order to better understand the competitive abilities of harmful cyanobacterial in brackish and marine waters.
- More research is needed to determine the role(s) of organic substances in the structure of the plankton community; in order to determine whether dissolved organic matter influences the formation and/or the toxicity of harmful cyanobacterial blooms.
- Reliable methods need to be developed to measure *in situ* grazing rates on harmful cyanobacteria in order to better define the role of grazing in the selection for these species.
- There is a need for quantitative studies on the uptake, accumulation and fate of cyanobacterial toxins in marine and estuarine food webs, using native aquatic organisms exposed to natural levels of toxins.
- Nutrient transformations in nutrient aggregates need to be quantified in order to understand the role of nutrient recycling versus anthropogenic nutrient loading in cyanobacterial bloom development and maintenance.
- Systematic experimental food web studies at the mesocosm scale are needed to resolve the role of grazing in the formation of harmful cyanobacterial blooms
- The complex interactions between harmful cyanobacterial species and other members of the marine and estuarine food webs cannot be understood without the use of ecosystem models, based on known physiological properties of the constituent organisms.
- Detailed toxicological studies, from population to molecular level, and performed with ecologically-relevant assemblages and organisms, are needed to provide better understanding of the effects of dissolved cyanobacterial toxins on the plankton community.

5.4 Chemical and physical environment

5.4.1 Current knowledge

Several experimental studies and field observations show a positive correlation between heavy cyanobacterial bloom occurrence and water temperature, calm weather conditions and strong stratification. Generally, these factors increase the time during which the plankton community can develop, leading to a top-down controlled system and a selection towards inedible and/or toxic species. In apparent conflict with this, exceptional cyanobacterial blooms have also been observed in late autumn in some coastal regions of the Baltic Sea. Furthermore, discharges of blooms of *Microcystis* spp., originally formed in European rivers, have occurred into estuaries without obvious reference to weather conditions.

The different light optima of algal bloom species may partly explain their abundance and survival at particular water depths, and mechanisms including the accumulation of photosynthetic ballast and formation/collapse of gas vacuoles, explaining vertical distributions of different species have been revealed for cyanobacteria (Oliver 1994).

Due to the competitive advantage provided by nitrogen fixation, nitrogen-fixing cyanobacterial populations are particularly responsive to phosphate availability and growth conditions are assumed to be better if the ratio of dissolved inorganic nitrogen to phosphorus (DIN:DIP) in water is low. In the Baltic Sea, cyanobacterial blooms form during periods when nutrients are the main limiting factor for phytoplankton growth. There is evidence that nitrogen-rich nutrient loading from land and the internal loading of mainly phosphate from the oxygen-depleted water layers and sediments control the areal distribution of blooms in this sea area. Thus the most intense blooms of nitrogen-fixing cyanobacteria occur in areas where the DIN:DIP ratio below the seasonal thermocline is lowest (Kononen and Leppänen 1997). In coastal zones the development of blooms of non-nitrogen-fixing cyanobacteria (e.g., *Microcystis, Ocillatoria*) or nitrogen-fixing species with high preference for other inorganic nitrogen-sources (e.g., *Aphanizomenon*) is obviously related to nitrogen and phosphorus loading from the land.

The impact of small-scale turbulence may affect cyanobacterial blooms both directly and indirectly. Turbulence may cause physical damage to cyanobacterial filaments, which would partly explain why these blooms are most intense in calm weather conditions. On the other hand, small-scale turbulence is known to increase the aggregation of marine particles. Cyanobacterial aggregates are known to be sites of high diversity of microbial organisms (bacteria, ciliates, and other algae) (Bursa 1968): thus turbulence may have an effect on community interactions.

Cyanobacteria can form HAB with high biomass and all hydrophysical processes affecting vertical nutrient transportation (including mixing, upwelling, intrusions and frontal activity) have an impact on their bloom formation (Kononen et al. 1996).

5.4.2 Gaps in knowledge

- For a better understanding of the survival strategies of cyanobacterial species in different light regimes and fluctuating light conditions, which in some cases are modified by hydrodynamic events, information is needed about the response of cyanobacteria to differences in irradiance, light quality and fluctuations in these factors. As cyanobacteria often form blooms extremely high irradiance; we need to better understand the mechanisms, which they employ to avoid and protect against photoinhibition and to repair photoinhibitory damage.
- There is inadequate knowledge the role of nitrogenous nutrients in the population dynamics of marine and brackish water nitrogen-fixing cyanobacteria, or of the capability of cyanobacteria to leach nutrients from organic substrates.
- Information is lacking on the relationships between ambient nutrient concentrations, ratios and fluxes and the bloom dynamics of marine non-nitrogen fixing cyanobacteria.

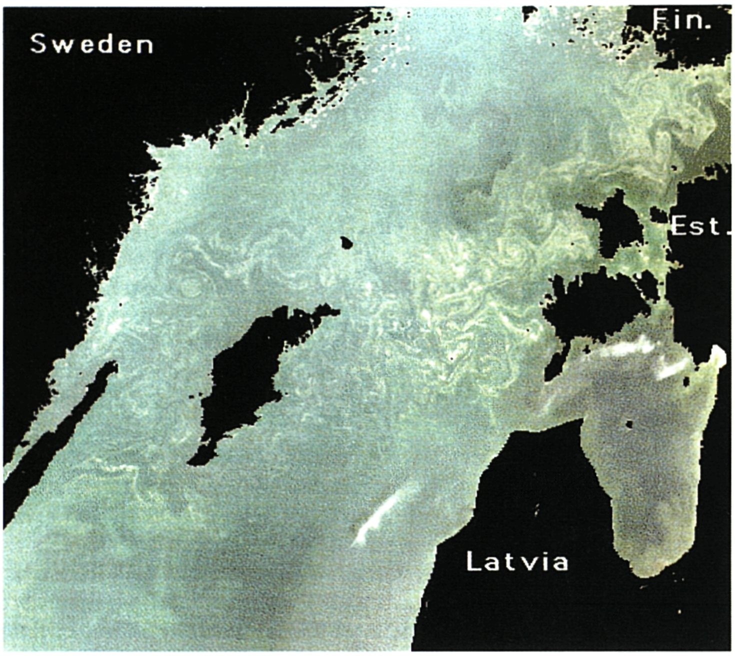


Fig. 17. Real colour satellite image (CZCS) of the Baltic Sea showing a *Nodularia spumigena* bloom in August 1983. Courtesy of Remote Sensing Group at CCMS Plymouth Marine Laboratory.

- Very little is known about the effect of small scale turbulence on cyanobacterial growth, physiology and aggregate formation and the consequent effects on community interactions.
- No quantitative information is available on vertical nutrient fluxes caused by various hydrodynamic processes through pycnoclines.
- The importance of nutrient fluxes between marine and estuarine sediments and water in relation to loading from land and atmosphere is not known.
- We do not know where, when and under which environmental conditions cyanobacterial akinetes are deposited and germinated and how near bottom currents distribute them in marine and estuarine environments.
- No tools for prediction of the accumulation of cyanobacterial rafts in different coastal regions are available.

5.4.3 Research needs and justification

Research is needed on the following:

- effects of small-scale turbulence on cyanobacterial growth and aggregate formation.
- the influence of mixing on fluctuations in the light regime and how cyanobacteria versus other phytoplankton respond to this in marine and estuarine waters.
- hydrodynamic mechanisms near the sea bottom, that may resuspend and transport cyanobacterial akinetes up into the euphotic layer
- mechanisms that affect nutrient flows between sediment and water, the vertical movement of nutrients, and the quantitative importance of these intrusions in total nutrient flow for the development and persistence of cyanobacterial blooms.
- the mechanisms of cyanobacterial raft accumulation in coastal regions and the ability to predict accumulation.

5.5 Research benefits

The research needs identified on the production, properties, significance and control of harmful cyanobacterial blooms are part of the overall aims of the EUHAB Plan to meet Fifth Framework objectives, with reference to Europe's estuarine and marine waters. Specific benefits with reference to cyanobacterial blooms include:

- effective prediction of the production, magnitude, potential toxicity, distribution and duration of blooms through understanding of the genetic diversity, growth physiology and population biology of cyanobacteria, with reference to the hydrology of their environment.
- understanding of the relative importance of natural and anthropogenic nutrient loading as causes of cyanobacterial bloom formation, leading to rational decision-making on nutrient discharge policies and HAB reduction.
- understanding of the toxicity of blooms, enabling the formulation of appropriate policies for health protection, based on hazard characterisation, knowledge of exposure levels and risk assessment. Decision-making to benefit would include policies on the recreational use of beaches and coastal waters, and shellfisheries affected by cyanobacterial blooms.
- understanding of the environmental significance of cyanobacterial toxins to contribute to the formulation of appropriate measures and standards for the protection of biodiversity.
- knowledge of the molecular, toxicological and pharmacological actions of cyanobacterial toxins and related compounds with potential medical and industrial applications.

6. Field studies of physical-biological interactions

6.1 Introduction

HAB phenomena occur in natural systems, which involve a variety of climatologically and meteorologically driven hydrodynamical processes. These processes occur within a broad range of spatial and temporal scales. A schematic presentation of the important scales determining the cyanobacterial blooms in the Baltic Sea is given as an example in Fig. 18.

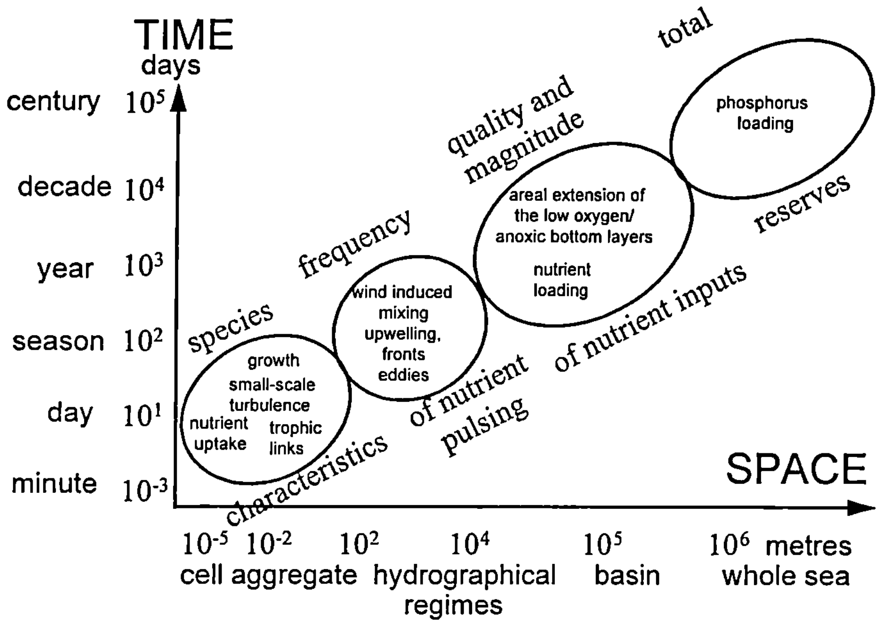


Fig. 18. A schematic presentation of the spatio-temporal scales of mechanisms regulating cyanobacterial blooms in the Baltic Sea (Redrawn from: Kononen and Leppänen 1997).

It is not possible to understand the bloom dynamics without investigating possible links between the species' ecophysiology and the hydrodynamical processes forcing its population dynamics. Field studies are necessary to extrapolate the information obtained from laboratory and mesocosm experiments to the natural scale of any HAB. It is essential that the design of field experiments is based on analysis of meteorological time scales and spatio-temporal scales of crucial hydrodynamical events.

The impact of small-scale turbulence may affect HAB species both directly and indirectly (Kiørboe 1993). Turbulence may cause physical damage of cells, colonies or filaments. It is known to effect growth and mortality of some dinoflagellate species. On the other hand, small-scale turbulence is known to increase aggregation of marine particles, e.g., cyanobacterial aggregates are known to be sites of high diversity of microbial organisms (bacteria, ciliates, other algae), thus turbulence may have an effect on community interactions. Simulating turbulence in mesocosms has serious limitations. Therefore it is essential to validate in vitro experiments on the effect of turbulence by field observations of turbulence and physiological indexes of the species of interest. Very little is known about these processes.

Mixing in the upper layer creates fluctuations in the light regime experienced by HAB species. These fluctuations are connected to stratification patterns and meso-scale dynamics of water masses (e.g., buoyant plumes). These differences in level and fluctuation of the irradiance may select for some species, including HAB species.

Many HAB species concentrate around the pycnocline in very thin layers down to the 0.5 meter scale (Gentien et al. 1995). The factors governing the establishment, maintenance, and dispersion of these layers are largely unknown. It has been shown in a few cases that the confinement into thin layers limits the diffusion of the population therefore allowing a build up of high cell density. It is crucial to determine what are the reasons leading to this aggregation. They probably involve a compromise situation between physical, chemical and behavioural characteristics. They may result from sensitivity to high shear, chemotropism or specific growth factor and avoidance strategies of grazers. These questions have to be clarified by multidisciplinary field studies.

In the mesoscale, hydrodynamical processes affect vertical nutrient transport and horizontal transport of HAB populations. Several observations of enhanced production across hydrophysical boundaries suggest that mixing due to various processes (hydrodynamical fronts, eddies, upwelling, intrusions etc.) creates non-nutrient-limited growth conditions for pelagic ecosystems. There are examples showing that certain HAB benefit from these hydrodynamical processes. Accumulations of motile HAB species are often found in frontal convergence zones, due to the ability of motile species to maintain their position in the surface layer through swimming. In several systems, such as the Iberian upwelling system, the increase or decrease of HAB may not be related to growth, but to physical accumulation/ dispersion process (Figueiras et al. 1998). These mechanisms can only be revealed through multidisciplinary field studies.

Hydrodynamic processes near the bottom affect the resuspension and transport of resting stages (cysts of dinoflagellates, akinetes of cyanobacteria) of HAB species. These processes determine location of the seed beds and inoculation efficiency of HAB from a given cyst/akinete density.

6.2 Research questions

- How does the small-scale turbulence affect growth, physiology and aggregate formation of HAB species and how do these effects modify community interactions of HAB?
- How does mixing regulate the fluctuations in the light regime, and how do HAB species vs. other phytoplankton respond to this?
- What are the physical, chemical and behavioural characteristics leading to the formation, maintenance and dissipation of subsurface thin layers of HAB?
- What are the hydrodynamical mechanisms near the sea bottom, which may resuspend and transport resting stages of HAB species?
- Which are the time spans of mechanisms that affect the accumulation and dissipation of HAB?
- Which are the mechanisms that affect nutrient flows between sediment and water, and on vertical transport of nutrients, and what is the quantitative importance of these intrusions in the total nutrient flow for HAB?

6.3 Benefits

The parameterisation of critical processes and the scenarios of the HAB dynamics arising from multidisciplinary field studies are a major input for model development. Comparative multidisciplinary field studies in different systems in European waters will provide evidence regarding the relative importance of anthropogenic impact vs. natural variability of HAB.

7. Tools and Technology

7.1 Introduction

Harmful Algal Bloom species, with the exception of those generating high biomass, are relatively minor components of the plankton. The natural occurrence of HAB species is often episodic and highly unpredictable. Toxicity can be expressed at relatively low cell abundances, often with a catastrophic impact on the ecosystem as a whole. At such low abundances, identification, isolation and estimation of the species present and the toxins they produce can be very difficult and time consuming. Moreover, strains brought into culture tend to lose their toxicity in a relatively short time. Polymorphism and indistinguishable life cycle stages among HAB species are more the rule than the exception. All of these factors pose great difficulties to the scientific community dealing with HAB species and limit the effectiveness of routinely applied techniques in marine plankton (HAB) research.
Molecular biological tools have, however, greatly enhanced our ability to identify species, to determine the expression of genes regulating key cellular processes, and to estimate gene flow and distribution of species in time and space. We are only just beginning to see routine and innovative uses of these methodologies as applied to HAB species and their interactions with other members of the community.
Modern techniques to determine and quantify the production and fate of the various toxins produced by HAB are evolving rapidly. Unfortunately, they still have not reached the stage of routine application for laboratory or field use where toxins may have a selective effect on certain organisms comprising in the pelagic or benthic food web.
Remote sensing from airborne or spaceborne sensors can provide on-line information at a range of spatial and temporal scales relevant to HAB. A first sensor (SeaWiFS) is currently available and more are to come. Nevertheless, low abundance, sub-surface blooms and large cloud cover in particularly the northern parts of Europe limit the usefulness of this approach. In this respect (automated) detectors in drifting buoys should provide additional information/data.
Similar to the restrictions causing limitations in bio-chemical analysis, field-modelling attempts should address the specific character of HAB. The presence of life cycles and species-specific behaviour implies severe restrictions on most available models.
The aim of this chapter is to identify and improve the tools and technology that are necessary to address issues at the cellular/organismal up to the ecosystem level as related to HAB development.

7.2 Cellular/Organismal

7.2.1 Taxonomy

7.2.1.1 Identification

Problem: Identification of the species causing a HAB event is the first critical step needed to design or implement effective management and mitigation strategies. Many harmful algal species closely resemble benign or other harmful algae that respond very differently to environmental conditions and human-derived changes in the marine ecosystem. Accurate identifications still remain extremely time-consuming and difficult because traditional procedures are limited to analysis of cellular features using light or electron microscopy. These 'classical' techniques generally do not consider (1) that genetically distinct but morphologically similar lineages may have overlapping geographic distributions or (2) that many harmful algae are remarkably polymorphic, often with species previously regarded as separate, being different morphs of the same species. Comparison of identifications with different techniques often fail to take account of these features, which generally occur as species cross gradients of salinity, nutrient availability, temperature and other factors.
Existing Technology: Light microscopy, including epifluorescence, Nomarski and phase contrast procedures, is useful to determine certain cellular features, but generally can be insufficient for species

identifications. Scanning electron microscopy can be used on its own or in conjunction with other techniques to identify some harmful species. For weakly armoured dinoflagellates (having thin deposits or 'plates' of cellulose beneath surface membranes), standard techniques do not reveal the cellular features needed for identification with scanning electron microscopy. For very small organisms, such as (harmful) haptophytes, transmission electron microscopy is required. New techniques, such as confocal microscopy, are also useful in discerning the presence and three-dimensional structure of internal cellular features that may aid in identification. Recently, DNA and RNA probes, cell-surface antibodies and other molecular and biochemical techniques have also come into more widespread use for identification of a limited number of harmful algal species. These techniques should be used collectively to verify and cross-corroborate identifications made using classical procedures.

Future Needs:

- Establish a network of expert taxonomists to aid in species identifications when HAB events occur,
- Test whether harmful algae of similar appearance that have been classified as separate species using classical procedures are, in fact, distinct species or ecotypes,
- Develop molecular probes and other techniques to identify all life history stages,
- Genotype all local populations,
- Utilise Artificial Neural Networks and image analysis systems to determine morphological features that provide optimal discrimination.

7.2.1.2 Cultures

Problem: Our current state of knowledge concerning HAB is mainly based on laboratory cultures and these are not representative of natural populations. Much of what is known about controlling influences of environmental conditions, nutrient, pollution, and other factors on the growth and toxicity of harmful algal species has been derived from work with these species in laboratory cultures. Our ability to make rapid progress in gaining insights about the growth, physiology, and controlling influences on some harmful algal species has been greatly impeded from the use of culture collection material that is not maintained to yield virulent toxic populations. Moreover, it is essential that reference material from HAB is identified, characterised, validated, securely deposited, maintained in a virulent form and made available for research and regulatory purposes.

Existing Technology: Culture collection centres, in general, have been created to provide algal cultures for research to better understand algal physiology, growth and responses to changing environmental conditions. However, many harmful algal species lose their ability to produce toxins after prolonged culture conditions. Isolates should be obtained on a frequent basis if it is demonstrated that species lose toxicity under culture conditions. Subjection of harmful algal species to highly artificial culture conditions over time also prevents normal transformations among life history stages, so that life histories of these species remain, for the most part, poorly understood. Confusion is created when the eco-physiological responses of harmful algal species from old, aberrant cultures conflict with those obtained from fresh isolates of the same species. Furthermore, nearly every clonal isolate of a single species has demonstrated variability in each phenotypic expression tested. Culture collections generally supply unialgal, non-clonal cultures, with species identifications that may be erroneous, making them unacceptable for HAB type of eco-physiological work.

Future Needs:

- Ensure that the facilities and means are available to provide clonal algal reference collections of known identification and demonstrated virulence or continued toxin-production capability,
- Re-check all toxic clones for continued virulence or capacity to produce toxins,
- Obtain fresh isolates of species that cause HAB, or are harmful to aquaculture or other human uses of European marine waters on a regular basis,
- Develop culture techniques that simulate *in situ* growth conditions so that the algal species are able to complete life history transitions,

- Maintain archived samples from HAB events in European waters and from the higher trophic levels that they contaminate.

7.2.2 Molecular Biology

7.2.2.1 Phylogenetic affinities

Problem: Certain harmful algal taxa, such as the *Alexandrium tamarense* species complex, *Pseudo-nitzschia* spp., *Phaeocystis* spp., *Chrysochromulina polylepis* and *Prymnesium* spp. have been extensively characterised using molecular genetic method (Scholin et al. 1995, Medlin et al. 1994, Edvardsen and Medlin 1998, Larsen and Medlin 1997). In these studies, fine scale genetic resolution reflecting geographic origin of strains has resulted in the redefinition of morpho- species vs. genotypic species in nearly every case. However, these kinds of data are totally lacking or insufficient for most other HAB species. There undoubtedly remains many species complexes, relict floras and cryptic species yet to be resolved among harmful algae, especially in European waters.
Existing Technology: Sequence data from coding and non-coding regions of the genome are routinely used to reconstruct phylogenetic affinities. Data from ribosomal RNA genes are especially suitable for the design of species-specific gene probes. As an alternative, mono- and polyclonal cell-surface antibodies can distinguish between closely related species. Although rRNA and antibody probes are available for some taxa (Box 1.7, see review in Medlin and Simon 1997 and various papers in Reguera et al. 1998), a platform that allows for a routine automated application of these probes to identify HAB species in field and laboratory studies is not yet available (Tyrrell et al. 1997, Miller and Scholin 1998). There are also limited data on the applicability/sensitivity of these gene probes in field situations when target numbers are low or cells are senescent (Rhodes et al. 1998).

Future Needs:
- Continue/enhance efforts to characterise key HAB species using molecular methods,
- Accelerate the application of these new methods of taxon identification for routine laboratory and field use,
- Enhance precision and sensitivity of the molecular probes.

7.2.2.2 Isolation of toxin genes

Problem: Although toxin production is a key feature of nearly all HAB species, some algal toxins remain unknown or poorly characterised chemically, such as those from *Pfiesteria* or *Chrysochromulina*. There is no biosynthetic pathway completely defined for any toxin. No gene or suite of genes directly involved in toxin production has been isolated (but see Taroncher-Oldenburg and Anderson 1998). The determination of the genetic basis for toxin production will provide a better understanding of the mechanisms involved and the reasons for toxin production by the alga.
Existing Technology: Novel methodologies, such as differential display of messenger RNAs, offer exciting possibilities to isolate genes/proteins that are preferentially expressed during maximum periods of toxin production in the cell cycle. These methods are labour intensive requiring the establishment of subtraction libraries and the sequencing of all genes differentially expressed.

Future Needs:
- Characterise unknown toxins biochemically,
- Isolate and characterise genes involved in toxin production,
- Develop functional gene probes for any toxin gene identified to determine when toxin genes are being expressed

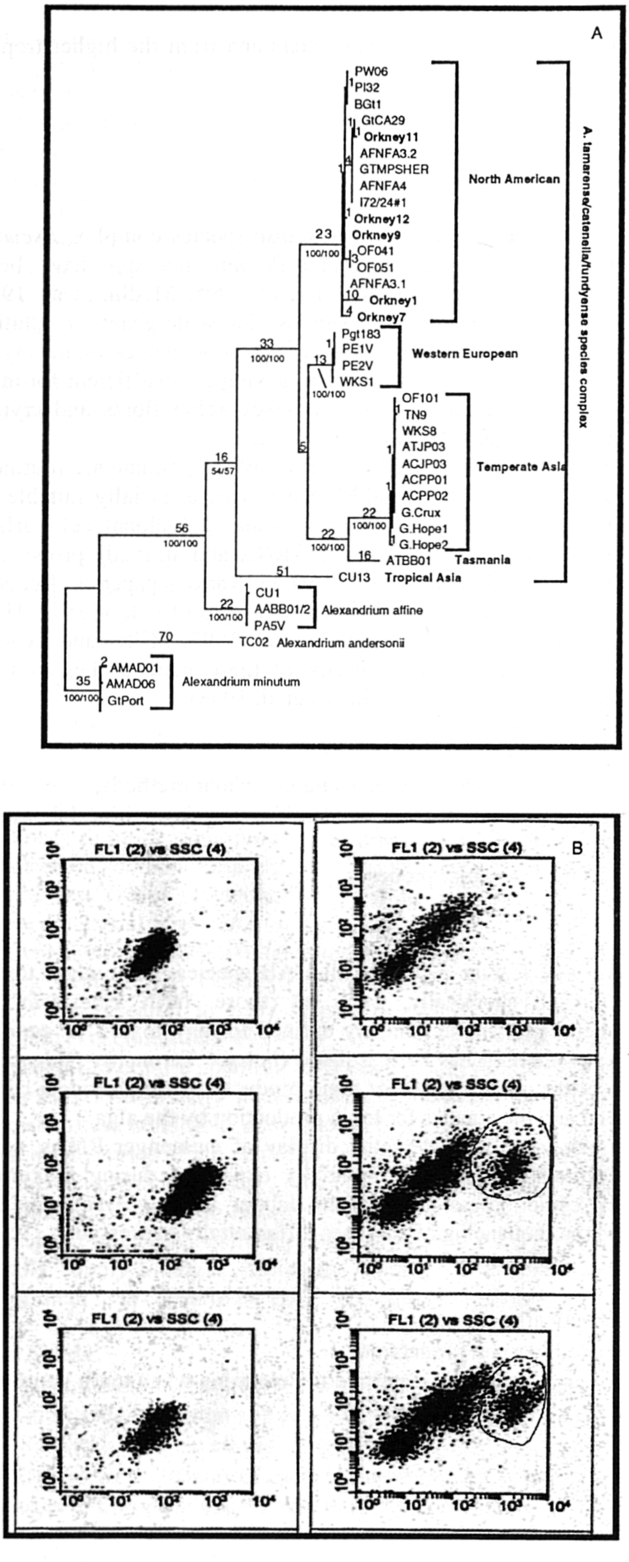


Fig. 19. (A) Phylogenetic reconstructions of *Alexandrium* spp. using the D1/D2 region of the LSU rRNA gene showing the placement of the Orkney Island isolates within the North American clade, redrawn from Medlin et al. 1998, TEPS. Previous molecular analyses of western European isolates have indicated that the non-toxic European strains of *Alexandrium tamarense* belong to a different clade. (B) Molecular probes based on ribosomal RNA using the SSU gene for the *Alexandrium tamarense* species complex (Brenner et al. unpubl., TEPS) and the D1/D2 region of the LSU for the toxic North American clade (Miller & Scholin 1998) can detect toxic *Alexandrium tamarense* cells in natural populations with in-situ hybridisation of the probes and detection by flow cytometry. Left panel shows the flow cytometric detection of a culture of a non-toxic Western European isolate of Alexandrium tamarense hybridised with no probe = top box, SSU species level probe = middle box, LSU North American probe = bottom box, and compared with isolates from the Orkney Islands (right panel) hybridized with the same series of probes.

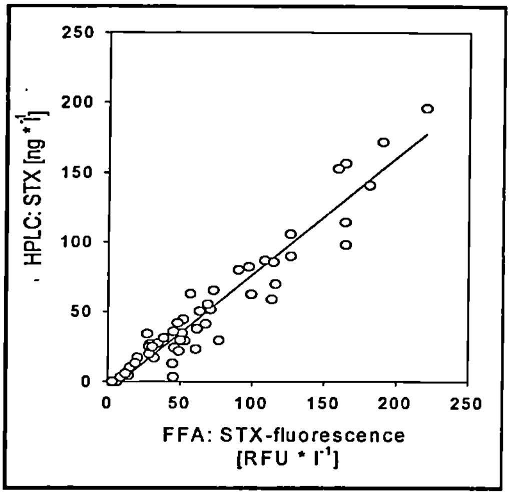


Fig. 20. Detection of saxitoxin (STX) in natural *Alexandrium sp.* blooms in the waters of the Orkney Islands and the Firth of Forth (May-June 1998): Comparison of fast fluorimetric assay (FFA) with HPLC. Linear regression (r^2 = 0,916) of relative fluorescence units (RFU) as measured by FFA (Exc. 333 nm, Em. 390 nm) versus STX concentration as measured by HPLC (Gerdts et al., 1999; TEPS-Project, courtesy of Gunnar Gerdts).

7.2.2.3 Genetic Diversity:

Problem: Little is known of the genetic diversity of marine phytoplankton populations and how it changes in space and time, except for a limited number of allozyme studies and RAPD fingerprinting analyses (see review in Medlin and Simon 1997). These studies have shown extensive genetic diversity on both temporal and spatial scales for some species. Such data underline the point that no single clone can be considered truly representative of an algal species. Thus, we are unable to provide estimates of how the population dynamics of HAB species are controlled or influenced by fluctuating levels of genetic diversity.

Existing Technology: At present, studies on microalgal genetic diversity must rely on the establishment of extensive clonal isolates. This requirement has undoubtedly hampered the advance of our knowledge in this area and obviously will bias estimates of genetic diversity and gene flow because of the selective nature of culturing. Nevertheless, these cultures are essential to establish the tools necessary for population genetic studies. Microsatellites, a single locus fingerprinting technique, are quickly replacing isozymes and RAPDs as the tool of choice for studies of genetic diversity. Presently, these tools can only be used with considerable amounts of target DNA available from culture material.

Future needs:

- Develop population genetic markers for key HAB species,
- Develop means of applying these molecular techniques to single cell isolates.

7.2.3 Cellular composition and physiology

7.2.3.1 Analytical Chemistry: toxin detection, quantification and standards

Problem: Several techniques exploiting characteristic molecular features of marine toxins are available for both field and laboratory work. Nevertheless, problems still exist with respect to reproducibility, sensitivity, and comparability of different assays. It is therefore mandatory to improve the selectivity and sensitivity of existing methods of toxin analysis. Furthermore, new and rapid toxin specific methods need to be developed for fieldwork.

Although several marine toxin standards are commercially available, there is a need for certified reference materials. These should not only be chemically pure but also be calibrated against a standard biological test, such as the mouse bioassay. In addition, regular intercalibration exercises are required.

Existing Technology: In the analytical chemistry of HAB toxins, detection and quantification may be accomplished by (1) analysis of effects on biochemical systems, (2) selective extraction followed by spectroscopic analysis, (3) non-selective extraction followed by selective spectroscopic analysis and (4) extraction followed by chromatographic separation. The first three approaches are more or less non-specific. Whereas the first allows for the determination of total toxicity or rather total activity against a target system, the next two provide an assessment of total toxin concentration in terms of a reference compound.

(1) Enzyme inhibition tests are routinely applied for several toxins. However, when these are to be used for quantification of toxin concentration/activity, matrix effects have to be taken into consideration and standard additions performed. Furthermore, non-selective enzyme inhibitors can bias these tests. These tests can be performed either in batch mode using suitable model substrates or via appropriate bio- or enzyme sensors. Fluorescence detection increases the sensitivity of the assay by orders of magnitude.

(2) Selective extraction of toxins is rarely feasible because in living cells a wide range of compounds of similar chemical nature is present.

(3) Selective detection of toxins in extracts usually requires some kind of sample clean-up to avoid interference from co-extracted matrix compounds and should only be used as a first attempt to test for toxins.

(4) So far all marine phytoplankton toxins that have been structurally characterised have been shown to be either of high molecular weight or highly polar in nature. Yet in nature, toxins are present in mixtures rather than single compounds. Thus, the method of choice for chromatographic separation is high performance liquid chromatography (HPLC), although gas chromatographic separation (after suitable derivatisation) of some toxins has been described.

Standardised HPLC protocols with UV or electrochemical detectors exist for ASP, PSP and DSP toxins as well as for cyanobacterial and other toxins. For extracts derived from biological samples, usually a clean-up step is necessary prior to HPLC analysis. Derivatisation techniques to enhance detectability or specificity in detection have so far not been exploited to any great extent in toxin HPLC, although pre- and post-column reactions have been employed in some cases. Other detection systems not investigated include light scattering or infrared detectors.

Although the combination of HPLC and a mass spectrometer is generally not used in routine analysis, this technique is so far the only one that will provide unambiguous identification of the compounds of interests. In all other cases positive identification requires either a second HPLC run under different conditions or another detection system. Capillary zone electrophoresis (CZE) might eventually become a feasible alternative/addition to HPLC analysis. Biomarkers, e.g., pigments, fatty acids or steroidal compounds, can be used as an alternative approach for species identification. This approach assumes that the toxic species produces these specific compounds throughout its entire life cycle.

Future Needs:

- Develop biosensors that allow unattended deployment for longer time, e.g.,, moored or drifting automatic surveillance systems,
- Develop biosensors for ion channel blocking compounds because a number of phytoplankton toxins act on ion channels,
- Develop sequential extraction methods coupled with an intermediate derivatisation that changes e.g., polarity to increase selectivity of total analysis,
- Develop toxin analysis for shipboard operation,
- Characterise unknown toxins using sophisticated HPLC techniques coupled with mass spectrometry,
- Develop and calibrate international toxin standards.

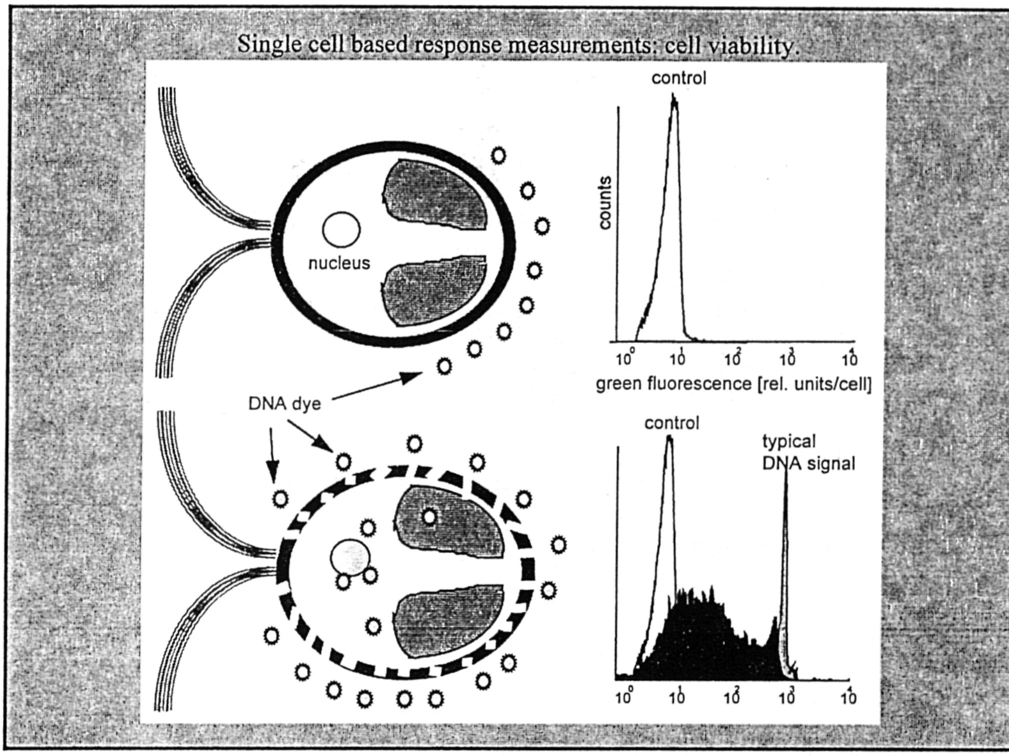


Fig. 21. Innovating techniques: viability of plankton can be examined on the level of individual cells, e.g., by examining cell wall permeability using DNA-specific dyes (Veldhuis, unpublished results). In healthy cells, dye can not enter the cell (top panel). In contrast, in cells with reduced viability dye passes the cell wall membrane and DNA in the nucleus is stained (bottom panel).

7.2.3.2 Biological Assays: toxin detection and quantification

Problem: In several cases biological assays are to be preferred over chemical assays. They are easier, often cheaper to apply, do not require complex instrumentation and can handle large number of samples. Nevertheless, they never can replace the need for detailed chemical analyses. All existing methods are laboratory-based and there is an urgent need to develop and adapt these methods for on-line (field) implementation requiring a minimum amount of laboratory skills.
Existing Technology: PSP can be measured using three different bioassays. The mouse neuroblastoma cell assay is a functional assay based on the detection of sodium channel blocking activity. Two other methods include a receptor binding assay based sodium channel blocking activity and an ELISA system. DSP is measured using a protein phosphatase assay, cell cyto-toxicity assays or ELISAs. Finally, ASP is measured using a receptor binding technique (functional assay) or ELISA.

Future needs:

- Construct biosensors for field operations additional to laboratory-based methods
- Construct biosensors based on probes for the genes coding the various toxins.

7.2.3.3 Eco-physiology

Problems: A major problem associated with HAB is their low abundance relative to that of the total plankton. This severely limits the use of bulk measurements to assess the specific role of HAB in the ecosystem. The inability to maintain representative cultures of toxic species are a limiting factor in all analyses and stresses the need for tools for single cell analyses.

Existing Technology: Several techniques are available, such as flow-cytometry, CLM and "image in flow" microscopy, but none of these are as yet widely applied. Standard or specially designed flow cytometers can detect rare cell events in mixed communities or large cells (chains and colonies) in marine phytoplankton. Species selective probing is of additional value to this type of classification (Vrieling and Anderson 1996). Sorting options and 'image in flow" systems allow accurate identification.

Moreover, there has been significant progress in determining (*in situ*) growth rates on the level of individual species applying the mitotic index or DNA-cell-cycle methods (Van Bleijswijk and Veldhuis, 1995). With the exception of *Phaeocystis* or dense populations of cyanobacteria, the low cell abundance of certain HAB species may reduce the usefulness of these methods.

Similarly, a wide variety of functional probes and stains are presently available to determine the cellular chemical composition, physiological status (nutrition), enzyme activity, info-chemicals etc. at the single cell level. These assays can be used to examine the direct effect of stress factors on the species of interests or the impact of this species on the ecosystem (e.g., phytoplankton-bacteria-viruses-parasite-interactions, Veldhuis et al, submitted).

Future Needs:

- Develop inexpensive and easy to handle detection systems for identification and sorting in the field,
- Improve and develop single-cell-based analyses for rapid detection of physiological status in laboratory and field applications.

7.3 Ecosystems

7.3.1 Detection/Monitoring

Problem: In order to improve our understanding of HAB dynamics at the ecosystem level, factors affecting their initiation and development need to be measured at a wide range of temporal and spatial scales. To develop early warning systems to guide scientific sampling, to enable avoidance or mitigation strategies, or to inform management decisions and so reduce the socio-economic impact, there is a need to integrate and further refine a number of existing tools and monitoring technologies.

Existing Technology: Remote sensing from airborne or spaceborne sensors can provide repeated observations at a range of spatial and temporal scales relevant to HAB. Ocean colour, the spectral variation of light reflected out of the sea surface, can be related to near-surface phytoplankton pigment concentrations through the selective absorption of downwelling sunlight by the algae. The first operational ocean colour sensor, SeaWiFS, was launched as recently as August 1997 and new sensors such as ESA's MERIS and NASA's MODIS are due for launch in the next few years and will provide improvements and continuity in ocean colour observation. Ocean colour data have been used for observing high biomass blooms such as *Phaeocystis globosa* off the Dutch coast, but in many cases toxic blooms cannot be distinguished from the phytoplankton community and they also may occur in subsurface layers. Satellite data can be affected by cloud cover, particularly in northern European regions, and may provide insufficient resolution in marine environments: these limitations can be partly overcome by airborne sensors. Satellite SST images can show the physical environment of HAB, distinct water masses with different phytoplankton population and phenomena, such as eddies of current that may advect HAB into regions of concern.

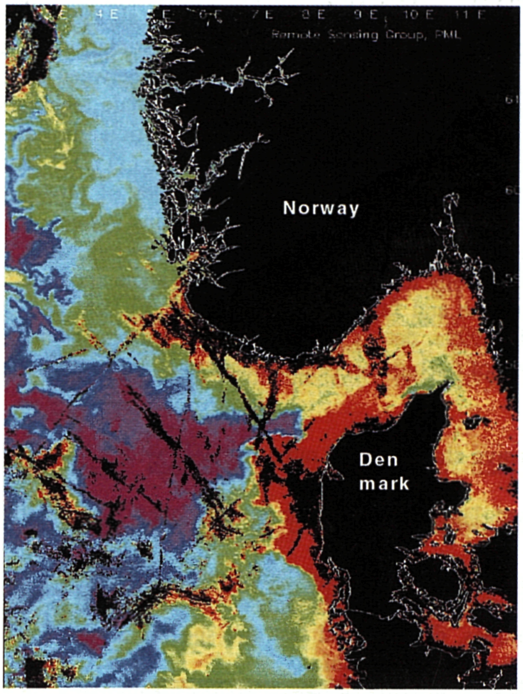


Fig. 22. Remote sensing from airborne or spaceborne sensors can provide repeated observations at a range of spatial and temporal scales relevant to HAB. This example shows a bloom of *Chatonella verruculosa* indicated by the red colour off Denmark and Norway, in May 1998. Courtesy of NASA SeaWIFS, processed by Remote Sensing Group at CCMS Plymouth Marine Laboratory.

Future needs

- Monitor algal scattering, absorption, fluorescence for characterising photosynthetic properties of water with *in situ* bio-optical sensors, and to partly overcome cloud cover problems with remote sensing and to detect sub-surface blooms,
- Utilise opportunities of continuous or repeated near-real time monitoring on moorings, drifters or ships of opportunity.
- Address problems associated with differentiation of phytoplankton pigments, coloured dissolved organic matter or suspended particles using bio-optical models or neural network approaches,
- Develop further algorithm for retrieval of bio-optical parameters,
- Investigate biological-physical or composite algorithms with multi-parameter sensors,
- Develop data assimilation tools for remotely sense of data on surface winds, roughness, surface elevation, and rainfall or surface irradiance to provide inputs to models investigating the initiation and development of a bloom,
- Integrate diverse data from different sources e.g., satellite and *in situ* together with model output into decision support systems.

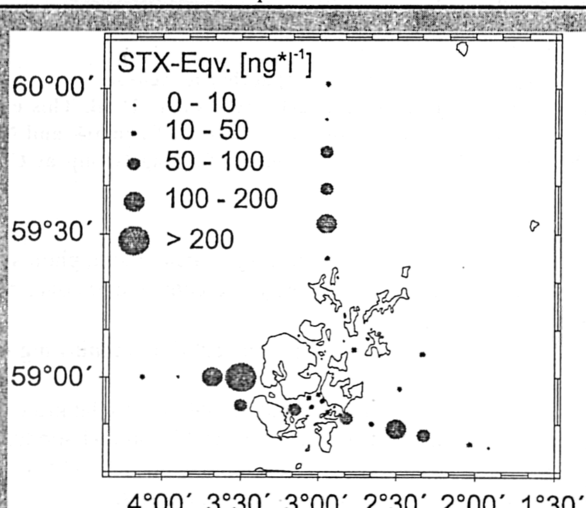
7.3.2 Ship board

Problem: Research vessels have been used in the past mainly for collecting samples for HAB research. Fortunately, there is presently more emphasis on the functional aspect of the ecosystem, so more experimentation is to be performed at sea. An intensified use of research vessels may bridge the gap between the more general information acquired from remote sensing systems, the necessary meteorological, physical, chemical and biological data and the laboratory experiments within HAB. Up to now there are only a few approaches on-board ship, to estimate the abundance and *in situ* concentration of PSP-toxins by HPLC-analysis (Hummert et al. 1999; Fig. 23). These first results emphasise the importance of this approach, a real-time estimation of the complexity of HAB (occurrence, toxic species, trigger mechanisms and interactions between the different trophic levels.

Existing Technology: Currently, an entire suite of oceanographic techniques is available for water column and benthic research. Different fields of research sufficiently cover the full need for basic physical, chemical and biological measurements. These include (1) CTD probes for hydrographic data and chlorophyll determinations for monitoring depth profiles of phytoplankton, (2) Recorder for meteorological, hydrographic, oceanographic data, (3) Sampling systems for water and sediments, (4) Specific sampling systems for planktonic organisms, (phytoplankton, bacteria, filter feeders and meso-zooplankton) (5) Light microscopy for taxonomic studies of algae zooplankton, filter feeders, (6) PSP toxin analysis in phytoplankton and filter feeders by HPLC, ELISA, and by a new fast fluorimetric assay (FFA) (Gerdts et al.; 1999a, details in Fig. 20), (7) Drifters for drift experiments, which may lead to answers of the origin of the blooms, (8) Sediment traps for studies of cyst occurrence.

Future needs:

- Request more extensive ship time for rapid response with a (inter)national, multi-disciplinary research teams,
- Adapt currently used land-based laboratory techniques for specific conditions on research vessels,
- Use flow cytometry systems with cell sorters, coupled with *in situ* hybridisation techniques for on-board community analysis of algal blooms and its associated bacterioplankton,
- Develop new techniques, devices, and fast and specific assays for detection and analysis of DSP, ASP and new toxins on-board ship.



STX-equivalents in a natural toxic *Alexandrium sp.* bloom (May-June 1997) measured on ship board by HPLC in the waters of the Orkney Islands
(Hummert et al., 1999, TEPS-Project Figure provided by Gunnar Gerdts .

Fig. 23. Ship board measurements of toxin production of field population of toxic *Alexandrium sp.* bloom and associated microbial community structure.

7.3.3 The use of C stable isotopes for in situ monitoring of algal bloom development.

Problem: One nutrient frequently over-looked in the study of HAB is carbon dioxide (CO2). As blooms occur the concentration of dissolved inorganic carbon (DIC) will decline. Whereas the relative decline in DIC may not be great compared to other nutrients, it can, in otherwise nutrient replete cultures, influence growth rates and the photosynthetic physiology and hence the cellular requirement for other nutrients. This process is very common in laboratory culture of marine phytoplankton, where cell densities are often orders of magnitude higher than in the natural environment. To be able to relate laboratory observations to field data, it is absolutely necessary that the conditions of cell culture more realistically reflect the natural environment, i.e., we need to ensure that laboratory cultures are maintained under near normal air-equilibrium (Johnston and Kennedy 1998).
Existing Technology: Whereas measurements of pH and alkalinity are useful indicators of the carbonate system, they cannot be used as an *in situ* measure of photosynthetic status of a bloom. Mass spectrometric analysis of DIC and the cellular organic carbon gives a measure of the fractionation of the photosynthetic process. It is possible to estimate the specific growth rate from the concentration of dissolved CO_2 and the fractionation observed between source and product $^{13}C/^{12}C$. This relationship appears to be fairly robust for light and nutrient-limited diatom laboratory cultures.

Future needs:

- Compare isotopic methods of estimating growth rates with traditional methods and more recently developed methods,
- Use stable isotopes to ensure laboratory cultures are maintained in a physiologically useful state,
- Apply stable isotope techniques to monitor the development of HAB in mixed water and sub-surface HAB in stratified waters.

7.3.4 Community Interactions

Problem: Generally, interactions between members of the plankton community are poorly understood because most of the organisms involved are micro-organisms and therefore escape direct observation. There is now some scattered evidence that these interactions are far more complex than simple predator-prey relationships. Micro-organisms have a diverse, though poorly recognised, array of chemically communicating with other organisms. In the light of harmful blooms one might expect intensive signalling of specific organism groups. Essentially, production and release of toxins or excessive polysaccharide production can also be seen as a release of a chemical signal by a particular organism targeted against another organisms.
A major future topic is the elucidation of the natural community structure of algal blooms and its associated bacterioplankton. Within this complex unresolved questions are (i) the role of bacteria in producing toxins by themselves, (ii) effects of bacteria on the algal toxicity, (iii) effects of bacteria on HAB dynamics and algal growth, (iv) taxon-specific relationships between bacteria and toxic algal strains.
Existing technology: To study competition for nutrients, laboratory studies are usually performed using batch, semi-continuous or continuous culture techniques. These cultures can be xenic or axenic. A number of plankton species cannot be grown under axenic conditions indicating their dependence on interactions with other organisms (mainly bacteria).
Grazing experiments with algae can be performed by a variety of techniques, such as using radiotracer techniques, serial dilution series, or fluorescent-labelled organisms coupled with flow cytometry. The preferred method depends on the organisms involved and on the specific questions addressed. These methods can generally be used for bacterio- as well as phytoplankton and mixo- or autotrophic protists.
Denaturing Gradient Gel Electrophoresis (DGGE) profiling provides a sensitive tool to determine taxa-specific relationships between bacteria and algae. DGGE data of a toxic *Alexandrium* bloom in waters of the Firth of Forth and the Orkney's strongly suggest stable bacterial communities associated with toxic algal blooms (Gerdts et al. 1999b; Fig. 24).

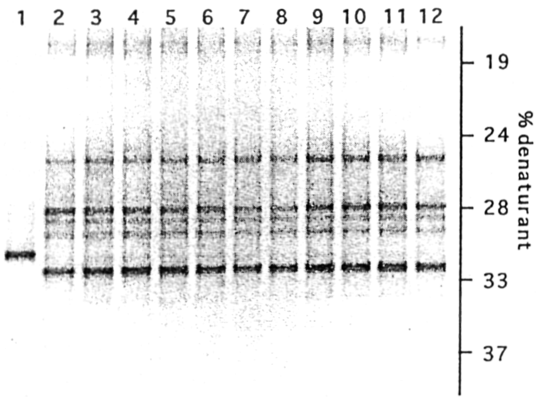


Fig. 24. DGGE analysis of 16S rDNA fragments indicate stable bacterial communities associated with a toxic *Alexandrium sp.* bloom east of Firth of Forth (May 1998) obtained after PCR amplification with the eubacterial primers P2 and P3 (negative image). Fraction < 3 µm > 0,2 µm. Lane 1: *E. coli* J53 (positive control); Lane 2 - 12: Different stations (Gerdts et al., 1999; TEPS-Project). Courtesy of Gunnar Gerdts.

Future needs:

- Develop new technology to study infochemicals,
- Develop functional probes for the gene expression of infochemicals,
- Develop group and strain specific gene-probes for bacteria associated with algae,
- Refine existing methods to determine grazing pressure,
- Increase the used of DNA based detection methods (e.g., DGGE) to study community interactions

7.3.5 Modelling

7.3.5.1 Physical/biological models

Problem: Physical/biological models have so far not been able to account for the effect of climatic and hydrodynamic forcing in relation to ecosystems dynamics across temporal and spatial scales. The role of climatic oscillations, such as El Niño (ENSO) and the North Atlantic Oscillation (NAO) as a possible climate forcing linked to HAB formation, occurrence and duration have not been considered. Although 3D physical/biological models of HAB have been developed, they remain poorly resolved spatially, and poorly constrained by data. The existing models can not be used for regional and large/scale resolution and single modelling strategies can not provide a better understanding of physical/biological coupled processes at different ecological scales.

Existing Technology: A number of models are currently available and in routine use. Circulation models (GCM 2D, 3D) are mainly hydrodynamic models existing for different basins. Atmospheric/ Climatic models can be regarded as tools to investigate climate variability and prediction. Coupled physical/biological models can help to understand and evaluate the coupling between physical and biological processes, at different spatial and temporal scales relevant to specific studies. Biogeochemical models have the potential to be coupled with GCM models and allow a comparison with remote sensing data, as well as the study of biological/chemical processes at mesoscale levels.

Future needs:

- Integrate existing Lagrangian circulation models with statistical models in order to use information from time series data sets and allow to include biological information into physical circulation models (Woods and Barkmann, 1994),
- Use acoustic data to investigate patch dynamics and the effect of turbulence and eddy dynamics in HAB blooms duration and formation,

- Improve and compare the outputs from existing purely mechanistic models with phenomenological models,
- Improve high-trophic resolution ecosystem models by including hydrodynamic and climate forcing (Bryant et al. 1997, Heath et al. 1997),
- Develop nesting procedures to couple high-resolution physical/biological models with large-scale GCM models.

7.3.5.2 Ecological models

Problem: The scenario of models used in ecological research covers a wide spectrum from purely mechanistic to phenomenological models. There is a need to use the existing information from long/short term time series data to improve our modelling effort. A novel approach is needed to take into account both endogenous and exogenous dynamic factors at a population and community level. The outputs from different modelling approaches has not been compared to allow a better understanding of complex dynamics in HAB and improve our capability to make prediction about HAB formation and duration at different temporal and spatial scales. Community analysis using multivariate statistical methods to relate species-environment associations and trend analysis in species composition have not been performed using the novel approaches that are available. Novel modelling approaches have not yet been proposed for improving our knowledge in basic and applied ecological HAB research leading to a better management of marine areas in a socio-economic and ecosystems health context in relation to human health in Europe.

Existing Technology: A variety of models and their potential applications include: (1) Density-independent population growth models in continuous time for problems concerning age structure at a population level, (2) Density dependent growth models for understanding what factors may be regarded as causes and consequences regulating population growth and presence of chaotic dynamics and metapopulation models, (3) Single locus models with and without selection as potential tools for studying polymorphism and life histories in relation to behavioural and evolutionary studies, (4) Predator-prey models for elucidating predator-prey interactions as well as investigating complex dynamics, (5) Nicholson-Bailey models for studying host-parasitoid interactions and what factors allow them to persist, (6) Epidemic models for addressing questions concerning the duration of epidemics and the persistence of diseases as well as understanding the dynamics of epidemics at a population level, (7) Cell cycle and life cycle models for investigating the behavioural response to nutrients uptake and availability, and (8) High-trophic resolution ecosystem models for studying of the coupling between physical/chemical /biological processes and for studying food web dynamics, (9) Multivariate statistical methods, such as Canonical Correspondence Analysis (CCA) and combined CCA-PLS, for the analysis of species-environment associations, (10) statistical methods for detecting trends in multispecies assemblages.

Future needs:

- Examine LTS full use for periodicity/chaotic behaviour,
- Use existing time series data to investigate the presence chaotic dynamics and population dynamics at a single species level (Ellner and Turchin 1995, Little et al. 1996),
- Fit models to long term and short term time series to detect periodicity, account for both endogenous and exogenous factors and gain new information for setting up novel field and laboratory experiments,
- Compare/calibrate spatial models with remote sensing to integrate ecological processes across scale,
- Use non-linear nonparametric statistical methods to identify climatic and environmental variables that could be used as predictor for the occurrence of HAB in oceanic, coastal and brackish waters and to reduce statistical uncertainties of existing models,
- Improve the existing single locus models with and without selection to better understand the maintenance of polymorphisms and frequency dependent selection (Hastings and Harrison 1994, Gavrilets and Hastings 1994),
- Investigate the possible use of predator-prey and metapopulation models in relation to HAB population dynamics,

- Improve cell cycle models in relation to nutrient uptake and availability (Pascual and Caswell 1997),
- Use Nicolson-Bailey models and epidemics models for studying host-parasitoid and pathogens dynamics in HAB,
- Use Artificial Neural Networks to identify variables that may be responsible for controlling the fluctuations at a population level and their potential use as a predictive tool,
- Use novel statistical methods for detecting trends in composition of phytoplankton assemblages in relation to HAB occurrence,
- Re-analyse existing historical data sets,
- Combine novel modelling efforts in relation to ecosystem health, management of marine waters and human health in Europe,
- Use CCA-PLS methods to study species-environment relationships and predict the species data from environmental data in accordance with a supposed causal flow in the sense that species respond to the environment (Ter Braak and Juggins 1993),
- Use novel statistical methods to extract trends in species assemblages (Solow 1994a, 1994b).

8. MITIGATION OF HAB AND THEIR IMPACTS: PRACTICAL APPLICATIONS OF FUNDAMENTAL SCIENCE

8.1 Introduction

The goal of all HAB research is to protect public health, fisheries resources, ecosystem structure and function, and marine aesthetics. This requires an understanding of the factors that regulate HAB dynamics and how they cause harm, although by itself, this knowledge does not provide protection. Management and mitigation strategies are needed to reduce impacts by avoiding the blooms or minimising their effects (impact prevention), or by actions that directly target bloom populations (control). Examples of impact prevention strategies include moving fish cages from the path of an HAB, altering the chemical composition of fish food to reduce their susceptibility to a bloom, or reducing pollution inputs in an effort to decrease the number or size of bloom events. Examples of control efforts would be direct application of chemicals or biological control agents that kill or disrupt HAB cells during blooms.

The term "mitigation" is broad and includes many different types of activities that can reduce HAB impacts. Scientists and society easily accept some of these activities, and others are highly controversial. The former include bloom or toxin monitoring programmes, site selection for aquaculture based on HAB incidence and the use of aquaculture species which have reduced susceptibility to HAB.

The status of HAB mitigation science is uneven and inadequate in comparison to advances made in terrestrial programmes to minimise terrestrial pest impacts (Anderson 1997). Some progress has been made in protecting aquaculture resources from HAB. Much less attention has been given to mitigation strategies for blooms that affect large areas or widespread resources.

This chapter identifies areas where fundamental research can potentially lead to HAB mitigation and control strategies. There are clearly mechanisms in HAB that are critical elements of bloom dynamics, which are logical areas to target for research. The main focus of EUROHAB should be on factors that regulate bloom dynamics, but the rationale for that research should be to provide the scientific basis for possible mitigation strategies. Practical aspects of designing and testing mitigation strategies are beyond the immediate scope of EUROHAB, but would be a logical extension of its activities.

8.2 Impact prevention

One level of impact prevention relies on HAB detection and mapping, and the prediction of their movements. The tools needed to accomplish these tasks described in Chapter 7. Here we highlight the need for these technologies and provide practical justification for the research, which will bring them to the application stage.

8.2.1 Prediction and early warning

8.2.1.1 Modelling

Some effects of HAB on living marine resources could be restricted or avoided if it were possible to predict bloom occurrence or movement. This requires detailed understanding of bloom dynamics and conceptual or numerical models of bloom transport. Bloom modelling and prediction capabilities are not sufficient to achieve this goal at present, so there is a clear need to expand this capability. This needs not only the development of 3-dimensional hydrodynamic models of areas subject to HAB outbreaks, but also the incorporation of biological features into those models. Resolution of various rate processes integral to population dynamics (e.g., input and losses due to growth, grazing, encystment, excystment and physical advection) has not been accomplished, but is essential in model simulations. Large-scale, 3-dimensional numerical models of each area impacted by HAB are needed and these do not exist for most European waters. The end result is that despite the proven utility of models in so many oceanographic disciplines, there are no predictive models of HAB development, transport and toxin accumulation in Europe.

We are not presently capable of predicting the occurrence, distribution, movement, toxicity, and environmental response of HAB. There is therefore a clear need to develop realistic physical models for regions subject to HAB events, and to parameterise these models with biological data needed to simulate the behaviour and population dynamics of the relevant HAB species.
Monitoring and management systems for HAB and their toxins are not optimised to provide the data needed for prediction or model development and the modes of action of some HAB toxins are not sufficiently understood to guide intervention efforts on impacted fish or other resources. Furthermore, functions that define the manner in which shellfish become toxic from given HAB are not known.

8.2.1.2 Bloom detection

Management of resources impacted by HAB requires that toxic cells and blooms be detected and mapped rapidly, accurately and over broad scales. At the simplest level, monitoring programmes can accomplish this by detecting and enumerating toxic species using water samples and microscopic observations. The EUROHAB programme recognises the need for accurate bloom detection through plankton analysis and seeks to ensure that monitoring is recognised as an important element in HAB mitigation strategies.

The standard monitoring approach by light microscopy is quite effective but can be limited by the number of samples that must be examined, toxic or harmful species identification difficulties and the time required for cell counts. To accelerate this process, molecular probes, which are specific for HAB species are under development, but direct application to natural HAB populations, remain few (Anderson 1997). These probes can be used in the laboratory and are amenable to automation, and could be deployed on moorings for semi-continuous monitoring purposes. Clearly, additional development effort is required to bring these powerful tools into full application in HAB detection.

Our ability to detect and track blooms of harmful algae over large scales is limited. Because detection is an essential component of any early warning or prediction system, efforts must be directed to the development of technologies to detect cells, blooms, or toxins on scales necessary for early warning strategies to be implemented.

Optical sensors show great promise for *in situ* monitoring of HAB dynamics. Radiometric sensors on moorings, profilers or undulating instrument packages can measure ocean colour and sunlight penetration to characterise water components of, including phytoplankton. Instruments that measure spectral absorption, scatter and fluorescence *in situ* can provide continuous information on algal pigments with excellent spatial resolution. The potential for resolving certain taxonomic groups is good, and specialised instruments with high spectral resolution may distinguish important species or functional groups. Some instrument packages are commercially available, but their direct utility for HAB remains to be proven.

Considerable development work on the optical properties of specific HAB and the instrumentation needed to detect them are high priorities among efforts to develop early warning strategies.

On larger scales, satellite remote sensing has great promise in HAB mitigation if it could be used to detect and map blooms over scales that are not possible with standard ship-based or mooring-based observation programmes. Blooms can sometimes be identified by their optical properties, and water masses containing blooms can be detected and followed through time using temperature or salinity sensors. However, HAB detection using remote sensing remains an elusive goal because of the limitations of available sensors, the lack of distinctive "optical signatures" for HAB species, and the inability of existing algorithms to deal with the optical complexity of marine waters. There is great promise, nevertheless, that should be exploited to achieve the goals of EUROHAB.

HAB are often sporadic in nature on restricted spatial and temporal scales, which will be missed by traditional fixed-station, infrequent monitoring. An alternative approach is to use Continuous Plankton Recorders (CPRs) on commercial ferry lines or other ships of opportunity (Chapter 5). Coupled with the technologies of satellite imagery and optical moorings, CPR's and research vessel observations can provide means to observe surface and subsurface blooms with the resolution needed for HAB detection. To fully exploit the CPR approach to early warning, we need: 1) technical development of the methodologies as well as installations of towed nets and/or flow-through systems on commercial ferries and moorings; and 2) data assimilation protocols, intercalibrations and assessments. New communication technologies are also needed for information transfer from ships of opportunity to the decision- makers.

8.2.1.3 Research needs and justification

In order to develop new and improve existing mitigation strategies it is recommended that:

(a) Realistic physical models are developed for regions subject to HAB events. These models must be parameterised with biological data needed to simulate the behaviour and population dynamics of the relevant HAB species.
Justification: At present our capability to predict occurrences, distribution, movement, toxicity and environmental response of HAB is very limited. Linking several independently operating research efforts together that use different tools can greatly enhance our capability to develop improved predictive models.
(b) Our limited ability to detect and track HAB over large scales is improved by directing research and development efforts towards technologies that detect cells, blooms and toxins on scales necessary for early warning strategies to be implemented.
Justification: Because detection is an essential component of any early warning or prediction system, the need for these techniques is obvious. Existing tools are still too slow, time- consuming, inaccurate and expensive to be used cost-effectively on a large scale and often are useful only to assist in reactive monitoring.
(c) CPR approaches are being exploited on a trial/research basis to develop early warning systems while specifically focussing on two priority areas: (1) technical development of the methodologies as well as installations of towed nets and/or flow-through systems on commercial ferries and moorings; and 2) data assimilation protocols, intercalibrations and assessments.
Justification: HAB should be observed at an early stage of development to prevent consequences. Whereas CPRs do not provide data online but with a considerable time delay, linkages to other recording systems may improve our understanding of area-specific frequencies of occurrences, which is particularly helpful for identifying designated areas of ballast water exchange. New instruments, such as the fast-repetition-rate fluorometer (FFR) can provide the link of observations of plankton particles (cells, pigments, etc.) to growth process of the plankton community. The FFR may be used on board ship, in vertical hauls or in undulating instrument packages.

8.3 Impact reduction

Another category of mitigation strategies involves those that seek to reduce HAB impacts through actions which will take years or decades to show results (e.g., pollution reduction policies), and sometimes through direct actions that target the affected resource (e.g., shellfish depuration enhancement). These activities are far less controversial than direct bloom control and offer significant benefits. Many require targeted research to bring them to full fruition, however.

8.3.1 Nutrient reductions

One of the explanations given for the increased incidence of HAB outbreaks worldwide is that these events caused by increased pollution and nutrient loading in coastal waters. HAB species may increase in abundance due to nutrient enrichment but remain as the same relative fraction of the total phytoplankton biomass (i.e. all phytoplankton species are affected equally by the enrichment).

Alternatively, a selective stimulation of particular HAB species can occur due to the changes in nutrient supply ratios from human activities (Smayda 1990). Regardless of the mechanism, there is no doubt that HAB have increased in certain European waters where pollution has also increased and linkage is real, but less evident in areas where pollution is more gradual and unobtrusive.
It follows that that a reduction in pollution, or a change in the ratios in which major nutrients are supplied to marine waters may lead to a decrease in HAB frequency or magnitude. An example of this type of mitigation strategy was seen in the Seto Inland Sea of Japan, where pollution increased nutrient loadings dramatically between 1970 and 1978, during which time visible red tides more than tripled. Legislation was passed that mandated a reduction in industrial and domestic effluents, and several years later, the number of red tides began to decrease. This example highlights the connection between human pollution and HAB incidence. It is, however, not clear how may blooms actually reflect this linkage.
Scientists are frequently asked whether a reduction in pollution or effluent discharges will result in a decrease in HAB incidence. To address this important and common societal issue, we must first clarify the relative importance of anthropogenic inputs in the development of specific HAB, since this will directly determine the mitigation strategy to be applied. This requires laboratory and field investigations of the response of HAB species and their associated communities to nutrient inputs.

Issues: We must determine how changes in the magnitude and elemental ratios of nutrient inputs to marine ecosystems can influence ecological responses, especially those that favour HAB. This in turn requires the characterisation of an organism's response to the environmental factors that govern bloom dynamics.

8.3.2 Transfer of HAB species (e.g., via ballast water and intentional introductions through aquaculture)

8.3.2.1 Ballast water transmission

Ships have long been recognised as a major vector for the transfer of exotic and harmful organisms (Rosenthal, 1980; Carlton, 1985). The intensity of shipping and the structure of fleets, however, have changed greatly in the past decade. Recent invasions and population explosion of exotic species in various parts of the world are causing environmental and economic damage (Carlton and Geller 1993, Carlton et al. 1995).
Dredging in harbours and river estuaries has also changed tidal patterns in marine habitats leading to increased chances for survival of exotic species. Risk assessment of ecosystem disruption by non-indigenous species must consider new criteria for quantifying survival probabilities of exotics in order to define appropriate management strategies to minimise these risks to coastal resource-users (e.g., aquaculture, fisheries and tourism). Reasons for growing concern also include the rapidly changing patterns of human use of coastal habitats with increasing pressure on natural resources (Chamberlin and Rosenthal 1996). Scenarios affecting the likelihood of ballast water-meditated biodiversity changes in marine habitats that can result in the transmission of parasites, disease agents and HAB species include:

- Increasing number of aquaculture activities (more sites at risk)
- Increasing density of units near shipping routes (more chances for transfer)
- Increasing sea traffic (number of ships and routes, larger critical mass)
- Increasing speed of ships (shortened transfer time, higher survival chance)
- Increasing size of ships (larger ballast volumes, more oxygen available)
- Changing strategy of ballast water management (cleaner water)
- Changing human population density in the coastal zone (more activities)
- Increasing poverty in small coastal communities (rapid local transmission)
- Lack of satisfactory hygienic conditions in harbours (higher health risks)
- Changing donor and receiving environments (often higher survival potential)

A recent estimate indicates that 80% of the world's cargo is transported via ships and the volume of ballast water released into coastal waters may be 10 to 12 billion tonnes per year. A minimum of about 3000 aquatic species is transferred by ballast water intercontinentally and daily. The transfer of microalgae (including HAB) via ballast water is no exception. It has caused damage to a variety of aquaculture operations and well-documented cases include species such as *Scrippsiella trochoidea, Cochlodinium sp., Gymnodinium galatheanum, Gymnodinium mikimotoi,* and *Prymnesium parvum)* Ballast water is mainly released in harbours or nearby areas. Inshore waters are the major areas where aquaculture facilities are located. These are at particular risk and suffer when HAB species are released in their vicinity, creating a scenario for bloom outbreaks.

Little is known about the fate of HAB species in receiving waters after different retention times in ballast tank sediments. These sediments accumulate in the tank bottom, despite high flushing rates, when ballast water is exchanged because the tanks are constructed with many baffles (for stability reasons), offering numerous sites where sediments easily settle(Fig. 25). At intervals, however, tanks are emptied completely and sediments are washed out. Besides the risk of transferring viable cells, concern is expressed that massive transfer of dormant cysts with ballast water sediment releases may affect receiving waters with subsequent serious consequences to receiving ecosystems, nearby aquaculture operations and local fisheries. Such harbours and designated ballast water exchange areas in the open sea may become subsequent donor areas through other distribution mechanisms (e.g., boating, fishing gear, tides and currents). Little research has been done to evaluate the potential of such vectors.

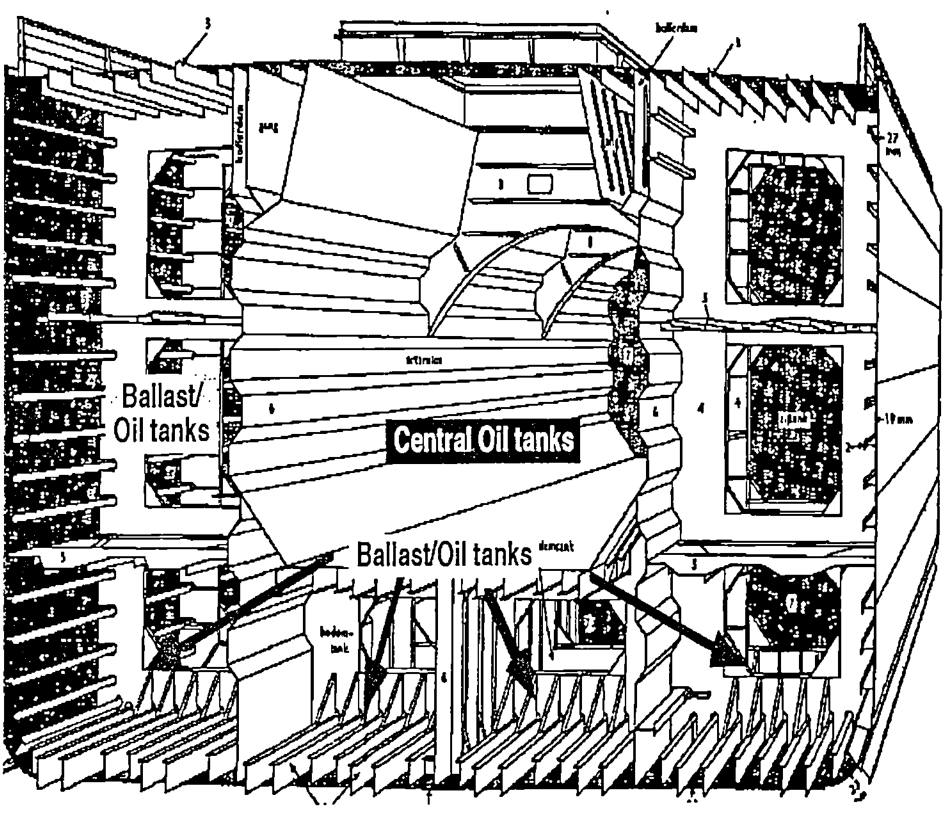


Fig. 25. Cross-section of an oil tanker, indicating various tanks dimensions, including ballast water tanks, while also showing numerous baffles that provide extensive settling corners where permanent cysts can be imbedded in the sediment.

Concern is also raised to that so-called "cosmopolitan" species of HAB may have different physiological capabilities and, when released in a distant area, may give rise to massive growth within the native population of the same species. There is a need to develop methodologies that study these aspects of transfer consequences. The results will have drastic effects on management strategies to be recommended for ballast water handling by the shipping industry.
Modern phytoplankton identification tools (e.g., RAPD-PCR, Bolch et al, 1998, see Fig. 26; Chapter 7) are under development in many countries. These will hopefully provide the means to study such scenarios. Although the validity of the findings is presently controversially discussed, the example presented in Fig. 26 is a visualisation of the possible utility of such tools in identifying the origin of populations of the same species, which might have been introduced into different areas.

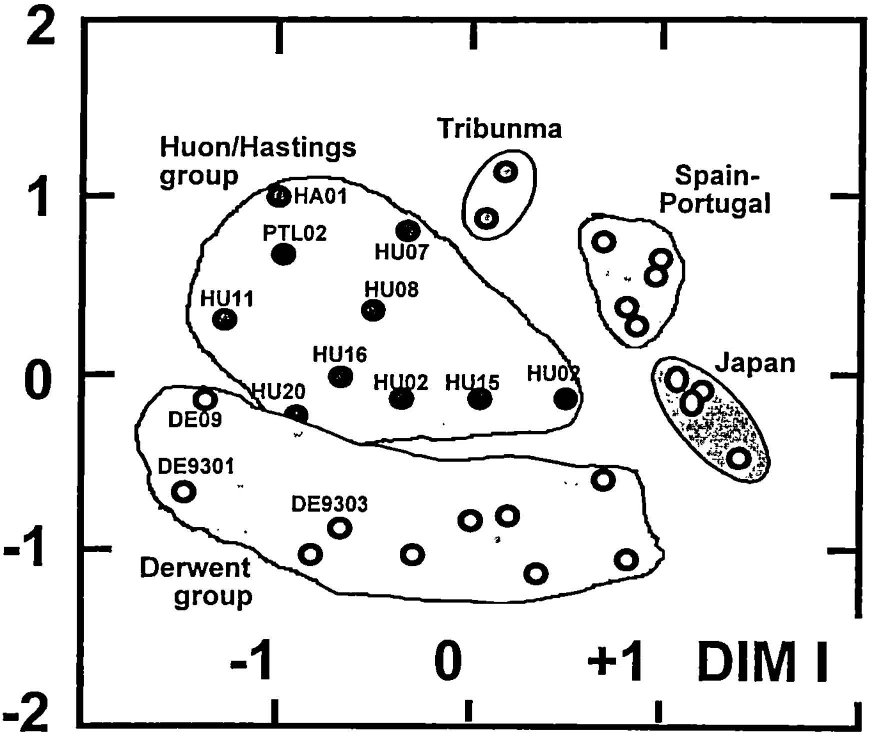


Fig. 26. Identifying the variation among different global populations of *Gymnodinium catenatum.* Isolates of *G catenatum* were analysed by RAPD-PCR (Randomly Amplified Polymorphic DNA - Polymerase Chain Reaction) and clustered to show similarities. With this method it is possible to differentiate strains of different species and thus potentially document and trace the source of accidental introductions of HAB species (modified after Boalch 1998).

Recent attempts to mitigate ballast water-meditated effects in coastal environments call foropen ocean ballast water exchange. The IMO has prepared a voluntary guideline for this procedure, which was adopted in 1993 (IMO 1993). The advantage is seen in the possibility that algae from inshore areas may be less able to survive in offshore, generally low-nutrient environments, whereas offshore fauna and flora may easily adapt to harsh near- and inshore conditions. Although this might be true to some extent, the fact that large blooms of microalgae do occur offshore, covering huge areas, appears to be ignored. Great concern is expressed that the suggested offshore ballast water exchange strategy will not achieve its aim because of the likelihood of high biomass blooms of micro-algae occurring in the designated exchange areas. So far, we have little information on their occurrence and distribution.

Research is needed to develop tools to guide the industry to areas where HAB are not present at times when ballast water exchange is needed.

8.3.2.2 Aquaculture as a vector of transfer of HAB

There are documented cases of unintentional introductions and transfers of non-indigenous algal species via aquaculture in coastal and marine waters. Whereas most aquaculture activities e.g., finfish farming, tight national regulations and international Codes of Practices (ICES and EIFAC, FAO) exist in Europe to reduce the risks of transfer of associated fauna, the present regulations within the EU dealing with live shellfish transfers are unsatisfactory and have already lead to additional transfers of exotics with shellfish seed. No concern, however, has yet been expressed and few scientific studies have been performed to assess the risk of HAB species transfer with the live transport of seed oysters. Subsequent studies on the fate of these algae in the receiving waters are also lacking. Transfer of seed oysters has become a common practice. The potential for local and regional consequences of non-indigenous HAB species release in Europe is real and of growing concern to the mussel and oyster farming industry, as well as to those concerned with the conservation coastal ecosystem communities. There is a need to provide guidance through adequate research on the subject. Much can be learned, however, from past events on shellfish disease transfers such as the MSX disease in *Crassostrea virginica* along the Atlantic east coast of the United States and the introduction of *Bonamia ostrae* and *Marteillia fringens* into European oyster farms. Little scientific information is available on similar case histories related to HAB.

8.3.3 Riverine and watershed management

Watershed and riverine management may increase the periodic input of nutrients, trace elements, metals, etc into coastal/estuarine nutrient regimes, favouring sporadic plankton growth. Operations that aim to regulate water flow to the sea or waterflow direction (filtering effect of land omitted) may increase the intensity of nutrient/trace elements etc pulses to the sea and thus affect marine HAB dynamics. The timing of nutrient pulses may be critical to bloom development.

8.3.4 Aquaculture siting and operational guidance

Coastal fish farm siting is presently practised by selecting areas with appropriate hydrodynamic and morphodynamic habitat characteristics through mass-balance modelling of nutrients whereas other site selection criteria are concerned with determining oxygen budgets and sediment accumulation under cages in relation to the size of the proposed operation. These criteria aim to minimise environmental interactions between the farm and the local ecosystem and also consider the carrying capacity of the entire system through predictive modelling (e.g., fjords in Norway and sea-lochs in Scotland). The industry and regulatory authorities in several EU countries have made some progress in safeguarding the industry whilst also protecting the environment through Coastal Zone Management policies (e.g., LENKA and MOM Programmes in Norway). However, few site selection strategies have included the cysts of microalgae in the sediments of potential sites into site selection criteria (e.g., in Denmark and Faroe Islands).

With reference to operational guidance, few studies have been made to determine the fate and understand the risk associated with HAB cysts accumulated in sediments under and around fish farm cages. Modern aquaculture management strategies also consider site rotation (fallowing periods) to allow for recovery of the benthos. Little consideration has yet been given to the interaction of HAB species with these sediments during recovery periods or during harsh weather conditions when re-suspension of sediments might occur.

Shellfish culture in EU waters is traditionally practised in many member states using the same sites and areas for decades. New site allocations are occurring to some degree. So far, site selection criteria do not include the frequency or density of occurrences of HAB. Whereas most sites are selected either for best growth or best settling of seed, only a few studies exist (e.g., British Columbia) where Habitat Suitability Indices have been developed on the basis of productivity and larval settling of oysters. Further studies are needed to help the industry by incorporating the potential for HAB occurrence in decision-making.

Existing regulations on the transfer of aquaculture species within the EU open market do not sufficiently cover the need to control accompanying fauna and flora in shellfish such as seed oysters and mussels and this has somewhat weakened the tight protocol being developed over many years in the ICES Code of Practice (ICES 1997). Little is known of the viability of HAB species transported in shellfish for commercial practice (relaying of seed on culture grounds) or for market supplies (e.g., life storage in harbours with flow-through systems). Season, transport conditions and storage time may influence viability. Studies are needed to identify the potential risk of successful transfer of HAB species with live shellfish to new areas. These would help to understand the effects of these practices and to provide advice on mitigation strategies.

Finfish species produced by cage farming along coasts respond differently to HAB. When considering new fish species as potential candidates for aquaculture, sensitivity to HAB should be included in the criteria for species selection. Within the EU, interest has increased in developing "artificial reefs" as coastal structures that serve many purposes in the Coastal Zone: extensive aquaculture, stock enhancement, recreational fisheries, prevention of trawling in protected areas. Decisions for siting such facilities in shallow waters should include HAB considerations.

With regard to toxins, several countries require extensive testing with adequate certification prior to shipment of live shellfish. The industry in some countries is well aware of the benefits of control mechanisms to maintain consumer confidence and prevent market disruption. However, toxin testing is often costly, time-consuming and not yet straightforward to implement for all relevant toxins. When precautionary closure is applied, this causes tension between farmers and regulatory authorities. Delays in toxicity assessment cause loss of shelf life and therefore of value of the products, adversely affecting the competitiveness of the European market. There is a need to develop rapid analytical methods, preferably including low cost test kits for HAB toxins, which can effectively be used by the industry and the control agencies in EU countries. Intercalibration of improved analytical tools will also be required.

There is a need to improve HAB and environmental monitoring capabilities. The option of involving the aquaculture industry directly in these exercises can be very useful. Aquaculture operations need to monitor their environmental conditions on a regular basis for site management and regulatory purposes. With some guidance, aquaculture operators can be trained to record valuable basic data on a regular basis, which can assist researchers to obtain a broader picture on occurrences and events outside or nearby their study area. One example where such monitoring is successfully practised is British Columbia, Canada, (Gaines and Taylor 1986) where regulatory authorities, farmers and scientists have worked closely with the best sources of expertise to bear on practical mariculture problems.

Studies are also needed to assess the fate of permanent cysts in sediments under cages

8.3.5 Research needs and justification

Recommendation: Monitoring of HAB species should be expanded to involve aquaculture operations for both shellfish and finfish directly, providing opportunities to gain better area coverage and high frequency data.

Justification: Aquaculture in European coastal areas is growing rapidly, increasing the number of stakeholders who are directly affected by HAB and who need effective advice to minimise their effects. Involving farmers through training programmes and provision of equipment is one of the cost-effective means to expand the database in support of long time- series studies being developed by other programmes. Further, cooperation with the aquaculture industry would also largely remove the tension between regulatory authorities and industry (marketing needs) once action has to be taken to reduce human health risks.

Recommendation: Studies should be initiated to understand the fate of HAB cysts and other viable units accumulated under cage farms, including the effects of fallowing periods and the potential of cysts and other resting stages, for bloom inoculation under given environmental circumstances.

Justification: Although most new licences for cage culture systems require an EIA with predictive modelling on benthic deposition of suspended solids in order to set limits on farm size to avoid anoxia underneath cage flotillas while allowing for certain bioturbation, there is still a change that such habitats may act as reservoirs for dormant cysts. Studies in this area should identify whether there is such a risk through cage farming while assisting to improve predictive modelling in order to minimise the risk of bloom inoculation.

Recommendation: Aquaculture system design studies should be supported that allow for effective control and collection of waste feed and faeces to reduce the accumulation of sediments, thereby lessening the build up of conditions that support the accumulation and survival of HAB resting stages.

Recommendation: The potential of HAB species transmission in ship ballast water needs urgent attention and studies need to be initiated to identify the dimension of the problem in Europe, while addressing in particular (a) survival capabilities of species while in transit to EU waters and (b) assessing the risk arising from ballast tank bottom sediments containing dormant cysts and other resting stages of HAB.
Justification: ballast water volumes transmitted intercontinentally increase steadily. The intended offshore ballast water exchange strategy presently promoted by IMO through MARPOL, seems not only to reduce some risks associated with transfer but to pose new threats when carrying bloom species to other areas. Studies should be initiated to follow the fate of HAB species after release into new habitats, including inshore and offshore bloom species. The number of cysts in ballast tank bottom sediments is astronomical.

8.4 Control

Control methodologies can be categorised as "direct" or "indirect" depending on whether the effort targets the HAB specifically, or strives to reduce their impacts, such as through bloom prediction or through alteration of pollution inputs, which can stimulate bloom growth. The latter have been discussed above under impact prevention or impact reduction. General categories of direct control include chemicals or additives to kill algal cells in the water column, physical removal of the cells by mechanical means, flocculant addition to scavenge cells and transport them to the sediments, or biological control via the introduction of a predator or other pathogenic agent which can destroy HAB cells.
Direct control strategies are by far the most controversial means of mitigation, due to concerns about environmental impact, as discussed above. As a result, very little research has been conducted in this area. With increasing pressures from aquaculturists, regulatory agencies, and the general public to reduce the impacts of HAB phenomena, it is important that aspects of direct control be explored at a fundamental level. This will provide information needed to evaluate the efficacy of proposed mitigation strategies. At present, these evaluations are based more on "gut-feeling" reactions than on sound science.

8.4.1 Biological control

Predation and mortality of HAB species are critical elements of bloom dynamics, but are also routes to explore for potential control strategies. Zooplankton, which graze on HAB species, have been proposed for bloom control (Shirota 1989). Zooplankton grazing rates have been investigated in laboratory and field studies several HAB species, but this knowledge needs to be expanded. Similarly, studies on bivalve filtration rates and long-term effects of HAB species upon bivalve feeding behaviour are needed to elucidate aspects of HAB dynamics and to identify opportunities to exploit these predator-prey interactions to mitigate or control blooms. For example, the establishment of populations of benthic filter feeders might be an effective strategy to control populations of HAB in certain areas.
Viruses could be highly specific and effective control agents. They are abundant in marine systems, replicate rapidly and tend to be host-specific, suggesting that a single algal species could be targeted - the ultimate "magic bullet". In reality, however, viruses are sometimes so host-specific that they are unable to infect different genetic strains of the same host species, and we know that HAB can be genetically heterogeneous. Clearly, the nature of viral-HAB interactions needs to be explored, not only with respect to the impacts on bloom dynamics in natural waters, but also in the context of control or mitigation.

Similarly, a variety of parasites infect marine phytoplankton, including HAB species. As with the viruses, a key issue is that of host specificity, but this is an area where little is known with respect to HAB species. Resolution of these specificity issues is needed.

Bacteria could also be employed to control HAB (Doucette et al., 1998). An intriguing example is a *Gymnodinum mikimotoi* - killing bacterial strain, which exhibits strong and specific algicidal activity. Cultures of *G. mikimotoi* were completely destroyed within 24-38 hours of exposure to the bacterium due to an unknown "killing substance" produced in response to exudates from the dinoflagellate. The compound's algicidal activity was restricted to *G. mikimotoi* and another closely-related species but had no effect on other algae tested. Studies of such bacteria have thus far been confined to basic investigations of the nature of the interaction. No field applications have yet been attempted.

Issues: It is clear that members of the planktonic microbial community as well as larger grazers (both planktonic and benthic) can have profound impacts upon HAB population dynamics, but we have little knowledge of the underlying mechanisms, nor do we have quantitative estimates of their impacts on bloom dynamics. Such knowledge is critical to our understanding of HAB, but is also necessary if these approaches are to be explored for mitigation. A scientific assessment of the feasibility of biological control of this type requires investigations into HAB population dynamics, with emphasis on these microbial-and grazer-mediated mortality or loss factors.

8.4.2 Physical/chemical control

The only large-scale application of a chemical to control an HAB was in 1957 when copper sulphate was spread over 16 square miles of a red tide bloom in the Gulf of Mexico (Rounsefell and Evans 1958). Evaluation after the fact led to the conclusion that the treatment was too expensive and non-specific. Chemical control has been largely ignored ever since because of strong environmental objections and negativism and a conviction that a "magic chemical bullet" that will somehow kill only a specific, targeted HAB species probably does not exist. This line of inquiry should not be dropped entirely, as chemicals are used successfully in the attack on terrestrial pests, especially in the context of integrated pest management, in which biological and chemical approaches are used in tandem to minimise the impacts of each. Similar strategies might someday be useful against HAB.

One control strategy that has considerable promise involves the use of flocculants - materials that, when added to water, precipitate and scavenge co-occurring particles as they fall to the sediments below (Shirota 1989). The surface charge on phytoplankton cells attracts them to opposite charges on the flocculants, forming aggregates, which will eventually fall to bottom sediments. Removal efficiencies of 95-99% have been achieved in laboratory cultures using clay, and small- and large-scale field trials near fish farms have also been successful, though not well-documented. The prospects for this mitigation strategy look good, but considerable research is first needed, especially at the ecosystem or community level. Critical unknowns include the fate and effects of sedimented cells and toxins on bottom-dwelling animals and the collateral mortality of co-occurring planktonic organisms.

Other direct bloom interventions should also be considered. Physical removal of cells from the water using screens or air sparging is possible using a number of different devices, but none are presently capable of this at a scale applicable to most HAB. Development efforts at this engineering level may lead to improvements in capabilities and ultimately to practical applications on natural blooms in the future.

Issues: A number of physical/chemical strategies have potential for HAB mitigation, but considerable development effort is needed to bring these to the application stage. There is also a need to assess the ecological and environmental effects of these strategies as well.

8.5 Conclusions

It is evident that our ability to mitigate the impacts of HAB is limited and inadequate at present. To enhance this capability in the future, we first need to increase our understanding of the mechanisms underlying HAB and the manner in which they affect ecosystems and fisheries resources. For example, direct bloom control will not be possible without a thorough understanding of the critical "control points" of HAB population dynamics, which could be exploited in mitigation strategies. Some HAB are small or localised, either permanently or during key stages of development. A widespread marine bloom that would be neither economically nor logistically feasible to control might, on closer inspection, be localised and accessible at an earlier point in time, such as at the stage in an HAB organism's life cycle when cysts germinate as a bloom inoculum.

In addition to this need for understanding of HAB dynamics, we must also invest in research on a variety of methods for impact prevention (such as models and early warning systems) as well as for impact reduction (depuration technologies, pollution control strategies). There are many different types and scales of mitigation strategies, and all are poorly supported by fundamental research at this time. Targeted studies are needed on many of the specific approaches, emphasising the fundamental science behind the strategy. Studies of grazer- or pathogen-induced mortality as a factor in HAB decline can provide information needed to determine whether direct biological control of an HAB would ever be feasible. Useful and fundamental science can be targeted on these important questions, leading ultimately to practical results. In this manner, the emphasis in EUROHAB will be on investigations of solutions to HAB problems, not simply investigations of the problems.

9. OTHER NATIONAL AND INTERNATIONAL ACTIVITIES

There are a large number of potential linkages with other international, national and regional programmes and initiatives.

Indeed over the past ten years initiatives for improved understanding of the processes underlying HAB occurrences, improved monitoring, management and mitigation, and improved training and capacity building, have been taken at the national, regional and international level. These initiatives vary significantly in impact and many of them are still in a preliminary phase. International activities have in particular focused on enhanced HAB research and monitoring capabilities in developing countries.

The HAB Programme of the Intergovernmental Oceanographic Commission (of UNESCO) includes scientific, educational and operational elements. Concrete results are accessible at http://ioc.unesco.org/iocweb/activities/ocean_sciences/oslr.htm.
Scientific working groups on HAB have in particular been established by, alone or jointly with the IOC, the Scientific Committee on Oceanic Research (SCOR) and the International Council for the Exploration of the Seas (ICES).

A new initiative is the international SCOR-IOC science programme called GEOHAB, the Global Ecology and Oceanography of Harmful Algal Blooms. The scientific goal of GEOHAB is to: determine ecological and oceanographic mechanisms underlying the population dynamics of harmful algae, by the integration of biological and ecological studies with chemical and physical oceanography, supported by improved observation systems. GEOHAB research projects will have to be funded through national or regional funding sources.

Another relevant international undertaking is the establishment of the Global Ocean Observing System (GOOS) a co-ordinated international system for gathering data about the oceans and seas in order to meet the needs of the world community of users of the oceanic environment. Harmful Algal Blooms (HAB) have been identified as a major problem by the Coastal (C-GOOS), the Health of the Oceans (HOTO), and the Living Marine Resources (LMR) panels. In particular, the priorities of C-GOOS include the design and implementation of HAB observation systems on regional to global scale.

A regional activity which is relevant in terms of strengthening monitoring networks and to provide quality assured data is the phytoplankton network under HELCOM. Within this activity, a time series of phytoplankton species composition, including HAB species, over the last two decades has been collected from the open sea areas of the Baltic Sea. In addition to species data, the HELCOM data include measurement data on several environmental factors relevant for HAB dynamics.

In the United States several funding agencies have joined forces to establish a programme on research of harmful algae: ECOHAB (The Ecology and Oceanography of Harmful Algal Blooms)- A National Research Agenda. This programme started in the autumn 1997, and is expected to run for a 10 years period. The agencies supporting this research programme are: the National Science Foundation (NSF), National Oceanic and Atmospheric Administration (NOAA), Environmental Protection Agency (EPA), National Aeronautics and Space Administration (NASA), and Office of Naval Research (ONR).

The Global Ocean Ecosystem Dynamics (GLOBEC) Program, established by SCOR and the IOC in 1991, addresses the need to understand how changes in the global environment will affect the abundance, diversity and production of animal populations comprising a major component of the ocean's ecosystems. HAB are often associated with a switch in phytoplankton composition which have significant effects on the trophic dynamics linking nutrients and primary production to zooplankton and fisheries.

The United Nations World Health Organisation (WHO) has prepared Guidelines for Safe Recreational-water Environments. Guidelines are provided for health authorities and the general public on the risk in coastal and fresh waters of human health hazards due to algal toxins as well as precautionary measures. The Guidelines have been prepared by the European Centre for Environment and Health, WHO Rome Division, and European expertise have contributed significantly to their preparation.

Accidental transfer of HAB species by human activities have been addressed in conjunction with general considerations on introductions of aquatic organisms. ICES has through its long-standing 'Working Group on Introductions and Transfers of Marine Organisms' developed an international 'Code of Practice' to minimise ecological risks arising from the introductions.

The International Office of Epizooties (IOE) has over the past years also been working with the transfer of non-indigenous aquatic species. IOE has established a data-base including reference to HAB species.

Transfer by ballast of ships of harmful aquatic organisms and pathogens, causing injury to public health and damage to property and the environment has been recognised as a major problem internationally. Harmful marine microalgae is one group of organisms of concern in this respect. Accordingly the International Maritime Organisation (IMO) has adopted international Guidelines for the Control and Management of Ships' Ballast Water to Minimise the Transfer of Harmful Aquatic Organisms and Pathogens (Resolution A.868(20)). IMO is currently preparing legally binding provisions in this field.

The research proposed by EUROHAB would utilise, coordinate and expend the expertise developed by European scientists within a number of recent and ongoing projects supported by the EU and European science agencies. These projects include NUTOX, DOMTOX, Project on Ballast waters and COMWEB (MAST III Programme) focused on monitoring and control of harmful toxic algal blooms, EHUX and MEICE, focused on algal physiology, EULIT and CLEAN, on controlling factors of harmful blooms dynamics, NIRO on the modelling of algal bloom formation, as well as BASIC, PHAEOCYSTIS and EROS 21 (ENVIRONMENT&CLIMATE Programme).

10. REFERENCES

Anderson D.M. 1994. Red Tides. Scient. American 271:52-58

Anderson D.M. 1995. Identification of harmful algal species using molecular probes: an emerging technology. In: Harmful Marine Algal Blooms. Eds. Lassus P., Arzul G., Erard E., Gentien P. and C. Marcaillou. Lavoiser Science Publishers, Paris. pp. 3-13.

Anderson D.M. 1997. Turning back the harmful red tide. Nature 338:513-514.

Anonymous 1998. Report of the ICES-IOC Working Group on Harmful Algal Bloom Dynamics. ICES-CM/1998/C:4, 80.

Arzul G., Erard-Le Denn E., Belin C. and Nézan E. 1995. Ichthyotoxic events associated with *Gymnodinium cf. nagasakiense* on the Atlantic coast of France. Harmful Algae News 12/13: 8-9.

Bell S.G. and Codd G.A. 1996. Detection, analysis and risk assessment of cyanobacterial toxins. In: *Agricultural Chemicals and the Environment.* Eds. Hester R.E. and Harrison R.M., The Royal Society of Chemistry, Cambridge, 109-122.

Berlin R. 1948. Haff disease in Sweden. Acta. Med. Scand. 129: 560-572.

Botsford L.W., Castilla J.C. and Peterson C.H. 1997. The management of fisheries and marine ecosystems. Science 277: 509-515.

Bryant A.D. Heath M., Gurney W.S.G. and Beare D.J. 1997. The seasonal dynamics of *Calanus finmarchicus*: development of a three-dimensional structured population model and application to the northern North Sea. J. Sea Res. 38,3-4: 361-379.

Bursa A. 1968. Epicenoses on *Nodularia spumigena* Mertens in the Baltic Sea. Acta Hydrobiol. 10: 267-297.

Bury N.R., Codd G.A., Wendelaar Bonga S.E. and Flik G. 1998. Fatty acids from the cyanobacterium *Microcystis aeruginosa* with potent inhibitory effects on fish gill Na^+/K^+-ATPase activity. J. Exp. Biol. 201: 81-89.

Buskey E.J., Montagna P.A., Amos A.F. and Whitledge T.E. 1997. Disruption of grazer populations as a contributing factor to the initiation of the Texas brown tide algal bloom. Limnol. Oceanogr. 42: 1215-1222.

Cadée G.C. and Hegeman J. 1993. Persisting high levels of primary production at declining phosphate concentrations in the Dutch coastal area (Marsdiep). Neth. J. Sea Res. 31: 147-152.

Carlton J.T. 1985. Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. Oceanogr. Mar. Biol. Ann. Rev. 23: 313-371.

Carlton J.T. and Geller, J.B. 1993. Ecological roulette: The global transport of non-indigenous marine organisms. Science (Wash.) 261(5117): 78-82.

Carpenter E.J., Janson S., Boje R., Pollehne F. and Chang J. 1995. The dinoflagellate *Dinophysis norvegica*: biological and ecological observations in the Baltic Sea. Eur. J. Phycol. 30: 1-9.

Christoffersen K. 1996. Ecological implications of cyanobacterial toxins in aquatic food webs. Phycologia 6: 42-50.

Codd G.A. 1998. Cyanobacterial blooms and toxins in fresh-, brackish and marine waters. In: *Harmful Algae*. Proceedings of the VIII International Conference on Harmful Algae. Eds. Reguera B., Blanco J., Fernandez M.L and Wyatt T., Xunta de Galicia, Intergovernmental Oceanographic Commission of Unesco, 13-17.

Dahl E. and Tangen K. 1990. *Gyrodinium aureolum* bloom along the Norwegian coast in 1988. In: *Toxic marine phytoplankton.* Eds. Granéli E., Sundström B., Edler L. and Anderson D.M., Elsevier Sci. Publish., New York, 123-127.

Dahl E., Lindahl O., Paasche E. and Throndsen J. 1988. The *Chrysochromulina polylepis* bloom in Scandinavian waters during spring 1988. In: *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms.* Eds. Cosper E.M., Bricelj M. and Carpenter E.J. Springer-Verlag, Berlin. 383-405.

Dale B. and Yentsch C.M. 1978. Red tide and paralytic shellfish poisoning. Oceanus 21: 41-49.

Dale B., Madsen A., Nordberg K. and Thorsen T.A. 1993. Evidence for prehistoric and historic 'blooms' of the toxic dinoflagellate *Gymnodinium catenatum* in the Kattegat-Skagerrak region of Scandinavia. In: *Toxic Phytoplankton Blooms in the Sea* Eds. Smayda T.J. and Shimizu Y., Elsevier, New York, 47-52

Dale B., Thorsen T. A. and Fjellså A. in press. Dinoflagellate cysts as indicators of cultural eutrophication in the Oslofjord, Norway. Est. Coastal Shelf Science.

Delgado M., Garces E., Vila M. and Camp J. 1997. Morphological variability in three populations of the dinoflagellate Alexandrium taylori. J. Plankton Res. 19: 749-775.

Edvardsen B. and Paasche E. 1998. Bloom dynamics and physiology of *Prymnesium* and *Chrysochromulina.* In: *Physiological Ecology of Harmful Algal Blooms.* Eds Anderson D.M., Cembella A.D. and Hallegraeff G.M., Springer-Verlag, Berlin. NATO ASI Series, Vol. G41, 193-208.

Edvardsen, B. and Medlin, L.K. 1998. Genetic analysis of authentic and alternate forms of *Chrysochromulina polylepis* (Haptophyta). Phycologia, 37: 275-283.

Egmond van H.P., Aune T., Lassus P., Speijers G.P.A. and Waldock M. 1993. Paralytic and diarrhoeic shellfish poisons: occurence in Europe, toxicity, analysis and regulation. Jour. Nat. Toxins 2: 41-83.

Ellner S. and Turchin P. 1995. Chaos in a noisy world – new methods and evidence from time-series analysis. Am. Nat. Vol. 145,3: 343-375.

Figueiras G.F., Alvarez-Salgado A., Castro G.E. and Villarino M.L. 1998. Accumulation of *Gymnodinium catenatum* Graham cells in western Iberian shelf waters in response to poleward flowing slope currents. In: *Harmful Algae.* Eds, Reguera B., Blanco J., Fernandez M.L. and Wyatt T. Punta de Galicia and IOC of Unesco, 114-117.

Fonda Umani S., Ghirardelli E. and Specchi M. 1989. Gli episodi di "mare sporco" nell'Adriatico dal 1729 ai giorni nostri. Regione Autonoma del Friuli-Venezia Giulia. Pubb. Direz. Region. Ambiente, Trieste, 178 p.

Fraga S. 1987. Government-science-aquaculture relationship: Case history - Spain. In: *Proceedings: International Conference and Workshop on the Problems of Toxic Dinoflagellate Blooms in Aquaculture.* Eds. Dale B., Baden D. G., Mck. Barry B., Edler L., Fraga S., Jenkinson I R., Hallegraeff G. M., Okaichi T., Tangen K., Taylor F. J. R., White A.W., Yentch C. M. and Yentch C. S., Sherkin Island Marine Station, Sherkin Island, Co. Cork, Ireland, 26-27.

Fraga S., Anderson D.M., Bravo I., Reguera B., Steidinger K.A. and Yentsch C.M. 1988. Influence of upwelling relaxation on dinoflagellates and shellfish toxicity in Ria de Vigo, Spain. Estuar. Coast. Shelf Sci. 27: 349-361.

Franca S. 1991. Dinoflagellate toxicity: harmful effects, warning measures and research trends in Portugal. In: *Proceedings of Symposium on Marine Biotoxins.* Eds. Fremy J.M., Maisons-Alfort, C.N.E.V.A., 229-233.

Gavrilets S. and Hastings A. 1994. Dynamics of genetic-variability in 2-locus models of stabilizing selection. Genetics. 138,2: 519-532.

Gentien P. 1998. Ecophysiology and bloom dynamics of the *Gymnodinium mikimotoi* species complex. In: *Physiological ecology of harmful algal blooms* . Eds.. Anderson D.M, Cembella A.D. and Hallegraeff G.M., Springer-Verlag, Berlin, NATO-ASI series Vol. G 41.

Gentien P., Lunven M., Lehaitre M. and Duvent J.L. 1995. In situ depth profiling of particle sizes. Deep-Sea Res. 42: 1297-1312.

Gerdts G., Hummert C., Luckas B. and Schütt C. 1999a. A fast fluorimetric assay for the detection of saxitoxin in natural dinoflagellate blooms(in prep.)

Gerdts G., Wichels A., Pahler S., Müller W.E.G. and Schütt C. 1999b. Bacterial diversity in toxic *Alexandrium tamarense* blooms of the Orkney Islands and the Firth of Forth as described by DGGE (in prep.).

Granéli E., Anderson D.M., Carlsson P. and Maestrini S.Y. 1997. Light and dark carbon uptake by *Dinophysis* species in comparison to other photosynthetic and heterotrophic dinoflagellates. Aquat. Microb. Ecol. 13: 177-186.

Green J.C. and Leadbeater B.S.C. (eds.) 1994. *The Haptophyte Algae.* Systematics Association Special Volume No. 51. Clarendon Press, Oxford. 446 pp.

Gussmann H. and Bicks B. 1985. Vergiftungen bei Jungrindern durch die Blaualge *Nodularia spumigena* Monatsch. (Poisoning of young cattle by the blue-green alga *Nodularia spumigena* Monatsch. Veterinaermedizin 40: 76-79 (in German).

Hallegraeff G.M. 1993. A review of harmful algal blooms and their apppparent global increase. Phycologia 32: 79-99.

Hallegraeff G.M. 1998. Transport of toxic dinoflagellates via ships' ballast water: bioeconomic risk assessment and efficacy of possible ballast water management strategies. Mar. Ecol. Prog. Ser. 168: 297-309.

Hastings A. and Harrison S. 1994. Metapopulation dynamics and genetics. An. Rev. Ecol. Syst. 25: 167-188.

Hayes K.R. 1998. Ecological risk assessment for ballast water introductions: A suggested approach. ICES J. Mar. Sci. 55: 201-212.

Hayes P.K and Barker G.L.A. 1997. Genetic diversity within Baltic Sea populations of *Nodularia* (Cyanobacteria). J. Phycol. 33: 919-923.

Heath M., Robertson W., Mardaljevic J. and Gurney. 1997. Modelling the population dynamics of Calanus in the Fair Isle Current off northern Scotland. J. Sea Res. 38,3-4: 381-412.

Herndl G.J. 1992. Marine snow in the northern Adriatic Sea: possible causes and consequences for a shallow ecosystem. Mar. Microb. Food Webs 6: 149-172.

Holligan P.M., Aarup T. and Groom S.B. 1989. The North Sea satellite colour atlas. Continent. Shelf Res. 9: 667-765.

Honjo T. 1994. Overview on prediction and prevention of red tides causing death of marine life. Farm. Japan 28: 9-15.

Honjo T., Imada N., Oshima Y., Maema Y., Nagai K., Matsuyama Y. and Uchida T. 1998. Potential transfer of *Heterocapsa circularisquana* with pearl oyster consignements. In: *Harmful Algae.* Eds. Reguera B., Blanco J., Fernandez M.L. and Wyatt T., Xunta de Galicia, Vigo; IOC-UNESCO, Paris, 224-226.

Hummert C., Gerdts G., Schütt C. and Luckas B. 1999. On board measurments of PSP toxicity and PSP profiles in natural algae communities by application of an automated HPLC system. (in prep.)

ICES, 1998. Report of the Working Group on Environmental Interactions of Mariculture. ICES CM 1998/F:2, 1-61 pp plus Appendices (Mariculture Committee, Ref. Advisory Committee on the Marine Environment, Environmental Quality Committee).

IMO 1993. Guidelines for preventing the introduction of unwanted aquatic organisms and pathogens from ships' ballast water and sediment discharges. IMO Assembly, 18th Session, A18/RES. 14 pp.

Johnston A.M. and Kennedy H. 1998. Carbon stable isotope fractionation in marine systems: Open ocean studies and laboratory studies. In: *Stable Isotopes and the integration of biological, ecological and geochemical processes.* Ed. Griffiths H. BIOS Scientific Publishers Ltd, Oxford. pp. 239-256.

Jones H.L.J., Leadbeater B.S.C. and Green J.C. 1993. Mixotrophy in marine species of *Chrysochromulina* (Prymnesiophyceae): ingestion and digestion of a small green flagellate. J. Mar. Biol. Ass. Unit. Kingdom 73: 283-296.

Kaas H., Larsen J., Mohlenberg F. and Richardson K. 1991. The *Chrysochromulina polylepis* bloom in the Kattegat (Scandinavia) May-June 1988. Distribution, primary production and nutrient dynamics in the late stage of the bloom. Mar. Ecol. Prog. Ser. 79: 151-161.

Kahru M., Horstmann U. and Rud O. 1994. Satellite detection of increased cyanobacteria blooms in the Baltic Sea: natural fluctuation or ecosystem change? Oceanology 23: 469-472.

Kiørboe T. 1993. Turbulence, phytoplankotn cell size and the structure of pelagic food-webs. Adv. Mar. Biol. 29: 1-72.

Kononen K. and Leppänen J-M. 1997. Patchiness, scales and controlling mechanisms of the cyanobacterial blooms of the Baltic Sea: applications of a multiscale research strategy. In: *Monitoring Algal Blooms: New Techniques for Detecting Large-Scale Environmental Change.* Eds. Kahru M. and Brown C.W., Landes Bioscience, Austin Texas, 63-84.

Kononen K., Kuparinen J., Makela K., Laanmemets J., Pavelson J. and Nommann S. 1996. Initiation of cyanobacterial blooms in a frontal region at the entrance to the Gulf of Finland, Baltic Sea. Limnol. Oceanogr. 41: 98-112.

Lancelot C. 1995. The mucilage phenomenon in the continental coastal waters of the North Sea. Science Total Environ. 165: 83-102.

Lancelot C. and Billen G. 1985. Carbon-nitrogen relationships in nutrient metabolism of coastal marine ecosystems. Adv. Aquat. Microbiol. 3: 263-321.

Lancelot C., Billen G., Sournia A., Weisse T., Colijn F., Veldhuis M.J.W., Davies A. and Wassman P. 1987. *Phaeocystis* blooms and nutrient enrichment in the continental coastal zones of the North Sea. Ambio 16: 38-46.

Lancelot C., Panin N. and Martin J.-M. 1998. The northwestern Black Sea: a pilot site to understand the complex interaction between human activities and the coastal environment.. Third European Marine Science and Technology Conference, Lisbon 23-27 May 1998. Project Synopses, volume II: Strategic Marine Research. 38-46.

Larsen A. and Medlin L.K. 1997. Inter- and intraspecific genetic variation in twelve *Prymnesium* (Haptophyta) clones. J. Phycol. 33: 1007-1015.

Lassus P. 1988. Plankton toxique et plancton d'eaux rouges sur les cotes europeennes. IFREMER, Nantes.

Lehtimäki J., Moisander P., Sivonen K. and Kononen K. 1997. Growth, nitrogen fixation and nodularin production by two Baltic Sea cyanobacteria. Appl. Env. Microbiol. 63: 1647-1656.

Lindholm T. 1998. Algfenomen och algproblem (Algal phenomena and algal problems). CIP Cataloguing and Publishing, Abo, Finland 168pp (in Swedish).

Little S., Ellner S., Pascual, M. and Neubert, M. 1996. Detecting nonlinear dynamics in spatio-temporal systems nples from ecological models. PHYSICA D. 96,1-4: 321-333.

McMahon T. and Silke J. 1998. Algal blooms and algal toxicity in Irish coastal waters. In: *Eutrophication in Irish Waters.* Ed. Wilson J.G., Royal Irish Acadely, Dublin, 106-114.

Medlin L.K. and Simon N., 1997 Molecular phylogeny of the phytoplankton. In: *Molecular analysis of the Oceans.* Ed. Cooksey, K. Chapmann and Hall. 161-186.

Medlin L.K., Lange M. and Baumann M.E.M. 1994. Genetic differentiation among three colony-forming species of *Phaeocystis.* Phycologia 33: 199-212.

Merceron M. 1987. Mortalités de poissons en baie de Vilaine (juillet 1982). Causes - Mécanismes - Propositions d'action. IFREMER, DERO-87.14-EL, 100.

Miller P.E. and Scholin C.A. 1998. Identification and enumeration of cultured and wild *Pseudo-nitzschia* (Bacillariophyceae) using species-specific LSU rRNA-targeted fluorescent probes and filter-based whole cell hybridization. J. Phycol. 34: 371-382.

Moestrup Ø. 1994. Economic aspects:'blooms', nuisance species, and toxins. In: *The Haptophyte Algae.* Eds. Green J.C. and Leadbeater B.S.C., Clarendon Press, Oxford, 265-285.

Nehring S. 1993. Mortality of dogs associated with a mass development of *Nodularia spumigena* (Cyanophyceae)in a brackish lake at the German North Sea coast. J. Plankton Res. 15: 867-872.

Oliver R.L. 1994. Floating and sinking in gas-vacuolate cyanobacteria. J. Phycol. 30: 161-173.

Ono K., Arakawa O., Onoue Y., Matsumoto S., Shihara H., Takeda K., Nakanishi M., Yamaji T., Uemura K. and Ogawa Y. 1998. Synthetic surfactants for protecting cultured fish against toxic phytoplankton. Aquacult. Res 29: 569-572.

Ozaka Y. 1985. Mutsu Bay (in Japanese). In: *Toxic Dinoflagellates - Implication in shellfish poisoning.* Ed. Fukuyo Y. Bull. Jap. Assoc. Sci. Fish., 56, 59-70.

Paerl H.W. 1996. A comparison of cyanobacterial bloom dynamics in freshwater, estuarine and marine environments. Phycologia 6: 23-35.

Pascual M. and Caswell H. 1997. From the cell cycle to population cycles in phytoplankton-nutrient interactions. Ecology. 78,3: 897-912.

Pauly D., Christensen V., Dalsgaard J., Froese R. and Torres F. Jr. 1998. Fishing down marine food webs. Science 279: 860-863.

Pazos Y. and Maneiro J. in press. Algal bloom detection, monitoring and prediction in the Galician Rias (NW Spain). Algal Bloom Detection, Monitoring and Prediction, Third Workshop, Instituto Superiore di Sanita, Roma 16-18 Febryary 1998, EC Contract ENV5-CT96-0355 (DG12-EHKN).

Pedini M. and Shehadeh Z.H. 1997. Global outlook. Review of the state of world aquaculture. FAO, Roma, FAO Fisheries Circular, 886: 30-37.

Penna N., Rinaldi A., Montanari G., Di Paolo A. and Penna A. 1993. Mucilaginous masses in the Adriatic Sea in the summer of 1989. Wat. Res. 27: 1767-1771.

Rana K.J. 1997. Trends in global production, 1984-1995. Review of the state of world aquaculture. FAO, Roma, FAO Fisheries Circular, 886: 3-6.

Reguera B., Blanco J., Fernandez M.L., Wyatt T. 1998. Harmful Alga. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO 1998. 635 pp.

Rhodes L., Scholin C., Garthwaite I., Haywood A. and Thomas A. 1998. Domoic acid production *Pseudo-nitzschia* species educed by whole cell DNA probe-based and immunochemical assays. In: *Harmful Algae.* Eds. Reguera B., Blanco J., Fernandez M.L., Wyatt T. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO 1998. 274- 277.

Riegman R., Colijn F., Malschaert J.F.P., Kloosterhuis H.T. and Cadée G.C. 1990. Assessment of growth rate limiting nutrients in the North Sea by the use of nutrient-uptake kinetics. Neth. J. Sea Res. 26: 53-60.

Rodger H.D., Turnbull T., Edwards C. and Codd G.A. 1994. Cyanobacterial (blue-green algal) bloom associated pathology in brown trout, *Salmo trutta* L. in Loch Leven, Scotland. J. Fish. Dis. 17: 177-181.

Rosenberg R., Lindahl O. and Blanck H. 1988. Silent Spring in the Sea. Oceanology 17: 289-290.

Rosenthal H. 1980. Implications of transplantations to aquaculture and ecosystems. Mar. Fish. Rev. 42(5): 1-14.

Rounsefell G.A. and. Evans J.E. 1958. Large-scale experimental test of copper sulfate as a control for the Florida red tide. U.S. Fish Wildlife Serv. Spec. Sci. Rep. 270.

Sætre M.M.L., Dale B., Mohammed I.A. and Sætre G-P 1997. Dinoflagellate cysts as potential indicators of industrial pollution in a Norwegian fjord. Marine Environmental Research 44 (2): 167-189.

Sandén P. and Hakansson B. 1996. Long-term trends in Secchi depth in the Baltic Sea. Limnol. Oceanogr. 41: 346-351.

Scholin C.A., Hallegraeff G.M. and Anderson D.M. 1995. Molecular evolution of the *Alexandrium tamarense* 'species complex'(Dinophyceae): dispersal in the North American and West Pacific regions. Phycologia 34: 472-485.

Schöllhorn E. and Granéli E. 1996. Influence of different nitrogen to silica ratios and artificial mixing on the structure of a summer phytoplankton community from the Swedish west coast (Gullmar Fjord). J. Sea Res. 35: 159-167.

Shirota A. 1989. Red tide problem and countermeasures (2). Int. J. Aq. Fish. Technol. 1:195-293.

Sivonen K. 1996. Cyanobacterial toxins and toxin production. Phycologia 6: 12-24.

Sivonen K. 1998. Toxins produced by cyanobacteria. In: *Mycotoxins and Phycotoxins*. Eds. Miraglia M., van Egmond H., Brera C. and Gilbert J. Alaken Inc., Fort Collins, Colrado USA, 547-568.

Sivonen K., Kononen K., Carmichael W.W., Dahlem A.M., Rinehart K.L., Kiviranta J. and Niemela S.I. 1989. Occurrence of the hepatotoxic cyanobacterium *Nodularia spumigena* in the Baltic Sea and the structure of the toxin. Appl. Env. Microbiol. 55: 1990-1995.

Smayda T.J. 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol. Oceanogr. 42: 1137-1153.

Solow A.R. 1994a. Detecting change in the composition of a multispecies community. Biometrics. 50,2: 556-565.

Solow A.R. 1994b. On the Bayesian-estimation of the number of species in a community. Ecology. 75,7: 2139-2142.

Taroncher-Oldenburg G. and Anderson D.M. 1998. Identification by differential display of genes expressed concurrently with saxitoxin biosynthesis in the red dinoflagellate *Alexandrium fundyense*. In: *Harmful Algae*. Eds. Reguera B., Blanco J., Fernandez M.L., Wyatt T. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO 1998. 331-332.

Ter Braak C.J.F. and Juggins S. 1993. Weighted averaging partial least-squares regression (WA-PLS) – an improved methods for reconstructing environmental variables from species assemblages. Hydrobiologia. 269: 485-502.

Thiel T. 1994. Genetic analysis of cyanobacteria. In: *The Molecular Biology of Cyanobacteria*. Ed. Bryant D.A. Kluwer, Dordrecht, 581-611.

Thorsen T.A. and Dale B. 1998. Climatically influenced distribution of Gymnodinium catenatum during the past 2000 years in coastal sediments of southern Norway. Palaeogeogr., Palaeoclimatol., Palaeoecol. 143: 158-177

Tobiesen A. 1991. Growth rates of *Heterophrys marina* (Heliozoa) on *Chrysochromulina polylepis* (Prymnesiophyceae). *Ophelia* 33: 205-212.

Tyrrell J.V., Bergquist P.R., Saul D.J., MacKenzie L. and Bergquist P.L. 1997. Oligonucleotide probe technology as applied to the study of harmful algal blooms. N. Z. J. Mar. Freshwat. Res. 31: 551-560.

Underdal B., Skulberg O.M., Dahl E. and Aune T. 1989. Disastrous bloom of *Chrysochromulina polylepis* (Prymnesiophyceae) in Norwegian coastal waters 1988 - Mortality in marine biota. Oceanology 18: 265-270.

Van Bleijswijk J.D.L. and Veldhuis M.J.W. 1995. *In situ* gross growth rates of *Emiliania huxleyi* in enclosures with different phosphate loadings revealed by diel changes in DNA content. Mar. Ecol. Prog. Ser. 121: 271-277

Veldhuis M.J.W., Kraaij G.W. and Timmermans K.R. (submitted) Viability of phytoplankton: are they all alive ?

Verity P.G. and Smetacek V. 1996. Organism life cycles, predation, and the structure of marine pelagic ecosystems. Mar. Ecol. Prog. Ser. 130: 277-293.

Vrieling E.G. and Anderson D.M. 1996. Immunofluorescence in phytoplankton research: applications and potential. J. Phycol 32: 1-16.

Walsby, A.E. 1994 Gas vesicles. Microbiol. Rev. 58: 94-144.

Wilhelm S.W. 1995. Ecology of iron-limited cyanobacteria: a review of physiological responses and implications for aquatic systems. Aquat. Microb. Ecol.9: 265-303.

Woods J. and Barkmann W. 1994. Simulating plankton ecosystems by the lagrangian ensemble method. Phil. Tran. Roy. Soc. B. Lon. 343,1303: 27-31.

Wulff F., Stigebrandt A. and Rahm L. 1990. Nutrient dynamics of the Baltic Sea. Ambio 19: 126-133.

Yasumoto T. and Satake 1998. New toxins and their toxicological evaluations. In: *Harmful Algae* Eds. Reguera B., Blanco J., Fernandez M.L. and Wyatt T. Xunta de Galicia Intergovernmental Oceanographic Commission of UNESCO, 461-464.

Yasumoto T., Underdal B., Aune T., Hormazabal V., Skulberg O.M. and Oshima Y. 1990. Screening for hemolytic and ichtyotoxic components of *Chrysochromulina polylepis* and *Gyrodinium aureolum* from Norwegian coastal waters. In: *Toxic Marine Phytoplankton*. Eds. Granéli E., Sundström B., Edler L. and Anderson D.M. Elsevier, New York, Lund, Sweden.

APPENDIX 1. LIST OF TERMS

akinete	resting stage of cyanobacterial cell
algicidal	causing algal mortality
allelopathy	negative interaction against functionally similar organisms
allozyme	different variants of the same functional enzyme
amino-acids	group of organic molecules containing nitrogen - building blocks for proteins
ammonification	the recycling of nitrogen-containing organic compounds causing the release of ammonium
antibody	a molecule produced by the immunosystem in direct response to an antigen and which combines specifically and reversible with antigen which caused its formation.
aquaculture	cultivation of aquatic organisms, e.g., fish, mussels, oysters
artificial neural networks	a rapid classification method for multivariate date
autotrophic	needing only inorganic nutrients for growth
benthos, benthic	life forms that are restricted to the bottom
bio-active compounds	compounds that have a negative effect on biological processes
bio-optical sensor	a sensor that measures the optical properties of water with the phytoplankton and associated detritus
bioassay	quantitative procedures in which a given organism is used for assay purposes. Used to measure trace amount of substances; this given the fact that this substance is essential or harmful to the organism.
bloom	relative dense accumulation of algae
brackish waters	water with salinity of 2 - 10 per mille
capillary electrophoresis	analytical tool to separate dissolved substances on the basis of electrical charge
cyanobacteria	bacteria with a plant-like way of live
cyst	a specialised microbial cell produced in response to adverse environmental conditions or as a part of the normal life cycle
cytotoxins	toxins that act on a cellular level
Decision Support System	system that takes diverse information and sorts in order of relevance to the topic under investigation. Some systems can query the nature or lack of the information provided, and make weighted predictions based on input information. An example is SimCoast.
denitrification	a net removal of biologically available nitrogen by conversion of nitrate to nitrogen gas
DNA	Deoxyribonucleic acid; contains the genetic code in every living organism
DNA-cell-cycle	the sequence of phases in genome size which constitute the cycle of vegetative growth and cell division
DNA/RNA probes	short strands of genetic material complementary to target regions on the DNA or RNA to recognise specific taxa
estuary, estuarine	the gradual transition of fresh- to sea-water in a river outlet
euryhaline	a species is euryhaline when it can accomodate to a wide range of salinities
eurythermal	a species is eurythermal when it can accomodate to a wide range of temperatures
eutrophic	loaded with a high concentration of nutrients
fecundation	reproduction capacity
filamentous	thread-forming
flow-cytometry	procedure in which individual cell in suspension are characterized
frontal activity	the edge between two different water types

functional gene probes	probes that target gene expression either at the mRNA level or the protein level
gas chromatography	analytical tool to separate volatile substances on the basis of polarity
gene flow	the exchange of genes between different populations
genotype	genetic constitution of an organism: i.e. the content of genetic information, either in total or with respect to one or more particular named alleles
haplodiploid	having a life cycle consisting of haploid and diploid stages
haploid	having only one set of chromosomes
hepatocytes -	specialized liver cells
hepatotoxins -	toxins acting on hepatocytes
heptapeptides	polymer of six amino acids
heterotrophic	using organic forms of nutrition
high-performance liquid chromatography	analytical tool to separate dissolved substances on the basis of polarity
immunodiagnostic -	using diagnostic tool which specifically acts on certain molecules by using antibodies
intrusion	mixing of two different water types
life-cycle	the sequence of phases of a cell which constitute the cycle of vegetative growth, cell division and sexual reproduction
lipopolysaccharide	polymer of sugars and fatty acids
mass spectrometry	analytical tool to separate substances on the basis of molecular mass
mesocosm	meso-scale experimental set-up (usually 100 - 1000 liters) for microbial foodweb studies
methylated	containing a methyl (CH_3-) group
microcystins	toxins formed by the cyanobacterium *Microcystis aeroginosa*
mixotrophy	the ability of a cell to meet its nutritional requirements using various substrates (organic and inorganic nutrients, detritus and living particles) together with photosynthesis
mutualistic	beneficial for all participants
neurotoxins	toxins acting on the nerval system of animals
nitrification	the bacteria-mediated conversion of ammonium to nitrite and finally nitrate
nitrogen fixation	the biochemical process where nitrogen gas from the atmosphere (N_2) is converted into a biologically available form
nodularin	toxin formed by the cyanobacterium *Nodularia spumigena*
nuclear magnetic resonance	analytical tool
organoleptic	using sensory organs
pelagic	the aquatic environment in deeper fresh or marine waters which is not influenced by the bottom
pentapeptide	polymer of five amino acids
phenotype	the observable characteristics of an organism, either in total or with respect to one or more particular named characteristics. The phenotype of an organism is the manifestation of gene expression in that organism.
phototactic	the ability to detect and move along a gradient of light intensity
phytoplankton	microscopically small organisms with a plant-like way of life
picoplanktonic	smallest plankton fraction (0.2-2 µm)
plankton	free floating, usually microscopic, aquatic organisms living in the pelagic environment
polymorphism	the ability of an organism to occur in two or more morphologically distinct forms (morphotypes), depending on e.g., environmental conditions or on the stage in the life cycle
primer	piece of genetic material that acts as a "start code" for molecular analyses of the genetic material

protein phosphatases	key enzymes that regulate the activity of other enzymes in cells of all organisms.
pycnocline	in a water column, the pycnocline is the layer of maximum density gradient
pyrogenic	causing fever
RAPD finger printing	Randomly amplified polymorphic DNA uses a single oligo-nuclucoeotide primer to obtain fragments of DNA; the banding patterns of these fragments represent a genetic fingerprint of an individual
RNA	ribonucleic acid; a molecule that translates the genetic code (DNA) to functional proteins
serine	an amino acid
species-specific-gene probes	RNA or DNA oligonucleotide probes that recognise a single species
threonine	an amino acid
toxin	any poison derived from a plant, animal or microorganism
turbulence	irregular motion of a fluid
upwelling	the transport of nutrient-rich deep waters to the surface, where there is enough light available for algae to grow
zooplankton	animal plankton

APPENDIX 2. ACRONYMS

ANN	artificial neural network
ASP	Amnesic Shellfish Poisoning
CZCS	Coastal Zone Color Scanner (NASA ocean colour 'proof of concept' satellite mission 1978-86)
CZE	capillary zone electrophoresis
DIC	dissolved organic carbon
DNA	Deoxyribonucleic acid: a nucleic acid consisting of deoxyribonucleotides each which contains on of the bases adenine, guanine, cytocine or thymine. DNA is repository of genetic information in all cells
DSP	Diarrhoeic Shellfish Poisoning
ELISA	enzyme-linked- immunosorbent assay
ENSO	El Niño Southern Oscillation
ESA	European Space Agency
ESA	European Space Agency
GCM	Global Circulation Model
HAB	Harmful Algal Bloom(s)
HPLC	High performance-liquid-chromatography
MAST	Marine Science and Technology Programme
MERIS	MEdium Resolution Imaging Spectrometer
MODIS	MODerate-resolution Imaging Spectrometer
NAO	North Atlantic Oscillation
NASA	National Aeronautics and Space Administration
PSP	Paralytic Shellfish Poisoning
RNA	Ribonucleic acid: a nucleic acid consisting of ribonucleuotides, each which contains on of the bases adenine, guanine, cytocine or uracil.
rRNA	the ribosomal RNA: major component of the ribosome.
SeaWiFS	Sea-viewing Wide-field of view Sensor
SST	Sea Surface Temperature

APPENDIX 3. LIST OF PARTICIPANTS

Participants of the Wokshop on Marine Harmful Algal Blooms: Research in Europe, EUROHAB

Don M. Anderson Woods Hole Oceanographic Institute Biology Department MS#32 Redfield 3-32, Woods Hole MA 02543 USA Tel:+1-508-289-2351 Fax: +1-508-289-2134 danderson@whoi.edu	Per Carlsson Deptartment of Marine Science Institute of Natural Sciences University of Kalmar Box 905, S-391 29, Kalmar Sweden Tel: +46-480447316 Fax: +46-480447305 per.carlsson@ng.hik.se
Andrea Belgrano Kristineberg Marine Research Station S-450 34 Fiskebäckskil Sweden Tel: +46-523-18500 Fax: +46-523-18502 a.belgrano@kmf.gu.se	Geoffrey A. Codd Department of Biological Sciences Univinersity of Dundee DDI 4HN Dundee U.K. Tel: +44-1382-344272 Fax: +44-1382-344275 g.a.codd@dundee.ac.uk
Dolors Blasco Instituto de Sciences del Mar P. Joan de Borbó s/n ES-08039 Barcelona Spain Tel: +34-93-2216416 Fax: +34-93-2217340 blasco@cucafera.icm.csic.s	Barrie Dale Department of Geology University of Oslo, P.O. Box 1047 Blindern N-0316 Oslo 3 Norway Tel: +47-22-854214 Fax: +47-22-854215 barrie.dale@geologi.uio.no
Franz Brümmer Univiversity of Stuttgart Biology Institute Pfaffenwaldring 57 D- 70550 Stuttgart Germany Tel: +49-711-6855083 Fax: + 49-711-6855096 bruemmer@po.uni-stuttgart.de	Bente Edvardsen Department of Biology/Marine Botany University of Oslo P.O. Box 1069 Blindern N-0316 Oslo Norway Tel: +47-228-54741 Fax: +47-22854438 bente.edvardsen@bio.uio.no
JoAnn Burkholder North Carolina State University Deptartment of of Botany Box 7612, NCSU, Raleigh NC 27695-7612 USA Tel: +1-919-5152726 Fax: +1-919-5153436 joann_burkholder@ncsu.edu	Henrik Enevoldsen IOC Science and Communication Centre on Harmful Algae University of Copenhagen Øster Farimagsgade 2D DK-1353 Copenhagen K Denmark Tel: +45-33-134446 Fax: +45-33-13-4447 henrike@bot.ku.dk

Susana Franca Lab. Microbiologia Experimental Instituto Nacional de Saude Ricardo Jorge Av-Padre Cruz, PT-1699 Lisboa Codex Portugal Tel: +351-1-7519200 Fax: +351-1-7590441 lmeinsa@mail.telepac.pt	Edna Granéli Department of Marine Science Institute of Natural Sciences University of Kalmar Box 905, S-391 29 Kalmar Sweden Tel: +46-480-447307 Fax: +46-480-447305 edna.graneli@ng.hik.se
Susan Gallacher Marine Laboratory Fisheries Research Services PO Box 101, Victoria Rd Aberdeen AB9 8DB U.K Tel: +44-1224-876544 Fax: +44-1224-295511 Gallachers@marlab.ac.uk	Steve Groom Remote Sensing Group CCMS Plymouth Marine Laboratory Prospect Place, West Hoe Plymouth, Devon PL1 3DH U.K. Tel: +44-1752-633150 Fax: +44-1752-633101 sbg@ccms.ac.uk
Patrick Gentien IFREMER/Centre de Brest. BP 70, F-29280 Plouzané France Tel: +33-2-98224324 Fax: +33-2-98-224548 pgentien@ifremer.fr	Åke Hagström Department of Marine Science Institute of Natural Sciences University of Kalmar Box 905, S-391 29 Kalmar Sweden Tel: +46-480-28392 Fax: +46-480-447314 ake.hagstrom@ng.hik.se
Lars-Åke Gisselson Department of Marine Science Institute of Natural Sciences University of Kalmar Box 905, S-391 29 Kalmar Sweden Tel: +46-480-447319 Fax: +46-480-447305 lars-åke.gisselson@ng.hik.se	Per Juel Hansen Marine Biology Laboratory University of Copenhagen Strandpromenaden 5, DK-3000 Helsingør Denmark Tel: +45-49-213344 Fax: +45-49-26-1165 mblpjh@inet.uni2.dk
Eli Gomes Department of Marine Science Institute of Natural Sciences University of Kalmar Box 905, S-391 29 Kalmar Sweden Tel: +46-480-447327 Fax: +46-480-447305 eli.gomes@ng.hik.se/eatgomes@acd.ufrj.br	Gerhard J. Herndl Netherlands Institute for Sea Research P.O. Box 59, 1790 AB Den Burg, Texel The Netherlands Tel: +31-222-369-507 Fax: +31-222-319-674 herndl@nioz.nl
John Graffman Nutek Liljeholmsv. 32 S-117 86 Stockholm Sweden Tel: +46-8-6819100 Fax: +46-8-196826 john.graffman@nutek.se	Lydia Ignatiades NRC, Demokritos Nat. Centre of Scient. Res., Inst. of Biology PO Box 60228, Aghia Paraskvi, GR-153 10 Athens, Greece Tel: +30-01-6503529 Fax: +30-01-6511767 igna@mail.demokritos.gr

	Sven Janson Department of Marine Science Institute of Natural Sciences University of Kalmar Box 905, S-391 29 Kalmar Sweden Tel: +46-480-447310 Fax: +46-480-447305 sven.janson@ng.hik.se
Andrew M. Johnston CEP, SCRI (Scottish Crop Research Institute) DD2 5DA Dundee UK Tel: +44-1382-562731 Fax: +44-1382562426 ajohns@scri.sari.ac.uk	Jane Lewis School of Biosciences, University of Westminster 115, New Cavendish Street London W1M 8JS U.K Tel: +44-171-9115000 Fax: +44-171-9115087 lewisjm@westminster.ac.uk
Chatarina Karlsson Department of Marine Science Institute of Natural Sciences University of Kalmar Box 905, S-391 29 Kalmar Sweden Tel: +46-480-447325 Fax: +46-480-447305 chatarina.karlsson@ng.hik.se	Gerd Liebezeit Terramare Research Centre Schleusenstr. 1 D-26382 Wilhelmshaven Germany Tel: +49-4421-9440/+49-4421-944100 Fax: +49-4421-944199 gerd.liebezeit@terramare.de
Kaisa Kononen Maj and Tor Nessling Foundation P. Hesperiankatu 3 A 4 FIN-00260 Helsinki Finland Tel: +358-9-406616 Fax: +358-9-406684 Kaisa.kononen@nessling.fi	Elisabeth Lipiatou DG XII MAST. Rue de la Loi 200 B-1049 Bruxelles Belgium Tel: +32-2-2966286 Fax: +32-2-2963024 Elisabeth.Lipiatou@dg12.cec.be
Jorma Kuparinen Finnish Institute of Marine Research P.O. Box 33 FIN-00931 Helsinki Finland Tel: +358-0-61394550 Fax: +358-0-61394494 jorma.kuparinen@fimr.fi	Bernd Luckas Institute of Nutrition and Environment University of Jena Dornburger Str. 25, D-07743 Jena Germany Tel: +49-3641-949650 Fax: +49-3641-949652 B5belu@rz.uni-jena.de
Catherine Legrand Department of Marine Science Institute of Natural Sciences University of Kalmar Box 905, S-391 29 Kalmar Sweden Tel: +46-480-447309 Fax: +46-480-447305 catherine.legrand@ng.hik.se	Serge Y. Maestrini CREMA L'Houmeau CNRS-IFREMER, BP5 F-17137 L'Houmeau France Tel: +335-46-500621 Fax: +33-5-46-500600 smaestri@ifremer.fr

Christiane Lancelot GMMA, Université Libre de Bruxelles Campus de la Plaine, CP 221, Boulevard du Triomphe, B-1050 Bruxelles Belgium Tel: +32-2-6505988/89 Fax: +32-26505993 lancelot@ulb.ac.be	Linda K. Medlin Alfred Wegener Institut Postfach 120161 D-27570 Bremerhaven Germany Tel: 49-471-4831-443 Fax: +49-471-4831-425 lmedlin@AWI-Bremerhaven.DE
M. Teresa Moita Instituto de Investigaçao das Pescas e do Mar Av Brasilia, PT-1400 Lisboa Portugal Tel: +351-1-3027000/+351-1-3021360 Fax: +351-1-3015948 tmoita@ipimar.pt	Christian Schütt BAH, Marine Station Helgoland D-27483 Helgoland Germany Tel: +49-04752-819-225 Fax: +49-4651-956200 cschuett@awi-bremerhaven.de
Sheila Newcombe Remote Sensing Group Department of APEME University of Dundee DD1 4HN Scotland Tel: +44-1382-344933 Fax: +44-1382-345415 s.k.newcombe@dundee.ac.uk	Kaarina Sivonen Department of Appl. Chem. & Microb. University of Helsinki, P.O. Box 56 Biocenter Viikki FIN-00014 Helsinki Finland Tel: +358-970859270 Fax: +358970859322 Kaarina.Sivonen@helsinki.fi
Renata Panosso Department of Limnology/Marine Ecology Ecology Building S-223 62 Lund Sweden Tel: +46-46-2228370 Fax: +46 -46 2224536 renata.panosso@marinecol.lu.se	Willem Stolte Department of Marine Science Institute of Natural Sciences University of Kalmar Box 905, S-391 29 Kalmar Sweden Tel: +46-480-447308 Fax: +46-480-447305 willem.stolte@ng.hik.se
Beatriz Reguera Centro Oceanográfico de Vigo Instituto Espanol de Oceanografia, Apartado 1552 SP-362 80 Vigo Spain Tel: +34-986-492111 Fax: +34-986-492351 beatriz.reguera@vi.ieo.es	Urban Tillmann Forschungs-und Technologiezentrum Westküste, Hafentoern D-25761 Büsum Germany Tel: +49-4834-604206 Fax: +49-4834-604299 tillmann@ftz-west.uni-kiel.de
Harald Rosenthal Institut für Meereskunde an der Universität -Kiel Düsternbrooker Weg 20, D-24105 Kiel Germany Tel: +49-431-5973916 Fax: +49-431-5973917/+49-431-565876	Marcel J.W. Veldhuis NIOZ, Landsdiep 4 Den Burg, PO Box 59 NL-1790 AB The Netherlands Tel: +31-222-369300/+31-222-369512 Fax: +31-222-319674 veldhuis@nioz.nl

Egil Sakshaug Trondhjem Biological Station, NTNU Bynesveien 46 N-7018 Trondheim Norway Tel: +47-73-591583 Fax: +47-73-591597 egil.sakshaug@vm.ntnu.no	Adriana Zingone Stazione Zoologica "A Dohrn" Villa Comunale I-80121 Napoli Italy Tel: +39-81-5833295 Fax: +39-81-7641355 zingone@alpha.szn.it

Publications to date in the *Research in Enclosed Seas Series* :

- Air-Sea-Ice Interaction – Scientific Report (W. Oost and E. Lipiatou, Eds.), EUR 18638 EN, (in press, 1999).

- Marine Research and Policy Interface – Links, Interdisciplinary Co-Operation, Availability of Results, Case Studies (M. Cornaert and E. Lipiatou, Eds), 1999

- Catalogue of publications from Marine Science and Technology projects – Mediterranean Sea, Volume 2 (E. Lipiatou, Ed.) EUR 18226 EN, 1999

- Scientific Report of the First European Conference on Progress in Oceanography of the Mediterranean Sea (E.Lipiatou, R. Mosetti, S. Heussner, A. Tselepides, J. Tintoré, R. Santoleri & A. Monaco, Eds.) EUR 18312 EN, 1998.

- Catalogue of publications from MAST projects in the Mediterranean Sea – results from EU research, Volume 1 (E. Lipiatou, Ed.) EUR 18021 EN, 1997

- Interdisciplinary research in the Mediterranean Sea : A synthesis of scientific results from the Mediterranean Targeted Project (MTP) phase I, 1993-96 (E. Lipiatou, Ed.) EUR 17786 EN, 1997

European Commission

EUR 18592 — Harmful algal blooms in European marine and brackish waters

Luxembourg: Office for Official Publications of the European Communities

1999 — VIII, 94 pp. — 17.6 x 25 cm

ISBN 92-828-6612-2

Price (excluding VAT) in Luxembourg: EUR 16.5

Venta • Salg • Verkauf • Πωλήσεις • Sales • Vente • Vendita • Verkoop • Venda • Myynti • Försäljning

BELGIQUE/BELGIË

Jean De Lannoy
Avenue du Roi 202/Koningslaan 202
B-1190 Bruxelles/Brussel
Tél. (32-2) 538 43 08
Fax (32-2) 538 08 41
E-mail: jean.de.lannoy@infoboard.be
URL: http://www.jean-de-lannoy.be

La librairie européenne/
De Europese Boekhandel
Rue de la Loi 244/Wetstraat 244
B-1040 Bruxelles/Brussel
Tél. (32-2) 295 26 39
Fax (32-2) 735 08 60
E-mail: mail@libeurop.be
URL: http://www.libeurop.be

Moniteur belge/Belgisch Staatsblad
Rue de Louvain 40-42/Leuvenseweg 40-42
B-1000 Bruxelles/Brussel
Tél. (32-2) 552 22 11
Fax (32-2) 511 01 84

DANMARK

J. H. Schultz Information A/S
Herstedvang 10-12
DK-2620 Albertslund
Tlf. (45) 43 63 23 00
Fax (45) 43 63 19 69
E-mail: schultz@schultz.dk
URL: http://www.schultz.dk

DEUTSCHLAND

Bundesanzeiger Verlag GmbH
Vertriebsabteilung
Amsterdamer Straße 192
D-50735 Köln
Tel. (49-221) 97 66 80
Fax (49-221) 97 66 82 78
E-Mail: vertrieb@bundesanzeiger.de
URL: http://www.bundesanzeiger.de

ΕΛΛΑΔΑ/GREECE

G. C. Eleftheroudakis SA
International Bookstore
Panepistimiou 17
GR-10564 Athina
Tel. (30-1) 331 41 80/1/2/3/4/5
Fax (30-1) 323 98 21
E-mail: elebooks@netor.gr

ESPAÑA

Boletín Oficial del Estado
Trafalgar, 27
E-28071 Madrid
Tel. (34) 915 38 21 11 (Libros),
913 84 17 15 (Suscrip.)
Fax (34) 915 38 21 21 (Libros),
913 84 17 14 (Suscrip.)
E-mail: clientes@com.boe.es
URL: http://www.boe.es

Mundi Prensa Libros, SA
Castelló, 37
E-28001 Madrid
Tel. (34) 914 36 37 00
Fax (34) 915 75 39 98
E-mail: libreria@mundiprensa.es
URL: http://www.mundiprensa.com

FRANCE

Journal officiel
Service des publications des CE
26, rue Desaix
F-75727 Paris Cedex 15
Tél. (33) 140 58 77 31
Fax (33) 140 58 77 00
URL: http://www.journal-officiel.gouv.fr

IRELAND

Government Supplies Agency
Publications Section
4-5 Harcourt Road
Dublin 2
Tel. (353-1) 661 31 11
Fax (353-1) 475 27 60

ITALIA

Licosa SpA
Via Duca di Calabria, 1/1
Casella postale 552
I-50125 Firenze
Tel. (39) 055 64 83 1
Fax (39) 055 64 12 57
E-mail: licosa@ftbcc.it
URL: http://www.ftbcc.it/licosa

LUXEMBOURG

Messageries du livre SARL
5, rue Raiffeisen
L-2411 Luxembourg
Tél. (352) 40 10 20
Fax (352) 49 06 61
E-mail: mail@mdl.lu
URL: http://www.mdl.lu

NEDERLAND

SDU Servicecentrum Uitgevers
Christoffel Plantijnstraat 2
Postbus 20014
2500 EA Den Haag
Tel. (31-70) 378 98 80
Fax (31-70) 378 97 83
E-mail: sdu@sdu.nl
URL: http://www.sdu.nl

ÖSTERREICH

Manz'sche Verlags- und Universitätsbuchhandlung GmbH
Kohlmarkt 16
A-1014 Wien
Tel. (43-1) 53 16 11 00
Fax (43-1) 53 16 11 67
E-Mail: bestellen@manz.co.at
URL: http://www.manz.at/index.htm

PORTUGAL

Distribuidora de Livros Bertrand Ld.ª
Grupo Bertrand, SA
Rua das Terras dos Vales, 4-A
Apartado 60037
P-2700 Amadora
Tel. (351-1) 495 90 50
Fax (351-1) 496 02 55

Imprensa Nacional-Casa da Moeda, EP
Rua Marquês Sá da Bandeira, 16-A
P-1050 Lisboa Codex
Tel. (351-1) 353 03 99
Fax (351-1) 353 02 94
E-mail: del.incm@mail.telepac.pt
URL: http://www.incm.pt

SUOMI/FINLAND

Akateeminen Kirjakauppa/
Akademiska Bokhandeln
Keskuskatu 1/Centralgatan 1
PL/PB 128
FIN-00101 Helsinki/Helsingfors
P./tfn (358-9) 121 44 18
F./fax (358-9) 121 44 35
Sähköposti: akatilaus@akateeminen.com
URL: http://www.akateeminen.com

SVERIGE

BTJ AB
Traktorvägen 11
S-221 82 Lund
Tfn (46-46) 18 00 00
Fax (46-46) 30 79 47
E-post: btjeu-pub@btj.se
URL: http://www.btj.se

UNITED KINGDOM

The Stationery Office Ltd
International Sales Agency
51 Nine Elms Lane
London SW8 5DR
Tel. (44-171) 873 90 90
Fax (44-171) 873 84 63
E-mail: ipa.enquiries@theso.co.uk
URL: http://www.the-stationery-office.co.uk

ÍSLAND

Bokabud Larusar Blöndal
Skólavördustig, 2
IS-101 Reykjavik
Tel. (354) 551 56 50
Fax (354) 552 55 60

NORGE

Swets Norge AS
Østenjoveien 18
Boks 6512 Etterstad
N-0606 Oslo
Tel. (47-22) 97 45 00
Fax (47-22) 97 45 45

SCHWEIZ/SUISSE/SVIZZERA

Euro Info Center Schweiz
c/o OSEC
Stampfenbachstraße 85
PF 492
CH-8035 Zürich
Tel. (41-1) 365 53 15
Fax (41-1) 365 54 11
E-mail: eics@osec.ch
URL: http://www.osec.ch/eics

BĂLGARIJA

Europress Euromedia Ltd
59, blvd Vitosha
BG-1000 Sofia
Tel. (359-2) 980 37 66
Fax (359-2) 980 42 30
E-mail: Milena@mbox.cit.bg

ČESKÁ REPUBLIKA

ÚSIS
NIS-prodejna
Havelkova 22
CZ-130 00 Praha 3
Tel. (420-2) 24 23 14 86
Fax (420-2) 24 23 11 14
E-mail: nkposp@dec.nis.cz
URL: http://usiscr.cz

CYPRUS

Cyprus Chamber of Commerce and Industry
PO Box 1455
CY-1509 Nicosia
Tel. (357-2) 66 95 00
Fax (357-2) 66 10 44
E-mail: demetrap@ccci.org.cy

EESTI

Eesti Kaubandus-Tööstuskoda (Estonian Chamber of Commerce and Industry)
Toom-Kooli 17
EE-0001 Tallinn
Tel. (372 [illegible]
Fax (372 [illegible]
E-mail: e[illegible]
URL: htt[illegible]

HRVATSKA

Mediatrade Ltd
Pavla Hatza 1
HR-10000 Zagreb
Tel. (385-1) 481 94 11
Fax (385-1) 481 94 11

MAGYARORSZÁG

Euro Info Service
Európa Ház
Margitsziget
PO Box 475
H-1396 Budapest 62
Tel. (36-1) 350 80 25
Fax (36-1) 350 90 32
E-mail: euroinfo@mail.matav.hu
URL: http://www.euroinfo.hu/index.htm

MALTA

Miller Distributors Ltd
Malta International Airport
PO Box 25
Luqa LQA 05
Tel. (356) 66 44 88
Fax (356) 67 67 99
E-mail: gwirth@usa.net

POLSKA

Ars Polona
Krakowskie Przedmiescie 7
Skr. pocztowa 1001
PL-00-950 Warszawa
Tel. (48-22) 826 12 01
Fax (48-22) 826 62 40
E-mail: ars_pol@bevy.hsn.com.pl

ROMÂNIA

Euromedia
Str. G-ral Berthelot Nr 41
RO-70749 Bucuresti
Tel. (40-1) 315 44 03
Fax (40-1) 314 22 86

ROSSIYA

CCEC
60-letiya Oktyabrya Av. 9
117312 Moscow
Tel. (7-095) 135 52 27
Fax (7-095) 135 52 27

SLOVAKIA

Centrum VTI SR
Nám. Slobody, 19
SK-81223 Bratislava
Tel. (421-7) 54 41 83 64
Fax (421-7) 54 41 83 64
E-mail: europ@tbb1.sltk.stuba.sk
URL: http://www.sltk.stuba.sk

SLOVENIJA

Gospodarski Vestnik
Dunajska cesta 5
SLO-1000 Ljubljana
Tel. (386) 613 09 16 40
Fax (386) 613 09 16 45
E-mail: europ@gvestnik.si
URL: http://www.gvestnik.si

TÜRKIYE

Dünya Infotel AS
100, Yil Mahallessi 34440
TR-80050 Bagcilar-Istanbul
Tel. (90-212) 629 46 89
Fax (90-212) 629 46 27
E-mail: infotel@dunya-gazete.com.tr

AUSTRALIA

Hunter Publications
PO Box 404
3067 Abbotsford, Victoria
Tel. (61-3) 94 17 53 61
Fax (61-3) 94 19 71 54
E-mail: jpdavies@ozemail.com.au

CANADA

Les éditions La Liberté Inc.
3020, chemin Sainte-Foy
G1X 3V Sainte-Foy, Québec
Tel. (1-418) 658 37 63
Fax (1-800) 567 54 49
E-mail: liberte@mediom.qc.ca

Renouf Publishing Co. Ltd
5369 Chemin Canotek Road Unit 1
K1J 9J3 Ottawa, Ontario
Tel. (1-613) 745 26 65
Fax (1-613) 745 76 60
E-mail: order.dept@renoufbooks.com
URL: http://www.renoufbooks.com

EGYPT

The Middle East Observer
41 Sherif Street
Cairo
Tel. (20-2) 392 69 19
Fax (20-2) 393 97 32
E-mail: mafouda@meobserver.com.eg
URL: http://www.meobserver.com.eg

INDIA

EBIC India
3rd Floor, Y. B. Chavan Centre
Gen. J. Bhosale Marg.
400 021 Mumbai
[illegible]

ISRAËL

ROY International
41, Mishmar Hayarden Street
PO Box 13056
61130 Tel Aviv
Tel. (972-3) 649 94 69
Fax (972-3) 648 60 39
E-mail: royil@netvision.net.il
URL: http://www.royint.co.il

Sub-agent for the Palestinian Authority:

Index Information Services
PO Box 19502
Jerusalem
Tel. (972-2) 627 16 34
Fax (972-2) 627 12 19

JAPAN

PSI-Japan
Asahi Sanbancho Plaza #206
7-1 Sanbancho, Chiyoda-ku
Tokyo 102
Tel. (81-3) 32 34 69 21
Fax (81-3) 32 34 69 15
E-mail: books@psi-japan.co.jp
URL: http://www.psi-japan.com

MALAYSIA

EBIC Malaysia
Level 7, Wisma Hong Leong
18 Jalan Perak
50450 Kuala Lumpur
Tel. (60-3) 262 62 98
Fax (60-3) 262 61 98
E-mail: ebic-kl@mol.net.my

MÉXICO

Mundi Prensa Mexico, SA de CV
Río Pánuco No 141
Colonia Cuauhtémoc
MX-06500 Mexico, DF
Tel. (52-5) 533 56 58
Fax (52-5) 514 67 99
E-mail: 101545.2361@compuserve.com

PHILIPPINES

EBIC Philippines
19th Floor, PS Bank Tower
Sen. Gil J. Puyat Ave. cor. Tindalo St.
Makati City
Metro Manilla
Tel. (63-2) 759 66 80
Fax (63-2) 759 66 90
E-mail: eccpcom@globe.com.ph
URL: http://www.eccp.com

SRI LANKA

EBIC Sri Lanka
Trans Asia Hotel
115 Sir chittampalam
A. Gardiner Mawatha
Colombo 2
Tel. (94-1) 074 71 50 78
Fax (94-1) 44 87 79
E-mail: ebicsl@itmin.com

THAILAND

EBIC Thailand
29 Vanissa Building, 8th Floor
Soi Chidlom
Ploenchit
10330 Bangkok
Tel. (66-2) 655 06 27
Fax (66-2) 655 06 28
E-mail: ebicbkk@ksc15.th.com
URL: http://www.ebicbkk.org

UNITED STATES OF AMERICA

Bernan Associates
4611-F Assembly Drive
Lanham MD20706
Tel. (1-800) 274 44 47 (toll free telephone)
Fax (1-800) 865 34 50 (toll free fax)
E-mail: query@bernan.com
URL: http://www.bernan.com

ANDERE LÄNDER/OTHER COUNTRIES/ AUTRES PAYS

Bitte wenden Sie sich an ein Büro Ihrer Wahl/ Please contact the sales office of your choice/ Veuillez vous adresser au bureau de vente de votre choix

Office for Official Publications of the European Communities
2, rue Mercier
L-2985 Luxembourg
[illegible]

15 CG-NA-18592-EN-C

NOTICE TO THE READER

Information on European Commission publications in the areas of research and innovation can be obtained from:

RTD info
The free of charge European Commission's magazine on European research, with the focus on project results and policy issues, issued every three months in English, French and German.
For more information and subscription, contact:
DG XII Communication Unit, 200 rue de la Loi, B-1049 Brussels
Fax + 32-2-295.82.20; e-mail: info@dg12.cec.be

Proceedings of the workshop sponsored by the European Commission's Marine Science and Technology Programme, DG XII, reviewing current European research on harmful algal blooms, held in Kalmar, Sweden, 5 to 7 November 1998. Organised by the University of Kalmar in collaboration with an international organising committee, the workshop aimed to establish a more generic approach to the problem, in order to understand better the mechanisms underlying the occurrence and dynamics of algal blooms. The workshop was timely, in view of the upcoming Fifth RTD Framework Programme, and in particular the research theme "Human impacts on the marine environment". It is felt that it is particularly important to coordinate research at an international level since the water bodies containing harmful algal blooms are shared by several European countries. In order to address these issues, the workshop had the following objectives:

- Identification of the scope of the problem;
- Reviewing the impact of anthropogenic activities on the functioning of marine ecosystems;
- Identification of key research issues needed at European level.

Price (excluding VAT) in Luxembourg: EUR 16.5

ISBN 92-828-6612-2

OFFICE FOR OFFICIAL PUBLICATIONS
OF THE EUROPEAN COMMUNITIES

L-2985 Luxembourg

9 789282 866122