Harmful Algae Management and Mitigation



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Harmful Algae Management and Mitigation



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PREFACE

Harmful algal blooms (HABs) occur throughout the world. They have occurred from antiquity, although in many locations their frequency and severity appear to be increasing. HABs threaten human health and the productivity of fisheries - both open sea and aquaculture - by making seafood toxic to consumers and by direct effects on marine life.

Coping with these challenges requires money, personnel, and other resources. But governments face many other obligations. While HAB toxins in seafood can and do cause illness and death, many other things - malaria, infant diarrhea, motor-vehicle accidents - cause far more. How much should be allocated to dealing with HABs in the face of these competing demands? How can these often scarce resources best be employed?

HABs have significant economic impacts, both within economies and on the trade among them. On the one hand, concern over the presence of algal toxins in seafood, or the proliferation of harmful algae, can be a source of tension among trading partners. On the other hand, programs implemented to deal with these problems can themselves, if not harmonized, become unintended impediments to trade.

The Harmful Algae Management and Mitigation (HAMM) conferences, in contrast to the many excellent scientific conferences on HABs, focus primarily on exploring practical, currently available options for dealing with HABs and HAB toxins. The goal of this volume, like the conferences, is to assist in the development of a consensus with a solid technical and practical basis that will provide for policy makers a spectrum of management options applicable to the needs of diverse economies and cultures, faced with an array of HAB problems and societal constraints.

This volume has been developed from the first two conferences on Harmful Algae Management and Mitigation, held 10-14 May 1999 in Subic Bay, Philippines (HAMM99) and 12-16 November 2001 in Qingdao, China (HAMM2001).

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CASE STUDIES – IMPACTS AND PROGRAMS

CHINA'S MARINE RED TIDE MANAGEMENT AND DISASTER MITIGATION

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ABSTRACT

Red tide is a global marine issue as well as one of the major marine disasters in China. Management and disaster mitigation have not only aroused widespread attention around the world, but is considered of great importance in China.

While the marine development and the coastal economy in China are advancing rapidly, the problems of the marine environment are facing us. With yearly increases of nutrient and organic compound pollution in the sea, marine red tide disasters have also occurred frequently.

In this article, the author gives information concerning with harmful algae bloom management and mitigation in China, including red tide occurrences in China, heavy loss caused by the red tide disasters in China, and the efforts made by the Chinese government with respect to the red tide management and disaster mitigation,.

INTRODUCTION

Red tide is a global marine issue as well as one of the major marine disasters in China. Management and disaster mitigation have not only aroused widespread attention around the world, but is considered of great importance in China.

Boasting a continental coastline of over 18,000 km and an insular coastline of over 14,000 km, China is a major marine country. China's seas stride across tropic and temperate zones. They have a broad shallow sea and tidal flat, a multitude of coastal bays, favorable physical environments and resources. The ocean occupies an important strategic position in China's national economy and social development. From the early 1980s to the late 1990s, the gross output value of China's major marine industries increased 32 fold with an annual increase rate of 25%. In 1999, the gross output value of major marine industries reached 365 billions yuan (RMB) and the added value 202 billion yuan (RMB). The rate of development of marine economy is much higher than the average rate of development of the national economy as a whole.

However, while the marine development and the coastal economy in China are advancing rapidly, the problems of the marine environment are facing us. With yearly increases of nutrient and organic compound pollution in the sea, marine red tide disasters have also occurred frequently.

RED TIDE STATUS IN CHINA

Up to the year 2000, more than 400 cases of marine red tide have been recorded in China. According to the records made by the State Oceanic Administration, 20 cases of red tide were reported in the 1970s, 75 cases in the 1980s and 234 cases in the 1990s. The statistics of the recent two years have indicated that, since 2000, the trend of the frequent occurrence of red tides in the China's coastal seas is still very grave.

In the 1980s, red tides were mainly found in small semi-enclosed bays. Since the 1990s, however, the occurrence of red tide has been recorded mostly in China's near shore sea area. Bohai Sea, the Yangzi River mouth (including the Hangzhou Bay) and the Pearl River mouth waters are the areas suffering most from red tide disasters. The influent scope of individual red tide has expanded from several km^2 to thousands km^2.

To summarize the situation of China's red tides, it appears that the duration and frequency for their occurrence have increased, their scope of influence has expanded, and their harmful effects have aggravated.

HEAVY LOSS CAUSED BY THE RED TIDE DISASTERS IN CHINA

Economic Summary

The red tide brings about great harm to marine fisheries and results in an enormous economic loss. In recent years, the loss incurred in the disaster has risen from nearly 100 million yuan (RMB) in the early 1990s to about 1 billion yuan (RMB) in the late 1990s. Following are some examples.

In July-September, 1989, an extensive red tide occurring in the coastal Bohai Sea caused a direct economic loss of over 300 million yuan (RMB).

The red tide that occurred in the Helen Bay in Raoping of Guangdong in the middle ten days in November 1997 went on for half a month with a total economic loss of 180 million yuan (RMB).

From March to April 1998, occurred in the Pearl River mouth, a red tide incurred a loss of 350 millions yuan (RMB).

From September to October 1998, a red tide occurred in the Bohai Sea. It extended all over the Liaodong Bay, Bohai Bay, Laizhou Bay and part of the central Bohai Sea with the maximum cover area of over 5000 km^2, lasted for more than 40 days and caused a direct economic loss of about 500 million yuan (RMB).

According to incomplete statistics, in the three years of 1997-1999, the direct economic loss resulted from the red tide in the East China Sea reached over 2 billions yuan (RMB).

Direct losses from the entire sea area of China in 2000 and 2001 would amount to several billion yuan (RMB). Marine fishery is the prop industry in China's marine economy and its output value occupies about 54.7% of the gross output value of the national marine

economy. So, the direct consequences of the frequent occurrence of red tides would pose a grave threat to the development of China's fishery.

Impacts on Human Health

Red tide poses a grave threat to human health. The toxic red tide would result in food poisoning. Nowadays, every year throughout the world, there is food poisoning accidents due to accidental eating of the aquatic products polluted by the red tide toxins. Previously in China there were also some records of poisonings or even deaths of people because of red tide toxins. In recent years, as the result of strengthening the red tide monitoring and the supervision and management of seafood, no record of deaths of people from poisonings has been recorded. But there is always the possibility that the toxic red tide may occur and, therefore, its potential threat to human health still exists.

Impacts on Environment

The occurrence of red tide brings about anomalies in the seawater hydro chemical environment and marine ecological process. It seriously affects the normal physiological activities and productive processes of marine life, and leads to the sharp decrease of marine animals. A few highly enclosed inner bays are in a serious anoxic state due to the occurrence of red tide. The decomposition of organic matter produces large amounts of gas, such as hydrogen sulfide, deteriorates the atmospheric and water environments, and further does a great harm to the eco-environment of local sea areas.

According to the experts' predictions, in the coming years, under the influence of the marine environmental condition, China will still see the peak period of red tide occurrence. If effective measures were not taken immediately, the harmfulness incurred by the red tide would further increase.

The frequent occurrence of marine red tides and the damage arising there-from has aroused more and more extensive concern. In recent years, China has carried out the following work with respecting to the marine red tide management and disaster mitigation:

EFFORTS WITH RESPECT TO THE RED TIDE MANAGEMENT AND DISASTER MITIGATION

Government's Administrative Support

The Chinese government has fully realized the importance of red tide managing and mitigation. Wen Jiabao, vice-premier of the State Council, has made some special instructions on red tide managing and mitigation these years. The Tenth "Five-Year Plan of the People's Republic of China for National Economic and Social Development" also asks to "strengthen protection of water quality in the near shore and research on the prevention and control of red tides".

The State Oceanic Administration (SOA) of China has made a great deal of effects on red tide research, monitoring, managing, and disaster mitigation in active cooperation and coordination with the sectors of science and technology, education, environmental protection, agriculture, etc. So far, the monitoring and surveillance over the red tide has been strengthened, the scientific and technological research on red tides has been supported, the legislation and planning for the red tide management has been intensified, experiments have been conducted in the red tide prediction and forecast, the release of red tide information has been standardized, the historical data of red tide have been organized, and popular science books, monographs, electronic information products on red tide have been compiled and published.

Meanwhile, under the unified planning of the Central Government, the coastal local governments have positively organized and carried out the work on red tide disaster mitigation. Various localities such as Zhejiang, Fujian, Liaoning, Shandong, Hainan, Shanghai and Tianjin are establishing the red tide monitoring and early warning system step by step, constructing the red tide prevention and control coordination organization or the red tide emergency response system. All efforts above-mentioned have made a foundation for the effective red tide management and disaster mitigation.

Strengthening Red Tide Research

Since 1990s, China has made a great deal of research on red tide. Researches have been conducted successively in the fields of ecological dynamics of red tide populations; process, genesis and mechanism of the red tide generation and decline; interactions between the red tide algal species and between the algae and bacteria; red tide toxin and its mechanism; biological and chemical control methods for the red tide; evaluation of eutrophication in the offshore area; technologies for the red tide monitoring, prediction and assessment; ecological numerical simulation of the red tide and the application of the enclosure ecosystem experiments, etc. Achievement in researches above-mentioned has played an important role in preventing and controlling red tide.

Intensifying Red Tide Monitoring and Surveillance

Red tide monitoring is one of the important parts of China's marine environmental monitoring. At present, the SOA has established a three-dimensional marine environmental monitoring system. This system composes of many monitoring methods as coastal station, buoy, ship, radar, aerial remote sensing and satellite remote sensing. In recent years, the State has also strengthened red tide monitoring and surveillance of the, especially over the sea areas such as the culture and stock enhancement zone, estuarine area, sea areas adjacent to the major outlets for discharging sewage and marine recreational zone. Many large-scale red tide incidents have been observed in time through monitoring and surveillance conducted by using satellites, airplanes and ships in the seasons when the red tide frequently occurs.

Demonstration on Red Tide Prediction and Disaster Mitigation

In recent years, while strengthening the red tide monitoring and surveillance, we have attempted to carry out red tide prediction and forecasting. SOA makes a comprehensive analysis on red tide-prone zones and disaster occurrence each year, provides information to the central and coastal local governments and economic entities for the early planning of disaster prevention and mitigation. Starting from 2001, SOA has also carried out the demonstration work on red tide prediction. In the Bohai Sea and East China Sea, we selected some areas at where red tide occurs frequently as the pilot areas for red tide monitoring and prediction. On the basis of the experience in the long-term marine environmental monitoring and the large amounts of monitoring data, we make short-term prediction on the trends of red tide occurrence and development, and put forward concrete measures for prevention and mitigation red tide disasters. Now we have established a demonstration system on red tide monitoring, early warning and management. This system is composed of early-stage monitoring of the red tide occurrence, emergency ship monitoring, aerial remote-sensing monitoring, satellite remote-sensing monitoring after the occurrence of red tide, release of monitoring and early warning information, taking emergency response measures and damages evaluation.

MAJOR PROBLEMS ENCOUNTERED

China has done a great deal in the field of red tide management and disaster mitigation, and possessed some conditions and basic capacity. However, we still face many problems. For instance, we are still weak in the operational monitoring capability and the early warning and forecast capability for marine red tides, we are short of professional and technological personnel, and our instruments and equipment are relatively outdated. The integrated manage system for marine red tides and the relevant laws and regulations are not perfect, and the national plan for the prevention and control of red tides has not yet been promulgated. The research on marine red tides still lags behind the requirements of the red tide prevention and mitigation, the practical technologies have not been developed adequately, and the operational transformation of the related scientific and technological results is slow.

In this connection, we shall take the following measures to solve these problems in the near future:

Further Developing Red Tide Monitoring, Prediction and Emergency Response System

In the coming 3-5 years, we shall establish and perfect the red tide monitoring, surveillance, early warning and forecast system in order to feed the requirements of China's social and economic development; perfect the red tide disaster information system; carry out the red tide monitoring and surveillance over the near shore of China, and early warning and forecast of the large-scale red tide in the local sea areas; release in time circulars on the red tide disaster warnings and the monitoring results of red tide toxins so as to provide better service for the sustainable development of society and economy and raising the living standards of the people in the coastal region.

Strengthening Legislation and Planning, Perfecting the Red Tide Management System

An overall plan for the marine red tide monitoring and management with coordination between the State and the localities is to be made to guide and administer all-round development of the work on marine red tides. Laws, regulations, and various rules concerning to red tides prevention and mitigation will be established and perfected. A coordination mechanism will be established to promote red tide monitoring, information sharing and management.

Intensifying the Effort on Ecological Improvement

Reducing land-based pollution and lowering the level of seawater eutrophication are important measures for red tide disasters mitigation. In the future 3-5 year, China will make the efforts on centralized treatment and offshore discharge of sewage, gradually prohibit to sale and use phosphorus-containing detergents in cities. Practice total amount controlling on pollutants discharged into sea areas, strengthen the integrated improvement of the regional environment, rationally plan the development of aquiculture and gradually improve the eco-environmental quality in the near shore sea area.

Promoting Scientific Research

China will further promote the research on red tide outbreak mechanisms, the relationship between red tide and eutrophication, the key technologies on red tides prevention and mitigation, red tide disaster prediction and forecast, and red tide emergency handling. Meanwhile, bioengineering of preventing and controlling red tide disasters will be developed so as to provide scientific and technological support for red tide disasters prevention, monitoring and controlling.

Strengthening Public Education

Propaganda and education on marine red tides will be disseminated throughout the country by means of TV, broadcast, newspaper, and internet in order that the public, fishermen and government officials at all levels would get knowledge of red tide, and then play positive roles in red tide management and disaster mitigation. At the same time, the mechanism of volunteers monitoring will be established, special reporting channels in the coastal provinces and municipalities have been established.

Strengthening International Cooperation

China will take an active role in conducting extensive international cooperation and exchange on red tide management and disaster mitigation.

In a word, China is a major developing marine state. Red tide management and disaster mitigation has become an important historic mission at the present stage and over a very long period of time in future. China will take effective measures to speed up planning and management, enhance capacity building, promote scientific and technological development, increase the awareness of the public and push on international cooperation so as to usher in a new historical period on marine red tide management and disaster mitigation.

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NEW ZEALAND MANAGEMENT PROGRAMME: TEN YEARS OF PROGRESS IN THE CONTROL AND MONITORING OF BIOTOXINS

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ABSTRACT

During 1993 New Zealand experienced its first marine biotoxin event. Over 100 persons reported illness fitting the case description for neurotoxic shellfish poisoning (NSP) [1]. During the ensuing investigation, amnesic shellfish poisoning (ASP), paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP) and NSP toxins were detected in shellfish [2]. This presented unique problems, as the internationally recognised test methods were not developed to manage such a complex array of toxins in a cost-effective manner. Standard methods were used for several years for the detection of ASP [3] and PSP [4]. A mouse bioassay screen test was developed for DSP and NSP, based on the Yasumoto mouse bioassay method [5]. Confirmation of DSP was by DSP Check ELISA Kit and NSP by the American Public Health Association (APHA) NSP method [4]. The management of marine biotoxins became more complex between 1993 and 1998 with the identification in New Zealand shellfish of dinophysistoxin-3 (DTX-3), okadaic acid diol esters, pectenotoxins (PTX), yessotoxins (YTX) and the bioactive compound, gymnodimine. In 1999/2000, samples were being sent to Australia for confirmation by liquid chromatograph-mass spectrometry (LC-MS) of PTXs and YTXs. This resulted in the purchase of an LC-MS/MS system by a marine biotoxin testing laboratory in New Zealand and the development of LC-MS methods for the ASP and DSP groups of toxins. Concurrently, a guide for the validation of new test methods was developed to ensure that international protocols were complied with. In November 2001, approval was issued for the laboratory involved to use the LC-MS/MS for domoic acid (DA) and the DSP toxins (okadaic acid and dinophysistoxins, and also pectenotoxins, yessotoxins and azaspiracid). The ability to determine DSP by LC-MS/MS as an alternative to the DSP/NSP mouse bioassay screen test required a review of alternative ways of managing NSP. At the same time in New Zealand, significant progress was being made in ELISA development, particularly for YTX, PTX, okadaic acid (OA) and DA - and this will present more management options in the future. In November 2003 a second laboratory received an interim approval to use LC-MS/MS for domoic acid, okadaic acid, dinphysistoxin-1, gymnodimine, pectenotoxin-2 and yessotoxins (YTX and 45 OH YTX). These new approaches have a common beneficial outcome for the shellfish industry and regulators - improved cost-effective management of a small country's complex biotoxin situation.

KEY WORDS: biotoxins, management, ELISA, LC-MS/MS, test methods

INTRODUCTION

New Zealand is a small country with a population of around 4 million. The shellfish industry is mainly farmed Greenshell® Mussel (*Perna canaliculus*) and Pacific Oyster (*Crassostrea gigas*). While these are generally farmed on 3-5 hectare farms, shellfish companies are now applying for 8-10, 000 hectare offshore farms. Over 90% of New Zealand's NZ$230 million molluscan bivalve shellfish are exported - to over sixty countries. This means that our marine biotoxin standards must provide assurance to these countries that our standards are equivalent to theirs. To achieve this, New Zealand must have clearly written marine biotoxin regulatory standards that can be readily understood and audited by overseas government agencies.

Midway through the 1993 marine biotoxin event, the New Zealand Government formed a Marine Biotoxin Management Board, comprising a senior representative from the New Zealand Fishing Industry Board, the Ministry of Agriculture and the Ministry of Health. This Board formed a Marine Biotoxin Surveillance Unit, which contained a marine biologist, health protection adviser and a technical support officer and was based in the Ministry of Agriculture under the National Manager Shellfish. This Unit developed and managed the marine biotoxin programme and just before it was dissolved in 1996, it produced the New Zealand National Marine Biotoxin Management Plan (also known as the "Red Book").

Also early in 1993, a Toxic Shellfish Co-ordination Committee, which included regulators, shellfish industry, laboratory analysts, toxicologists, oceanographers, phytoplankton taxonomists and researchers was formed, initially met weekly, and then at less frequent intervals, and provided advice to the regulators. In 1994, this Committee was dissolved and replaced by the Marine Biotoxin Science Workshops which have been held by the Marine Biotoxin Technical Committee via the Food Safety Authority, twice a year. The Marine Biotoxin Science Workshops have been the backbone to the development and progress of the New Zealand marine biotoxin programme - they are open to all parties interested in any aspect of marine biotoxins with open invitations for 20-minute presentations on recent developments in New Zealand or overseas. The proceedings are informally collated, bound and distributed to attendees.

The National Marine Biotoxin Management Plan required each commercial shellfish growing area to have

a written marine biotoxin management plan developed by the local health official in consultation with the shellfish industry. This plan is required to include detailed maps, showing: growing area boundaries; hydrography and current flows in the area; sample site location; species and location of shellfish (especially important when several species are grown on a farm); local, regional and national contact details; early warning procedures; frequency of phytoplankton and toxin sampling for each of the toxins; sampling procedures; recall and detention procedures; surveillance procedures for growing areas when closed to deter illegal harvesting; early warning indicator arrangements; arrangements with laboratories; and result notification procedures.

TESTING REQUIREMENTS

Between 1993 and 1996, all commercial shellfish growing areas were being tested weekly for ASP, DSP, NSP and PSP. In 1997, after some 80,000 analyses, the Marine Biotoxin Technical Committee conducted a major review of the marine biotoxin programme. As part of the review, all phytoplankton information in New Zealand, especially phytoplankton activity associated with the detection of toxins, was reviewed. A decision was made to introduce phytoplankton monitoring as part of the regulatory programme, with the following conditions: a person in the laboratory was required to have attended an Intergovernmental Oceanographic Commission (IOC) taxonomy course; the laboratory was to provide a twenty four hour turnaround time from receipt of the sample to despatch of the results to the health officer; the laboratory was required to have ISO 17025 accreditation; phytoplankton samplers were required to be trained and certified; the laboratory was required to provide lists of proven and possible toxin producers in New Zealand; phytoplankton sampling was required to be at least weekly; and a 'Phytoplankton Action Level Table' was to be developed by the Marine Biotoxin Technical Committee in conjunction with the laboratory, with the "Table" showing "trigger" levels for each of the known toxigenic species - the "trigger" levels were set conservatively- and in some cases set at the level of detection – 100 cells L^{-1}. This Table has been amended several times over the years, as more information became available. If the "trigger" level is exceeded and the shellfish have not undergone toxin analysis, then a shellfish sample is required to be taken within one day for analysis for the relevant toxin.

The difficulty in differentiating between toxigenic and non-toxigenic *Pseudonitzschia* sp. resulted in collaboration between the Cawthron Institute in New Zealand and the Monterey Bay Aquarium Research Institute in California to develop whole cell DNA probes [6]. While these probes are currently used in the non commercial marine biotoxin programme and by the shellfish industry to make farm management decisions, they cannot be used in the regulatory programme for commercial shellfish growing areas until validation material has been provided to gain New Zealand Food Safety Authority (NZFSA) approval. Whole cell DNA probes have also been developed for PSP and NSP toxin producing phytoplankton.

New Zealand requires all laboratories (including shellfish toxin and phytoplankton) conducting testing to meet regulatory requirements to have ISO 17025 accreditation [7]. In addition, laboratories are required to have a minimum of two signatories, use NZFSA approved methods and undergo at least an annual evaluation (as a performance based laboratory evaluation system is in place) by the NZFSA Laboratory Evaluation Officer.

TEST METHOD REGIME

The detection of ASP, DSP, NSP and PSP in 1993 presented serious problems with developing a cost-effective test method regime. Initially, an acetone-dichloromethane mouse bioassay (with 24 hour observation time) was used to screen for DSP and NSP with confirmation of DSP with the SCETI DSP Check ELISA and confirmation of NSP with the APHA ether method. However, by 1998 DTX-3, diol esters, PTX and YTX had also been detected, requiring a review of how the DSP group was being managed. Between 1998 and 2000, PTXs, YTXs and DTX-3 were being detected in more areas, probably due to increased research and improved analytical techniques. Less consistency in local management was also observed. However, the key problem was when a positive result on the mouse bioassay screen was detected and the confirmatory DSP and NSP tests were negative. A review of the test method management in 2000 resulted in several new requirements to address these anomalies: in order to address the possible presence of DTX-3 and diol esters, hydrolysis was required prior to the DSP Check ELISA or Protein Phosphatase test; for YTX, HPLC or LC-MS analysis was required; and for the PTXs, LC-MS was required. With limited access to one LC-MS in Queensland Health Scientific Services, Australia and one at the police forensic laboratory at the Institute of Environmental Science and Research in New Zealand, the analytical costs and time to get toxin results were becoming unacceptable to the regulators and shellfish industry. It was in this environment that the shellfish industry, regulators, researchers and laboratories decided that a new approach to toxin testing was required.

In considering new approaches to marine biotoxin testing, the New Zealand Shellfish Quality Assurance Circular 1995, the 1980 Memorandum of Understanding on Shellfish with the United States Food and Drug Administration, Council Directive 91/492/EEC and Commission Decisions 2002/225/EC and 2002/226/EC (when finalised) and the need for a new approach had to be considered. In August 2000 the New Zealand Marine Biotoxin Technical Committee received two proposals for the use of LC-MS for shellfish toxin testing and agreed that laboratories could proceed in developing LC-MS methods.

VALIDATION AND APPROVAL

In November 2000, the New Zealand Food Safety Authority conducted a one-day validation workshop with regulators, shellfish industry, laboratories and test method researchers. While the main emphasis of the workshop was on LC-MS method validation, AgResearch Ltd was making significant progress in New Zealand on the development of ELISA methods with the possibility of an ELISA screen for all marine biotoxins. Hence we were weary of getting a mindset on developing one particular method. To ensure that the new test methods being developed were "fit for their intended purpose" and were reasonably equivalent to the methods being replaced, an internationally acceptable "Guide for the Validation of New Test methods" was developed. A draft of this Guide was presented to the USA Interstate Shellfish Sanitation Conference Laboratory Methods Review Committee. The Guide was based on several internationally accepted validation guides which are referenced in the Guide. In particular the Guide was strongly influenced by the Eurachem Guide [8] and the Joint FAO/IAEA Expert Consultation [9]. While consideration was given to adopting one of the international guides, in light of the uniqueness of LC-MS validation of marine biotoxin methods, a decision was made to prepare a guide primarily for LC-MS methods, but which could also be used for other methods. While new ground was being broken for regulatory marine biotoxin test methods, an open mind was maintained and the Guide made it clear that the laboratories would need to demonstrate that the LC-MS methods could be "fit for their intended purpose" without certified reference standards. The Guide requires that accuracy, precision, sensitivity, uncertainty, ruggedness, working and linear ranges, the Horrat value and stability of standards be addressed in accordance with the stated requirements. Some specific issues needed to be addressed, including dealing with analogues, standards, recovery rates and hydrolysis. While purists might say that LC-MS cannot be used for the DSP, YTX and PTX toxin groups as there are not certified standards for each of them, as public health regulators an alternative approach was accepted to the quantitation of some of the toxins and the rationale provided.

During the validation process, which was fully funded by the developing laboratory, periodic meetings were held between the regulatory agency and laboratory to ensure that the validation was on the right track. Initially, an Interim Approval was issued by the NZFSA to the Cawthron Institute followed by full approval in November 2001 for the use of LC-MS for domoic acid, isodomoic acid, okadaic acid, DTX-1, PTX-1, PTX-1SA, PTX-2, PTX-2SA, PTX-6, YTX, 45 OH-YTX and the laboratory had the ability to detect but not validate methods for DTX-2, AZA-1, AZA-2, AZA-3 and homo-YTX. This method uses a standard methanolic extraction protocol as published by Japanese workers and these workers have also previously described the electrospray ionisation and fragmentation of DSP toxins [10].

While the development and approval of LC-MS methods for the larger DSP group was a challenging achievement on its own, the splitting of DSP testing from the DSP/NSP mouse bioassay screen method meant that the management of NSP needed to be reviewed. The intention was that until a LC-MS method was developed for the NSP toxin group, NSP would be managed by weekly phytoplankton monitoring and the APHA ether method for NSP toxins in the shellfish.

A full review of NSP was conducted by the Cawthron Institute [11], including eight years of toxin analyses, phytoplankton data and data from researchers in New Zealand and overseas. From this a list of brevetoxins identified in NZ shellfish was produced and a list of phytoplankton found to have produced brevetoxins. The Cawthron Institute is currently developing a LC-MS method for the detection of brevetoxins and this will soon be submitted with the supporting validation documents to the NZFSA for approval.

In November 2003 the AgriQuality Lab Network was issued interim approval for a LC-MS method for domoic acid, okadaic acid, dinophysistoxin-1, gymnodimine, pectenotoxin-2 and yessotoxin (YTX and 45 OH YTX), thus there are now two laboratories in New Zealand with approval to analyse shellfish for marine biotoxins using LC-MS.

CONCLUSION

In concluding, while the development and approval of LC-MS methods for shellfish toxin testing has broken new ground internationally, it has not been without a number of issues, with some, such as hydrolysis, analogues, and lack of certified reference standards still being addressed - but being managed in the interim. The progress could not have occurred without a strong co-ordinated approach by the research, shellfish industry, laboratory and regulatory groups in New Zealand and the assistance from many groups and individuals overseas.

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MONITORING OF BIOTOXINS ON THE SOUTH AFRICAN COAST

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ABSTRACT

South Africa with its broad range of oceanographic conditions experiences a variety of harmful algal bloom phenomena. Monitoring of wild shellfish is undertaken to provide a warning and information system to the public and shellfish industry, but is not designed as a comprehensive system of public health assurance. Monitoring of shellfish from aquaculture establishments is undertaken in accordance with a Biotoxin Management Plan to provide guarantees as to the safety of these products to foreign buyers and local consumers. Sampling is conducted at differing levels of intensity as determined by the shellfish species of concern, the history of contamination with the specific biotoxin, and the region where cultivated. Monitoring requirements and plans of action during the routine and non-routine phases of the Biotoxin Management Plan are described.

INTRODUCTION

Harmful algal blooms (HABs) are common phenomenon on the South African coastline which is influenced by two major boundary current systems – the cool Benguela on the west and the warm Agulhas on the east. The juxtaposition of these two systems creates a broad range of oceanographic conditions leading to regional disparity in problems relating to harmful algae. The West Coast, as a component of the southern Benguela upwelling system, is subjected to a disproportionately high incidence of harmful events whereas blooms in subtropical waters on the East Coast are seldom reported.

HAB events on the South African coast can be broadly grouped into those that create problems owing to their high biomass and those that produce toxins [1]. The harmful effects of high biomass blooms result typically from anoxia following the decay of dinoflagellate dominated blooms, often leading to massive faunal mortalities, whereas public health is impacted by the high incidence of paralytic (PSP) and diarrhetic shellfish poisoning (DSP).

PSP is caused by the dinoflagellate *Alexandrium catenella* which appears confined to the West Coast and often associated with high biomass blooms in stratified coastal waters over the Namaqua shelf. Despite the recent detection of another *Alexandrium* spp. on the South Coast, PSP remains undetected in this region owing in all likelihood to the observed low concentrations of this species and to its very low cellular toxicity [2].

DSP is typically associated with either *Dinophysis acuminata* or *Dinophysis fortii* which are prevalent on both the West and South Coasts. Determination of the specific toxicity of *D. acuminata* and *D. fortii* from the South African coast, has in both cases revealed moderate toxicity attributable to okadaic acid and the derivative DTX 2B [3].

Amnesic shellfish poisoning (ASP) has not been reported on the South African coast, although extensive blooms of *Pseudo-nitzschia australis* have been recorded particularly on the West Coast [4]. Neurotoxic shellfish poisoning is also unknown on the South African coast, but a newly described fish-killing dinoflagellate *Karenia cristata* [5] has been found to produce aerosol toxins similar to those produced by *Karenia brevis*.

In South Africa Marine & Coastal Management (M&CM), a branch of the Department of Environmental Affairs and Tourism, is the lead agency responsible for the detection of HAB events and the management of harvesting closures.

BIOTOXIN MONITORING

Monitoring of Wild Shellfish Utilized in Recreational and Commercial Fisheries

A HAB monitoring programme was implemented on the South African coast by M&CM in 1989. This programme has remained basic, comprising the investigation of all reported red tides, daily collection of water samples for enumeration of phytoplankton at a limited number of stations, and irregular testing for toxins based on the presence of toxic species. The primary objective of the programme was to provide a warning and information system to the public and the aquaculture and fishing industry. The intention of the programme was to identify the species responsible for harmful blooms and to establish the problems associated with each. It was not intended to serve the purpose of ensuring seafood safety. Information obtained on the incidence, distribution and duration of blooms, together with improved insight into the problems and risks associated with blooms, has however permitted better planning and management options in different coastal regions.

Monitoring of Shellfish from Aquaculture Establishments

As part of the South African Molluscan Shellfish Monitoring and Control Programme, biotoxins and biotoxin-producing algae are monitored in each growing area in accordance with a Biotoxin Management Plan. The management plan serves to clearly identify the agencies responsible for and the procedures necessary to undertake the actions listed below:

- Monitor toxin producing plankton and the geographical distribution thereof on a routine basis. At present shellfish toxicity tests are performed in

conjunction with plankton monitoring, at least until there is sufficient confidence in using plankton as a proxy.

- Develop a contingency plan that provides an emergency response when a potential problem is detected. The plan defines those administrative procedures and resources necessary to: initiate emergency shellfish and water sampling, close areas to harvesting, prevent commercial harvesting from closed areas, and recall and embargo contaminated shellfish.
- Provide assurance that certain shellfish species, or products, can be safely excluded from the contingency plan. This requires collection of sufficient supporting data and regular review.

The Programme document is currently in the process of being ratified as an official M&CM operations manual.

BIOTOXIN MANAGEMENT PLAN

Each growing area management plan includes a map showing the sampling positions, which are determined on the basis of "key stations" and "critical species" from assessment of historical data collected by M&CM.

Sampling is conducted at differing levels of intensity as determined by the shellfish species of concern, the history of contamination with specific biotoxins, and the geographical area under consideration.

Table 1. Recommended methods for biotoxin detection and criteria used for closure to harvest.

BIOTOXIN TEST	TEST METHOD	STANDARDS
PSP	Mouse bioassay [6]	<0.8 mg kg^{-1} edible flesh
DSP	Mouse bioassay [7] - a broad range detection method regarded by the EU as providing the highest level of sanitary protection	Mouse mortality (EU): ≤5 hours: high toxicity; 5-24 hours: less toxic; reject if any positive findings within 24 hours
ASP	Canadian HPLC-UV method for domoic acid [8]	<20 mg kg^{-1} edible flesh

Toxin levels as determined by mouse bioassay (PSP and DSP) in the edible portions of shellfish provide the present basis for regulatory action. This constitutes the whole animal except for scallops when the final product is the adductor muscle (with or without roe) and abalone that are cleaned and eviscerated prior to placing on the market. The biotoxin limits and recommended methods of analysis are provided in Table 1. Analyses for domoic acid are not currently being conducted on a routine basis due to a lack of analytical capability in South Africa. However, one laboratory is in the process of being accredited for the method. It is envisaged to include regular testing for ASP with the development of this capability.

Routine Monitoring Phase

Routine monitoring is conducted year round. In open water systems, a sample of the upper water column is taken by the farmer daily and promptly delivered to M&CM for phytoplankton identification and enumeration. For land-based systems a water sample representative of intake water is used for phytoplankton analyses. These water samples are composited on a weekly basis for microscope counting. Should the presence of toxic algae be indicated in the water or in shellfish flesh, the individual daily samples around the event are counted. It is anticipated that these data will ultimately lead to establishing critical concentrations for certain species that could be used in a regulatory context in the future. Such information, together with biotoxin testing of shellfish flesh, is essential for establishing detoxification curves.

Shellfish samples for bioassay are collected in accordance with Table 2. A composite sample of shellstock currently under harvest or intended for the next harvest is taken at the required interval by independent samplers. At present inspectors from the South African Bureau of Standards (SABS) or other personnel officially sanctioned by M&CM are employed for this purpose. In the future it is intended to utilize the local health authorities to assist with sampling and inspection requirements. All costs involved with sampling are recoverable from the farmers.

Table 2. Maximum allowable time between biotoxin test and shellfish harvest.

	West of Cape Point		East of Cape Point	
	Filter feeders	Non-filter feeders	Filter feeders	Non-filter feeders
PSP	48 hr*	2 weeks	1 month	1 month
DSP	1 week	1 month	2 weeks	1 month
ASP	1 month	1 month	1 month	1month

*or twice a week for multiple harvesting

Intensive Sampling Phase

Intensive sampling is initiated following detection of biotoxins in shellfish meats, though at levels still below regulatory limits. Intensive sampling may also be instituted when toxic phytoplankton are present in the absence of shellfish intoxication. This will be based on the detection of biotoxin-producing phytoplankton in filter-feeder growing waters or the occurrence of toxic blooms in non-filter feeder production waters. With experience it may prove feasible to apply a critical concentration for a particular toxic species as the criterion for initiation of intensive sampling. Intensive sampling does not apply to areas with a history of low-level contamination with specific biotoxins.

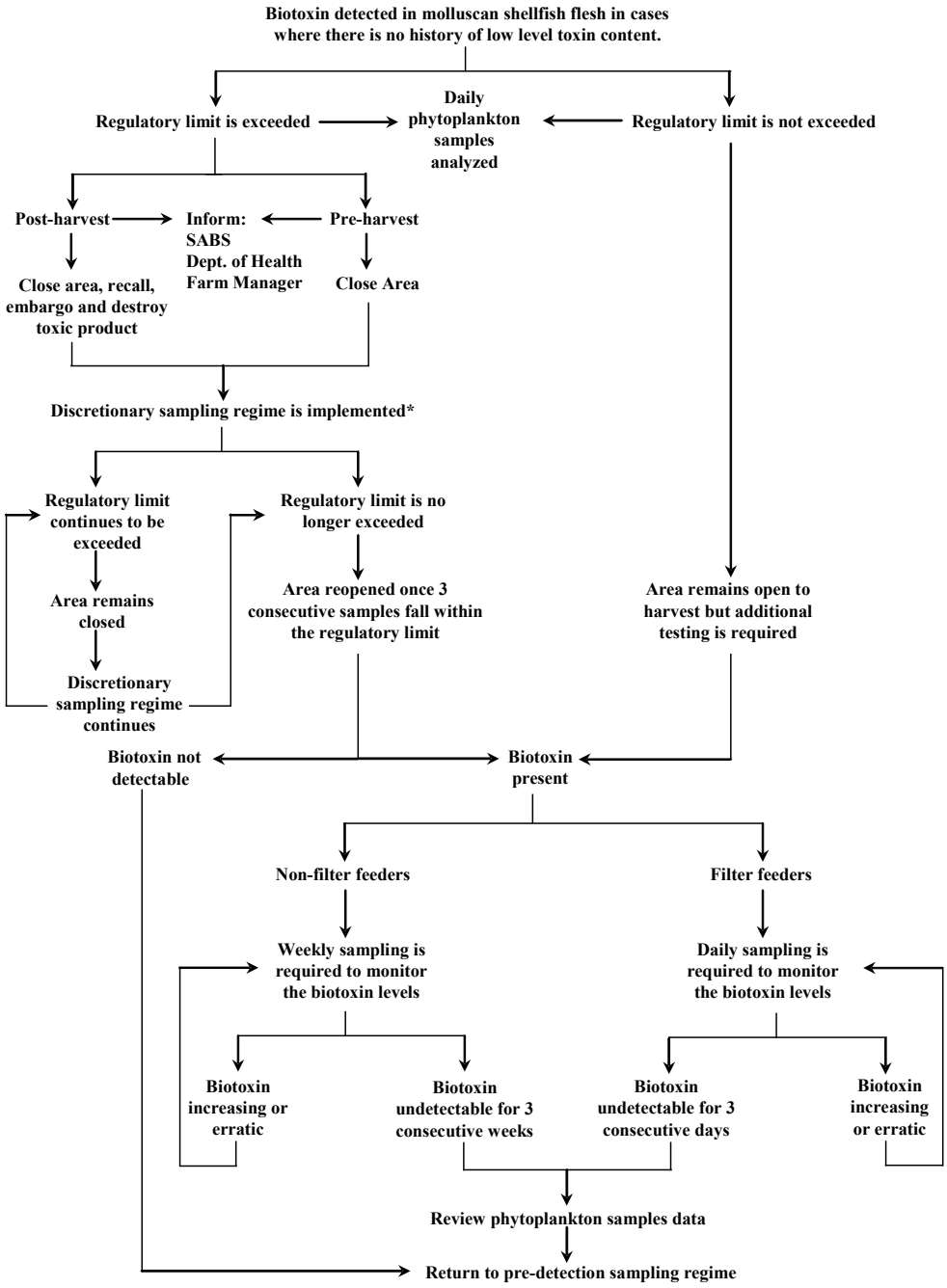

Figure 1. A flow diagram summarizing the course of action during non-routine sampling (*Frequency of sampling is at farm managers discretion, but no more than one sample may be submitted per day. Multiple samples on the same day will be considered one sample).

This exclusion applies specifically to a few abalone farms on the West Coast. Cultivated abalone from these areas often have measurable PSP toxins in their flesh though below the regulatory limit. A flow diagram summarizing the course of action during non-routine sampling is given in Figure 1. Intensive sampling involves:

- Daily testing of filter-feeder shellfish meats and weekly testing of non-filter feeder meats for the relevant biotoxin(s) at an increased number of sampling points.
- Daily analysis of phytoplankton samples.

Routine sampling is re-established when biotoxin levels are undetectable for 3 consecutive samples.

Quarantine Phase

Quarantine is brought into effect immediately following detection of biotoxin in shellfish meats at levels sufficient to cause a public health hazard. The following actions are undertaken during quarantine:

- M&CM places a ban on harvesting in the area.
- The closed status of the area is communicated to the shellfish industry and other regulatory authorities.
- Contaminated products are recalled, embargoed and destroyed under the supervision of a health officer responsible for the area.
- M&CM compliance officers are instructed to ensure the embargo is maintained.
- Analysis of phytoplankton samples and testing of shellfish meats continue, though at a frequency determined by the farm manager in consultation with M&CM.

Re-Opening Phase

A shellfish growing area closed due to biotoxins will only be re-opened to harvesting when M&CM has determined that the criteria justifying this action are met. Areas will only be re-opened when biotoxin levels are below the regulatory limit for a minimum of 3 consecutive samples. Sampling intensity following re-opening is dictated by toxic algal presence. This may involve intensive sampling in the continued presence of toxic species even though the shellfish have attained sub-quarantine levels of biotoxins. Information regarding detoxification curves will assist in adjusting this criterion in the future. The re-opened status is communicated to all relevant parties.

CONCLUSION

Monitoring of biotoxins on the South African coast presently targets aquaculture installations with the aim of complying with the stringent sanitation requirements imposed by certain countries for molluscan shellfish. Current shellfish exports of predominantly abalone are to eastern markets. However, it is the intention of the industry to explore additional markets in Europe and elsewhere. This will require inspection and audit of the broader system of molluscan shellfish sanitation in South Africa prior to entering into an agreement between the respective authorities. At present, the sampling and testing requirements for biotoxins are enforced through mariculture permit conditions issued by M&CM. All shellfish exports are certified by the SABS, the competent authority for fishery products in South Africa. The harvest of wild shellfish is not subjected to the same level of scrutiny as the cultivated stock. There is thus a need to extend the present Biotoxin Management Programme to include commercial and recreational harvests in a more systematic manner.

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STUDY ON HARMFUL ALGAL BLOOMS AND MITIGATION IN THE YANGTZE RIVER ESTUARINE AREA AND ITS ADJACENT WATERS

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ABSTRACT

In this paper, we describe algal composition, dominant species, and distribution of harmful algal blooms (HABs) in the Yangtze River estuarine area and its adjacent waters. Traditional species of red tide in these waters were *Noctiluca scintillans*, *Skeletonema costatum* and *Pseudo-nitzschia pungens*, but in recent years, more toxic species such as *Alexandrium* spp. and *Prorocentrum* spp. occurred frequently. The distribution of HAB events has expanded yearly. Some HAB poisoning events happened frequently and were, in some cases, fatal. Control, counter-measures, and suggestions are given.

INTRODUCTION

Harmful Algal Blooms represent one of the major natural disasters of the oceans. HABs have been spreading and increasing along the coast of China Sea in the past two decades, causing damage to the marine environment and threatening human health (Zhu 1992; Qi et al. 1993; Tseng et al. 1993; Zhu et al. 1995). In recent years, more HAB events have occurred in the estuarine area of Yangtze River and its adjacent waters, where there is an important fishing ground, Zhoushan fishing ground, and major marine culture waters.

Yangtze River is a longest river in China. It has abundant water resources. The East China Sea receives huge amounts of run off from many rivers of mainland Eastern China, including the run off of almost one hundred million $\times 10^4$ tons per year, delivering abundant nutrients into the estuary and affecting the composition and dynamic distribution of phytoplankton.

Eastern China is an important economic area whose rapid economic development and increased population in recent years has led to large values of waste water and sewage discharge into rivers and the sea. It has led to serious problems and frequent events of red tides in recent years. These HABs harm the ecosystem, fisheries resources, public health and especially marine culture in this region. In recent years more HAB events have been occurring in the estuarine area of Yangtze River and its adjacent waters. Seventy-eight species of red tide organisms were identified. Key species were *Noctiluca* sp., *Skeletonema costatum* (Greville) Cleve, *Pseudo-nitzschia, Alexandrium affine* (Inoue & Fukuyo) Balech, *Prorocentrum* spp., and *Gymnodinium* sp.

Distributions of some algal groups and dominate species of HABs in the East China Sea:

Dinoflagellates:

- Spring (1998): Dinoflagellates were widely distributed almost in the whole East China Sea with highest densities located in coastal water off Zhejiang province (Fig. 1).
- Winter (2000): Higher concentrations were distributed in the Yangtze River estuarine area and Zhoushan fishing ground. Highest values were located near 124°E (Fig. 2).
- Autumn: Dinoflagellates were widely distributed in the Yangtze River estuarine area with higher values found at Zhongshan fishing ground (Fig. 3).

Noctiluca scintillans:

- Spring: This species was rarely distributed in the East China Sea, except for high values that appeared near the southern part of Zhejiang province (Fig. 4).
- Summer: Its distribution was similar to spring, which was near the coastal water of Zhejiang province; the mean value was 1.3×10^4 m^{-3} (Fig. 5).
- Winter: This species was distributed in the whole East China Sea. High concentrations were off of the Yangtze River estuarine area; the mean value was 0.13×10^4 m^{-3} (Fig. 6).

Cyanophytes:

Four species of *Trichodesmium* were identified in the surveying water, all of which are tropical Oceanic species. They occurred in summer and autumn. Typically high values appeared in summer (mean value of 3.7×10^4 m^{-3}) with lower concentrations in autumn (0.67×10^4 m^{-3}).

- Summer: *Trichodesmium* appeared about 67.59% of the time. It was widely distributed in the whole East China Sea, especially in Krushio water and Taiwan warm current water (Figs. 7-9).

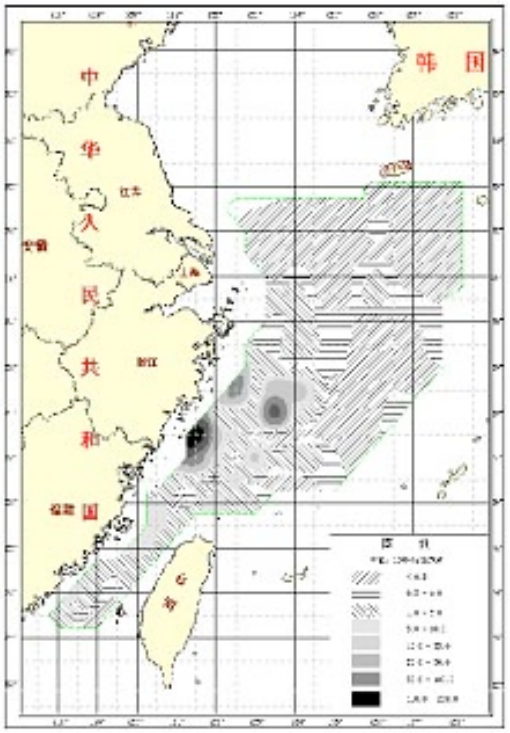

Figure 1. Dinoflagellate distribution, spring 1998.

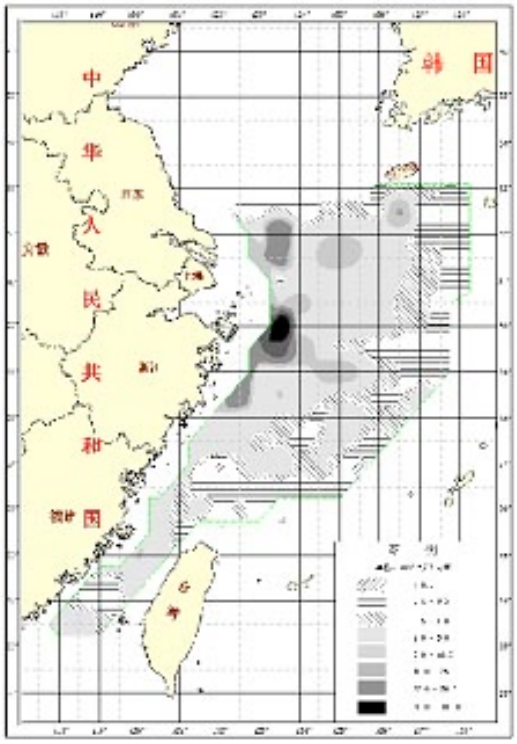

Figure 2. Dinoflagellate distribution, autumn 1997.

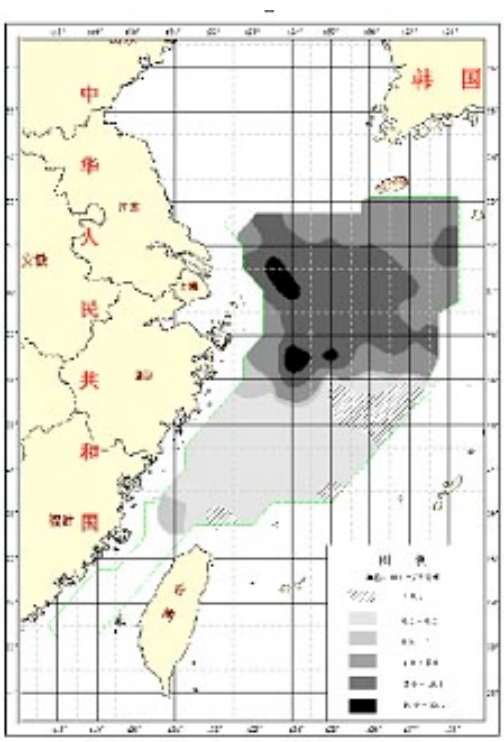

Figure 3. Dinoflagellate distribution, winter 2000.

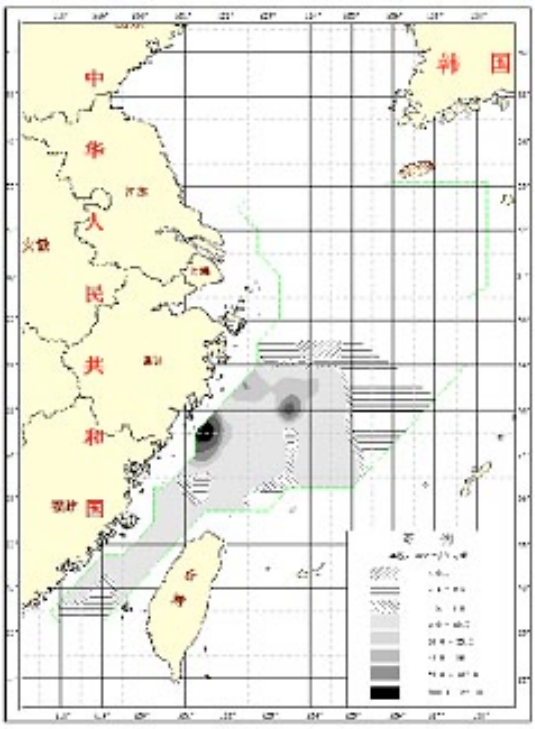

Figure 4. *Noctiluca* sp. distribution, spring 1998

- Autumn: It appeared about 73.86% of the time. Its distribution area was similar to summer, but its concentration was less. In most of the stations its value was $<10^4$ m^{-3}.

Ceratium fusus:

This coastal, temperate species is widely distributed. It appeared in the East China Sea throughout the year.

- Summer: Its value was the highest at 0.3×10^4 m^{-3}.
- Spring: Its value was the lowest at 0.02×10^4 m^{-3}.
- Winter and autumn: Concentrations were 0.19×10^4 and 0.12×10^4 m^{-3}, respectively.

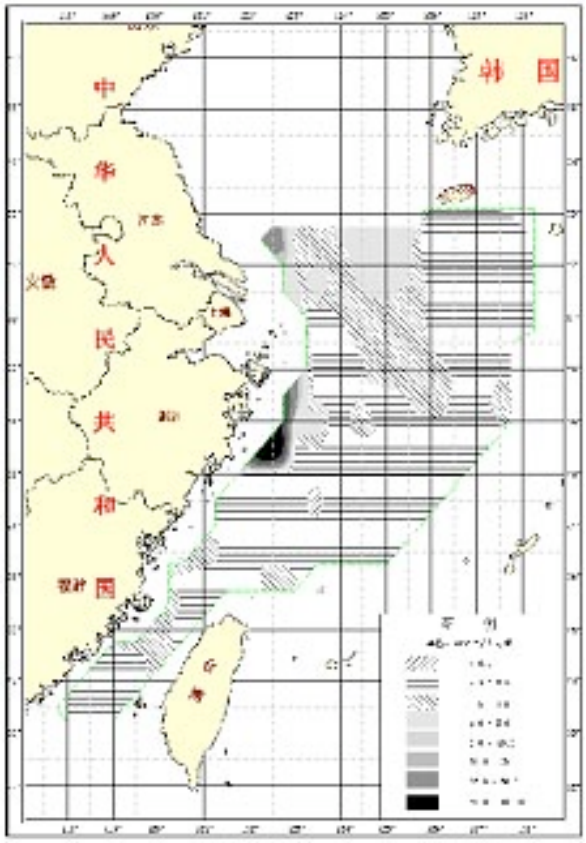

Figure 5. *Noctiluca* sp. distribution, summer 1999.

Most of the HAB events covered under 100 km^2. But in 2001, the largest event was >7000 km^2. Until now of this year about 50 events were recorded, most of which occurred in the Yangtze River area and Zhoushan fishing ground. Most events occurred during May to August, but in Xingshan Bay, HABs used to appear in the winter season. Some HAB events can be seen in Figures 10 and 11. Locations of most HABs occurred near 123°E longitude.

ECOLOGICAL CHARACTERISTICS OF HABS

Distribution

Most of the HAB events were distributed in the Yangtze River estuarine area and coastal water off Zhejiang province. HAB events in this region have also occurred more frequently in recent years. The spatial extent of blooms appears to be expanding, with scales up to 7000 km^2 observed.

Composition structure changed with more toxic species occurring such as *Alexandrium* and *Prorocetrum*. Events of poisoning such as paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP) happened more frequently, and some fatalities resulted.

HABS IN THE YANGTZE RIVER ESTUARINE AREA AND ITS ADJACENT WATERS

Dinoflagellates

High concentrations were located around longitude 123°E, possibly related to physical features. Cell densities in the surface layer reached 6.8×10^5 dm^{-3}. Potential HAB species were *Prorocentrum* sp., *Alexandrium catenatum, Alexandrium* spp., *Dinophysis* spp., *Karenia* spp.and *Noctiluca*.

Prorocentrum sp. is a key dinoflagellate species commonly distributed around 123°E and forms a high-density region. *Alexandrium* sp. was mainly distributed near the estuarine water of the Yangtze River with the highest density of 6×10^4 dm^{-3}in April 2002. *Karenia breve* was mainly distributed in Dust, located in the southern part of 31°N, 123°E. Its cell density reached 3.7×10^3 dm^{-3}. *Dinophysis* spp. was only occasionally found in a few stations.

Diatoms

Eighty-eight species (33 genus) had been identified in 105 samples, including species of *Coscinodiscus*, *Nitzschia* and *Psuedo-nitzschia*. The dominant species were *Pseudo–nitzschia delicatissima* and *P. pungens*.

Pseudo-nitzschia delicatissima was distributed in both the Yangtze River estuarine area and off Hangzhou Bay and Zhoushan fishing ground. High concentrations (75×10^3 L^{-1}) were located offshore of Hangzhou Bay.

Pseudo-Nitzschia pungens was widely distributed in the surveying water that is in the Yangtze River estuarine area and coastal water off Zhejiang province (29-32°N, 122-123°50E). A high density of 72×10^3 L^{-1} was located in the southern waters off Zhejiang province.

Melosira sulcata was widely distributed in this surveying area. The high density was located in Yangtze River estuarine water, where concentrations were $>2\times10^3$ L^{-1}. Cell densities of 1-2×10 L^{-1} were in Yangtze River estuarine water and Zhoushan waters.

SUGGESTIONS TO CONTROL HABS

- Strengthen the monitoring of the environment, HABs, and shellfish (especially for PSP and DSP);

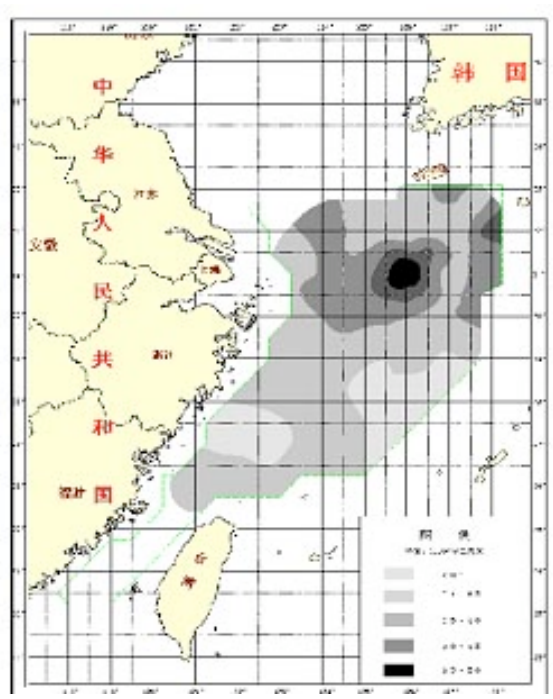

Figure 6. *Noctiluca* sp. distribution, winter 2000.

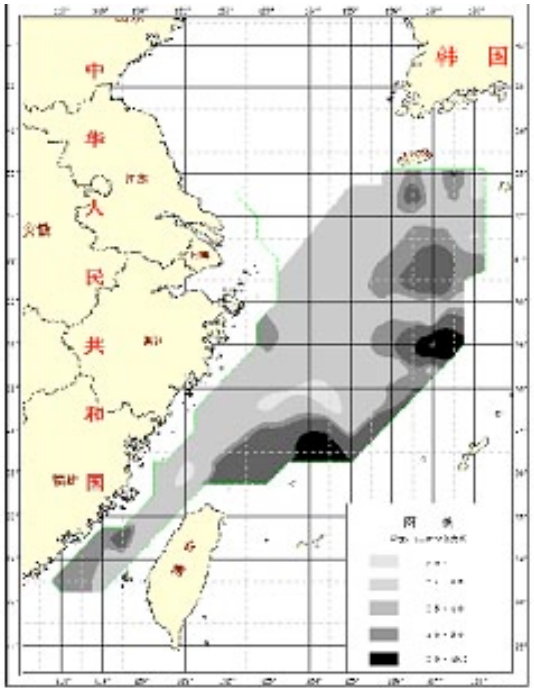

Figure 7. *Trichodesmium* sp. distribution, spring 1998.

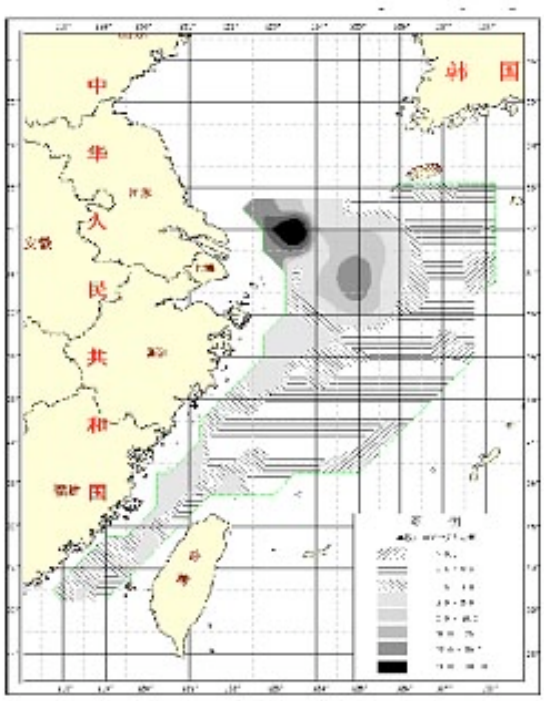

Figure 8. *Trichodesmium* sp. distribution, spring 1997.

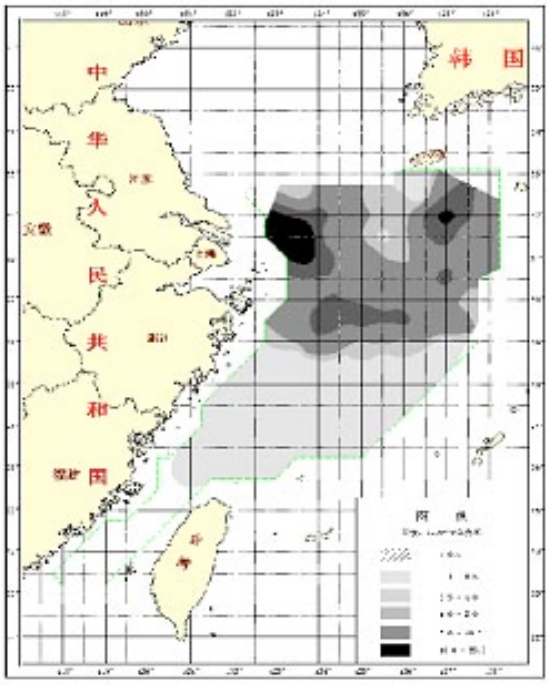

Figure 9. *Trichodesmium* sp. distribution, winter 2000.

- Control pollution - Government should pay more attention to reducing pollutants and discharge and waste-water and sewage should be treated well;
- Both monitor nets (system) SIO and fisheries environment monitor net should be combined and information exchanged as soon as possible;

- Strengthen information dissemination and education to prevent HAB disasters, especially in mariculture areas;



Figure 10. 1988 red tide occurring on a scale of 6600 km^2.

- Control capacity of mariculture and reduce pollution of mariculture;
- Strengthen the ability to examine and monitor for shellfish toxicity; and
- Ministry of Agriculture of China should strengthen shellfish management and monitoring (including mariculture environment), transportation and selling in the market.



Figure 11. 2000 red tide occurring on a scale of 4700 km^2.

ACKNOWLEDGEMENTS

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OVERVIEW OF HARMFUL ALGAL BLOOM MANAGEMENT IN HONG KONG SINCE 1998

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ABSTRACT

The unprecedented harmful algal bloom (HAB) in 1998 led to a review of the HAB management strategy established in 1983. A consultancy study confirmed that the established strategy which comprised an action scheme with a reporting network, quick risk assessment and prompt responses and a supportive scheme with research and education programmes for further enhancement of the action scheme is the correct approach but increase in coordination, communication and cooperation is needed. Following the recommendation of the study, the management effectiveness was upgraded through establishment of a management framework, enhancement of monitoring capabilities, improved public communication and education and targeted collaborative research. Based on Hong Kong's experience, the principles of red tide management strategy are discussed.

INTRODUCTION

Hong Kong lies on the coastal shelf of South China. The first scientific report of red tides in Hong Kong was in 1971 [1]. The Government's red tide record started in 1975. From 1975 to 2000, there were a total of 603 red tide incidents. With the exception of the 1988 peak, there appears to be a periodic cycle every 10 years ranging from 10 to 40 incidents annually (Fig. 1).

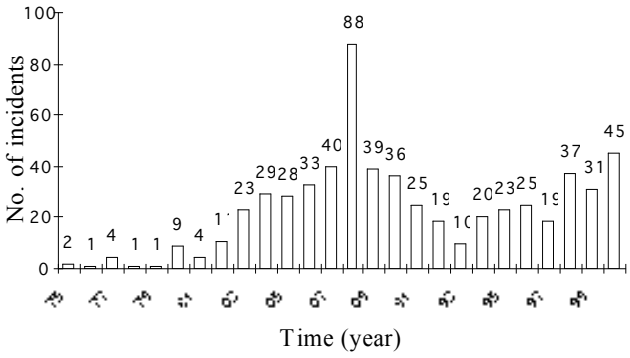

Figure 1. The number of red tide incidents in Hong Kong from 1975 to 2000.

So far 62 red tide causative species have been identified yet the majority of the species are harmless. Amongst the harmful species, a number can make shellfish toxic and a few are known to be toxic to fish. Some can cause anoxia leading to fish kills. Therefore, the potential impacts of HABs in Hong Kong were mainly mortality of cultured fish with associated loss to capture fishery due to avoidance of seafood by the public and health risk arising from consumption of algal toxin contaminated seafood.

RED TIDE MONITORING AND MANAGEMENT 1983-I998

In the early 1980s a few fish killing red tide incidents had prompted the Government to look into the harmful algal bloom problem. In 1983 to minimize the potential impacts of HABs in Hong Kong, a red tide management strategy was established. To enable prompt actions to be taken to minimize the impacts, it is necessary that the risks involved are assessed and the concerned parties get notified as soon as possible. Also in view of the limited information available and the lack of understanding of the fish farmers and the public about HABs, efforts on research and education are needed to improve the effectiveness of the response actions. Therefore, the management strategy was designed to include an action scheme with actions to be taken when HABs were sighted and a supportive scheme that included continuous research and education programmes (Fig. 2) [2].

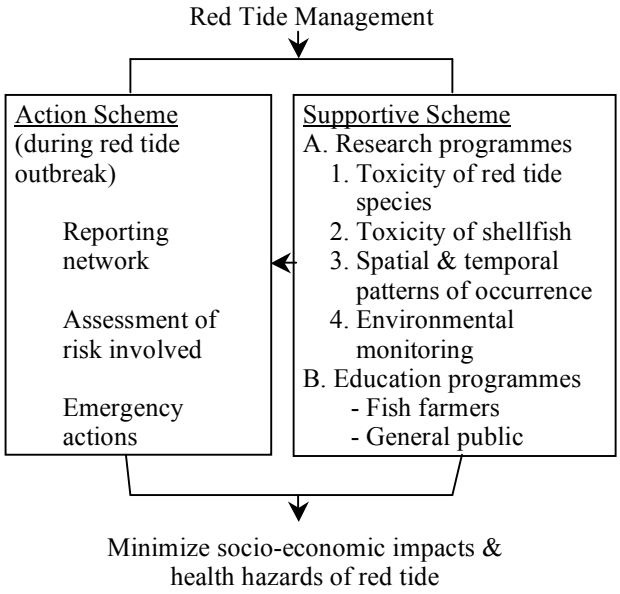

Figure 2. Red Tide Management Strategy (1983).

Under the action scheme, to facilitate early notification of the red tide incident to concerned parties such as the fish farmers and relevant government departments, a red tide reporting network which involved government staff carrying out routine work around Hong Kong waters, fish farmers and interested public reporting

sightings of discolored water to the Agriculture, Fisheries and Conservation Department (AFCD) was established. Water samples were taken for identification of the blooming algal species and assessment of the risk involved. Where necessary, a "mobile squad" would be deployed to conduct on site risk assessment. Nearby fish farmers would be advised to start aeration or relocate their raft according to the risk identified. The information of the red tide incident would also be forwarded to concerned departments for their necessary actions. When toxic algal species were detected in the water, the department in charge of food safety would enhance the seafood safety surveillance programme to check for algal biotoxin contamination. When red tide occurred in beaches, a red flag would be hoisted to alert beach-goers not to go into the water.

Under the supportive scheme, continuous effort was devoted to obtain information on all algal species found in Hong Kong waters including their taxonomic features, their toxicity and likely effects on fish, all of which are crucial to enable fast and accurate assessment of risk during a HAB incident. Laboratory techniques were also established to keep cultures of new algal species for toxicity testing. Based on past records, the patterns of occurrence of a number of harmful algal species were analyzed [3]. Results of shellfish toxicity surveillance together with occasional specific tests on shellfish and algal cultures indicated that the health risks due to HABs were not serious in Hong Kong and mainly involved paralytic shellfish poisons (PSP). These findings helped to direct shellfish toxicity surveillance to concentrate more on PSP. As most fish kills were caused by oxygen depletion, efficient aeration system for use on the fish raft was developed and extended to fish farmers since the late 80s.

This management strategy had proved to be effective for tackling red tide problems from 1983 to 1997. During this period, there were a total of 9 red tides with harmful effects, 8 associated with fish kills and one caused shellfish to become toxic [3]. All the fish kill incidents were caused by oxygen depletion and apart from the incident in 1987; all were relatively small scale involving only one to two farms.

The 1987 incident affected most farms at Yung Shue Au fish culture zone (FCZ), with a total loss of 120 tons of cultured fish valued at HK$4 million. The fish kill was caused by oxygen depletion resulting from the collapse of a dense bloom of *Heterocapsa circularisquama* (cell density up to 525,000 cells ml^{-1}). The reporting network, together with prompt confirmation that the fish kill was due to oxygen depletion, enabled fish farmers to sell off the freshly killed fish, which significantly minimized the impacts on the fisheries industry.

In 1989, an *Alexandrium catenella* bloom in Junk Bay caused green mussels in nearby FCZs to contain high levels of PSP toxin (10226–23645 MU kg^{-1}) which exceeded the World Health Organization (WHO) recommended safety limit (4000 MU kg^{-1}). At the same time, green mussels and clams from the northern waters and Lamma Island where the water did not turn red, were also found to contain unacceptable levels of PSP toxins (4779–9871 MU kg^{-1}). Toxicity tests were conducted immediately to enable prompt advice to fish farmers who was cooperative and surrendered voluntarily all the shellfish they collected. The impact was contained and no human intoxication resulted.

THE 1998 HAB

The HAB in 1998 was unprecedented in scale and caused enormous damage to the marine fish culture in Hong Kong wiping out most of the stock in the majority of the FCZs. The incident started at Kat O on 18 March 1998, followed by intermittent blooms in the northeastern waters for about two weeks. In late March to early April, blooms were found in Port Shelter. In early to mid April, various blooms occurred throughout the southern waters as well as in the Long Harbour and the Tolo Harbour (Fig. 3). According to the established red tide action plan, warnings were given to the fish farmers in FCZs in the northeastern region when red tide first affected Kat O. Most fish farmers, however, did not take heed to the warning, as red tides had not caused fish kills for many years.

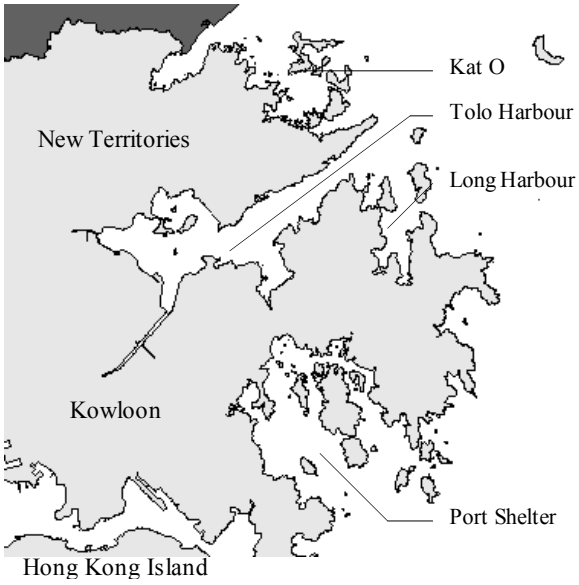

Figure 3. Map showing locations of red tide outbreak at the northeastern part of Hong Kong waters in March to April 1998.

The causative species was not described previously but was morphologically very similar to *Gyrodinium* (Fig. 4), so it was tentatively named as *Gyrodinium sp. HK98.* Later it was formally named as *Karenia digitata* [4]. This species was evidently a potent fish killer as cultured fish died quickly when they came into contact with the algal organism. Toxicity testing confirmed that this species did not carry PSP toxin. However, the mechanism by which it kills the fish is unknown. Therefore, the Government was unable to advise promptly whether the dead fish were suitable for human consumption. This generated serious criticism from the media and over reaction from the public to refrain from eating all seafood for a prolonged period.

The government responded quickly: stepped up monitoring of the bloom to give prompt warning to the fish farmers, helped to clean up the dead fish and provided financial assistance to affected fish farmers by issuing grants from the Emergency Relief Fund and low interest loans.



Figure 4. Red tide species, *Gyrodinium* sp. HK 98 (Scanning Electronic Microscope).

RED TIDE MANAGEMENT SINCE 1998

The devastating impact of the 1998 bloom on the mariculture industry had led to various criticisms on government's response: lack of alertness for crisis situation, lack of coordination among departments, confusing advice on seafood safety, and not equipped with modern technology (e.g., satellite remote sensing for red tide outbreak). There were also many suggestions made by the media, local academics and commercial firms but most were not practical or feasible in Hong Kong [5].

To better manage possible adverse impacts of HABs in the future, the Government considered that the existing management strategy should be reviewed and commissioned a consultancy study on "Monitoring and Management of Red Tide in Hong Kong" which was completed in 1999. The study confirmed that the existing strategy was an appropriate approach but there were areas for improvement. Recommendations for improvement included five major areas: management framework, monitoring programmes and facilities, mitigatory measures, public communication and education, and targeted research.

Management Framework

In accordance to the study's recommendation, a Red Tide Steering Group led by the Health, Welfare & Food Bureau (HWFB) has been established to address policy issues. For coordinating technical and operational matters during red tide events, AFCD takes a leading role in a Red Tide Interdepartmental Working Group involving a total of 9 departments: AFCD, Food and Environmental Hygiene Department (FEHD), Department of Health (DH), Leisure and Cultural Service Department (LCSD), Environmental Protection Department (EPD), Information Services Department (ISD), Marine Department (MD), Hong Kong Observatory (HKO) and Government Laboratory (GL), the first five of which are the key departments. Marine Police (MP) and Government Flying Services (GFS) also assisted in reporting red tide. An operational master plan (Fig. 5) with 24 hour contacts for all concerned parties coordinating the monitoring programmes and action plans of key departments has been drawn up.

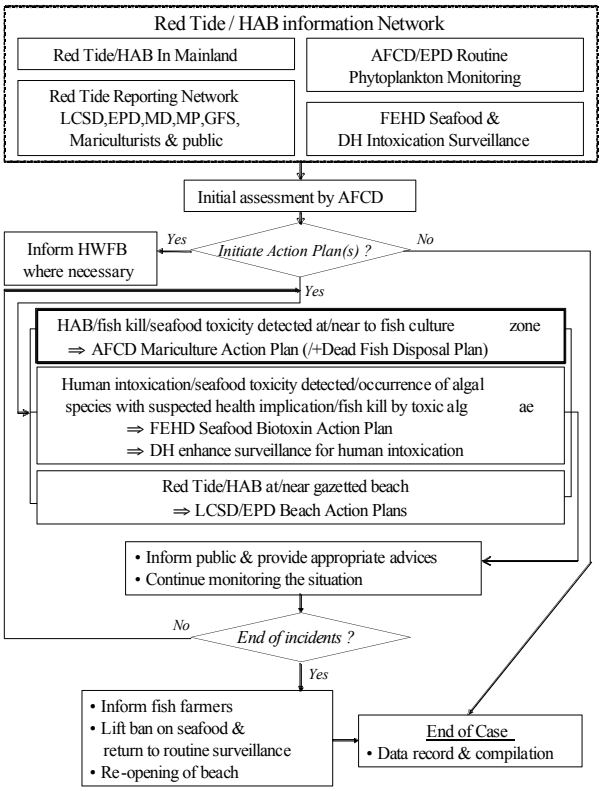

Figure 5. HAB Management Operational Master Plan (see text for abbreviations).

A contingency plan on dead fish collection and disposal is now in place. A Red Tide Expert Advisory Group has also been established with representatives from the departments involved in technological matters and experts in tertiary institutes and professional groups to enable better communication and cooperation between the management party and various research groups, so as to facilitate channeling of research efforts towards the more imminent needs in red tide management.

As the majority of the shellfish consumed in Hong Kong originates from Mainland China, for establishment of shellfish source identification/tracking system and biotoxin certification system, FEHD started some discussions with officials from the Mainland. Legislative control of harvesting/sale of biotoxin-contaminated seafood will be considered when testing facilities, as well as the biotoxin certification systems are in place.

Monitoring Programmes & Reporting Networks

In line with the study's recommendation, AFCD's phytoplankton monitoring programme was further modified to concentrate on key strategic sites to enhance its effectiveness and sustainability to provide early

warning of HABs to fish farmers. Water samples are taken weekly from 6 core stations and fortnightly from 5 offshore stations as well as 5 seasonal stations during red tide peak season for phytoplankton analysis (Fig. 6). Sampling frequency will be stepped up when harmful algal species or abnormally high phytoplankton concentrations are detected. An outreach team with clear guidelines for different scenarios is in place for rapid sampling and analysis upon receipt of red tide reports. Assistance of GFS to monitor the extent of red tides when needed was arranged. Fish farmers support groups set up in FCZs help disseminate HAB warnings to all fish farmers in the FCZ using a flag system (yellow: non-toxic bloom with risk of oxygen depletion; red: toxic bloom with risk of fish kill). A training course on water sampling and phytoplankton identification was conducted for fish farmers to help them monitor red tide in their own FCZs. Contacts with relevant official bodies in Mainland China in charge of coastal waters near Hong Kong have also been arranged for exchange of information on red tide outbreaks in neighboring waters.

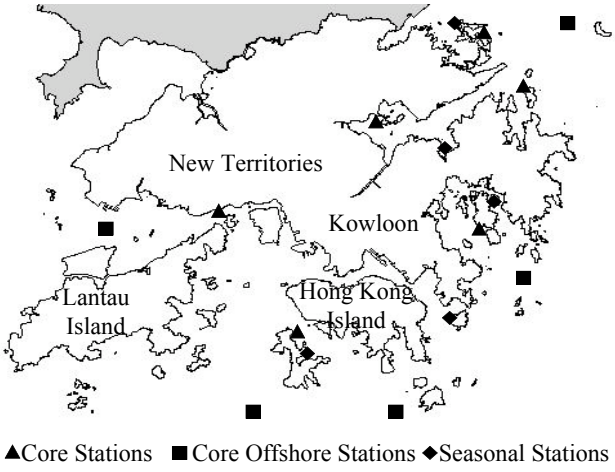

Figure 6. Locations of monitoring stations for Phytoplankton Monitoring Programme.

The shellfish toxicity surveillance programme was enhanced to identify the species and source of the shellfish samples after training of involved staff on shellfish identification. Upon receipt of report of toxic phytoplankton at a density >4 to 40 cells ml^{-1} near to known shellfish beds or FCZs, shellfish will be collected from the shellfish beds, FCZs or markets in the vicinity. On the other hand, any human intoxication case related to consumption of local shellfish in DH's intoxication surveillance will be reported to AFCD for possible correlation with occurrence of any toxic algae in the water. Regarding biotoxin testing facilities, since 1999 a private overseas service to conduct biotoxin testing in shellfish was contracted with test results being made available in seven to ten days or even shorter periods during an emergency. There are also plans to set up local biotoxin testing facilities within the government

A computer network with a Geographic Information System was established for mapping, recording, compiling and analyzing all the data obtained from the different monitoring and surveillance programmes as well as preparation of red tide reports. Also as recommended, an annual report is being prepared to serve as a basis for regular review of the data collected and operation of red tide monitoring and management programme during the year.

Mitigation

Regarding mitigatory measures to control red tide harmful effects, so far there is no single method. Fish kills due to oxygen depletion caused by algal blooms can be reduced by the efficient aeration system developed in the late 80s. For algal blooms that produce ichthyotoxins, so far the practical way is to avoid the red tide through raft relocation. To minimize impact of raft relocation on other marine activities or ecosystems, "no go" zones for fish raft relocation have been identified and a map showing location of these zones was provided to fish farmers. Arrangements were made to issue temporary permits to fish farmers whose rafts were relocated. Also to facilitate raft relocation, an improved mooring system that enabled fast detachment and reattachment of the moorings was designed and extended to fish farmers.

Following the consultancy study's recommendations, AFCD is planning to commission a study on the feasibility of using clay to control HABs in Hong Kong shortly [6, 7]. Also in-house tests on the effectiveness and practicality of applying a perimeter skirt with aeration using the Venturi nozzle to isolate fish from toxic red tides is being conducted.

Public Education & Communication

To help the general public in understanding the distinction between red tides and HABs, a poster and an information leaflet have been prepared and distributed to the public. To educate the public and the fish farmers that not all red tides will kill fish, information on red tide outbreaks is issued to the public through weekly press release and updates on the specific web page. Website:

http://www.hkredtide.org or
http://www.afcd.gov.hk/web/index.htm

Public lectures and educational seminars are conducted in collaboration with local interest groups or tertiary institutes. To publicize seafood safety, FEHD has incorporated information on shellfish poisoning in a number of leaflets and advised during regular health talks and seminars conducted for food handlers, public, teacher and students to avoid eating the viscera of fish and shellfish. Health educational materials on seafood biotoxin poisoning are also available at DH's Central Health Education Unit homepage:

http://www.info.gov.hk/healthzone/mainpage.html

Targeted Research

Funding for research is available mainly from three channels: Government commissioned studies, University

Research Grants or other specific funding such as the Environment and Conservation Fund, Jockey Club Community Fund, etc.

The study recommended the use of perimeter skirt with aeration and clay treatment to be the two most promising mitigatory measures for Hong Kong. In view of this, planning was underway for AFCD to carry out a feasibility study of using clay to control red tide in Hong Kong and an in-house experimental study on the practicality of perimeter skirt with aeration in isolating fish cages from red tide.

Based on a list of priority research topics provided by the study, a number of research projects on red tides were initiated by local tertiary institutes. Professor Joseph H. W. Lee of the University of Hong Kong has started a cross-disciplinary project on "Dynamics of Algal Blooms and Red Tides in Subtropical Coastal Waters." Joseph T. Y. Wong of the University of Science and Technology is applying various molecular techniques to develop probes for toxic algal species. Then there is a collaborative project between the City University and the Hong Kong University of Science and Technology on "Toxicities and Toxicity Mechanism of target species." A project to study monitoring of red tide occurrence with satellite remote sensing is also in progress by the University of Science and Technology.

CONCLUSIONS

As can be seen from the above, the basic strategy of red tide management involves detection of its occurrence and identification of risks followed by a series of action plans to control or minimize the impacts resulted and an on-going effort in research and education to support the action plans. The effectiveness of this strategy, however, depends very much on early detection, readily available information on the algal species and their toxicities, efficient testing facilities, good efficient communication and preparedness for cooperation amongst the different parties involved. Based on the Hong Kong experience, when HABs were small scale, a reactive approach with minimal resource input was adequate and improvement in effectiveness was gradually achieved through further information collection and educational programmes to improve communication and cooperation. However, for serious extensive HABs, a precautionary and proactive approach with substantial resource input would be necessary and effectiveness was greatly enhanced through toxic algal species monitoring to provide early detection, upgrading of toxicity testing facilities, and established action framework to ensure efficient communication and cooperation.

In recent years, red tides have become a global phenomenon and evidence is mounting that the nature and extent of the problem has been expanding [8]. Many countries/places have observed signs of increasing occurrences and impacts of HABs and are making plans or taking action to manage HAB impacts. The experience that Hong Kong has had in red tide management so far may be a useful reference for developing or improving HAB management strategy.

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HISTORICAL AND CURRENT STATUS OF RED TIDES AND SHELLFISH POISONINGS IN JAPAN

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ABSTRACT

Red tides have occurred frequently since the 1970's in Japan. In the Seto Inland Sea, the number of red tide events reached a peak of about 300 events per year in the middle of 1970's. They gradually decreased and recently remained stable at around 100 cases per year. The most noxious species are *Chattonella marina*, *C. antiqua* and *G..mikimotoi* which mainly kill finfish. *Heterocapsa circularisquama,* which shows a harmful effect on bivalves, is a new noxious species recorded in 1988. In addition, *H. akashiwo* and *Cocholodinium polykrikoides* and *Gonyaulax polygramma* are also noxious species.

The main species responsible for paralytic shellfish poisoning (PSP) in Japan are *Alexandrium tamarense*, *A. catenella* and *Gymnodinium catenatum*. PSP affected areas have shown a trend of expanding from northern to southwestern waters. One of the main causes of the PSP expansion is due to the occurrence of *G. catenatum* in the coastal waters of western Japan. Recently, PSP events by *A. tamiyavanichii* have also occurred in the coastal waters of western Japan. Meanwhile the diarrhetic shellfish poisoning (DSP) affected areas have not expanded from the northern waters and the main causative species are still *D. fortii* and *D. acuminata*.

The Fisheries Agency of the Ministry of Agriculture, Forestry and Fisheries (FA/MAFF) mainly plans the research and monitoring activities on red tides and shellfish poisonings in Japan. The Japan Fisheries Resource Conservation Association, universities, Fisheries Research Agency, and Prefectural Fisheries Experimental Stations, etc. carry them out with budget support from FA/MAFF.

RED TIDES

Historical Events on Red Tides

Noxious algal blooms associated with mass mortality of marine organisms, especially cultured finfish and shellfish, have occurred frequently since the 1970's in Japan. At the same time there has been development of heavy industry in the coastal terrestrial zone and fish aquaculture industry in marine embayments. Fish mass mortality incidents with serious economic loss also increased. Most of the noxious algal species cause water discoloration (i.e., red tides), but less than 20% of the red tides cause harmful effects. Until the 1980's efforts on management and scientific research have been devoted to the harmful species of raphidophytes *Heterosigma akashiwo*, *Chattonella marina*, *C. antiqua* and dinophyte *Gymnodinium mikimotoi* before 1980's. Recently, successive occurrences of novel harmful species such as dinophytes *Heterocapsa circularisquama* and *Gymnodinium* sp. type Imari have been observed.

Annual Changes of Red Tide Events and Economic Impacts

Annual changes in the number of red tides occurring in the western part of Japan - Seto Inland Sea, Kyushu area, Tosa Bay and Kumano-nada (Fig. 1) - are shown in Figure 2 [1, 2, 3]. In the Seto Inland Sea, red tide events were very rare in the 1950's. Then, the number dramatically increased and reached a peak of about 300 events per year in the middle of 1970's. After this period, they gradually decreased and recently remained stable at around 100 cases per year. This is thought to be mainly due to governmental regulations against eutrophication, such as "Law concerning special measures for conservation of the environment of the Seto Inland Sea (Seto Inland Sea Law)" enacted in 1973. On the other hand, in the other three areas, the number of red tide events has not decreased in these two decades. The ranges are 60 to 110 events per year in Kyushu area, 3 to 16 in Tosa Bay and 0 to 9 in Kumano-nada.

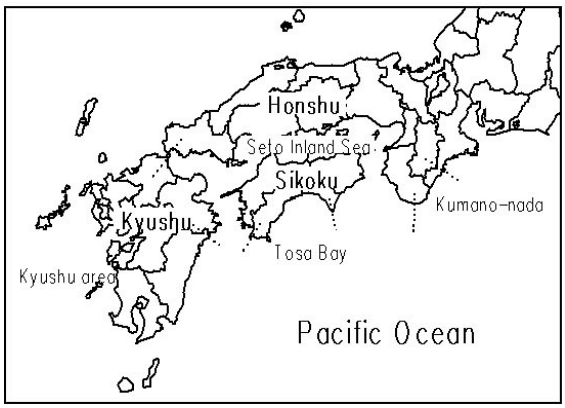

Figure 1. Map of western Japan.

The annual change in the number of red tides which caused fisheries damage in Japan is shown in Figure 3. In these three decades, it seems to be stable. The numbers of events range from 20 to 50. The annual economic losses caused by red tides in Japan are also shown in Figure 4. The maximum loss was recorded in the summer of 1972, when *Chattonella* red tides caused severe damage to cultured yellowtail in the eastern part of the Seto Inland Sea. In this case, the loss was about seven billion yen (about 70 million US$). Since then, even in

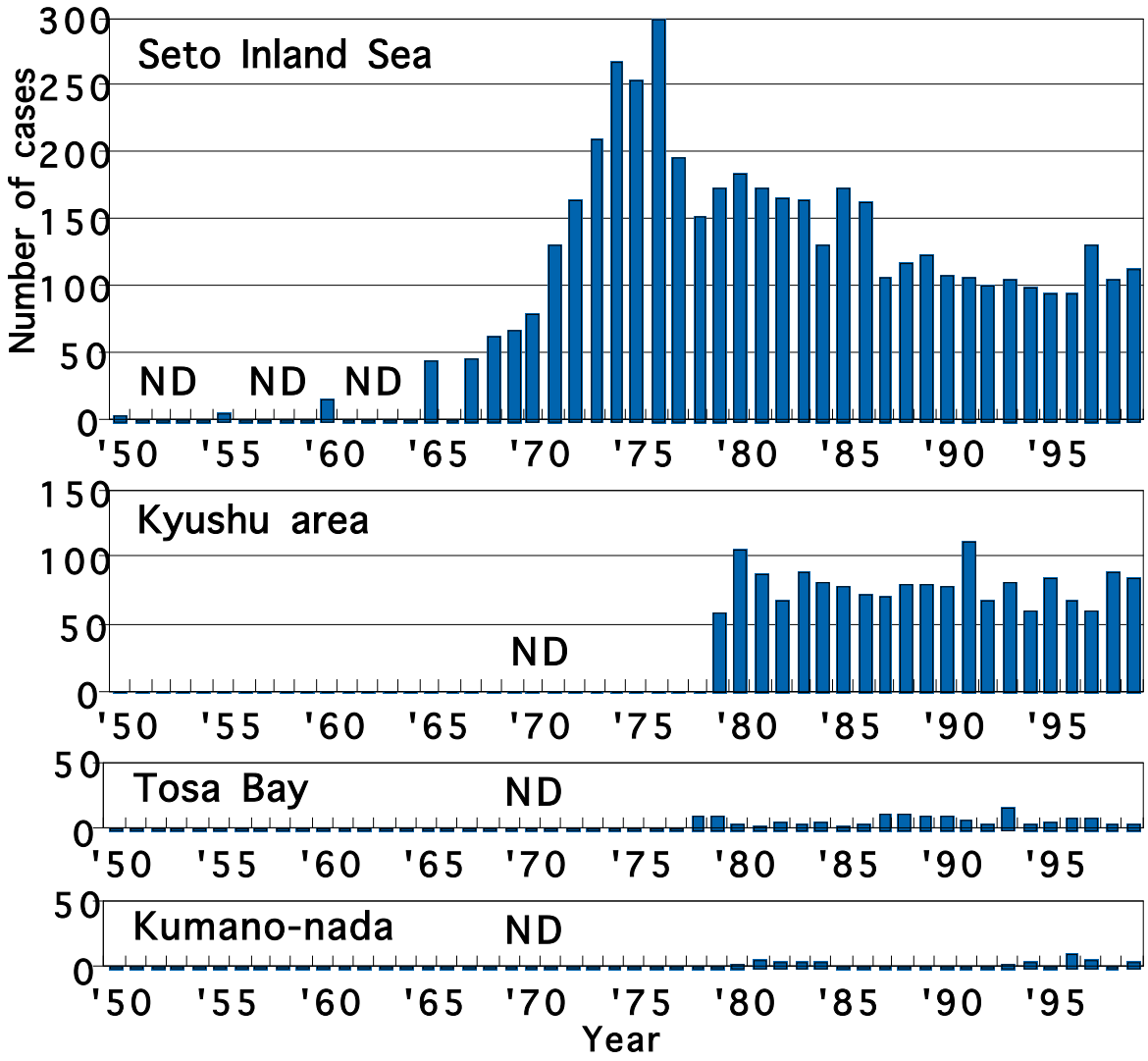

Figure 2. Annual changes of red tides in western Japan. Refer to Figure 1 for the names of areas.

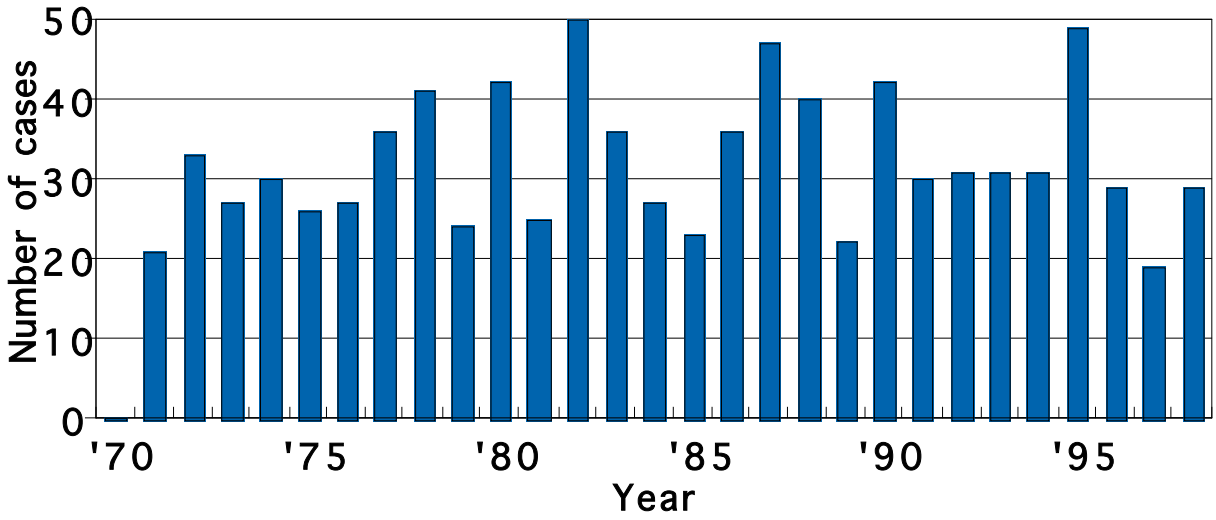

Figure 3. Number of red tides which caused fisheries damage in Japan.

the 1990's, severe fisheries damages were sometimes recorded.

Major Noxious Species and Their Harmful Effects

Table 1 shows major red tide causative species in Japan and the number of fisheries damages due to these species [4, 5]. The most noxious species are *Chattonella marina*, *C. antiqua* and *G..mikimotoi*. These three species mainly kill finfish and have frequently caused very severe fisheries damages worth more than 100 million yen (about one million US$). *Heterocapsa circularisquama,* recorded for the first time in a small semi-enclosed bay connected to Tosa Bay, was added to the noxious species list in 1988 [6]. This species shows a harmful effect only on shellfish, particularly bivalves such as oyster, pearl oyster and short-necked clam. Very severe fisheries damages caused by this species occurred four times in the last decade [7]. In addition, *H. akashiwo* and dinophytes *Cocholodinium polykrikoides* and *Gonyaulax polygramma* are also noxious species.

SHELLFISH POISONINGS

Historical Events on Shellfish Poisonings

In the early 1970s, techniques to mass culture the Yesso scallop (*Patinopecten yessoensis*) were developed, and the aquaculture industry spread mainly along the coasts of northern Japan. The occurrence of shellfish poisonings along the coasts of northern Japan became frequent since the late 1970s resulting in cases of human illness due to eating the contaminated shellfish [8]. To minimize the effects of the increases of the PSP (paralytic shellfish poisoning) and DSP (diarrhetic shellfish poisoning) outbreaks, administrative measures were set up to regulate the harvesting and shipping of shellfish. In 1978, the Fisheries Agency of the Ministry of Agriculture, Forestry and Fisheries (FA/MAFF) with the Ministry of Health and Welfare, Government of Japan set guidelines for monitoring the scallop culture grounds and marketing regulations for shellfish. Self-regulation of shellfish shipments by fishermen was implemented when the PSP or DSP contaminations in shellfish exceeded the quarantine levels of 4.0 and 0.05 MU g^{-1} edible parts, respectively. Since then, various administrative measures have been taken and monitoring programs for shellfish poisonings planned with the cooperation of the Prefectural Fisheries Departments and Sanitation Departments. As a result, a regional monitoring network has been functioning since 1981 in the northern waters and was expanded to cover the whole of Japan in 1984. Since then, the occurrence of poisoning events caused by marketing shellfish has been avoided [9].

The microalgae responsible for shellfish poisonings such as ASP (amnesic shellfish poisoning) and NSP (neurotoxic shellfish poisoning) are distributed in Japanese coastal waters. However, the occurrence of ASP and NSP events has not been officially recorded. Domoic acid contamination over the safe limit of 20 μg g^{-1} has not been detected in shellfish in spite of intensive surveys during the period 1993-1998 in Japanese coastal waters. Therefore, there have been no monitoring programs for those toxins, except in Mutsu Bay where ASP monitoring is carried out in the culture grounds of Yesso scallop.

Changes in the Areas and the Number of Shellfish Species Affected by PSP and DSP

The five yearly changes in the areas of coastal waters, which have been restricted for shipping and marketing affected bivalves and sea squirt due to PSP outbreaks, are shown in Figure 5. Shadowed areas indicate the coastal areas where the PSP quarantine limit of 4.0 MU g^{-1} in shellfish has been exceeded during each period, with the affected shellfish species. Between 1978-1982 and 1998-2001 July, the number of areas affected by PSP has increased from 10 to 32 and the number of affected shellfish species has increased from 8 to 12. Recently, PSP affected areas that have experienced toxin levels exceeding the quarantine limit have shown a trend of expanding from northern to southwestern waters [10].

Figure 6 shows the five yearly changes in the areas of restricted coastal waters for shipping and marketing affected bivalves due to DSP outbreaks. Shadowed areas also indicate the coastal waters where the DSP quarantine limit of 0.05 MU g^{-1} has been exceeded leading to the restrictions. Between 1978-1982 and 1998-2001 July, the number of areas and shellfish species affected by DSP has been almost constant.

Major Toxic Microalgae Responsible for PSP and DSP

The main species responsible for PSP in Japan are *Alexandrium tamarense*, *A. catenella* and *Gymnodinium catenatum*. Those responsible for DSP are *Dinophysis fortii* and *D. acuminata*. Initially, the PSP problems were involved with cultured scallop in northern Japan, but the areas affected by PSP have continued to expand to southwestern waters (Fig. 5). Based on the results from recent investigations, one of the main causes of the PSP expansion is considered to be due to the occurrence of *G. catenatum* in the coastal waters of western Japan. PSP events by *G. catenatum* have been increasing in the coastal waters of Kyushu and western Shikoku [11, 12]. Recently, two PSP events by *A. tamiyavanichii* have occurred in Japan. One was along the coasts of Kagawa and Tokushima Prefectures in 1999, and another was in Okinawa Prefecture in 1997 and 1998. While, the DSP affected areas have not expanded from the northern waters since the early 1980s (Fig. 6) and the main causative species are still *D. fortii* and *D. acuminata*.

Affected Marine Organisms by PSP and DSP

From the results of recent surveys, the bivalve shellfish affected by PSP have diversified. Now affected species include short-necked clam (*Tapes philippinarum*), equilateral venus (*Gomphina melanaegis*), noble scallop (*Chlamys nobilis*) and ascidian species of sea squirt

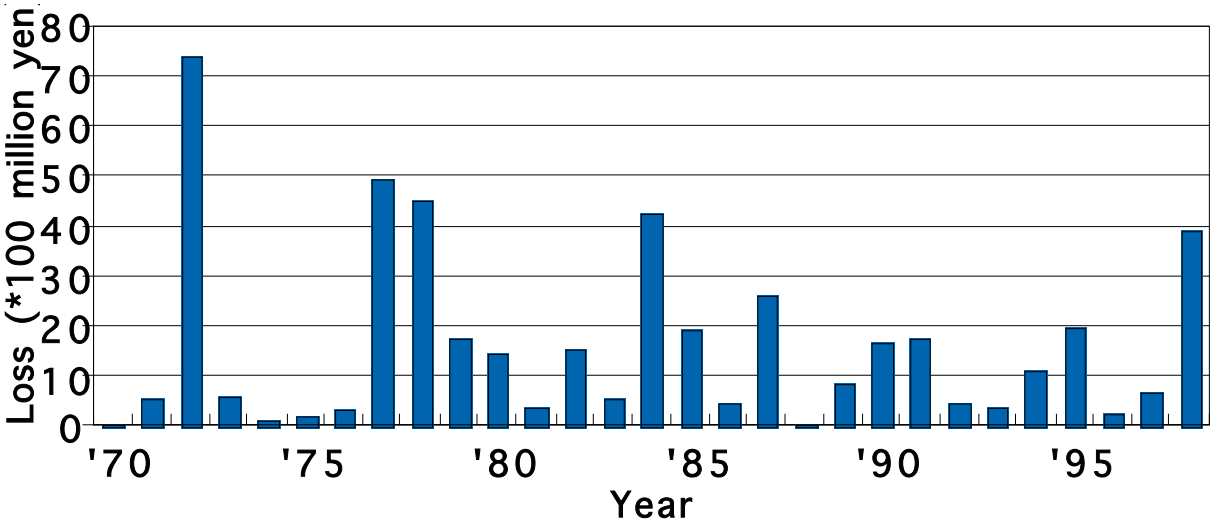

Figure 4. Economic losses caused by red tides in Japan.

(*Halocynthia roretzi*) as well as the initially affected bivalve species of scallops, mussels and oysters. The sea squirt, which is popular as seafood in Japan and is cultured mainly along the Pacific coasts in the northeastern Japan, is currently used as one of the monitoring organisms for the PSP and DSP.

The number of bivalve species which have been restricted for shipping by exceeding 4 MU g^{-1} of the PSP quarantine levels has reached 15 species at present (Table 2). The number of bivalve species restricted for shipping by DSP has remained at 9 species. Recently, the cultured green mussel (*Perna viridis*) in Shioya Bay, Okinawa Main Island, has been affected by PSP in 1997 and 1998.

ON-GOING ACTIVITIES AND RESEARCH PROJECTS ON RED TIDES AND SHELLFISH POISONINGS

The FA/MAFF plans the research and monitoring activities on red tides and shellfish poisonings in Japan. The Japan Fisheries Resource Conservation Association (JFRCA), universities, Fisheries Research Agency (FRA), and Prefectural Fisheries Experimental Stations (PFESs), etc. carry them out with budget support from FA/MAFF. There are three sections involved in the research on red tides and shellfish poisonings in the FRA, an Independent Administrative Institution in Japan, which was established in April 2001 and consists of nine research institutes. Two of the sections in the National Research Institute of Fisheries and Environment of Inland Sea (FEIS/FRA) are researching the physiology and ecology of phytoplankton causing harmful algal blooms. The other section in the Tohoku National Fisheries Research Institute (TNFRI/FRA) is researching the analytical methods to monitor shellfish toxins and the dynamics of the toxins in shellfish. Furthermore, the FA/MAFF, FEIS/FRA and TNFRI/FRA regularly organize meetings attended by prefectural officials for areas that experience harmful algae and shellfish poisonings to share information and techniques.

On-Going Activities Implemented Under the FA/MAFF

1) Support and reinforce measures against damage caused by red tides and shellfish poisonings

The production and distribution of standard toxins, which are commonly difficult to obtain and are indispensable for toxin analyses of shellfish poisonings with HPLC, are carried out, in order to promote the monitoring of shellfish toxins by local administrative governments.

Seminars on the research techniques of red tides and shellfish poisonings to train researchers in local administrative divisions are held annually. With clerical support from JFRCA and co-operation by university staff, FEIS/FRA trains officials from PFESs to help them with techniques for identification and investigation of harmful and toxic phytoplankton species. With regards to shellfish toxins TNFRI/FRA trains officials from PFESs and Prefecture Institutes of Public Health to help them with techniques for analysis of PSP and DSP toxins with cooperation of the university staff and the Japan Food Research Center. Teaching texts and videos to assist with plankton monitoring are issued, and teaching videos for fishermen are also issued by the JFRCA with cooperation and direction of the FEIS/FRA.

2) Urgent measures against Heterocapsa *red tides.*

To reduce the damage caused by the noxious species *Heterocapsa circularisquama* etc., this work aims to develop the techniques for predicting *H. circularisquama* blooms and for preventing damage by them.

3) Advanced use and development of an information network system on red tides and shellfish poisonings.

New systems to transmit a large amount of data collectively are being developed and added to the present information network system on red tides and shellfish poisonings, with a database that will enhance the system. Broad and general information on red tides and shellfish poisonings are being processed, and the functions offered to the users (e.g., administrative divisions) are being developed and improved.

Table 1. Major red tide causative species and the number of fisheries damages due to these species in Japan. SIS = Seto Island Sea ('70-'98); KA = Kyushu Area ('79-'98); TB = Tosa Bay ('78-'98); KN = Kumano-nada ('80-'98); T = Total.

Causative Organism (Genus)	More than 100 million yen					10-100 million yen					Species Name
	SIS	KA	TB	KN	T	SIS	KA	TB	KN	T	
Dinophyceae											
Prorocentrum	0	0	0	0	0	3(2)	1(0)	0	0	4(2)	*P. dentatum*
Cochlodinium	0	0	0	0	0	0	12(1)	0	0	12(1)	*C. polykrikoides*
Gymnodinium	16(2)	2(0)	1(0)	1(0)	20(2)	20(3)	9(1)	2(2)	0	31(6)	*G. mikimotoi*
Polykrikos	0	0	0	0	0	1(1)	1(0)	0	0	2(1)	*Polykrikos* sp.
Noctiluca	0	0	0	0	0	4(0)	0	0	0	4(0)	*N. scintillans*
Ceratium	0	0	0	0	0	1(1)	1(0)	0	0	2(1)	*C. fusus*
Alexandrium	0	0	0	0	0	0	1(0)	0	0	1(0)	*A. catenella*
Gonyaulax	2(0)	0	0	0	2(0)	0	0	0	0	0	*G. polygramma*
Heterocapsa	3(0)	1(0)	0	0	4(0)	1(0)	1(0)	0	0	2(0)	*H. circularisquama*
Rhaphidophyceae											
Chattonella	13(2)	5(0)	0	0	18(2)	11(1)	9(0)	2(1)	0	22(2)	*C. antiqua, C. marina*
Heterosigma	0	1(0)	1(0)	0	2(0)	7(3)	0	2(1)	0	9(4)	*H. akashiwo*
Chrysophyceae											
Distephanus	0	1(0)	0	0	1(0)	0	0	0	0	0	*D. speculum*

A dollar corresponds to 108 yen.
Figures in parentheses indicate the number of cases when more than two species caused the red tide (complex red tide).

4) Measures to maintain the safety of marine foods, such as bivalves, against shellfish poisonings

Studies to elucidate the mechanisms of toxication and detoxication in shellfish are being promoted. Easy and reliable screening techniques for processing a large amount of samples are being developed and these monitoring techniques will be validated. Procedures for the effective restriction by fishermen and for supplying the safe marine foods for consumers will be established in this work to prevent the incidents and to reduce the economic losses caused by shellfish poisonings.

5) Research to encourage environmental conservation in the coastal fishing grounds

Prompt reporting of the environmental information in the coastal fishing grounds to the fishery cooperatives and fishermen has been carried out to reduce the fishery damages by the red tide and shellfish poisoning, etc. It will be planned in this research to establish the long-term monitoring system on the environment factors such as nutrients and plankton etc. in the coastal fishing grounds.

6) Development of basic technology to control red tides and shellfish poisonings

The mechanisms by which red tides and shellfish poisonings occur and become an obstruction to the fishery environment will be elucidated. The development of basic technology to control red tides and shellfish poisonings will be planned in this sub-project, including the establishment of techniques for predicting the occurrence of red tides and shellfish poisonings.

7) Measures to enhance the monitoring systems in the coastal fishing grounds.

For the maintenance of good fishing conditions, plans to further enhance the efficiency of the monitoring system by the fishermen and to promote activities on waste removal in the coastal fishing grounds and the cleanup of beaches are performed in this work.

On-Going Research Projects Implemented by the FRA

1) Development of Techniques for Predicting Phytoplankton Blooms Responsible for Paralytic Shellfish Poisoning (Research period: FY 1998-2001).

The improvement of PSP monitoring systems, shellfish cultivation techniques and marketing systems based on the prediction of toxic phytoplankton blooms will reduce the economic losses to the shellfish fishery. In this study, (1) the physiological and ecological characteristics of PSP toxin-producing phytoplankton, (2) the relationships between PSP toxin-producing phytoplankton and coastal organisms, and (3) the mechanisms of toxin accumulation in bivalves will be clarified through field investigations, laboratory experiments, and analysis of toxins. Furthermore, we aim to develop a technique for predicting *A. tamarense* blooms in Hiroshima Bay. Organizations involved in this

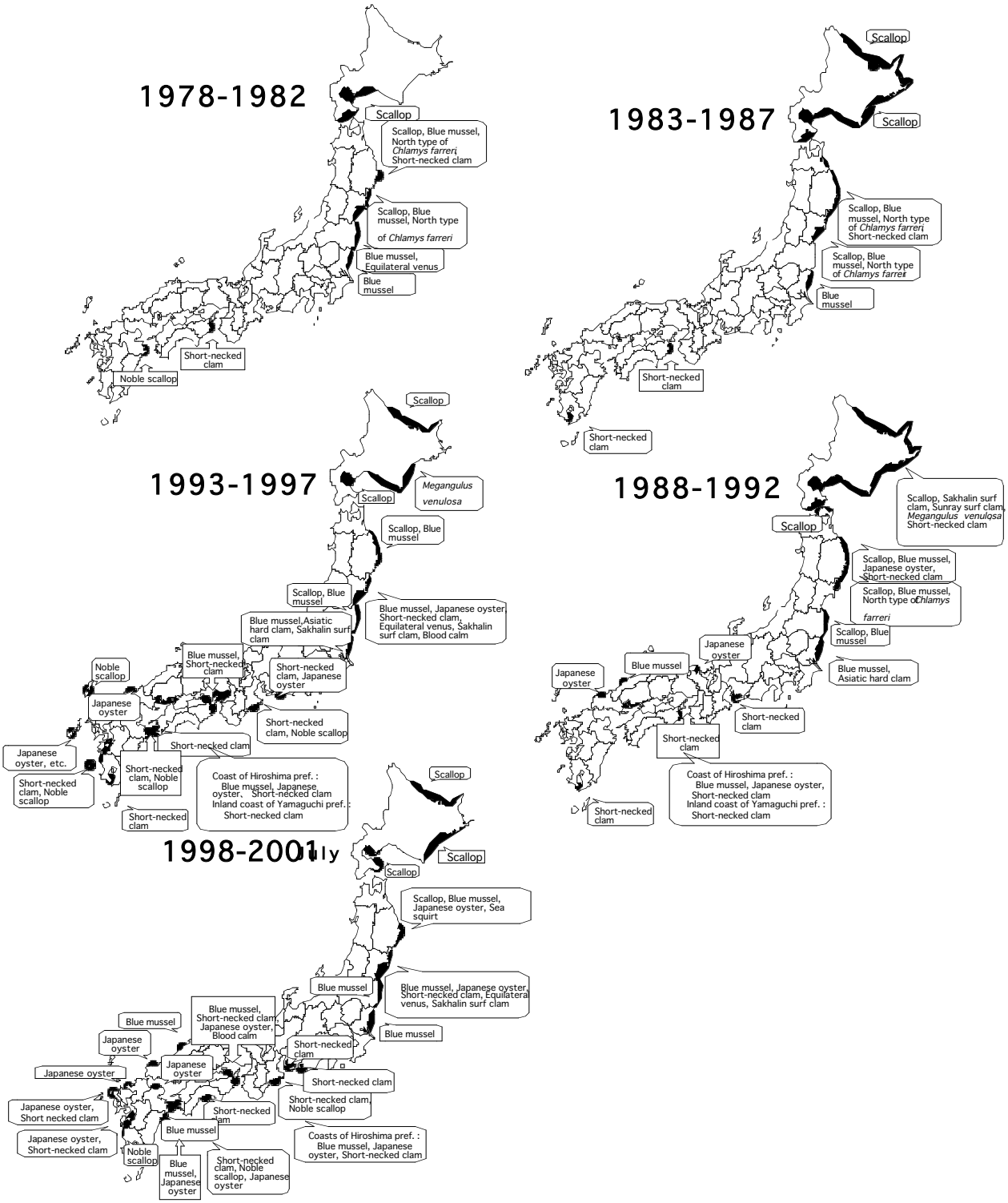

Figure 5. Changes on a five yearly basis of the restricted coastal waters for shipping and marketing bivalves and sea squirts due to PSP outbreaks.

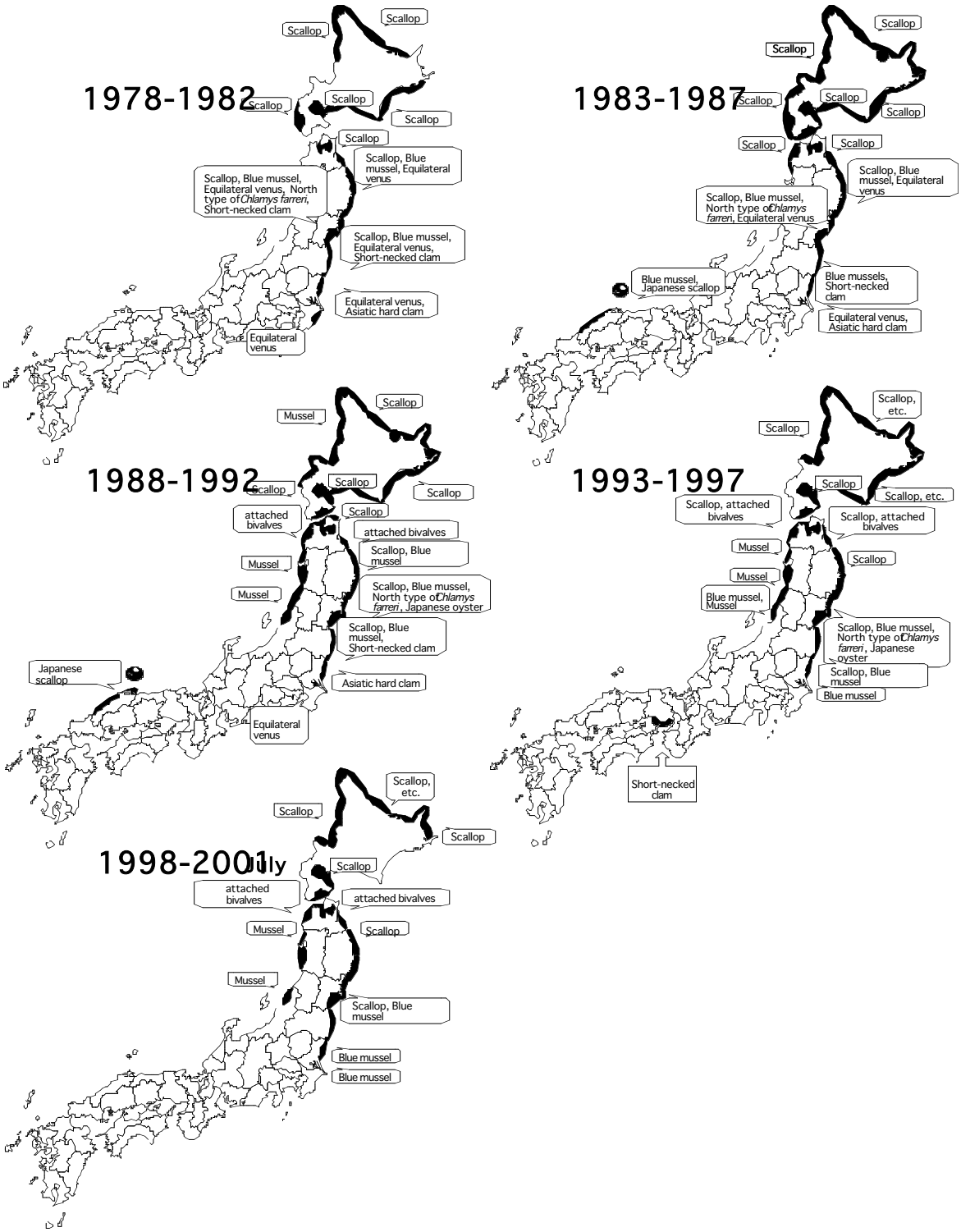

Figure 6. Changes on a five yearly basis of the restricted coastal waters for shipping and marketing bivalves due to DSP outbreaks.

Table 2. The bivalves which have been restricted from being marketed due to exceeding the quarantine levels of PSP and DSP in Japan during the period 1978 to 2001.

Toxins	Scientific name	English name	Japanese name
PSP, DSP	*Mytilus edulis*	Blue mussel	Murasakiigai
PSP, DSP	*Mytilus coruscus*	Mussel	Igai
PSP, DSP	*Crassostrea gigas*	Japanese oyster	Magaki
PSP, DSP	*Chlamys farreri nipponensis*	Farrer's scallop	Akazaragai
PSP	*Chlamys nobilis*	Noble scallop	Hiogigai
PSP, DSP	*Pecten albicans*	Japanese baking scallop	Itayagai
PSP, DSP	*Patinopecten yessoensis*	Yesso scallop	Hotategai
PSP	*Megangulus venulosa*	Great Alaskan tellin	Saragai
PSP	*Spisula polynyma*	Alaskan surf clam	Nagaubagai
PSP	*Pseudocardium sachalinense*	Sakhalin surf clam	Ubagai
PSP	*Scapharca broughtonii*	Ark shell, Blood calm	Akagai
PSP, DSP	*Tapes philippinarum*	Short-necked clam	Asari
PSP, DSP	*Gomphina melanaegis*	Equilateral venus	Kotamagai
PSP, DSP	*Meretrix lamarckii*	Asiatic hard clam	Chosenhamaguri
PSP	*Perna viridis*	Green mussel	Midoriigai

projective study are the Toxic Phytoplankton Section, Harmful Phytoplankton Section, Biological Environment Section of FEIS/FRA; Coastal Fisheries Promotion Section of TNFRI/FRA; Food Safety Section of NRIFS/FRA, and consignment partners in Kyushu and Tohoku Universities.

2) Techniques for the Prevention of Mass Mortalities of Bivalves due to the Harmful Dinoflagellate Heterocapsa circularisquama *and Preservation of the Marine Environment (Research period: FY 2000-2004).*

The dinoflagellate *Heterocapsa circularisquama* is one of the most harmful microalgae in Japanese coastal waters. Recently, the distribution of *H. circularisquama* has spread rapidly in the western part of Japan. The purpose of this study is to elucidate the mutual relationship between *H. circularisquama* and other phytoplankton and to promote predictive techniques of the occurrence of red tides, techniques to eliminate *H. circularisquama* and techniques to prevent mass mortalities of bivalves occurring due to *H. circularisquama.* Organizations in this projective study are the Harmful Phytoplankton Section, Toxic Phytoplankton Section, Biological Environment Section and Tidal Zone Ecology Section of FEIS/FRA.

3) Development of the techniques for detecting the vegetative cells and resting cysts of toxic phytoplankton species by fluorescent monoclonal antibodies (Research period: FY 1999-2001).

The fluorescent monoclonal antibodies, which react specifically to the vegetative cells and resting cysts of toxic phytoplankton species, are investigated. By using these fluorescent monoclonal antibodies, easy, objective, and rapid techniques for detecting the vegetative cells and resting cysts of toxic phytoplankton species collected from the field under a fluorescent microscope are being developed. The toxic Phytoplankton Section of FEIS/FRA and Transgenic Corporation execute this study.

4) Development of a technique for eliminating Heterocapsa circularisquama *red tides by use of lytic viruses (Research period: FY 2000-2002).*

The objective of the study is the accumulation of scientific information that is necessary to develop the means for eliminating red tides by characterizing the viruses infecting the noxious red tide-causing alga *Heterocapsa circularisquama*. The Harmful Phytoplankton Section of FEIS/FRA undertakes this study.

PRESENT AND PROSPECTIVE SUBJECTS ON RED TIDES AND SHELLFISH POISONINGS IN JAPAN

(1) The practical use of new techniques in the field monitoring system on red tides and shellfish poisonings, e.g., newly developed oceanic instruments and the immunoassay technique for detecting the harmful algae.

(2) Positive introduction of molecular techniques into the studies of red tides and shellfish poisonings, e.g., the classification and identification of harmful microalgae and the determination of their physiology and ecology.

(3) Development and practical use of new technology to control red tides by using phytoplankton control factors, e.g., algicidal bacteria and lytic viruses which exist naturally in the coastal ecosystem.

(4) Urgent promotion of investigations on novel toxic algae such as *G. catenatum* and *A. tamiyavanichii*, to minimize the effects of any further outbreaks and expansion of PSP by these species in the coastal waters of western Japan.

(5) Establishment of measures against ASP outbreaks in the future, and the accumulation of scientific information concerning ASP and the causative diatom species.

(6) Establishment of guidelines for the careful monitoring and restriction on shellfish poisonings according to each shellfish species, each fishery ground and each causative species.

(7) Reinforcement of public education and announcements on shellfish poisonings for avoiding the occurrence of poisoning due to contaminated bivalves collected by the public for personal consumption.

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RED TIDE, ITS DILEMMA AND STRATEGIES: MONITORING & MITIGATION PROGRAM ON COASTAL WATERS OF THE SOUTH CHINA SEA

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ABSTRACT

Environmental conditions of coastal waters in the South China Sea have gotten worse in the past two decades and red tide outbreaks from diverse species are frequent. Dealing with red tide problems has become an imminent issue, especially for the government. Thus, a red tide monitoring and management program is urgently needed. The objectives and organization of harmful algal bloom management and monitoring programs are described.

RED TIDES OF THE SOUTH CHINA SEA

Environmental conditions of coastal waters in the South China Sea have gotten worse in the past two decades due to untreated sewage discharge, over-fishing, intensive aquaculture, coastal development, over land use, and international maritime traffic. The consequences have been coastal eutrophication and alterations in hydrography and land use patterns in coastal areas. Apparently, red tide outbreaks are frequent and their impacts are worsening.

Red tide outbreaks from diverse species are frequent in the South China Sea. The first report on a South China Sea red tide incident was a *Noctiluca scintillans* bloom in Hong Kong [1]. The first toxic dinoflagellate bloom, due to *Alexandrium catenella,* occurred in 1989. During the bloom, shellfish toxicity increased from 2280 MU to 16500 MU kg^{-1}. Finally, a toxic Raphidophycean species, *Chattonella marina,* bloomed in Dapeng Bay in 1991 [2] and caused a fish kill. Since 1980, the number and magnitude of red tides have increased dramatically in Daya Bay, Dapeng Bay, Shenzhen Bay, Zhangjiang Port, the Pearl River estuary, the Guangdong coasts, and Hong Kong waters.

Recent examples of destructive red tides in China include: a *Phaeocystis globosa* bloom (Fig. 1a) in 1997 on the Guangdong coast causing caged-fish mortality with an estimated loss of over ten million U.S. dollars and a 1998 massive *Karenia mikimotoi* and *Karenia digitata* (Fig. 1b) [3] bloom in Guangdong and Hong Kong coastal waters, which devastated the mariculture industry, and caused a total claimed loss of almost $50 million.

There is a great diversity in the causative organisms of red tides in China's coastal waters (Table 1), including: *Alexandrium tamarense*, *A. catenella*, *Karenia catenatum*, *Scrippsiella trouchoides*, *Heterosigma akashiwo*, *Akashiwo sanguenea*, *Chattonella marina*, and *Phaeocystis globosa*.

The impacts of red tides have been diverse. However, the main aspects in China are damage to the mariculture industry and aquatic products safety relating to human health. The accumulation of algal toxins in shellfish is a notable human health issue. According to our investigation, paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP) and ciguatera fish poisoning (CFP) reached critical levels in some sea regions (Table 2).

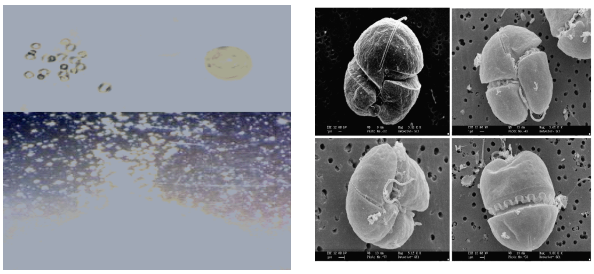


Figure 1. The causative organisms a) *Phaeocystis globosa* and b) *Karenia* spp.

Toxins identified (Table 3) from shellfish harvested from Daya bay show that this problem must draw greater attention for aquatic product consumption to protect human health. In addition, the incidence of ciguatera poisoning has increased dramatically in Hong Kong in the past decade (Fig. 2). Therefore, toxic algae-induced ciguatera is another area in need of increased concern.Dealing with red tide problems has become an imminent issue, especially for the government. Thus, a red tide monitoring and management program is urgently needed. The South China Sea Branch, SOA, and the Department of Marine & Fisheries of Guangdong Province must respond to this requirement for marine environmental protection of developing fisheries, by setting up new red tide management and monitoring (M&M) programs. The proposed system comprises the construction of red tide monitoring networks and a Center for red tide research in Guangdong province.

The objectives of HAB M&M programs are: (1) to determine regional red tide initiating mechanisms; (2) to set up a red tide forecast system to mitigate HAB adverse impacts, especially to reduce economic losses for the mariculture industry; and (3) to form a fast-reacting system, coupling all units for HAB incidents coordination action.

To carry out these tasks, red tide monitoring networks were formulated in the Marine and Fisheries Environmental Monitoring Center (MFEMC) of Guangdong, and the South China Sea Branch, SOA including the implementation of cruises every season in the South China Sea. The work of this organization

Table 1. Main red tide events in Guangdong coastal waters.

Time	Site	Red tide organisms	Consequences
March & April 1998	Guishan isle, Zhuhai, Guangdong and HK	*Karenia mikimotoi* *K. digitata*	Caged-fish Mortality; Economic losses estimated about $50 million
April 1998	Shengzhen Bay 100 km^2	*Gyrocinium instriatum*	Fish kill
May 1998	Daya Bay	*Karenia mikimotoi*	
September 1998	Daya Bay	*Scrippsiella trochoides*	
7 November 1998 March 1999	East Dapeng Bay	*Gonyaulax polygramma*	
10-23 July 1999	East Guangdong 400 km^2	*Phaeocystis globosa*	Economic loss of $200 thousand
28 March 2000 – 2 April 2000	Yangxi 0.2 km^2	*Prorocentrum* sp.	
8-20 August 2000	Huiyang 20 km^2	*Scrippsiella trochoides* *Protoperidinium quiquecorne*	Economic loss of $120 thousand
14-15 March 1999	Danpeng Bay	*Gonyaulax polygramma*	
10-23 July 1999	Raoping sea area 400 km^2	*Phaeocystis globosa*	Fish kill, $200 thousand loss
8-10 August 2000	Daya Bay 40 km^2	*Scrippsiella trochoides* *Protoperidinium quiquecorne*	Fish kill, $120 thousand loss
26 September – 10 October 2000	Dapeng Bay, Shengzhen 25 km^2	*Scrippsiella trochoides*	
8-12 January 2001	Houhai, Shengzhen Bay 2 km^2	*Thalassiosira* sp. *Alexandrium. catenella*	
6-23 February 2001	Dachan Bay 20 km^2	*Akashiwo sanguenea*	
March 2001	Yantian, Shengzhen 2 km^2	*Heterosigma akashiwo*	
22-28 March 2001	Nanao Port, Shengzhen 5-7 km^2	*Gonyaulax polygramma*	
18-23 April 2001	Xiaomeisha, Dapeng Bay 5 km^2	*Heterosigma akashiwo*	
23 April – 4 May 2001	Baguang, Shengzhen 5 km^2	*Fibrocapsa* sp. *Chattonella marina*	
28 April – 2 May 2001	Dapeng Bay	*Akashiwo sanguenea*	
13-14 May 2001	Dapeng Bay	*Noctiluca scintillans* *Gyrodinium instriatum* *Scrippsiella trochoides*	
8-10 May 2002	Yantian, Dapeng Bay 0.4 km^2	*Scrippsiella trochoides*	
4-12 June 2002	Shengzhen Bay 80 km^2 Sea area near to Hong Kong airport 40 km^2 Dapeng Bay 150-200 km^2 North to Macao airport 5 km^2	*Skeletonema costatum* *Cochlodinium* sp.	
10 December 2002 (lasted one month)	Shantou Port 100 km^2	*Phaeocystis globosa*	Natural larvae of eel, cultured oyster, and Porphyra damaged
9 June 2003	Shengzhen Bay 40 km^2	*Heterosigma akashiwo*	No damage
15-18 June 2003	Pingzhou isle, Shengzhen 50 km^2	*Karenia mikimotoi* *Gyrodinium insstriatum*	No damage
22 July 2003	Zheling, Raoping 400 km^2	*Phaeocystis globosa*	No damage
23 July 2003	Dapeng Bay	*Chattonella marina*	No damage
12 August – 2 September 2003	Daya bay 15 km^2	*Chattonella marina* *Scrippsiella trochoides*	Hazard to caged-fish, value $50 thousand

Table 2. PSP toxicity collected from the coast of Shenzhen area, Guangdong province.

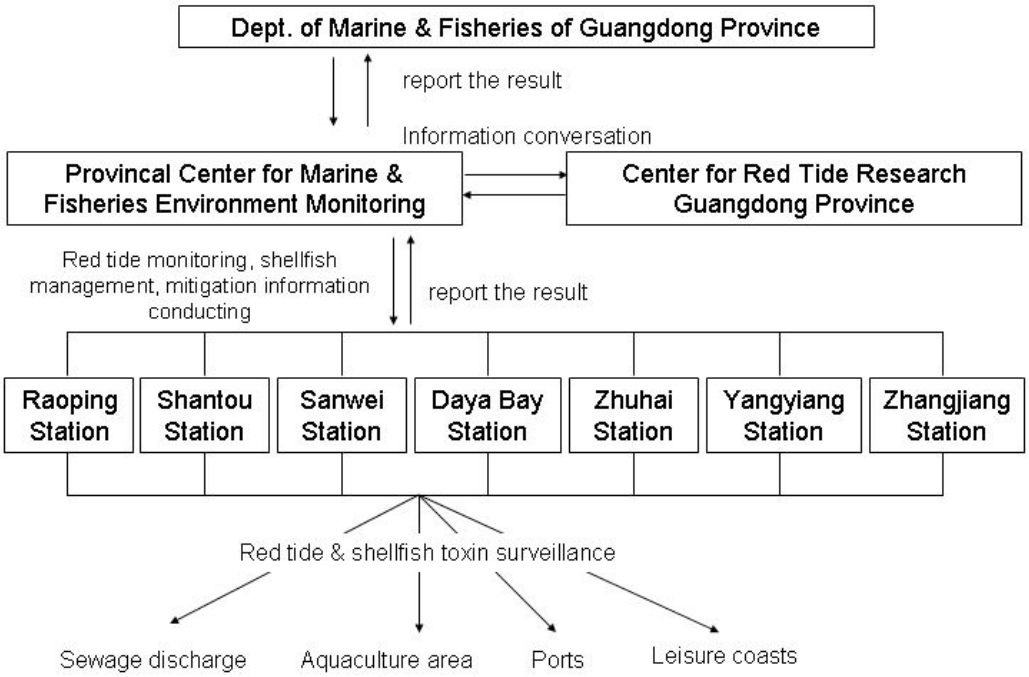
Sampling date	Shellfish species	Sampling station	Toxicity (MU g^{-1})	Growing Environment
25 Jan 2002	Perna viridis	Dongshan, Daya bay	0	Suspended culture
25 Jan 2001 31 Jan	*Chlamys nobilis*	Dongshan, Daya bay	0	Suspended culture
2002	*Chlamys nobilis*	Nanao, Dapeng bay	0	Suspended culture
21 Feb 2002	*Chlamys nobilis*	Dongshan, Daya bay	5.1	Suspended culture
21 Feb 2002	*Perna viridis*	Dongshan, Daya bay	0	Suspended culture
28 Feb 2002	*Chlamys nobilis*	Nanao, Dapeng bay	0	Suspended culture
26 March 2002	*Chlamys nobilis*	Nanao, Dapeng bay	0	Suspended culture
15 April 2002	*Chlamys nobilis*	Nanao, Dapeng bay	0	Suspended culture
24 April 2002	*Perna viridis*	Nanao, Dapeng bay	0	Suspended culture
24 April 2002	*Chlamys nobilis*	Nanao, Dapeng bay	1.4	Suspended culture
23 May 2002	*Chlamys nobilis*	Dongshan, Daya bay	0.28	Suspended culture
27 May 2002	*Perna viridis*	Dongshan, Daya bay	0	Suspended culture
27 May 2002	*Chlamys nobilis*	Dongshan, Daya bay	0.38	Suspended culture
27 May 2002	*Chlamys nobilis*	Nanao, Dapeng bay	0	Suspended culture
27 May 2002	*Chlamys nobilis*	Baguan, Dapeng bay	0	Wild areas
27 May 2002	*Perna viridis*	Baguan, Dapeng bay	0.41	Suspended culture
14 June 2002	*Perna viridis*	Dongshan, Daya bay	0	Suspended culture
14 June 2002	*Chlamys* sp.	Baguan, Dapeng bay	0.63	Wild areas
14 June 2002	*Chlamys nobilis*	Nanao, Dapeng bay	0.48	Suspended culture
14 June 2002	*Ostrea* sp.	Dongshan, Daya bay	0	Suspended culture
25 July 2002	*Chlamys nobilis*	Nanao, Dapeng bay	0.50	Suspended culture
30 July 2002	*Chlamys nobilis*	Nanao, Dapeng bay	0.57	Suspended culture
30 July 2002	*Perna viridis*	Dongshan, Daya bay	0	Suspended culture
20 August 2002	*Perna viridis*	Dongshan, Daya bay	0	Wild areas
20 August 2002	*Perna viridis*	Dongshan, Daya bay	0	Suspended culture
22 August 2002	*Ostrea* sp.	Houhai, Shenzhen bay	0	Suspended culture
22 August 2002	*Ostrea* sp.	Qianhai, Shenzhen bay	0	Suspended culture
26 August 2002	*Chlamys nobilis*	Nanao, Dapeng bay	0.35	Suspended culture
26 August 2002	*Perna viridis*	Nanao, Dapeng bay	0	Suspended culture
29 August 2002	*Ostrea* sp.	Neilingding, Shengzhen bay	0	Suspended culture

Table 3. Toxin profiles of scallop, *Chlamys nobilis* from Daya bay, Shenzhen.

Toxin profiles	Shellfish edible tissues			Shellfish digest gland tissue		
	nmol ml^{-1}	mole %	epimer %	nmol ml^{-1}	mole %	epimer %
C1	0.87	3.3	5.1	22.31	10.6	17.1
C2	0.49	1.8		13.80	6.5	
GTX1	1.01	3.8	6.7	61.28	29.0	38.9
GTX2	0.71	2.7	4.1	16.07	7.6	17.3
GTX3	0.37	1.4		20.41	9.7	
GTX4	0.78	2.9		20.91	9.9	
GTX5	3.02	11.4	11.4	42.21	20.0	20.0
neoSTX	16.12	60.8	60.8	7.87	3.7	3.7
dcSTX	2.00	7.6	7.6	1.89	0.9	0.9
STX	1.14	4.3	4.3	4.51	2.1	2.1
Total	26.51	100.0	100.0	211.26	100.0	100.0

* empimer % means the molar percentage of all epimers combined (C_1+C_2), (GTX1+GTX_4), (GTX_2+GTX_3) and total toxin content in tissues.

Table 4. A diagram of the red tide management network.



consists of the following: (1) to monitor regional red tides and shellfish toxins; (2) when red tides initiate, to issue information and offer materials to prevent and mitigate HAB consequences; (3) to put the Preventive Regulation of Shellfish Production's Environmental Sanitary Management into effect; (4) to build up a red tide information exchange network between neighbor provinces and Hong Kong, Macao; and (5) to increase communication with universities, colleges and institutions.

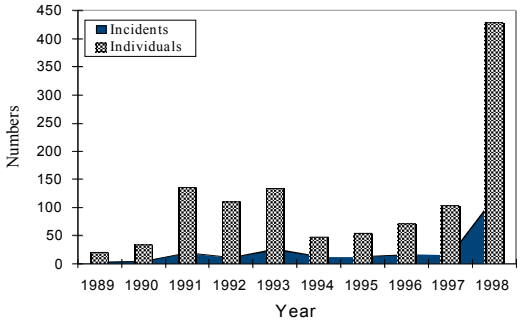


Figure 2. Historical data of ciguatera toxicity in HK (Data from DH, Hong Kong).

The following scheme was set up as a provincial mechanism for tackling red tide issues (Table 4). The networks will be focused on the following: (1) to monitor sea water quality; (2) to monitor plankton, its composition, concentration and seasonal distribution, especially for red tide causative species; (3) to inspect water pollution conditions; (4) to keep watch for invasive species introduced by mariculture; (5) to strengthen the communication to fisherpersons, to warn of HAB initiation; (6) to make ordinary metrological notes; and (7) to monitor shellfish toxins and their consequences.

Specialists from universities, colleges, and scientific institutions as well as officials of governments compose the Center for Red Tide Research of Guangdong. The main tasks of this center are to analyze data and information from the network and to give advice and guidance on forecasting HAB occurrences on mitigating any negative impacts of HABs.

Thus far, the action of the scheme of the provincial government for tackling red tide incidents has proven effective. By minimizing the possible damage of a *Phaeocystis globosa* bloom the strategy was shown to work well. Harmful blooms of *Phaeocystis globosa* have occurred on the northeast coast of Guangdong Province, China. Two of the biggest blooms happened in October 1997 and the summer of 1999. In the 1997 bloom, all fish and shrimp in about 4,000 culture cages died, resulting in millions of US dollars of economic losses. In contrast, during the 1999 bloom, in the same area and with the same cell density of *Phaeocystis globosa*, all fish cages were towed (raft relocation) from the embayments where the bloom developed to offshore open waters, which were not affected by the harmful bloom. Thus, only very small losses were caused by the bloom and accordingly the two blooms had markedly different results. This event provides a good demonstration that movement of cultured stock is a practical and cost effective mitigation measure for preventing the effects of harmful algal blooms and minimizing fish kills. Several lessons can be learned from this study: first, fishermen should have a good knowledge of specific harmful blooms, so that they can take action as soon as the bloom is initiated; second, fish pens should be smaller in size and easier to tow, so that they can meet the requirements for fast and cost effective action; and finally, proper and safe refuge areas should be selected.

Nowadays, people are more aware that for enhancing the ocean economy through mariculture and protecting human health and marine ecosystems, there must be further development in the control and mitigation of harmful red tides. An important step will be strengthening red tide monitoring and mitigation programs.

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BIOTOXINS IN EUROPEAN WATERS: OCCURRENCE AND MANAGEMENT

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ABSTRACT

Mussels, oysters and clams represent the dominant molluscan shellfish in the European industry, and the cultivation of these shellfish has major socio-economic importance. This paper describes a brief overview of the European industry and areas of future industry development, including constraints on future industry expansion as well as concerns and criticisms of proposed legislation for food hygiene. Improvements of biotoxin management are discussed.

INTRODUCTION

Although this paper focuses on biotoxin events and regulations, it is necessary to start with a brief overview of the European industry. In this paper emphasis is placed on the molluscan cultivation sector as opposed to total bivalve supplies, as this is where future development lies. Therefore, the first issue is to define 'cultivation' since bivalves are natural feeders; artificial feed or pellets are not supplied, as in finfish aquaculture. The FAO definition overcomes this difficulty by focusing on control of the environment and legal ownership of the stock. This allows a clear separation between cultivation and capture fishery supplies for analytical purposes.

Before assessing the challenges posed by biotoxins, it is essential to understand the current characteristics, scale and profile of the European cultivation industry. A relatively detailed picture of the industry is possible as a result of a comprehensive study of the aquaculture sector completed for the European Commission in 1999 by consultants *'MacAlister Elliott & Partners'*. Though some concerns regarding data accuracy have been expressed, the report provides a useful baseline of data presented on a comparable basis across the community. The report concluded that the production of cultivated shellfish in 1997 totalled some 665 thousand tons. The data indicated that this volume was dominated by mussels (515 thousand tons), which represented about three quarters of the European shellfish output (and allegedly equal to around 70% of global mussel production). Oyster production (*O. edulis* and *C. gigas*) reached some 100 thousand tons, 15% of the total EU cultivated shellfish output (representing 3% of world volumes). Clams accounted for around 50 thousand tons, representing 8% of European shellfish output (4% of global production).

With a first sale value of around Euro 600 million, cultivated shellfish was worth around 30% of the overall two billion Euro European aquaculture businesses. The dominant species, mussels, declined to 47% of the total, representing approximately Euro 270 million. The first sale values for oysters and clams totalled around Euro 150 million for each species (26-28%). Average prices ranged from just over Euro 500 per ton for mussels (Euro 400 for dredged product to Euro 1,600 for mussels grown in suspended cultivation in Scotland) to Euro 3,000 per ton for clams.

There is an equally skewed distribution of the location of activity across the Union. Shellfish production is geographically extremely concentrated, with 73% attributable to only three countries - Spain, the leader, with 198 thousand tons or 30% of the total, followed by Italy with 143 thousand tons (22%) and France at 141 thousand tons (21%). There is a similar pattern of concentration at the individual species level. Seventy-five percent of the mussels come from the top three producers - 189 thousand tons from Spain (NB *'Mexillon de Galicia'* estimate production at over 250 thousand tons), 103 thousand tons from Italy, and 93 thousand tons from the Netherlands. Ninety-four percent of the oysters originate from the top three producers - 87 thousand tons from France (the *'Comite National de la Cochyliculture'* estimate is 140 thousand tons), followed at a significant distance of 4.4 thousand tons from Ireland and 3.4 thousand tons from Spain. Ninety-six percent of clams come from the top three producers - 40 thousand tons from Italy, 5.6 thousand tons from Spain and 3.3 thousand tons from Portugal.

In addition to these production and value parameters, a further particularly significant attribute of shellfish farming, reflecting the relatively low-tech nature of operations, is the contribution it makes to employment. The sector is calculated to generate around 20,000 full time equivalent (FTE) jobs in the production segment alone, over 50% of total direct employment in the European aquaculture industry. The income generation opportunities created by shellfish cultivation have major socio-economic importance, as they are typically located in remote coastal regions where alternative employment is often lacking.

The European shellfish cultivation sector has historically lacked such a common voice in Brussels. This lack was remedied in 1999, with the creation of a community-wide organization to represent the sector's interests in Brussels, and to improve communication and dialogue with the European Commission. With the encouragement and support from the Commission, a number of national trade associations finally decided to form the *'European Mollusc Producers Association'* (EMPA). Initial membership includes trade associations from England & Wales, France, Greece, Ireland, Scotland and Spain, with an expectation of expansion to include additional Member States representatives in the future. Nevertheless, the six founding members form a representative cross-section of community producers, including examples of large scale and mature industries as well as smaller, more recently developed sectors, and

both Atlantic and Mediterranean operations. In addition, the combined production of EMPA national industries totals around 400 thousand tons, some 60% of European Union output. The Association is therefore a legitimate voice for the European-wide sector and enjoys formal recognition as the community-level representative for the industry. EMPA expects to be the focus for industry representation, interaction and communication with the Commission, the Parliament and other European institutions in future discussions concerning direct sectoral issues and associated development of policies, including food hygiene legislation.

FUTURE DEVELOPMENTS

As the data above clearly indicate, the major difference between national bivalve sectors is one of scale, with production dominated by the 'Big Four' of Spain, Italy, France and the Netherlands. But for three of these producers the best expectation for the future is for marginal increases; indeed there are those who argue that France, Spain and the Netherlands have already, in certain areas, exceeded the optimal biological carrying capacity. Italy is unique, in being the only current major producer with the potential for significant incremental production.

At the community level, future expansion is forecast to come largely from countries with limited current volumes but with strategic goals to expand output in future years, such as Portugal, Ireland, Scotland, Sweden and Greece. These peripheral EU suppliers will be joined by other expanding industries in Iceland, Norway, and potentially the nations of the Eastern coast of the Adriatic and the Black Sea. However, there are constraints on future increases in both groups of producers including:

- Availability of suitable sites;
- Bacterial pollution;
- Pollution 'crises', such as oil spillages;
- Climate change;
- Marine biotoxin events.

It is the opinion of some that biotoxin contamination is likely to prove the greatest threat to the future of the industry, and not simply in Europe, but worldwide. As the ICES maps of biotoxin events around Europe for the past decade indicate, the impact of algal toxins is extensive - what is not shown is that the extent of the events, their intensity and frequency appear to be increasing over time. Whether that is because we are monitoring with greater efficiency and analysing with improved accuracy, or because there is an underlying long-term trend towards a higher level of toxicity in the environment, is still subject to debate. Both explanations generate significant challenges for the future of the industry. But one certain result is that the consumer is becoming more aware of perceived dangers in consuming bivalves.

Although monitoring and harvesting controls are to safeguard consumers, closures frequently lead to "image erosion" as a result of unhelpful media interpretation. Our industry is perhaps suffering part of the backlash resulting from the apparently endless litany of food safety concerns of the past decade (e.g., BSE – bovine spongiform encephalopathy, dioxins, *Listeria* and *Salmonella* as well as chemical contaminants). There is no doubt that food safety legislation has been a lead issue within the European Community in recent years, with numerous Directives being implemented during the early 1990's. And there has been an apparently never ending series of food safety concerns over the past decade, partly because of the new legislation and the enhanced intensity of monitoring it has generated. The main objectives have been to harmonize standards (the 'Single Market') and improve food safety for consumers.

The Directive that has most affected our industry is undoubtedly Directive 91/492 (and the companion 'fisheries products' Directive, 91/493), "laying down the health conditions for the production and placing on the market of live bivalve molluscs," with its detailed End Product Standards covering bacterial, biotoxin and radionucloide levels and the definition of minimum requirements for documentation for harvesting areas and movements, depuration and despatch facilities and public health control systems (monitoring, closure mechanisms).

The industry has broadly supported the Directive and its aims. There is support for the objective of improving quality and safety of products and safeguarding public health. Harmonization of standards is similarly positively supported, along with any reduction in negative media reports.

In summary, the industry has been and remains in favour of effective hygiene legislation and acknowledges it as an essential prerequisite for future development. However, there are genuine concerns and legitimate criticisms:

- Are the Directives targeted at real risks and at improving consumer protection? Or is this all a bit of post-BSE paranoia?

- Have they been implemented in an appropriate and proportionate manner and with equal vigour in all member states?

- Industry worries that by highlighting potential risks, the consumer has been made more confused, concerned and worried about shellfish, unnecessarily and disproportionately to the real risk.

The food hygiene Directives are currently under review, with the potential for revision, as part of the consolidation of separate 'vertical' Directives to a single 'horizontal' food Regulation. The review process is on-going, but Commission officials have indicated that:

- As a Regulation rather than Directives, the legislation will focus on outcomes or targets rather than the method of achieving them, recognizing differences between Member States;

- There is unlikely to be any changes in the Harvesting Area Classification criteria;
- No changes should be expected on contaminants, viruses, etc;
- It is likely that some controls will be brought in for wild Pectinids;
- There will be a significant emphasis on post-primary production HACCP (hazard analysis and critical control points);
- There will be changes for biotoxins, e.g., there will be an unbundling of the DSP (diarrhetic shellfish poisoning) toxin complex to individual toxins (Yesso-, Pectino-, Azaspiracids, and Okadaic Acid);
- A Revised portfolio of testing methods will be created;
- There will be a tiered harvesting regime for scallops with ASP (amnesic shellfish poisoning), e.g., if the whole animal test exceeds 20 ppm but is below 250 ppm, then harvested scallops must be shucked to adductor and gonad, which must then be tested separately; if the sample of either part exceeds 4.6 ppm, then that part must be destroyed; each batch must also be tested for End Product Standards (20 ppm of ASP);
- With reference to imports from Third Countries, each company wishing to export must satisfy the hygiene requirements.

Publication of the revised proposed Regulation is expected at end-2002.

As mentioned earlier, biotoxin events are likely to continue to have a major restraining effect on our sector, especially if we fail to design innovative techniques to manage the impact of these occurrences. While one strand of management could be through polyculture of fish, shellfish and seaweeds, a second strand could be through a more holistic interpretation and implementation of Regulations. For example, following the massive ASP event in Scottish waters in 1999, which closed the scallop sector for virtually twelve months, discussions were initiated with the European Commission in an effort to mitigate the impact, by seeking a tiered marketing system. This would enable the industry to limp along on adductor meat sales, which would be better than nothing. To support the argument that such sales would not pose a threat to public health, a small-scale research project was organized. The results showed that toxins in the hepatopancreas tended to be 10 times greater than in the whole animal, while the toxin level in the adductor muscle tended to be less than one percent of the level in the whole animal.

Thus, we have been seeking agreement to introduce a tiered marketing regime, which would allow a phased response to an ASP event, reflecting the risk to consumer health. Surveillance would move from phytoplankton sampling to whole animal testing followed by separate part analysis, with the criterion for marketability remaining as the 20 ppm action level. Such an approach should satisfy the requirements of protecting public health without destroying the scallop industry.

However, the introduction of the tiered approach incorporating a 4.6 ppm trigger value, the recommendation of the Commission's 'ASP Expert Working Group', which is claimed to reduce the chance of any scallop having a toxin level of above 20 ppm to one-in-a-thousand, is likely to permanently close down the scallop sector.

The proposal effectively converts what was a population average (the 20 ppm average in the sample) to an absolute ceiling value. The effect will be to drive fresh supplies from "whole animal" harvesting to a short period of "roe-on" meats, then to an extended period of "roe-off" meats. The impact on revenues will be two-fold, firstly fewer kilos of product sold, and secondly, lower prices, as the white meat market is a global commodity market, with prices established by large volume frozen supplies from countries such as Chile, Argentina, and Thailand.

With the current cost structure of the NW European industry, it will be impossible to survive economically - jobs will effectively be exported to Third Countries as the EU sector is closed. In the absence of a realistic risk assessment, which takes into account individuals' consumption of these highly priced seafood products, average meal portion size, consumer body weight, etc, industry will remain incredulous of the scientific recommendations.

CONCLUSIONS

In addition to these biotoxin issues, today the European industry appears to be facing an uncertain future, characterized by mature, slow growing markets with narrow profit margins, capacity constraints in the traditional, large volume producers and apparently suffering from an unending series of problems from pollution, biotoxins and potential for exotic introductions, expensive hygiene legislation and the attentions of a zealous 'Food Police'.

In summary it appears that the industry suffers from a poor 'Risk/Reward Ratio', indeed, the worst possible combination of High Risk and, even when successful, Low Rewards. If we carry on with traditional attitudes, under a 'Business As Usual' scenario, the outcome will be a disparate industry, fragmented into national camps, isolated from other interest groups, failing to achieve environmental improvements and suffering from negative market perceptions, spiralling down to lower volumes and reduced political strength - suffering from a continued negative Risk/Reward Ratio.

Alternatively, the industry can acknowledge the challenges, such as biotoxin events and the requirement for Integrated Coastal Zone Management, and embrace the opportunities for multi-sectoral alliances (even with interest groups that we have historically opposed). Production can be expanded in peripheral countries, and new markets can be developed through innovative marketing of processed products.

The management and mitigation of harmful algal blooms and shellfish toxicity through multi-species

nutrient budgeting and the development of pragmatic Regulations based on risk assessment are central to the creation of a dynamic, expanding, profitable industry, one that is attractive to future generations of proud cultivators of the seas, and playing a major role in the sustainable development of the coastal zones of Europe and, by extension, of other countries around the planet. Policies from the EU and APEC, in the wider context of the WTO, are essential for the emergence of this positive vision, and a further crucial element of this approach is improved communication between industry, regulators and the scientific community.

Harmful Algae Management and Mitigation. 2004.
Hall, S., Etheridge, S., Anderson, D., Kleindinst, J., Zhu, M. and Zou, Y. (Eds.)
Asia-Pacific Economic Coooperation (Singapore): APEC Publication #204-MR-04.2

SEAFOOD TOXIN REGULATIONS IN JAPAN

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ABSTRACT

This paper describes how seafood toxins are regulated in Japan, and presents some historical data on the occurrence of paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP) in harvest areas over the last 10 years.

REGULATION OF SEAFOOD TOXINS

The important seafood toxin problems we have in Japan are paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP). PSP is assayed by mouse bioassay. This method is about the same as that of the AOAC [1], but without checking the conversion factor (CF) value with reference standard saxitoxin. Due to the difficulties associated with transporting saxitoxin (STX), only a few laboratories have STX and perform the AOAC method with standardization. But the CF value of a male ddY mouse, which is an albino closed-colony mouse and designated for use in the Japanese official method, has been found to be stable at around 0.20 in the past 6 years (unpublished data), so this is considered an acceptable approach for domestic use. Research to determine another useful substance to take the place of STX for bioassay standardization is now progressing (Personal communication with Prof. Y. Oshima).

DSP is assayed according to Yasumoto's method [2]. The mouse used in this assay is ddY or ICR, either of which is an albino closed-colony strain.

We have not yet experienced amnesic shellfish poisoning (ASP) domestically. If domoic acid is suspected to be present, the AOAC high performance liquid chromatography (HPLC) method or other validated method will be used for analysis.

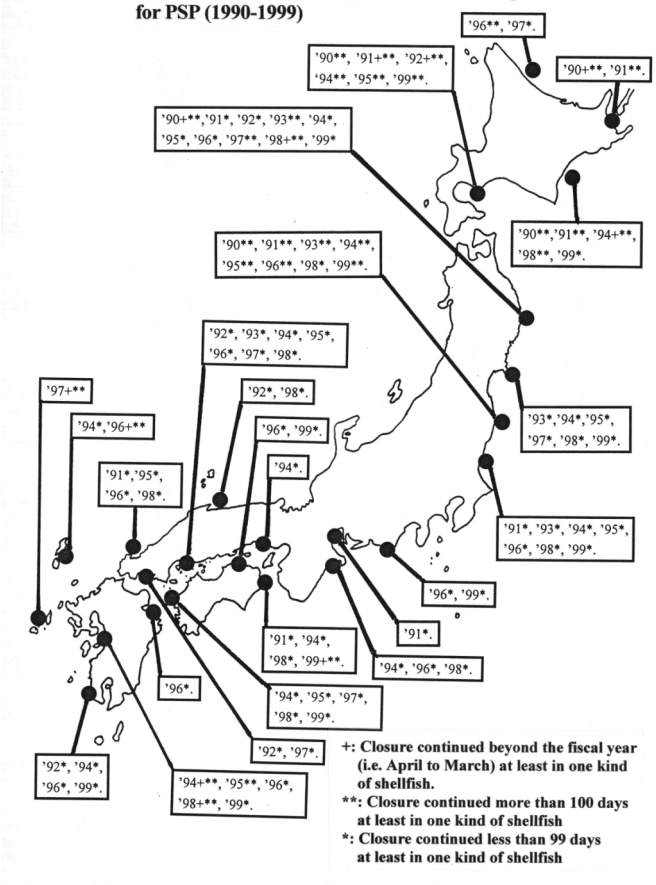
In the case of PSP, the regulatory value is 4 mouse units g^{-1} meat or, when standardized according to the AOAC method, 80 μg STX equivalent toxicity 100 g^{-1} meat. In the case of DSP the regulatory limit is 0.05 mouse units per gram of meat, and in the case of ASP, 20 micrograms domoic acid per gram of meat.

HISTORICAL DATA FOR HARVESTING AREA OVER THE PAST TEN YEARS

Figures 1 and 2 show the areas, years (indicated in the square) and length (days) of closure of shellfish harvesting areas for PSP and DSP, respectively [3]. The filled circle does not indicate a place but indicates a wide area around it.

These figures show that PSP is distributed more widely than DSP from north to south, with DSP mostly restricted to the northern part of Japan. There are some areas that experience both PSP and DSP, in some cases in the same year. On the other hand, there are a few areas that have experienced either PSP or DSP only once in these 10 years. These phenomena suggest long term continual investigation is very important and necessary.

Fig. 1 Occurrence of closure of Shellfish Harvesting area for PSP (1990-1999)



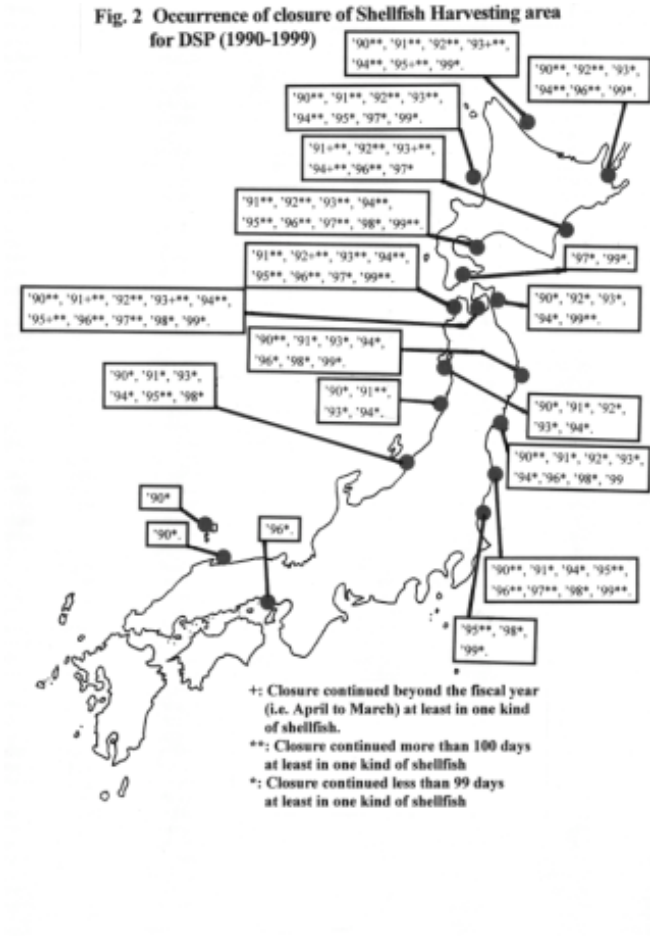


Fig. 2 Occurrence of closure of Shellfish Harvesting area for DSP (1990-1999)

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3. In: Report on the Project for the Safety of Marin Biotoxin in Bivalves by MAFF, 2001. Japan Fisheries Resource Conservation Assoc. ed., pp. 78-89.

Harmful Algae Management and Mitigation. 2004.
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Asia-Pacific Economic Coooperation (Singapore): APEC Publication #204-MR-04.2

THE CANADIAN SHELLFISH SANITATION PROGRAM AND MARINE TOXIN MONITORING IN BRITISH COLUMBIA, CANADA

Klaus Schallié

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ABSTRACT

The shellfish industry in British Columbia (BC) is a major economic contributor. This paper describes the Canadian Shellfish Sanitation Program, harmful algal blooms (HABs) that occur in BC, the marine toxin monitoring program, how marine toxin closures are handled, communications strategy for notification of closures, and current research on HAB related issues.

INTRODUCTION

The shellfish industry in British Columbia is dynamic, poised for growth, and increasingly significant as a contributor to BC economy. It provides a significant amount of employment in coastal areas. In 1996, the total wholesale value of the BC shellfish harvest was $163.1M, of which $14.5M was from farmed shellfish.



CANADIAN SHELLFISH SANITATION PROGRAM (CSSP)

Under the CSSP, the Department of Fisheries and Oceans (DFO) is responsible for fisheries management, including enforcement of closure regulations, enacting the opening and closing of areas, and resource (stock) management.

Environment Canada is responsible for growing water classification. It conducts shoreline sanitary surveys, monitors growing area water quality, and recommends classification of shellfish harvesting areas.

The Canadian Food Inspection Agency (CFIA) is responsible for fish inspection, including the control of handling, transportation, storage, processing and labeling, and thus the marine toxins control program. It recommends harvest openings and closures to DFO. The CFIA is the federal contact for exchanges with foreign governments.

Some concepts under the CSSP:

REGISTERED PLANTS: Risk is minimized by requiring all commercially harvested shellfish to be delivered to a federally registered shellfish processing plant prior to distribution. Record keeping (e.g., harvest location & date, product distribution) is mandatory; it facilitates product recalls if necessary.

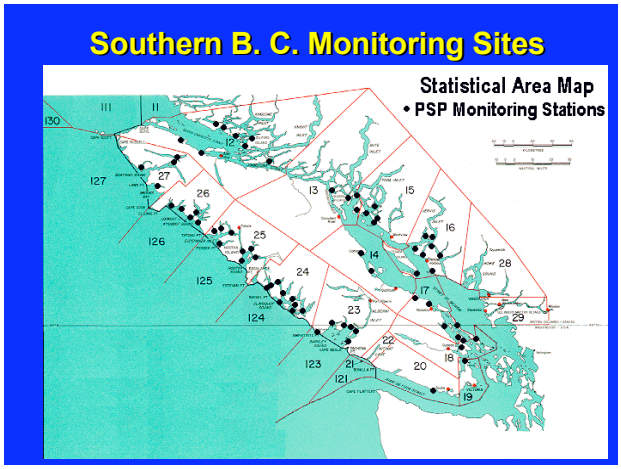
REGIONAL SHELLFISH CLASSIFICATION COMMITTEE: The Committee meets twice yearly to discuss Environment Canada growing area survey results and recommendations for growing water classification. It includes representatives from EC, DFO, CFIA, provincial government ministries. Observer status was recently given to industry associations and First Nations.

HARMFUL ALGAL BLOOMS

Harmful algal blooms (HAB's) are endemic and common in BC. Paralytic shellfish soisoning (PSP) is the most frequent type of HAB. Amnesic shellfish poisoning (ASP) blooms are less frequent.

HABs have also caused mass mortalities, which have seriously affected the salmon farming industry. Salmon farmers were monitoring for HABs at 26 sites in 2001. In recent years HABs belonging to the genera *Chaetoceros*, *Heterosigma*, *Cochlodinium*, and *Chrysochromulina* have been implicated in fish kills. The estimated loss to salmon farms since 1987 from HABs is $40M, with an estimated loss of $2M from *Cochlodinium* in 1999.

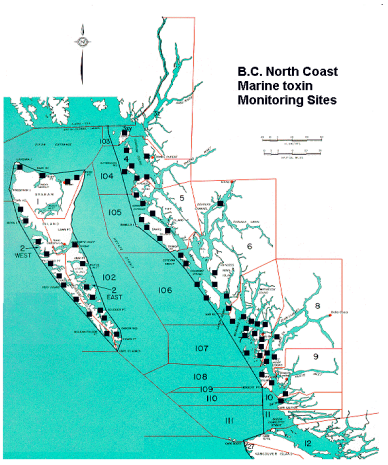
MARINE TOXIN MONITORING



Paralytic shellfish poisoning (PSP) toxins, the saxitoxins, from *Alexandrium* sp., are monitored by the mouse bioassay, with an action level of 80 µg 100 g^{-1} meat.

Amnesic shellfish poisoning (ASP) toxin, domoic acid, from *Pseudonitzschia* sp., is monitored by high performance liquid chromatography (HPLC), with an action level of 20 µg g^{-1} (ppm).

Northern B. C. Monitoring Sites



Monitoring Sites in Area 16

(showing Subareas)



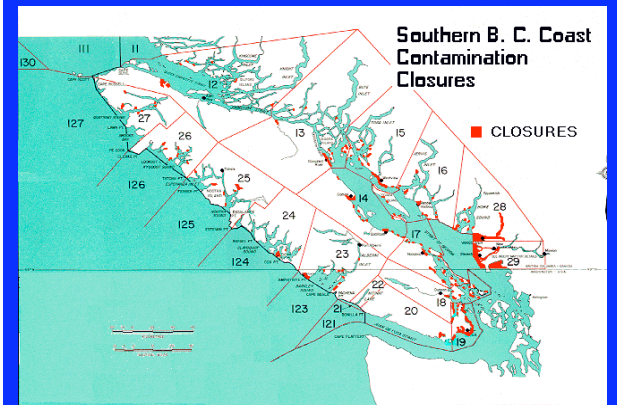
BC has a very extensive (~27,500 km) coastline with many remote areas that are often difficult to get to so that we rely on a sentinel species for our monitoring program. Mussels (*Mytilus californianus*) are used as our sentinel species as they acquire biotoxins more quickly than other species of bivalves. Samples of the other bivalve species, especially from commercial harvests, are used to supplement the program.

Plastic mesh sacks filled with mussels are hung in the water in major shellfish growing and harvesting areas. Samples are shipped to the CFIA Laboratory from these locations weekly from 1 May to 30 October and once every two weeks from 1 November to 30 April. The shellfish industry and First Nations are partners in our program, helping by providing samples to the CFIA. The CFIA administers the program, analyses samples, and provides toxin analysis results.

The annual number of marine toxin analyses conducted by the CFIA Burnaby, BC Laboratory are: PSP Mouse Bioassays, 3000-4000; ASP HPLC analyses, approximately 3000. DSP analyses are conducted by HPLC in the Halifax lab.

MARINE TOXIN CLOSURES

South Coast Contamination Closures

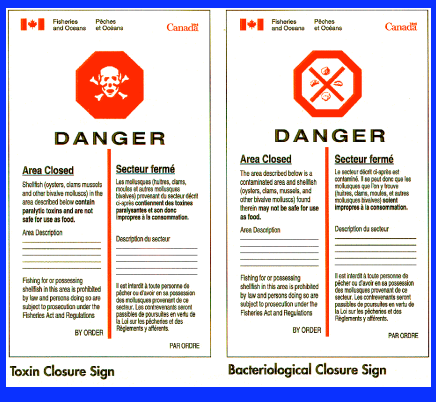


When the analysis results for a shellfish sample exceeds an action level, CFIA staff reviews all data and consults with DFO Fishery Managers. The extent of the area to be closed is determined. CFIA then recommends a harvest ban to DFO's Regulations Unit. The Regulations Unit prepares the Closure Order for signature of the DFO Regional Director General.

COMMUNICATIONS STRATEGY

CFIA sends faxes to other government agencies, all registered shellfish plants and partners in the monitoring program. DFO sends notification of closure by e-mail and fax to all DFO offices and non-government subscribers. Local DFO offices in affected areas are in turn responsible for notifying media, community organizations, etc. The closure information is on DFO & CFIA 24 hour phone lines and on DFO website.

Signs may be posted near harvest sites by DFO



Toxin Closure Sign | Bacteriological Closure Sign

RESEARCH

We are searching for alternatives to the mouse bioassay, especially for PSP. A number of different approaches are being investigated, including instruments (HPLC, LC-MS), synaptoneurosomes and neuroblastoma cells, and immunoassay. We are trying to identify causative species and mode of action, toxins produced, risk factors, etc. New toxic factors are being discovered (e.g., spirolides). Finally, we are trying to identify cause and effect relationships between environmental parameters and HABs. Such relationships may improve our predictive capability, and may indicate possible mitigation strategies such as reducing nutrient input or better waste water treatment.

Harmful Algae Management and Mitigation. 2004.
Hall, S., Etheridge, S., Anderson, D., Kleindinst, J., Zhu, M. and Zou, Y. (Eds.)
Asia-Pacific Economic Coooperation (Singapore): APEC Publication #204-MR-04.2

HARMFUL ALGAL BLOOMS IN THE CHESAPEAKE BAY, USA: COMMON SPECIES, RELATIONSHIPS TO NUTRIENT LOADING, MANAGEMENT APPROACHES, SUCCESSES AND CHALLENGES

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ABSTRACT

Harmful algal blooms in the Chesapeake Bay, USA, are not a new phenomenon, but may be increasing in frequency and diversity. Indeed, it is the diversity of such events that creates challenges for monitoring and management. The types of events that typically occur in the Chesapekae Bay range from toxic outbreaks of *Pfiesteria piscicida* and associated fish kills, to high biomass blooms such as *Prorocentrum minimum* and *Microcystis aeruginosa* that have varying impacts, including environmental damage caused by resulting low oxygen or habitat change. The Chesapeake Bay Monitoring Program measures a suite of key variables, including physical/chemical properties, nutrient inputs (inorganic and organic), sediment load, phytoplankton, zooplankton, and benthic organisms at numerous sites throuout the Bay and its tributaries on a regular basis. In addition, continuous monitoring of water quality conditions is ongoing at over 10 stations. These data have allowed long-term trends to be developed. Collaborations between academic institutions, state and federal agencies, and the medical community, have led to an understanding of how the Bay ecosystem functions in relation to anthropogenic and natural stresses, and has led to appropriate management actions, such as river closures, when necessary, and recommendations for long-term nutrient reduction strategies. Some successes have been achieved in nutrient reduction in the past several decades, leading to reductions in some high biomass blooms, but the challenges to achieve future successes are many.

INTRODUCTION: THE CHESAPEAKE BAY ECOSYSTEM

Located on the east coast of the USA, the Chesapeake Bay is the largest estuary in the USA and one of the most productive in the world in terms of fish and shellfish harvests (Fig. 1). Home to over 15 million people in its watershed, the Chesapeake Bay has been stressed in numerous ways from human activities and industrial production. One of the most significant issues for the Bay ecosystem is eutrophication and deteriorating water quality including the development of numerous harmful algal blooms.

The goal of this paper is to provide an overview of the problems of harmful algae in the Chesapeake Bay region, their relationship to eutrophication, and the management approaches that have been taken to address these problems. Management approaches include partnerships with academia and the medical community, leading to a better scientific understanding of processes and effects, as well as legislative efforts. Some successes have been attained, but many challenges still remain.



Figure 1. The Chesapeake Bay and the states comprising its watershed. The Chesapeake Bay is the largest estuary in the USA.

COMMON HARMFUL BLOOM SPECIES

The Chesapeake Bay has a range of harmful algal species thereby complicating the challenges of management. In contrast to systems in which specific species bloom year after year, in the Chesapeake and its tributaries, blooms of any of a number of species may occur. Some of the largest impacts are those from blooms that form high biomass; their effects include low oxygen, visible surface scums, and loss of submerged aquatic vegetation and benthic habitat. Other harmful species known to occur are the toxin producers, and they affect commercial species, and in some cases, human health. The management approach for one type of bloom may not necessarily be the appropriate management strategy for another type of bloom. Several species are of particular note.

Pfiesteria piscicida (Dinophyceae) is well known in the Chesapeake region because of its association with fish kills first observed on large scale in 1997 [10, 38]. *Pfiesteria* sp. is a heterotophic dinoflagellate that feeds on small algae, bacteria, and bits of fish tissue [10, 12]. This organism has a complex life cycle and spends much of its life in a benthic stage. In its zoospore form, it can retain the chloroplasts from its food and live in an autotrophic mode for a period of time, taking up inorganic and organic nutrients and carrying out photosynthesis [34, 35]. *Pfiesteria* has been well documented to produce toxins that are associated with

fish kills, and more importantly human health problems [43, 45]. Human health effects have been found for people in contact with the organism in the laboratory and in nature; symptoms include skin irritations, headache, eye irritations, and, more seriously, memory loss, confusion, and personality change [25, 28, 46, 55]. *Pfiesteria* sp., now recognized to be a several-species complex, has reoccurred in many Chesapeake Bay tributaries every year since it was first documented [21, 38, 39].

More widespread and more easily detected because of its tendency to form high biomass accumulations, the dinoflagellate *Prorocentrum minimum* is a frequent bloom-former in Chesapeake Bay [27, 24]. Typically delivered via subsurface transport from the south to the north in spring, *P. minimum* forms massive accumulations of chlorophyll [62, 63]. Well-known worldwide, this species is frequently found to bloom following freshwater flow events in the spring and early summer [13, 60, 26, 24]. Nutrient runoff in the freshwater flow following heavy rains is thought to fuel many of these blooms. Toxicity associated with *P. minimum* is uncertain for this system, but some evidence of toxicity to shellfish has been documented [49, 61, 64]. Of greater concern are effects of low oxygen, which result in local fish and shellfish stress and kills.

Microcystis aeruginosa (Cyanophyceae) often forms large, visible nuisance blooms in the low salinity portions of Chesapeake Bay and its tributary, the Potomac River, during summer months [57]. Blooms have been related to excess nutrients, and in particular phosphorus. These blooms have, in some instances, been shown to contain significant concentrations of the toxin microcystin. A potent hepatotoxin, microcystin has been associated with human illness and cattle death around the world [31, 20]. Such findings for the Chesapeake Bay led to precautionary beach closures to protect human health.

Aureococcus anophagefferens, a small Pelagophyte notorious for its effects off of Long Island, New York, has been found in the Chesapeake and Coastal Bays of Maryland [24, 37]. These blooms, like their New York counterparts, have also been linked with nutrient enrichment [17, 36, 4, 33, 37]. These cells, even at levels as low as 35,000 cells ml^{-1} may have sub-lethal effects on shellfish through interference with their feeding mechanisms. With larger blooms, the effects of these organisms also include the development of low oxygen and shading of seagrass beds [16, 9].

Although a rare event, when blooms of the dinoflagellate *Dinophysis* sp. occur, their effects can be severe both ecologically and economically. As a toxin producer (okadaic acid), shellfish contaminated with *Dinophysis* can lead to Diarrhetic Shellfish Poisoning (DSP), with symptoms of nausea, diarrhea, and intestinal pain [47, 2]. These blooms occur most often when salinity is unusually high due to low flow, and when sufficient nutrients are present for their rapid growth. In the Chesapeake, and the USA, only low levels of toxicity have been reported (unpub. data), so the actual threat from *Dinophysis* blooms remains unclear.

These and other harmful species which occur on a more limited scale, such as the dinoflagellate *Karlodinium micrum (=Gyrodinium galatheanum*), present a range of management issues. The conditions ideal for the outbreak of one harmful species may not be the same as the conditions leading to the outbreak of another. Thus, the strategy for management must not simultaneously promote a secondary species while controlling a primary one.

NUTRIENT LOADING TO THE BAY

Nutrient loading to the Bay is seasonally pulsed, with sharp gradients from the fresh, riverine sources, to the more saline waters in the mid- and southern reaches of the Bay [8, 18, 19, 41, 23]. The spring freshet can deliver nitrogen in excess of 100 μM nitrogen, the largest proportion of which is nitrate (Fig. 2).

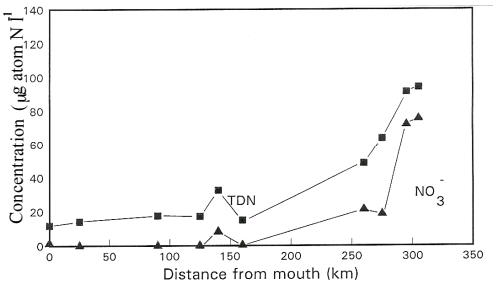

Figure 2. Concentrations of total dissolved nitrogen (TDN) and nitrate as a function of distance from the mouth of Chesapeake Bay in spring.

The majority of the nutrients are derived from non-point sources, particularly fertilizers and manures (Fig. 3). Also of significance are the atmospheric contributions of nitrogen, but their control is considerably more difficult at the local management level. The bulk of the non-point sources reflect the agricultural uses of the watershed, the use of animal manures for fertilizers, and the growing animal operations, especially chicken producers, particularly along the eastern shore of the Bay. The Chesapeake Bay eastern shore has some of the most intensive operations for poultry production anywhere in the USA (Fig. 4). The eastern shore, in fact, has been identified as one of the top watersheds in terms of vulnerability to manure leaching by the US Department of Agriculture (USDA). Agricultural management of nutrients is the primary challenge for nutrient reduction in the Chesapeake [6].

Long recognized as the primary cause of poor water quality (e.g. [48, 40]), excess nutrients are just beginning to be recognized as important in promoting certain HAB species (e.g., [58, 59, 1]. Three aspects of nutrient relationships are noted here.

First, nutrient quality and composition may be as important, if not more important, than total nutrient loading, in promoting certain species. The role of altered nutrient composition, in terms of nitrogen:phosphorus or nitrogen:silicon ratios, in population shifts from diatom to flagellates, has been well established for many regions of the world (e.g., [52, 53, 29]). Less well recognized is

the role of the composition of the respective nitrogen and phosphorus pools in promoting or maintaining blooms.

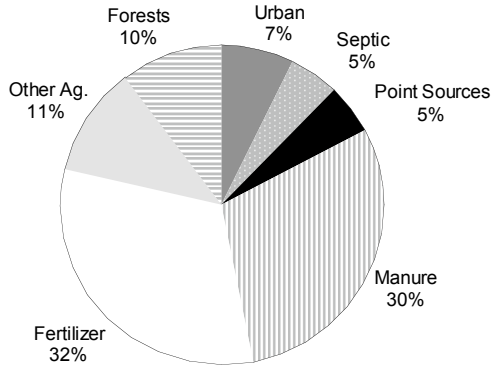

Figure 3. Sources of nutrients to Chesapeake Bay (excluding atmospheric). Source of data: Maryland Department of the Environment, Chesapeake Bay Program.

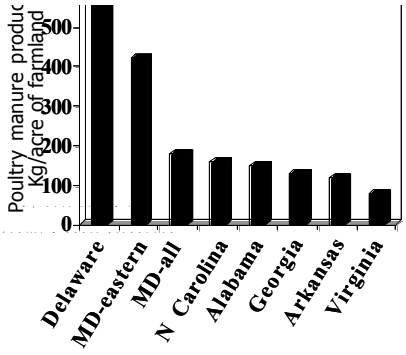

Figure 4. Poultry manure production per acre of farmland in the top poultry producing states in the USA. For Maryland (MD), the contribution is presented for the state as a whole (all) and for the eastern shore only. Source of data: US Department of Agriculture.

Many nuisance or harmful species have the ability to acquire their nutrients, and their carbon, via uptake of dissolved organic compounds or by ingestion of particles. Thus, for some species, particularly those with mixotrophic tendencies, the organic component of the nutrient pool may be more important to the development of particular harmful bloom species than the inorganic nutrient pool [50, 4, 22, 37]. In the Chesapeake Bay, for example, not only have outbreaks of *Pfiesteria piscicida* occurred under conditions of elevated organic carbon concentrations (Fig. 5), but the ratio of DOC:DON was found to be a better predictor of *P. piscicida*, *Prorocentrum minimum*, and *A. anophagefferens* in the Chesapeake than any inorganic nutrient ratio [24, 37].

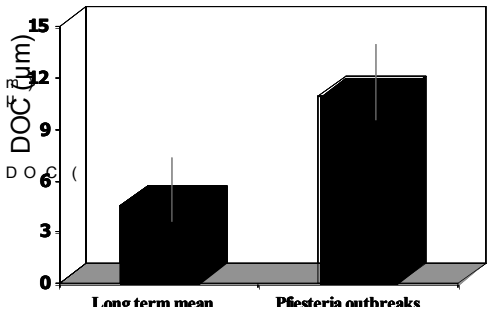

Figure 5. Comparison between ambient dissolved organic carbon concentrations (μM) for the long term mean for eastern shore tributaries and the concentration observed during *Pfiesteria* outbreaks in 1997.

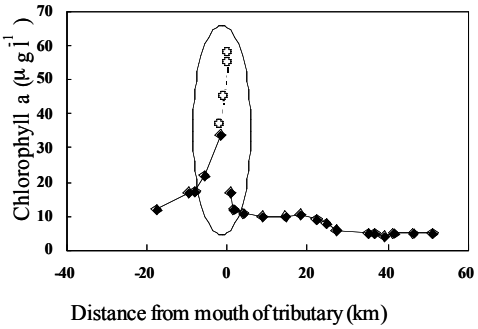

Figure 6. Chlorophyll concentrations in one of the tributaries of Chesapeake Bay during a mid-summer *Pfiesteria* sp. outbreak. The *Pfiesteria* outbreak occurred in the region defined by the oval, at the mouth of the tributary.

Second, the role of nutrients may be indirect. Species with such a complex nutrition as that of *Pfiesteria* sp., for example, may be stimulated directly by inorganic or organic nutrients, but more likely through the development of the food on which they preferentially graze [10, 11, 51]. Thus, while it has been shown that *Pfiesteria* production may be stimulated by nutrient pulses in the form of animal or human waste [10, 11], the strongest correlation seen for outbreaks of *Pfiesteria* in Chesapeake Bay is with chlorophyll. In one of the tributaries that has witnessed major *Pfiesteria* outbreaks, such events have been typically observed at the chlorophyll maximum (Fig. 6). Understanding the relationship between nutrient delivery and the development of chlorophyll biomass on which *Pfiesteria* grazes is critical for designing strategies for prediction and management of these systems. It is suggested that nutrients may indirectly stimulate *Pfiesteria* during spring when *Pfiesteria* is largely a grazer, but nutrients may directly stimulate *Pfiesteria* to a larger extent in summer when their food source is proportionately lower [35].

Third, the timing and intensity of nutrient loading may also be important in stimulating certain species. Nutrient quantity and quality must be considered in the context of other ambient conditions appropriate to the

growth of species, such as temperature and salinity, and to the growth of competitor or consumer species. For example, whether nutrients are delivered to the Chesapeake Bay in the spring with freshwater runoff, or in the case of unusually dry years in the summer, will impact total chlorophyll accumulation, as grazers are more likely to be poised for rapid chlorophyll consumption during the warmer months [42] (Fig. 7).

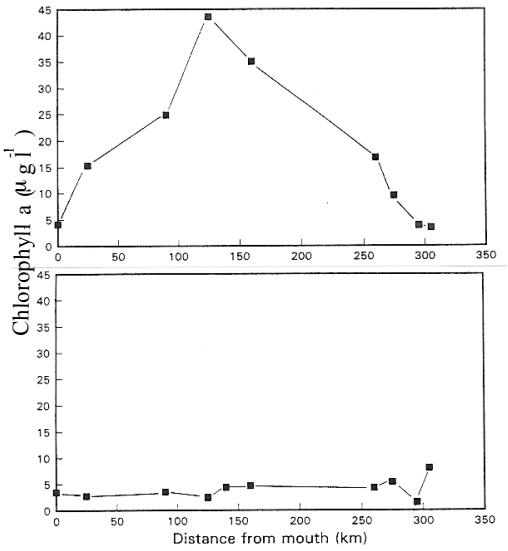

Figure 7. Concentration of chlorophyll a during spring along the main axis of Chesapeake Bay during a wet year (top panel) and during a dry year (bottom panel).

In addition, it may be significant to consider whether nutrients are delivered in a pulsed manner, such as that which would follow heavy rainfall, or a more continuous manner, such as may occur from continuous sewage discharge. Blooms of *Prorocentrum minimum*, in Chesapeake tributaries, for example, have been correlated with nitrogen delivery in the form of urea from heavy spring and summer rains [24]. In dry years, this source of nutrient is considerably less than during years with heavy spring rains, and blooms are correspondingly fewer (Fig. 8). Stimulation of *P. minimum* by organic material in freshwater runoff has also been observed in coastal waters of Japan [32] and Sweden [14].

In situ nutrient sensors are proving to be of use in quantifying ephemeral nutrient pulses, with time scales of hours to days, which are often missed in routine sampling but which may play an important role in promoting individual species. Such high frequency sampling permits an analysis of very short-term fluctuations in nutrient availability and in nutrient ratios. It is well known that phytoplankton have the physiological capability to respond rapidly to nutrient pulses; our ability to detect such events in the field has been limited with conventional sampling techniques.

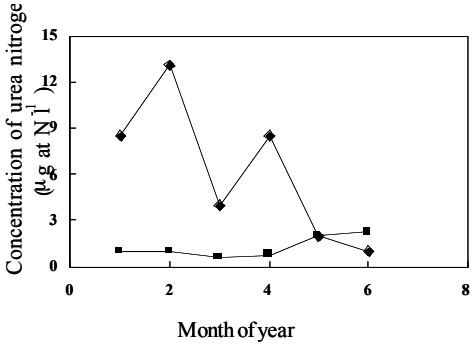

Figure 8. Comparison of concentrations of urea nitrogen in Chesapeake Bay tributaries during two spring/summer periods: a wet year (diamonds) and a significantly drier year (squares).

SCIENCE/MANAGEMENT PARTNERSHIPS

The scientific and management communities of the Chesapeake Bay region, in partnership, have addressed the problem of harmful algal blooms in several ways. The State of Maryland, having maintained a several decade monitoring program of key water quality parameters, has intensified this effort since the first outbreaks of the toxic dinoflagellate *Pfiesteria* in 1997. This effort now includes the monitoring of nine tributaries in addition to the mainstem of the Bay. During the seasons of peak nutrient delivery and algal growth, this monitoring encompasses the measurement of water quality parameters, including nutrients (inorganic and organic), dissolved oxygen salinity and temperature, phytoplankton composition and identification of harmful species, with traditional microscopic as well as newly developed molecular probe techniques (e.g., [7, 15, 54]), fish abundance and composition, frequency of abnormalities associated with fish species (lesions, etc), and toxicity in samples suspected of having a known toxin producer [39]. In addition, state agencies are now better positioned to respond rapidly should evidence of a harmful bloom outbreak become available [39]. With academic partners, additional tools have been brought to bear in this sampling, including automated sensors for water quality parameters such as chlorophyll, salinity, and nutrients, and molecular approaches for detecting a broad array of harmful species in the water and in the sediment. Academic research has also contributed to our understanding of the nutrition and physiology of these harmful species, the role of physical dynamics in their development, and the role of other biological factors such as trophodynamic relationships by species. A better understanding of the physiology of these species has led to adaptive sampling, such as increased monitoring of the organic pool of nutrients. Partnerships have also been important in improvements in understanding of the processes that contribute to these outbreaks and in the prediction of their likelihood for reoccurrence.

Further collaboration has been developed between the State of Maryland agencies and the medical community. The medical community has conducted cohort studies of individuals exposed to potentially toxic

waters (e.g., [44]), neurocognitive and neorobehavioral testing [55, 46], and other public heath testing as appropriate [3].

The State agencies, academic partners, and the medical community have formalized their relationship through the establishment of a standing Task Force. With representatives from each of these groups, the charge of the Task Force is to review the state of knowledge of harmful algal bloom events within the Chesapeake Bay, to improve sampling and methodological protocols, and make other recommendations as necessary. As the understanding of individual species, the environmental relationships, and the impacts of blooms, continues to grow, the agencies must adjust their sampling approaches to ensure that public health is adequately protected, fish health is sustained, and improvements in water quality are achieved. These partners have, also, had the responsibility for recommendations for specific river closures when the need has arisen.

MULTI-STATE AND LEGISLATIVE AGREEMENTS

The Chesapeake Bay region has been the site of several landmark agreements for the protection of habitat and improvements in water quality. With a watershed that encompasses the jurisdiction of six states plus the District of Columbia (Fig. 1), management cannot be successful if restricted to the local level. Multi-state agreements have thus been critical in increasing the efforts for improvements in water quality. These agreements have not been specifically targeted toward reduction in harmful species, but have focused on nutrient reduction and water quality. These efforts have resulted in some limited successes in harmful algal bloom reduction as well.

In 1987, the first such agreement was signed by the governors of the states in the Chesapeake watershed, plus the mayor of Washington D.C. This agreement committed to the reduction in nutrient loading by 40% by the year 2000. In some specific tributaries, this goal was achieved, but these were systems primarily dominated by sewage inputs, and the upgrades of these systems helped to meet the target goal. System-wide, however, the goal of the 1987 agreement was not attained, with overall reductions of roughly 30% for phosphorus and only 17% for nitrogen achieved during this time period.

The differential success in nutrient reduction within different regions of the Chesapeake Bay can be seen by comparing the changes in nitrogen concentrations by tributary from 1985 to 1995 (Fig. 9). While overall reductions were substantial for the western shore tributaries, there were significant increases in concentrations during the same period for the eastern shore region. These nutrient reductions resulted in the decrease in chlorophyll concentrations and in reductions of the frequency and intensity of *M. aeruginosa* blooms on the western shore [30]. Since 1995, however, these trends have not been consistently sustained due to continued pressure from population development.

In 2000, the governors of the Chesapeake Bay watershed states, as well as the U.S. Government signed a second agreement that recommitted their support to improvements in water quality and the reduction of nutrients. A comprehensive plan, with many components and goals, the 2000 agreement recognizes different strategies that are required for different tributaries in order to achieve these goals. For nutrients, the specific goal of the agreement is to, "Achieve and maintain the water quality necessary to support the aquatic living resources of the Bay and its tributaries and to protect human health" (Chesapeake Bay 2000 Agreement). To accomplish this, the 40% nutrient reduction targets are reiterated, but perhaps more significantly is the commitment to take actions on a tributary-by-tributary basis to achieve these goals.

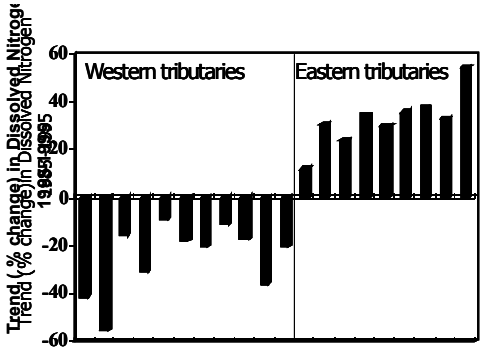

Figure 9. Percent change in dissolved nitrogen concentrations for western shore and eastern shore tributaries of Chesapeake Bay from 1985 to 1995. Source of data: Maryland Department of Natural Resources.

Other legislative actions ensure that efforts to achieve nutrient reductions are sustained. The Federal Clean Water Act, for example, establishes water quality standards for all water bodies and mandates the necessary action to ensure the assimilative capacity of these systems is not exceeded. Where water bodies have become impaired, total maximum daily loads for future nutrient loads are to be established.

CHALLENGES

In spite of legislative agreements and state and federal commitments to reduce nutrients and improve water quality, the challenges to achieve success are very large. Managing the problem of harmful algae in Chesapeake Bay will continue to be difficult due to the diversity of potentially harmful species found within the Bay ecosystem. Nutrient reduction goals will continue to be difficult to achieve because of continued population growth and development and the fact that non-point sources, especially from agriculture, are considerably more difficult to reduce than point sources. In addition, the Chesapeake Bay, like most coastal systems in the USA and around the world, will find funds for monitoring and research difficult to sustain, while clean-up efforts will be more and more costly. At least one estimate places the cost of Bay restoration at $8.5 billion over the next decade [5]. However, some success has been achieved in reducing nutrient levels and controlling harmful algal blooms. In order to build on this progress,

management practices must continue to be adaptive, with a willingness to act with the aid of scientific partnerships, agreements and legislation, even when there is much that we still do not understand about harmful algae and their relationships to nutrients. As noted over 20 years ago, but equally relevant today, "Because the natural fluctuations are so large and our data base so limited, it is exceedingly difficult to isolate the effects of people from those of nature. But if we wait until society's 'signals' can be separated from nature's 'noise', it may well be too late for effective planning and management" [56].

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ESTABLISHING A SHELLFISH MONITORING PROGRAMME FOR JAMAICA ON THE OFFSHORE PEDRO BANKS

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ABSTRACT

The presence of toxic phytoplankton species has not been well documented in the Caribbean region with the focus being on only a few species such as *Gambierdiscus toxicus* which occurs in coral reef areas and leads to ciguatera fish poisoning, and *Pyrodinium bahamense* which forms luminescent blooms and serves as major tourist attractions, e.g., in Oyster Bay, Jamaica. Toxic phytoplankton species are often linked to shellfish fishery because of the occurrence of associated human illnesses and fish kills. Numerous small-scale fish kills associated with red tide and other pollutants have occurred in Jamaican coastal waters. The causative or dominant species responsible have seldom been positively identified and when recorded are usually in numbers below the recognized harmful levels. Pedro Bank is the main harvesting area for Jamaica accounting for over half the amount of fisheries resource landed annually, and is the only area large enough to sustain a vibrant commercial conch fishery. Pedro Bank is a large submarine plateau located approximately 70 km southwest of Jamaica. The routine identification of toxic species in this area is expensive and not feasible as a management tool. A routine monitoring programme however, is more cost effective and not only assists in the sustainable management of all fisheries resources, but safeguards public health, decreases economic losses and fulfills the requirements set by some export markets such as the European Commission.

INTRODUCTION

Jamaica is located approximately 145 km south of Cuba and 161 km west of Haiti. The Island is 236 km long, between 35 and 82 km wide, with a total area of 10,940 km² and a coastline of approximately 885 km [1]. The fisheries resources are diverse and can be separated into two main groups:

1. Capture fisheries such as conch, lobster, finfish, shrimp, crab and oysters.
2. Aquaculture such as tilapia - the main cultured species with approximately 282,322 kg exported in 1997 [2]. Shrimp and lobster are also cultured on a smaller scale.

The fishing industry is important to the Jamaican economy and garners resources from 152 beaches located along the coastline, numerous near shore banks and two offshore banks (Fig. 1). While the industry supports over 3,224 registered fishers and 4,057 registered vessels, it is estimated that there are about 20,000 fishers and 9,000 vessels [3]. The main shellfish resource in Jamaica and the CARICOM (Caribbean Community and Common Market) region is the queen conch (a marine gastropod, *Stombus gigas*; Fig. 2), which is also one of Jamaica's main export earners.

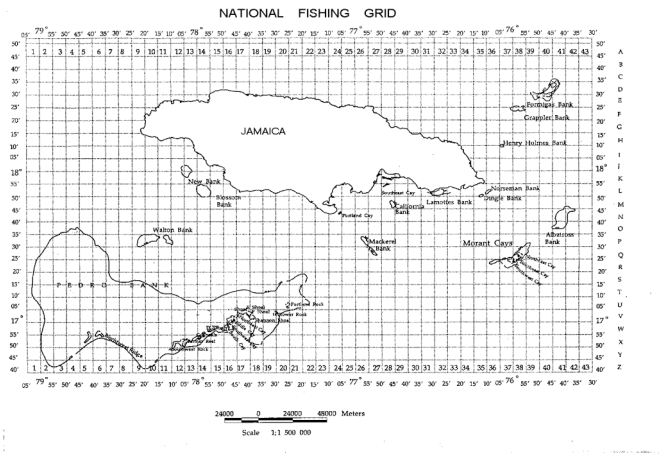

Figure 1. Map showing Fishing Banks around Jamaica.



Figure 2. Picture of a Queen conch shell *Strombus gigas*.

Conch is marketed at different levels of processing, mainly 85% and 100%, where the white meat 'fillet' can be cooked or eaten raw in salads. The levels of conch processing are categorized in Table 1.

Table 1. Conch processing classification.

PROCESS LEVEL	TISSUE LOSS
'Dirty'– unprocessed	None, animal simply removed from the shell
50%	Operculum ('claw') and viscera ('bag')
65% 'semi-fillet'	'Head' (eyes, stalks, and proboscis), 'claw' 'bag' and part of mantle ('skirt')
85%	All of the above plus verge, 'skirt' and most skin
100%	Pure white meat

As a result of overfishing occurring in coastal waters and fishers having to travel further away from traditional fishing grounds, the main conch beds are currently located offshore on Pedro Bank, a large submarine plateau located approximately 70 km south-west of Jamaica [4].

Toxic phytoplankton are microalgae capable of producing as the product of their metabolic processes, potent toxins that can be poisonous or harmful to other organisms [5, 6]. Plankton are grouped amongst the most important primary producers globally and can be found in all bodies of water [7]. The toxic or harmful algal species are capable of causing severe public health problems as they are responsible for illnesses such as: paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP) and ciguatera fish poisoning (CFP). Other species of algae may be non-toxic to humans but harmful to fish by damaging or clogging their gills thereby inhibiting respiration. In addition to public health concerns toxic algae can cause extensive economic losses to the fishing industry and impact the environment negatively [6].

In a survey done by the Centre for Marine Sciences of the University of the West Indies [8], Pedro Bank was found to display a low biomass borne out by low densities of phytoplankton cells with high species diversity indicative of oligotrophic waters. In this study diatoms were found to be dominant, comprising an average of 65% of the number of species at the sampling sites. Dinoflagellates were the second most important group making up 29% of the number of species present. Cyanophytes and Euglenoids were also present in the species composition. Pictures of some phytoplankton species from a water sample collected on Pedro Bank and preserved in Lugol's solution are shown in Figure 3.

Algal blooms have been reported periodically from coastal areas and cited as a contributor to small-scale fish kills. However, the causative organisms have seldom been identified and these events vary in magnitude and duration. The most severe and longest surviving algal bloom recorded in Jamaica, occurred on the eastern coast of the Island and lasted for five months [9]. Fish kills are presently investigated and monitored by the Coastal Zone Management Unit of the Natural Resources Conservation Authority as it not only affects the Fishing Sector but also Tourism where coastal areas have multiple uses. Apart from the beaches along the coast, Oyster Bay is a tourist attraction where a species of *Pyrodinium bahamense* that occurs in the Tropical Atlantic forms persistent luminescent blooms.

In order to protect consumers and gain access to international markets, the need exists for a comprehensive monitoring programme in all harvesting areas.

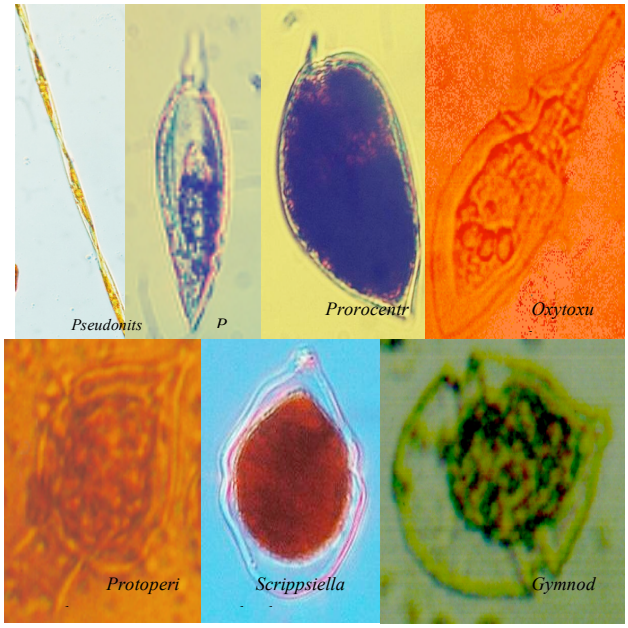

Figure 3. Pictures of phytoplankton species found in water samples from Pedro Bank.

MANAGEMENT OPTIONS

The Ministry of Agriculture, Veterinary Services Division (VSD) is the entity responsible for food safety in Jamaica. A major objective of VSD is to facilitate international trade of animals and animal products through the granting of permits and appropriate health certification. In order to trade certain food products on the international market, conformance with the requirements of a number of organizations is essential:

- World Trade Organization (WTO) Agreement on the application of Sanitary and Phytosanitary measures (SPS);
- CODEX Alimentarius Commission (CAC) - a joint FAO/WHO Food Standards Programme to safeguard consumers health and ensure fair practices in food trade by the development of international standards and guidelines;
- Food and Drug Administration (FDA) of the USA sets standards for imported fishery products;

- EU Council Directives provide standards for the production and processing of fishery products from third countries such as Jamaica. In particular, Directives 91/492/EEC and 91/493/EEC deal with the health conditions for the production and marketing of fishery products and live bivalve molluscs (including gastropods).

In addition to the various requirements of importing countries, several Acts and Regulations guide the activities of VSD. Two such pieces of legislations are the Aquaculture Inland and Marine Products and By-Products (Inspection, Licensing and Export) Act, 1999, and the accompanying Regulations that came into operation in 2000.

The Act provides for the inspection and certification of various categories of aquaculture, inland and marine products and their by-products intended for export, along with the monitoring of relay and production areas for such products, with the purpose of controlling malpractice, determining microbiological quality and detecting the presence of toxin-producing plankton, biotoxins and chemical contaminants in the products. It also governs all related matters such as licensing of persons and facilities engaged in production, harvesting, processing, handling, storage and transport for export of such products and by-products.

SAMPLING PROGRAMME

Approximately 95% of the conch harvested on Pedro Bank is exported [3]. The conch fishery comprises three categories of fishers, the primary category being the industrial fishers who target the export market. Data collected by the Fisheries Division indicates that conch are harvested between depths of 10–20 m and referred to as the industrial zone [10]. The sampling programme was designed to target this industrial zone, based on two parameters: 1) fishing pressure and 2) depth of the water column (safety for divers).

For sampling purposes the Pedro Bank was divided into five zones according to fishing pressure, where Zone 1 has the greatest fishing pressure and Zone 5 the least (Fig. 4). There are a total of 42 sampling stations located within the industrial zone (20 m contour), each represented by a 5 km grid. Zone 5 is used as a control site for water sampling only, as the depth is beyond 30 m and no industrial fishing activity occurs in this area. Sampling is done twice per month within the conch fishing season and once out of the fishing season, the duration of which is set by the Minister and published in the Gazette. The conch Fishing season normally lasts between 6–9 months per year.

On each sampling trip two grids or sampling stations are visited within a zone, where the relevant biological and supporting physico-chemical data are collected for analysis.

In order to adequately sample the water column three phytoplankton samples are taken at each station, one just below the surface, another at mid-depth and one deep (~1 m above seafloor). The samples are immediately fixed with Lugol's solution and stored in a cool, dark place for later analysis. The toxic phytoplankton species that are currently targeted under the monitoring program are listed in Table 2, along with the concentrations at which increased monitoring is triggered. Shellfish is usually taken from the same site or within a 1 km radius of the water samples. Six individual conch are taken from each site to cover the range of tests and yield sufficient hepatopancreas for DSP analysis via the Yasumoto mouse bioassay (1978) method. The types of samples collected and the tests performed are summarized below (Fig. 5).

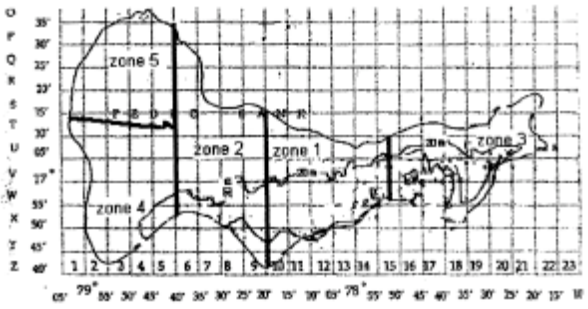

Figure 4. Map of Pedro Bank showing Sampling Zones.

Table 2. Cell Concentrations of Target Species for Shellfish Monitoring.

Target Species	Toxicity	Concentation cells L^{-1}
Alexandrium tamarense	PSP	500
Gonyaulax polyedra	PSP	> 500
Gymnodinium catenatum	PSP	500
Prorocentrum lima	DSP	100
Dinophysis acuminata	DSP	100
Dinophysis acuta	DSP	100
Dinophysis norvegica	DSP	100
Pseudo-nitzschia spp.	ASP	$1x10^5$

RESULTS & DISCUSSION

The shellfish monitoring programme is still in its embryonic stages, as it was implemented in 1999. The programme is overseen by the Residue and Biochemical Laboratory Unit (RBL) of the VSD. The RBL started out with three laboratory personnel and saw a 100% increase in staff by 1999. The inception of the shellfish monitoring programme was also in 1999, as reflected in the increase in biotoxin samples analyzed for that year (Table 3). The number of samples analyzed from 1997-2001 is shown in Figure 6. This reflects all the tests being carried out on all meats of animal origin [11]. A summary of the analyses per test from 1997 through to 2000 is recorded in Table 3.

Since the inception of the monitoring programme in 1998, positive samples have been detected and dealt with according to standard operational procedures where confirmatory testing is done before corrective measures are taken. The positive samples identified in Table 3, for antibiotics and heavy metal testing are from beef and mutton samples.

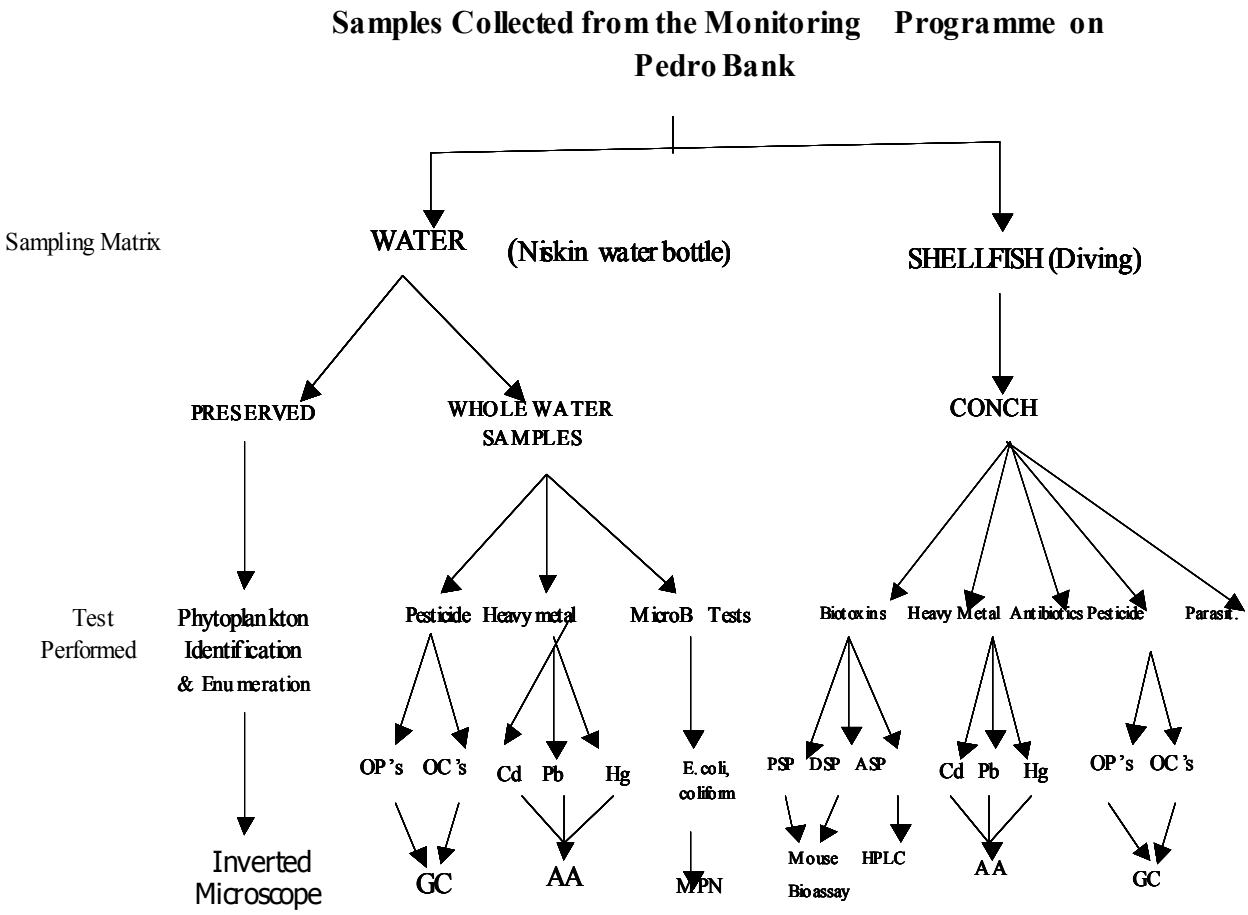

Figure 5. Samples collected by the Monitoring Programme on Pedro Bank.

Table 3. Summary of analyses done at the RBL 1997-2000.

Test	1997	1998	1999	2000
Antibiotics	230	285*	240*	1344
Heavy Metal	81	185*	176	532*
Pesticide	15	67	405	156
Biotoxins	0	12*	48*	18
Freshness	0	0	19*	0
Phytoplankton	0	0	2	5
Total	326	549	890	2055

*includes positive samples

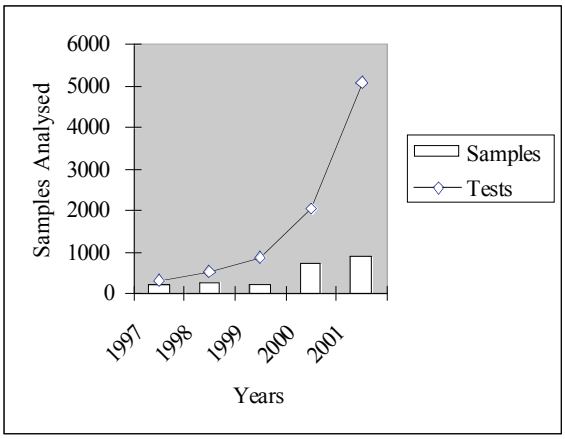

Figure 6. Graph showing number of analyses performed by RBL from 1997-2001.

In 1998, three out of the 12 samples analyzed for biotoxins, were found positive for DSP, using the rat bioassay method. The positive samples were sent to Jellett Biotek Ltd. in Canada for confirmation, these samples were reported as 'false positives' where a positive sample is reported without specifying an actual toxic component that may or may not be attributed to DSP. The method used by Jellett Biotek Ltd. was the adult mouse bioassay, at a dose of 5 g equivalent digestive tissue per mouse. The added dichloromethane clean up procedure specified by the EU was also performed. PSP testing was also done on the meat portions of the same samples and reported negative at a detection limit of <40 µg 100 g^{-1}. Again, in 1999 two samples were reported positive for PSP and DSP. These samples were sent overseas to the Department of Agriculture for Northern Ireland (DANI), which is a National Reference Laboratory, and were reported as being negative. The method used by DANI is the mouse bioassay for PSP and the rat bioassay for DSP, followed by analysis for okadaic acid using the HPLC.

From the phytoplankton samples collected and analyzed for Pedro Bank, toxic and potentially harmful species were identified but not in densities that would require closure of harvesting sites, some of the harmful species found are listed in Table 4.

CONSTRAINTS

As with any new programme there are a number of challenges to be met in order for it to be a success. Some of the challenges faced by the VSD are:

- Financial limitations lead to prioritization of programmes and implementation of programmes in stages. The availability of equipment and reagents for testing are also affected, ultimately impacting the type and frequency of tests performed.
- Human resources as it relates to competent personnel to perform relevant duties such as sample collection, inspections and analyses of samples.
- Lack of resources has also impeded the implementation of a programme to detect the occurrence of harmful algal blooms especially in coastal areas. Currently fishermen and residents in an area notify the Authorities, especially if there is a fish kill associated with the discoloration of the water.
- Distance of harvesting site from the mainland makes it difficult for intense monitoring. Inclement weather and mechanical problems with boats also interrupts monitoring of the offshore Pedro Bank.

Table 4. Harmful species found in preserved samples from Pedro Bank.

Harmful Species	**cells L^{-1}**
Amphidinium klebsii	10
Ceratium fusus	<1000
Chaetoceros concavicorne	1000
Gonyaulax diacanthe	1920
Gymnodinium sp.	690
Oscillatoria sp.	<1000
Prorocentrum spp.	20
Protoperidinium spp.	690
Pseudo-nitzschia sp.	4000
Trichodesmium thiebautii	<1000

CONCLUSIONS

In order to manage the shellfish resources on a sustainable basis while providing safe food for consumers, a number of activities need to be accomplished by the Veterinary Services Division: increasing of monitoring capability; improving testing capacity and traceability of samples; obtaining a strong commitment from the government towards food safety policies; developing additional regulation framework where necessary; training of staff to adequate competence levels, and developing a cost recovery scheme for regulatory testing and involvement in continuing research activities. If these activities are implemented with a credible quality assurance system, the main goal of achieving an ISO accreditation will be

easily attained and the required assurance provided for successful international trade.

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RED TIDE OUTBREAKS AND MANAGEMENT IN VENEZUELA

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ABSTRACT

Venezuela has registered episodes of red tides and toxicity since 1977, when 10 deaths and 256 people were intoxicated by paralytic shellfish poisoning (PSP) after consuming mussels. Since then, a program for detection of red tides is being carried out to prevent the risks of intoxication for consumption of mollusks. This program has four components: Monitoring, Research, Education and Information. The red tide action plan involves primarily FONAIAP, the Ministries of Health and Agriculture, the Venezuelan Coast Guard, and several kinds of mass media. The recurrence of the red tide events has caused serious health, social and economic impacts, and has also affected the tourism, fishing and aquaculture industries, and also some marine ecosystems. Other marine toxin - related events, like ciguatera, have affected the economy of the country. In October 1991, two people died and 51 were intoxicated after consuming fish contaminated with ciguatoxin in Sucre State. Cases of DSP and ASP have not been reported yet, but the causal organisms are present. Economic and social impact are particularly severe in the fishing communities whose economy is based on extraction and artisanal processing of mollusks from wild stocks. Some mussel aquaculture activities have been abandoned by the recurrence of red tide events in eastern Venezuela. The extension of the red tide outbreaks to areas not previously affected has caused irreparable damages to marine ecosystems. In 1996 a red tide occurred within Morrocoy Marine National Park (in the State of Falcón, western Venezuela), causing massive mortality of coral reefs and considerable losses to the tourism and the economy of the State. The program for toxin detection has minimized health risks; however the extension of the problem and its diversified causes require the investment of larger efforts and more resources however, to cover other potential areas and new research lines.

INTRODUCTION

Venezuela has a coastline of 2,813 km, of which 1,300 km corresponds to the northeastern region and 700 km to Sucre State. The latter is the portion of the marine coast that is most heavily affected by red tide and toxicity events. Their causes are probably associated with the physical and ecological characteristics of the area. There is upwelling along the coast from January through May, which results in dense phytoplankton blooms and highly productive fisheries. These conditions support dense populations of mollusks, such as: mussel (*Perna perna* and *P. viridis*), ark shells (*Arca zebra*), oysters (*Crassostrea rhizophorae*), and clams (*Asaphys* sp. and *Tivela mactroides*). There were also mussel aquaculture activities in the Gulf of Cariaco, where this species did not previously occur; unfortunately, they were abandoned because of recurrent red tide events. The economic and social impacts of red tides are particularly severe within the fishing communities whose economies are based on the collection and artisanal processing of mollusks.

OUTBREAKS AND IMPACTS OF DINOFLAGELLATES BLOOMS IN VENEZUELA

Paralytic shellfish poisoning (PSP) and ciguatera fish poisoning (CFP) are two kinds of illnesses from biotoxins affecting fish resources in Venezuela. DSP (Diarrheic shellfish poisoning) and ASP (amnesic shellfish poisoning) have not been reported yet, but the causal organisms are present.

The impact of PSP on public health has been known in Venezuela since 1977. In this year, two outbreaks of toxic dinoflagellate blooms occurred in northeastern Venezuela. The first one took place in the northwestern coast of Margarita Island in May 1977 and was caused by *Gonyaulax tamarense* var *excavata*. The second was detected in the northern coast of Sucre State in July-August and was caused by a chain-forming species of the genus *Cochlodinium*, followed by *Noctiluca scintillans* [1]. During the latter event, the authors reported 10 casualties and 171 intoxicated persons. Later, representatives of the Ministry of Health carried out a revision of cases and the number of intoxicated persons was raised to 257 cases.

The recurrence of harmful algal blooms and toxicity in mollusks has caused serious health, social and economic impacts, and has also affected the tourism, fishing and aquaculture industries, as well as some marine ecosystems [2]. The area of incidence of red tides in Venezuela extends from Cabo Codera to the Gulf of Paria. Since 1996, new areas have been affected, among them Morrocoy National Park (Falcon State) and the Lake of Maracaibo (Zulia State; Fig. 1). The record of fatalities and intoxicated persons by PSP are believed to be far below the actual number of cases, due to unreported and unconfirmed cases (Table 1).

The long history of toxicity events in the Gulf of Cariaco and the northern coast of Sucre State shows that toxicity events have longer duration in the Gulf, but fewer intoxications are registered from this area (Figs. 2, 3). This is possibly due to the easier management of prohibitions of bivalve consumption, since this zone had only cultured mussels. The northern coast of Sucre State has natural mussel beds, with easy access to any person, which would explain the high rate of intoxication and the difficulties to control the situation during toxic events. The toxicity events usually have a short duration period.



Figure 1. Map of Venezuela showing areas where blooms have been observed (\\\) and areas where PSP death cases have been reported (*).

Toxicity problems of PSP in mollusks originate in Venezuela from the constant blooms of *Alexandrium tamarense, Gymnodinium catenatum* and *Cochlodinium* cf. *catenatum*. Recently, another species belonging to the group *Alexandrium*, was involved in a toxicity case. *Pyrodinium bahamense* has been detected, but its toxicity has not been shown in the Venezuelan population. In 1989, the first red tide caused by *Gymnodinium catenatum* was observed in the northeast coast of the Sucre State. Eighteen people were intoxicated after consuming ark shells and mussels. This red tide caused toxicity in several kinds of mollusks. The recorded toxicity levels reached maximal levels of 1,309 µg STX 100 g^{-1} meat in mussel (*Perna perna*) and 546 µg STX 100 g^{-1} meat in Ark shells (*Arca zebra*) [3].

Table 1. Venezuela records of paralytic shellfish poisoning cases (C) and deaths (D) from 1977 until 1993.

DATE	PLACE	C	D	SPECIES
1977 May	Manzanillo (Nueva Esparta State)	12	1	*Alexandrium tamarense*
1977 July	Río Caribe (Sucre State)	230	9	*Cochlodinium*
1978 Sept.	El Morro (Sucre State)	12	--	*Alexandrium tamarense*
1980 Aug.	Carúpano y Chacopata (Sucre State)	9	1	*Alexandrium tamarense*
1989 Feb.	Carúpano (Sucre State)	18	--	*Gymnodinium catenatum*
1991 Aug.	Manzanillo (Nueva Esparta State)	24	--	*G. catenatum, Cochlodinium*
1993 Nov.	Chacopata (Sucre State)	5	--	*Gymnodinium catenatum*
1993 Nov.-Dec.	Carúpano (Sucre State)	5	--	*Gymnodinium catenatum*

In July 1991, an outbreak of PSP occurred in Margarita Island, northeastern Venezuela, where 38 persons were intoxicated. The victims had consumed mollusks from an area that was officially closed due to PSP. High levels of PSP (1,000 µg STX 100 g^{-1} meat) were found in mussel samples and an organism resembling *Gymnodinium catenatum* was found in plankton samples from the area [4].

Impacts on tourism and fishing resources, due to marine fish and invertebrate mortality caused by events of red tide in the northeastern region, are considerable. Bloom-forming species to note include dinoflagellates, rhaphidophyceans and protozoa, such as: *Alexandrium monilatum, Gonyaulax polygramma, Heterocapsa* sp., *Noctiluca scintillans, Prorocentrum gracile, P. micans, P. minimun, P. balticum, Protoperidinium* sp., *Pyrodinium bahamense*, *Chatonella* sp., *Mesodinium rubrum, Asterionella japonica, Coscinodiscus,* and *Thalassiosira*.

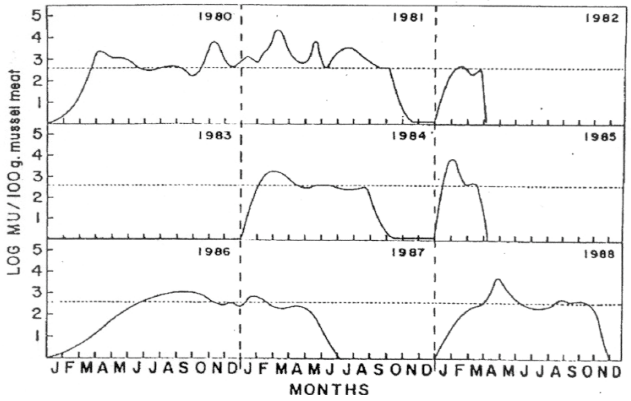

Figure 2. Annual variation in toxicity titre in the mussel *Perna perna* from the Gulf of Cariaco, Venezuela.

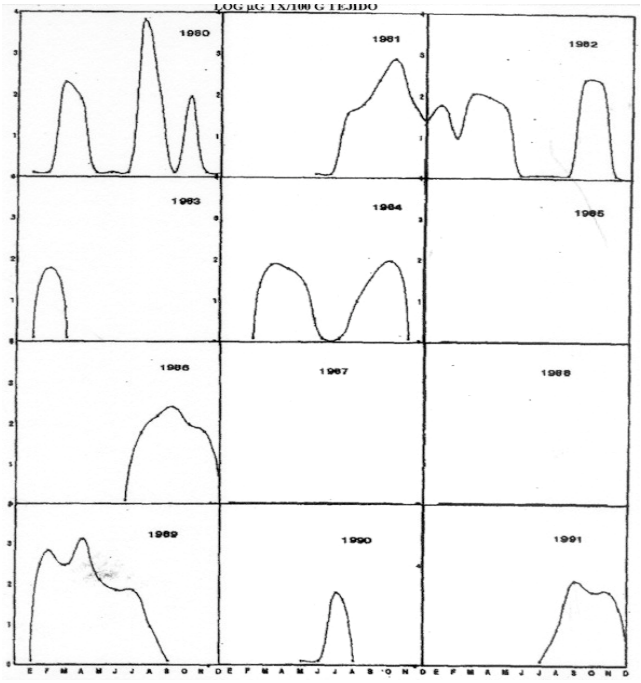

Figure 3. Annual variation in toxicity titre in the mussel *Perna perna* from the northern coast of Sucre State, Venezuela.

The extension of harmful algal blooms to areas not previously affected has caused irreparable damage to marine ecosystems. In 1996, a red tide occurred within Morrocoy Marine National Park (in the State of Falcon,

western Venezuela), causing massive mortality of coral reefs and considerable losses to the tourism industry and the economy of the State. Lake of Maracaibo has also been affected by red tide events, where mortality of larger fish has been observed.

In 1998, a red tide by *Prorocentrum minimun* covered an extensive area of a natural bank of the clam, *Tivela mactroides*, located in the northeastern sector of Sucre State, causing a severe mortality of the resource. Toxicity was not detected in the clams and consumption of the clam by fishermen did not induce any health discomfort.

CIGUATERA OUTBREAKS

In October 1991, two casualties and 51 persons intoxicated with ciguatera were reported in Cumaná, Sucre State [4]. The patients had consumed any of the following fish species: *Sphyraena* spp., *Sardinella aurita*, *Trachurus lathami*, and a non-identified species that was sold dry and salted. A sample of fish consumed by the victims was sent for analysis to the US-FDA, where ciguatera-like toxins were found. The symptoms reported in intoxicated patients were: reversion of temperature, headache, muscular pains, tachycardia and pain in the jaws. Local fishermen indicated having observed ciguatera before, but there is not official epidemiological information about previous cases.

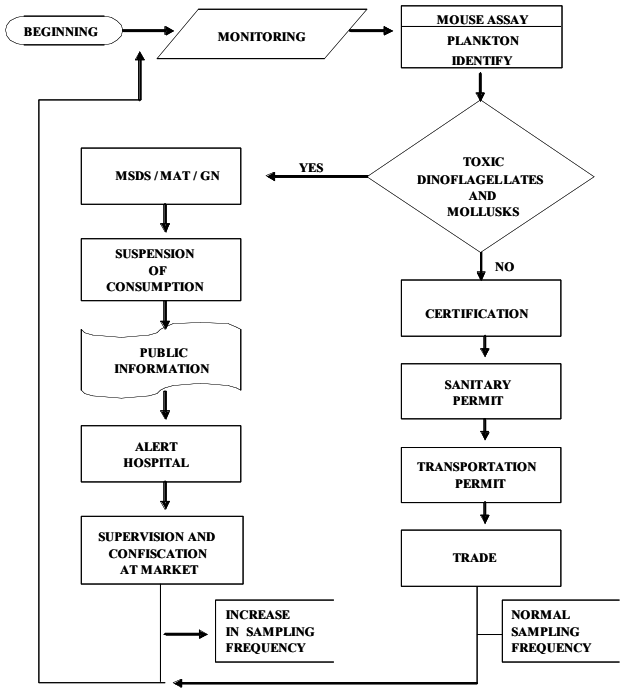

Figure 4. Surveillance program for marine toxins (PSP) in Venezuela.

MONITORING PROGRAM IN VENEZUELA

A surveillance program for detection and management of marine toxins was started in 1977 and continues to this day. The marine biotoxin program in Venezuela involves primarily INIA (Instituto Nacional de Investigaciones Agricolas) (institution that coordinates the program), the Ministries of Health and Agriculture, the Venezuelan Coast Guard, the National Guard and several kinds of mass media. The prohibition on consumption and commercialization of mollusks is the responsibility of the Ministry of Health. Decisions to close or to re-open an area for commercial exploitation are taken jointly by the institutions integrating the surveillance and management plan of marine biotoxins (Fig. 4).

Under this management program, fishermen and seafood processors are required by the Ministry of Agriculture to take samples of the products to be commercialized, and send them to the reference laboratory reporting the exact place of the harvest. If no PSP is detected, the laboratory issues a certificate to the fisherman or processor, valid for 15 days, assuring the safety of the product. For marine products to be transported within the country, health and transportation permits are also required.

THE MANAGEMENT PROGRAM ON RED TIDE AND TOXICITY IN VENEZUELA INVOLVES THE FOLLOWING ASPECTS:

A. Monitoring Strategies

The monitoring of toxic dinoflagellates and biotoxins in shellfish is conducted through monthly plankton samples and toxicity bioassay on shellfish, from the main locations where shellfish products are extracted. Sampling frequency increases to weekly during periods of blooms. The toxicity levels in the mollusks are tested using the standard AOAC mouse bioassay protocol. Five bivalve species are commonly consumed in eastern Venezuela: mussel (*Perna perna* and *P. viridis*), oyster (*Crassostrea rhizophorae*), ark shells (*Arca zebra*), clams (*Asaphys* sp. and *Tivela mactroides*) and Vieira. The mussels are the most likely species to accumulate toxins.

The periodic sampling of the causative organism in the plankton has had a positive effect in the monitoring program, since the bloom can be detected early. This helps the shellfish toxicity managers on the prompt casting of toxicity alerts.

B. Information Dissemination/Public Education

If the toxicity test is positive, the following actions are taken: the representatives of the Ministries of Health and Agriculture, the Venezuelan Coast Guard and the National Guard are informed immediately. The Ministry of Health restricts the sale and consumption of the product nation-wide and the general public is informed about the problem through mass media. An emergency plan is also set up, which includes an alert to the medical personnel of coastal hospitals, an increase of the sampling frequency and a check up of the affected areas. Once toxicity levels reach safe limits, the commercial activities are resumed.

The program has revealed a lack of information among medical doctors and nurses, as well as hospitals

and clinics in coastal areas, on aspects pertaining to intoxications caused by marine organisms. This situation has improved by means of conferences, workshops and courses. Furthermore, special forms to report PSP cases have been issued to people and institutions mentioned above. This last measure has facilitated identification of the type of intoxication and the existence of an updated record of cases.

In regards to education, workshops were organized for technicians from environmental institutions, and talks were organized for small-scale fishermen and representatives of the commercial fishing industry, on the risks involved in the consumption of seafood contaminated with marine biotoxins. Also for educational purposes, the work "Dinoflagellates of the northeast region of Venezuela" was published. Therefore, educating the population is an important component in the Alertness Program in marine biotoxins in order to prevent poisonings.

C. Regulatory/Control

Toxin concentrations are determined by mouse bioassay [4]. When the toxin level in samples exceeds the 80 µg STX eq 100 g^{-1} meat limit, or sometimes below this regulatory limit, the commercialization is closed by Ministry of Health. Licenses to transport and sell mollusks from the affected areas are also suspended during this period.

The control program has also allowed the early detection of toxicity in mollusks and the presence of toxigenic dinoflagellates in the water.

D. Research

INIA is conducting a research program on the prevention of toxic algal blooms, the study of shellfish toxicity, identification of potential areas for aquaculture and the evaluation of events of harmful algal blooms in relation to oceanographic factors. Most resources have been provided by the Government, but the extent of the problem and its diversified causes require the investment of larger efforts and resources to cover other potential areas, new research lines, and also to obtain modern equipment and trained personnel.

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HABS IN THE SOUTHWESTERN ATLANTIC OCEAN COAST: A GENERAL REVIEW

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ABSTRACT

An apparent increment in frequency and toxic events of harmful algal blooms (HABs) has been observed on a global scale. The South-western Atlantic Ocean coast has not been out of this situation. In this region harmful phytoplankton blooms (*Alexandrium tamarense*, *Gymnodinium catenatum*, *Dinophysis acuminata, Pseudo-nitzschia* spp., *Heterosigma akashiwo, Chatonella* sp., *Trichodesmium* spp. and others) and several toxins (paralytic shellfish poisoning, diarrhetic shellfish poisoning, and amnesic shellfish poisoning toxins and microcystin) occur from Tierra del Fuego to the north of Brazil (10º-55° S). *Chattonela* sp. and *Heterosigma akashiwo* represent a risk at intensive shrimp cultures and shellfish cultures, in Santa Catalina, Brazil.

Argentina has a National Monitoring program of mussel toxicity in each coastal province throughout its regional laboratories: Chubut and Buenos Aires provinces have phytoplankton monitoring and there is one fixed station at Mar del Plata. Brazil had a pilot monitoring during one year, but no national monitoring program has been established. Uruguay has a regular National Monitoring Program for mussel toxicity and toxic phytoplankton.

A Regional Science Planning workshop on HABs in South America supported by IOC is carried out every two years and the countries have an information network for toxic events.

KEY WORDS: HAB, South-western Atlantic Ocean, *Alexandrium tamarense*, *Gymnodinium catenatum*, *Dinophysis acuminata*, PSP, DSP, ASP, *Microcystis*, FANSA

INTRODUCTION

Interactions between the warm and oligotrophic Brazil Current, the nutrient-rich waters of the Malvinas Current, the position of the Subtropical Convergence and the continental runoff at Río de la Plata and Patos Lagoon estuaries are the principal forcing factors in the Southwestern Atlantic Ocean. These interactions are also responsible for the high biological production along the coastal zone, which can be seen by satellite images [1, 2].

An apparent increment in frequency and intensity of toxic events caused by algae species has been observed on a global scale [3]. This spreading has been explained as a function of increment in scientific awareness of toxic species, utilization of coastal water for aquaculture; stimulation of phytoplankton blooms by anthropogenic eutrophication and transport of algal resting cysts by ships' ballast water [4], but also by climatic and oceanographic factors when previous explanations cannot be applied [3]. The South-western Atlantic Ocean coast has not been out of this situation.

In this region, blooms of harmful species and toxic mussels by paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP) and other toxic effects have been observed occur from Tierra del Fuego to the north of Brazil (Fig. 1). The aim of this work is review the harmful algae events that occurred in the Atlantic coast of South America, from the north of Brazil to the south of Argentine (10º -55º S).



Figure 1. Map of the South-western Atlantic Ocean showing the countries, provinces and principal cities, the Rio de la Plata estuary, the Malvinas current (MC) and the Brazilian current (BC).

Table 1 synthesizes all the HAB events that have occurred in the South-western Atlantic coast, from the north in Pernambuco, Brazil to Tierra del Fuego, Argentina, including each province, toxins, associated species and its reference.

PSP

The first report of PSP was detected in Peninsula Valdés - Chubut, Argentina (42º S) in 1980 after a dramatic episode occurred where two members of the crew of the ship B/P Constanza died after eating mussels.

High toxic levels were detected (50,000 μg STX eq 100 g^{-1} tissue) in mussels, and *Alexandrium tamarense* was identified as the probable causative species (Fig. 2) [5]. Coincidentally, 60 persons were affected with neurotoxic symptomatology in Uruguay where PSP reached 1250 μg STX eq 100 g^{-1} tissue, and the identified dinoflagellate belonged to the genus *Gonyaulax* sp. [6].

Alexandrium tamarense recurrently causes blooms in south Argentina [7] and it has been suggested that northwards currents spread *A. tamarense* to south Brazilian waters [8]. *Alexandrium tamarense* is frequently

Table 1. HAB events in the South-western Atlantic coast, from the north in Pernambuco, Brazil to Tierra del Fuego, Argentina in each province, including toxins, associated species and its references.

Locality	Harmful effects	Species microalgae	References
BRAZIL	Febre de Tamandaré	*Trichodesmium erythraeum*	Satô *et al.,* 1964, [9]
Pernambuco Recife	Bird and fish mortalities by microcystins	cyanobacteria spp.	Ferreira, *et al*., 1999, [10] Domingos *et al*., 1999, [11] Magalhães *et al*., 1999 a, b [12,13].
Minas Gerais Bahia	Fish mortality	*Microcystis* and *Cylindrospermopsis*	Oliveira *et al*,. 1998, [14] Azevedo *et al*., 1994, [15]
Parana Rio de Janeiro	Fish mortality	*Synechocystis aquatilis* f *salina*	Oliveira *et al*., 1992, [16]
	Damage in tourism	*Tetraselmis* 1991/1992, 1996/1997 and 1999/2000. *Chatonella* sp. 1998	Tenenbaum *et al*., 1998, [17]
	"Febre de Tamandaré"	*Trichodesmium erythraeum* 1996, 1997, 1999.	Carvalho *et al*., 1999, [18]
	Mortality of *Echinometra lucunter*	*Prorocentrum* sp.	Ferreira *et al*., 1999, [19]
	okadaic acid	*Prorocentrum* sp.	Garcia per. com.
	anatoxina-a(s)	*Anabaena spiroides*	Monserrat, *et al*., 2001, [20]
	MCYST in plankton and fish meat	*Microcystis aeruginosa*	Azevedo *et al*., 1998 [21] Magalhães *et al*., 2001 [22]
São Paulo	PSP	*Cylindrospermospsis raciborskii*	Lagos, *et al*., 1999, [23]
	SXT	*Pseudomonas* spp.	Freitas *et al*., 1992, [24]
Santa Catarina	PSP	*Gymnodinium catenatum*	Proença *et al*., 1999, [8]
	DSP	*Dinophysis* spp.	Proença, *et al*., 1998[25]
Rio Grande Cassino Beach	Mollusc Mortality	*Gyrodinium* cf. *aureolum*	Machado, 1979 [26]
		Dinophysis acuminata and *Noctiluca scintillans*	Garcia *et al*., 1994 [27]
	Potentially Harmful spp.	*Heterosigma akashiwo*	Persich *et al*., 1998 [28]
		Alexandrium tamarense	Odrebrech *et al*., 1997, [29] Persich *et al*., 1998, [28]
		Pseudo-nitzschia spp.	Odebrecht *et al*., 2001, [30]
		Dinophysis acuminata	Persich et al., 1998, [28]
Lagoa dos Patos Estuary	MCYST	*Microcystis aeruginosa*	Yunes, *et al*., 1996 [31] Minillo *et al*., 1999 [32]
	anatoxina-a(s)	*Anabaena spiroides*	Yunes, *et al*., 2000 [33]
URUGUAY	PSP	*Alexandrium tamarense*	Brazeiro *et al*., 1997 [34]
		Gymnodinium catenatum	Méndez *et al*., 2003 [35]
	DSP	*Dinophysis acuminata*	Ferrari *et al*., 2000 [36]
	ASP	*Pseudo-nitzschia* spp.	Medina & Ferrari, 2002[37]
	MCYST	*Microcystis aeruginosa*	De León, 2001 [38]
ARGENTINA Buenos Aires	PSP	*Alexandrium tamarense*	Carreto *et al*.1985, 1986, 1998 [8, 39, 40]
		Gymnodinium catenatum	Akselman *et al*., 1998, [41].
	ASP	*Pseudo-nitzschia* spp	Montoya *et al*., 2000 [42]
Chubut	PSP	*Alexandrium tamarense*	Carreto *et al*., 1981 [5]
	DSP	Prorocentrum lima	Gayoso & Ciocco, 2001 [43]
Tierra del Fuego	PSP	*Alexandrium catenella*	Benavides *et al*., 1995 [44]

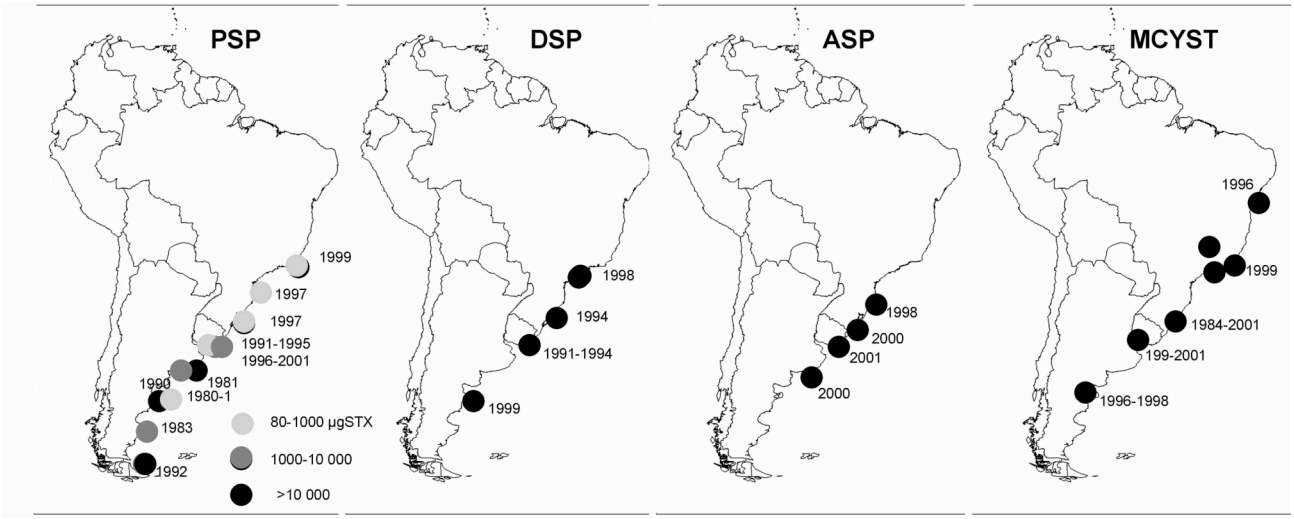

Figure 2. Distribution area, maximum toxicity and years of recorded for PSP, DSP, ASP and Microcystin.

reported in early spring on this area [45], and it has been associated to a frontal system [40].

Every year *A. catenella* blooms in the Argentine sector of the Beagle Channel (Tierra del Fuego) and shellfish toxicity is registered since 1992. The maximum reached has been 127,200 µg STX eq 100 g^{-1} tissue and it produced human intoxication [44], although Argentina has historic data that mentions massive mortalities of native people from Ushuaia in 1886 [46].

PSP outbreaks in Uruguay are also associated with *Gymnodinium catenatum* during summer since 1991 [35]. A bloom of *G. catenatum* reached a maximum density of 221 cells ml^{-1}. The maximum toxicity was observed at 1500 µg STX eq 100 g^{-1} with a density of 93 cells ml^{-1}. In coastal sediments high cyst densities of this species were reported [47]. In 1998 this species was observed off the coast of Santa Catarina State, Brazil (27º S), reaching a maximum of 103 µg STX eq 100 g^{-1} [8]. *Gymnodinium catenatum* outbreaks were also associated with autumn toxicity in Argentina [41]. In Uruguay, the hypothesis regarding the initiation of toxic blooms at Río de la Plata is that *G. catenatum* might be an endemic problem and its initiation is due to bottom cyst resuspension [35].

PSP has also been detected in the freshwater cyanobacteria *Cylindroespermopsis raciborskii*, but no human intoxication has been reported in Brazil [23].

DSP

Although several DSP potentially producing species as *Dinophysis acuminata, D. caudata*, *D. sacculus*, *D. acuta*, and *D. rotundata* have been registered in the area during the whole year, DSP in molluscs has been only reported associated with *D. acuminata, D. caudata* and *Prorocentrum lima* proliferations.

In 1990 several persons showed gastrointestinal distress and diarrhoea after eating mussels in Florianopolis, Brazil (27º S) and in Uruguay in 1991, (Fig. 2). In both countries, plankton analysis and mouse bioassay supported the evidence of DSP and *Dinophysis acuminata* presence [25, 36]. In Argentina *D. acuminata* and *D. forti* have been present but no toxicity was detected until 1999 when 40 persons were intoxicated in Patagonia, Argentina (40º S). *Prorocentrum lima* was confirmed in plankton samples and DSP was detected in mussels [43].

Mass mortality of yellow clams, *Mesodesma mactroides,* and clockles, *Donax hanleyanus,* occurred in the eastern coast of Uruguayan beaches contiguous to Brazilian southern beaches. The mousse bioassay gave positive results, associated with *D. acuminata* and *D. caudata* [48]. The same massive mortality happened in Brazilian southern beaches and in Argentina [49, 50]. It is not clear the relation between toxicity and the massive mortality.

ASP

Several ASP producing species of *Pseudo- nitzschia (P. australis, P. delicatissima P. pungens, P. multiseries, P. fraudulenta, P. pseudodelicatissima* and *P. turgidula)* have been previously reported for this region; some are domoic acid producers [51].

Recently, the most relevant were the ASP detections in winter 2000 at Mar del Plata, Argentina (38º S) and in Uruguay during spring of 2001. In Argentina, the dominant species was *Pseudo-nitzschia australis* and the toxin was detected in mussels and in the fish *Engraulis anchoita*. Two massive mortality episodes of sea birds were reported [42], whereas in Uruguay *P. multiseries* and *P. pungens* [52] were the associated species.

CYANOBACTERIA

Harmful cyanobacteria blooms are recurrent phenomena that have been increasing in the last years. Several *Microcystis aeruginosa* blooms in Lagoa dos Patos estuary, Brazil (30º S) were registered with microcystin detections [53]. The most shocking case happened in Pernambuco State in Brazil in 1996 when 54 persons died at a haemodialysis centre [54, 55] (Fig. 2). Afterwards, a new freshwater reglamentation for human consumption was introduced that included microcystin levels *(*toxic cyanobacteria in water: a guide to their

public health consequences, monitoring and management).

Microcystis aeruginosa commonly blooms during the summer periods in Uruguay. In February 2001, a very extensive bloom in different aquatic systems and in the coast of Río de la Plata was registered [56]. The microcystin-containing bloom of the cyanobacterium *Microcystis aeruginosa* was analysed, showing low levels of 155µg g^{-1} in the sample [38]. This episode was accompanied by other cyanobacteria species: *M. novacekii*, *M. flos-aquae*, *M. wessenbergii*, *Anabaena circinalis* and *A. spiroides*. This phenomenon caused public impact, fish mortality and problems in drinking water. Many people had skin problems, however none were reported to the Public Health authorities or to the Toxicity centre. The authorities of the City Hall of Montevideo closed the beaches affected by the algae and warned about harmful effects. The National Direction of Aquatic Resources authorities recommended not to consume fish in affected areas [56].

Blooms of the cyanobacteria *Trichodesmium* spp. were frequent in summer in tropical oceanic water and off the Uruguayan coast [58]; three species have been recorded as bloom forming: T. e*rythraeum, T. thiebautii* and *T. hildebrandtii. Trichodesmium* blooms were associated with a human disease known as Tamandaré Fever (name of the city where the disease was recorded) in the northwest of Brazil. Toxicity test from *T. hildebrandtii* gave negative results in mouse bioassay [57].

OTHERS SPECIES

Chattonela sp. and *Heterosigma akashiwo* represent a risk at intensive shrimp cultures and shellfish cultures in Santa Catalina, Brazil, [28].

There are many reports of water discoloration produced by algal blooms, but most of them are harmless [58]. In the Buenos Aires province coast, an intensive *Prorocentrum minimum* bloom was associated with toxicity and fish mortalities [59].

Several times there were reports of *Gymnodinium/Gyrodinium* spp. associated with respiratory diseases in bathers from Brazil [60].

In the Argentinean platform toxin transference into higher trophic levels was confirmed, as in heterotrophic zooplankton species [40] and in fishes (e.g., horse mackerel mortality and the ASP in anchovy [61, 62].

BALLAST WATER

Brazil was one of the six countries selected in a management and control of ballast water pilot program, GLOBALLAST, supported by GEF, IMO and ONU.

MANAGEMENT AND MITIGATION

Argentina has a National Monitoring program for mussel toxicity (SENASA, Servicio Nacional de Sanidad, calidad Agropecuaria y SAGPyA) in each coastal province throughout regional coastal laboratories (Río Negro, Chubut, Santa Cruz y Tierra del Fuego and Islas del Atlántico Sur). The exception is at Buenos Aires where it is conducted by SENASA. In Buenos Aires (INIDEP) and Chubut (Universidad Nacional de la Patagonia) provinces phytoplankton monitoring is also included [63].

Brazil had a pilot monitoring during one year, but no National Monitoring Program has been established since then. However, Uruguay has had a regular National Monitoring Program for mussel toxicity and toxic phytoplankton since 1980 [64, 65].

ECONOMIC IMPACTS

In this region algal blooms affect the commercialisation of bivalve molluscs, gastropods, and fish, public health and tourism. Commercial species affected by HABs in the South-western Atlantic coast are:

Argentina
- Bivalve molluscs
  - Mussels *Mytilus edulis platensis, Aulacomya atra atra*
  - Scallops *Zyochlamys patagonica, Aequipecten tehuelchus*
  - Clams *Amiantis purpurata, Ameghinomya antiqua*
  - Atlantic Geoduck *Panopea abbreviata*
  - Oysters *Ostrea puelchana*
  - Razor shells *Ensis macha*
  - Cockles *Donax hanleyanus*
- Gastropods
  - Snails *Zidona dufresnei*, *Adelomelon brasiliana*, *A. beckii*

Brazil
- Bivalve molluscs
  - Mussels *Perna perna*
  - Oysters *Crassostrea gigas*

Uruguay
- Bivalve molluscs
  - Mussels *Mytilus edulis*
  - Yellow clams *Mesodesma mactroides*
  - Cockles *Donax hanleyanus*
  - Scallops *Zyochlamys patagonica*
  - Clams *Pitar rostrata*
- Gastropods
  - Snails *Zidona dufresnei*

Table 2 presents the economic impacts of HABs in Brazil, Argentina and Uruguay. The economic losses for no mollusc commercialisation during a ban period, the human intoxications and the environmental effects in freshwater and marine waters are summarized. More information is included in the National report of each country to the Workshops reports of IOC-FANSA [65].

FANSA = HAB IN SOUTH AMERICA WORKING GROUP

An IOC supported Regional Science Planning workshop on harmful algal blooms in South America is carried out every two years, including Ecuador, Perú and Chile from the Pacific Coast and Brazil, Uruguay and

Argentina from the Atlantic coast. These countries have an information network for toxic events. The working group identifies the regional needs, the harmful algae species, the food regulation systems for marine toxins, and research priorities, and promotes contacts and information exchange between regional experts involved in control and research.

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Table 2. Economic impact of HABs in Brazil, Argentina and Uruguay. Loss of no mollusc commercialisation during a ban period, the human intoxication, and freshwater and marine environmental effects. n/d = not detectable.

		BRAZIL	ARGENTINA	URUGUAY
FRESHWATER ENVIRONMENT	*Economic Impacts*	*Microcystis* and *Oscillatoria* blooms, 4 ton d^{-1} $CuSO_4$ used as algaecide [66]	n/d	*M. aeruginosa* bloom, tourism impacted by no bathing allowed at affected beaches
	Human Intoxication	Pernambuco, 1988, *M. aeruginosa* bloom, 88 deaths and 200 intoxicated persons Pernambuco, hemodialysis centre 1996, *M. aeruginosa* bloom: 54 deaths and 123 intoxicated [21, 67] Minas Gerais, cyanobacteria bloom, toxins in fishes [14]	n/d	n/d
	Env. Effects	Rio de Janeiro, *Microcystis* bloom, zooplankton reproduction affected [68] Rio Grande, Lagoa dos Patos, *Microcystis* bloom, toxicity in shrimp [69]	n/d	n/d
MARINE ENVIRONMENT	*Economic Impacts*	Discolorations: Rio de Janeiro, *Chatonella* sp. bloom, heath problems and tourism impacts [17] Santa Catarina, *Trichodesmium erythraeum* and *T. thiebautii* spp., beach problems [58] Santa Catarina, PSP in *Perna perna* culture (10-thouthand tons yr^{-1} production) and PSP in Japanese oyster *Crassostrea gigas (*200 thousand tons yr^{-1} production); this activity involves 900 farmers	PSP in mussels, lost in two months of ban, 388 tons (37% of the year production); total harvesting losses: 1159.3 TM yr^{-1} Buenos Aires, Mar del Plata, 1999, *Prorocentrum minimum,* fish mortalities 1992, *Gyrodinoum cf. aureolum (=G. mikimotoi*), fish mortalities	Toxicity in mussels, (1991-1997) loss due to no commercialisation 901,802 US$ 1980-1992, fish commercialisation decreasing during the mussels bans, 7,668,000 US$
	Human Intoxication	*Trichodesmium erythraeum*, Fibre de Tamandaré or Tinguí fevers Santa Catarina, summer 1990, *Dinophysis acuminata*, diarrhetic intoxications [70]. *M. aeruginosa,* human and animal damage (Yunes *et al*., 1998).	PSP in mussels, *Alexandrium tamarense* and *A. catenella* blooms, total 43 intoxicated and 9 dead persons Chubut, 2000, DSP in mussels, *Prorocentrum lima* bloom, 40 intoxicated	PSP in mussels, intoxicated people, treatment estimated at 7,500 US$.
	Env. Damage	Rio Grande, *Gyrodinoum cf. aureolum., Synecocystis a. f salina,* a large list of fish and inter-tidal fauna mortality [71] Rio Grande, wild *Mesodesma mactroides* mortality associated with *Dinophysis acuminata* [49]	Fish mortalities Wild *Mesodesma mactroides* mortality [50] *Pseudonitszchia* sp. bloom, ASP in fishes, sea bird mortality	1994, bivalve mollusc mortality (*Mesodesma mactroides*) 27,000 US$ 1996, bird mortalities: penguins

REGULATORY ENVIRONMENT

EUROPEAN APPROACHES TO MARINE TOXIN CONTROL AND HARMONISATION

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ABSTRACT

In the European Union (EU), legislation on marine toxin control has existed since 1991. This legislation includes directives that specify limits for the paralytic shellfish poisoning (PSP) toxins, okadaic acid and dinophysis toxins, pectenotoxins, yessotoxins, azaspiracids and the amnesic shellfish poisoning (ASP) toxins. Imports of live bivalve molluscs from third countries into the EU are allowed if provisions are applied equivalently to those governing the production and the placing on the market of Community products. Animal testing, mainly with mouse assays, still plays a key role in official analytical methodology in the EU, except for the ASP toxins where high performance liquid chromatography (HPLC) methodology is prescribed. The *in vivo* tests can in principle be replaced by alternative methods, if there is analytical evidence through convincing validation studies that these alternatives provide an equivalent level of health protection. The increasing resistance in Europe towards the use of *in vivo* assays as well as relevant EU regulations on animal experiments has led to pressure on European researchers to develop alternative methods. A network of EU National Reference Laboratories on Marine Biotoxins exists in the EU, with the aim to eliminate inconsistencies among member states and to harmonize European trade. The network is coordinated by a Community Reference Laboratory in Vigo, Spain. Interactions between the laboratories of this network take place through annual meetings and by means of working groups hosted by the European Commission. Currently working groups exist about sampling, mouse bioassays and liquid chromatography/mass spectrometry (LC/MS) methodology. The reference laboratories cooperate with the Working Group on Biotoxins of the European Standardization Committee (CEN). This working group produces standard methods of analysis. CEN (pre-) standard methods now exist for some PSP, DSP (diarrhetic shellfish poisoning) and ASP toxins. The increasing demand for analytical quality assurance has recently led two European organisations (FAPAS® and Quasimeme®) to organize proficiency tests for marine biotoxins. First exploratory studies on PSP toxins and ASP toxins have taken place and it is expected that these programs will expand their activities in the near future.

INTRODUCTION

Within Europe, the European Union, currently made up of 25 countries (Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, the Netherlands, Portugal, Spain, Sweden, the United Kingdom, and the recently incorporated Cyprus, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Malta, Poland, Slovakia and Slovenia) is an area of free trade, with unrestricted circulation of merchandise, and with border control carried out only for imports from countries outside the EU. Along the last decade, a number of Directives and Decisions dealing with food safety legislation have been elaborated and implemented to ensure consumer protection, high quality of products and fair competition.

For molluscan shellfish, the process of harmonization of European legislation is at an advanced stage, and various relevant Directives and Decisions exist. EU Directive 91/492/EEC lays down the health conditions for the production and placing on the market of live bivalves [1] (European Commission, 1991a). This Directive establishes the control of the shellfish production areas and the different activities of harvesting, transport, depuration, storage, processing and marketing, as well as microbiological and chemical parameters and marine toxin content. EU Directive 97/61/EEC [2] (European Commission, 1997a) modifies and complements Directive 91/492/EEC concerning traceability of mollusc batches from harvesting to marketing and includes domoic acid control. According to Directive 91/492/EEC, a public health control system must be established by the responsible authority in order to verify whether the requirements laid down in the Directive are complied with. In relation to marine toxins, this control system must include:

1. Periodic monitoring of live bivalve molluscs and production areas in order to check the possible presence of toxin-producing phytoplankton in the waters and biotoxins in shellfish.
2. Sampling plans that will take into account possible variations in production and relaying areas in the presence of toxic phytoplankton. The sampling must be carried out periodically to detect changes in the composition of the toxic phytoplankton populations and their geographical distribution. Information leading to suspicion of accumulation of toxins in mollusc flesh must be followed by increasing the number of sampling points and the number of samples. Toxicity tests shall be carried out using the molluscs of the affected area that are most susceptible to contamination. If the results of a sampling plan show that marketing live bivalve molluscs may constitute a hazard to human health, the responsible authority must close the production area as regards molluscs until the situation has been restored.

Marketing molluscs from that area may not be re-authorized until new sampling has provided satisfactory test results.
3. Laboratory tests of the end product to verify that the level of marine biotoxins does not exceed safety limits.
4. Inspection of establishments at regular intervals.
5. Check on storage and transport conditions for consignments of live bivalve molluscs.

Traceability is another important aspect regulated in Directive 97/61/EC. This implies the implementation of a registration and labelling procedure, so that if a health problem occurs after placing shellfish on the market, the batch can be traced back to the dispatching establishment and the harvesting area of origin.

Regarding methods and limits for the control of PSP and DSP toxins, Directive 91/492/EEC states:

- The total paralytic shellfish poisoning (PSP) toxicity in the edible part of molluscs (the whole body or any part edible separately) must not exceed 80 μg per 100 g of mollusc flesh, in accordance with the biological testing method – in association if necessary with a chemical method for detection of saxitoxin – or any other method recognized in accordance with the procedure laid down in Article 12 of this Directive. If the results are challenged the reference method shall be the biological method.
- The customary biological testing methods must not give a positive result to the presence of diarrhetic shellfish poisoning toxins in the edible part of the molluscs (the whole body or any part edible separately).

The Directive is vague. Neither specific biological methods nor criteria of acceptability are mentioned for PSP and DSP control. In the case of DSP, the action limit depends on the detection limit of the biological method used. Since 2002 a new Commission Decision is in force for toxins of the DSP complex and associated toxins, which specifies different limits for various toxin groups and which provides details about the analytical methodology to be used (see section "DSP Control").

For ASP, Directive 97/61/EEC [2] (European Commission, 1997a) establishes that the total amnesic shellfish poisoning (ASP) toxicity in the edible parts of molluscs (the entire body or any part edible separately) must not exceed 20 micrograms of domoic acid per gram using the HPLC method. In this directive "the HPLC method" is not further defined in terms of performance and characteristics.

Concerning monitoring programs, the criteria for the design of the sampling plans, in relation to the ideal number and location of sampling stations, sampling points, sampling frequency and criteria to close and re-open the areas have not been established.

The need to eliminate inconsistencies among EU Member States and to harmonize European trade led the European Commission to nominate a National Reference Laboratory on Marine Toxins (EU-NRL) in each Member State and a Community Reference Laboratory (EU-CRL, Vigo, Spain), to co-ordinate the activities of the network of EU-NRLs and create a forum for discussion and agreements on analytical methods, performance criteria, and action levels for toxin control. Thus far, all European countries are represented except Luxembourg, which is currently represented by Belgium. In the course of 2004, the EU has expanded to 25 member states and the network is being supplemented by NRLs from the new EU member states. Currently these countries are in the process of organizing their NRL tasks and responsibilities.

In relation to countries outside the EU, Directive 91/492/EEC states that provisions applied to imports of live bivalve molluscs from third countries shall be at least equivalent to those governing the production and placing on the market of Community products. Inspections shall be carried out on the spot by experts from the Commission and the Member States to verify whether the conditions of production and marketing can be considered as being equivalent to those applied in the Community. In deciding on equivalency, particular account shall be taken of legislation of the country, organization of the responsible authority and inspection services, facilities for monitoring the implementation of their legislation, health conditions of production and marketing, monitoring of production areas in relation to microbiological and environmental contamination and the presence of marine biotoxins, information on toxic phytoplankton, in particular species not occurring in Community waters, and assurances on the compliance with the standards in Directive 91/492/EEC.

EU Decision 97/20/EC [3] (European Commission, 1997b) establishes a list of countries outside the EU that fulfil the requirements of equivalence concerning production of bivalve molluscs, echinoderms, tunicates and marine gastropods. This decision has undergone modifications in the last few years [4, 5, 6, 7, 8] (European Commission 1997c, 1998, 2000, 2001b, 2001c). Imports from the countries listed in the Decision are allowed and, after veterinary control at the border inspection posts, products can enter the Community.

The list of authorized countries can be updated if new agreements of equivalence are signed or if the current agreements are modified. Information about EU legislation currently in force or in preparation is available on the website (http://www.europa.eu.int/eur-lex).

EUROPEAN ACTIVITIES

The European National Reference Laboratories Network

The tasks of the EU-CRL are:

1) to inform NRLs on methods and intercomparative testing,
2) to coordinate the application of methods by organizing intercomparative testing,
3) to coordinate the investigation of methods and inform on advances in the area,
4) to organize training courses,
5) to collaborate with laboratories from third countries

6) to assist the EU Commission in case of discrepancies, and
7) to assist the NRLs on accreditation activities.

Typical examples of CRL-coordinated activities carried out in the last few years within the NRL network, include collaborative studies of methods to determine ASP [9] (CRL, 2003a) and intercalibration studies on DSP [10] (CRL, 2001) and PSP toxins [11] (CRL, 2003b). The tasks of the NRLs are:

1) to coordinate the activities of the national laboratories,
2) to assist the competent authority to organize the system for monitoring marine biotoxins,
3) to organize comparative tests between the various national laboratories, and
4) to disseminate the information supplied by the EU-CRL to the competent authority and national laboratories.

A typical example of an NRL-coordinated activity is the organization of a series proficiency test for PSP toxins among Dutch laboratories involved in analysis of marine biotoxins [12].

The interaction between the EU-NRLs network takes place through annual meetings, and by means of Working Groups (WGs) hosted by the EU Commission that deals with different marine toxin issues and that are integrated by experts from the network of EU-NRLs and also by experts outside the network that may join the WGs to improve the capabilities. The WGs might deal with items related to the activities of the EU-NRLs, or with specific issues under the request of the EU Commission or the EC's Standing Veterinary Committee. Examples of newly established WGs are a WG on sampling, a WG on mouse bioassay and a WG on LC/MS methodology. The outcomes of some of the WGs in the way of conclusions and/or recommendations have been used as pre-normative studies to elaborate new regulations. In the last few years, efforts have been done by the EU-NRL network and the EU Commission to achieve equivalent approaches for the control of marine toxins in shellfish.

PSP Control

For PSP sanitary control, experts of the EU NRLs on Marine Biotoxins agreed that the biological method mentioned in Directive 91/492/EEC should be the mouse bioassay, performed as described by AOAC [13]. As some recently developed technologies such as receptor binding assays, some immunoassays (MIST ®Alert) have shown that they may be suitable alternatives to the mouse assay, it is the intention of the EU-NRLs group to evaluate these methodologies with the goal to open a way of applying them in monitoring programs, if it can be demonstrated that they provide a level of protection equivalent to that of the mouse bioassay.

DSP Control

For DSP control in the European Union, EU Directive 91/492 establishes that the customary biological method must not give a positive result for the presence of DSP in the edible part of the molluscs, but it does not clarify the interpretation of a positive result and does not specify which biological method should be used. Therefore a WG performed a risk assessment on the basis of the available data on okadaic acid (OA) and dinophysis toxins (DTX), pectenotoxins (PTX), yessotoxins (YTX) and azaspiracids (AZA) with the goal of recommending allowance levels and suitable detection methods. The recommendations and conclusions of this WG [14] formed the scientific basis of the recently approved Decision 2002/225/EC [15] (European Commission, 2002a) that lays down detailed rules for the implementation of the Directive 91/492 as regards the maximum levels and the methods of quantification of OA and DTX, PTX, YTX and AZA in bivalve molluscs, echinoderms, tunicates, and marine gastropods. About maximum levels, the following has been stated:

A) The maximum level of okadaic acid, dinophysistoxins, and pectenotoxins, together shall be 160 µg of okadaic acid equivalents kg^{-1} edible part (the whole body or any part edible separately).

B) The maximum level of yessotoxins shall be 1 mg of yessotoxin equivalents kg^{-1} edible part (the whole body or any part edible separately).

C) The maximum level of azaspiracids shall be 160 µg of azaspiracid equivalents kg^{-1} edible part (the whole body or any part edible separately).

The new Decision states that in addition to biological testing methods, alternative detection methods such as chemical methods and *in vitro* assays should be accepted if it is demonstrated that the performance of the chosen method is not less effective than the performance of the biological method and that their implementation provides an equivalent level of public health protection. The compounds to be detected are: OA and Dinophysis toxins, PTX1 and PTX2, YTX, 45 OH-YTX, homo YTX and 45 OH homo YTX, AZA1, AZA2 and AZA3. However, if new analogues of public health significance are discovered they should be included in the determinations. Standards have to be available before chemical analysis will be possible and total toxicity will be calculated using conversion factors based on the toxicity data available for each toxin. The performance of these methods should be defined after validation following an internationally agreed protocol.

With respect to biological methods, it is stated that a suite of mouse bioassay procedures differing in the test portion and in the solvents used for the extraction and purification steps can be used. Sensitivity and selectivity depend on the choice of the solvents and this should be taken into account when making a decision on the method to be used in order to cover the full range of toxins. The mice are inoculated with extracts equivalent to 5 g of hepatopancreas or 25 g of whole body. The death of two out of three mice within 24 hours should be

considered as a positive result for the presence of one or more of the toxins of the groups regulated in the Decision at levels above those allowed. The full implementation of this new Decision on the basis of chemical methods and/or *in vitro* assays still requires effort in the development of toxin standards, toxicological studies, and development and validation of methods alternative to the bioassays. Nevertheless it represents a significant step towards harmonization of phycotoxin control approaches.

ASP Control

Extensive work was carried out by a NRL-WG on ASP in scallops to minimize the economic impact produced by the slow ASP detoxification process of certain *Pectinidae*. The efforts of these activities formed the basis of the recently approved Decision 2002/226/EC [16] (European Commission, 2002b). This decision allows the harvesting of *Pecten maximus* and *Pecten jacobeous* with levels of domoic acid (DA) higher than 20 mg kg^{-1} and lower than 250 mg kg^{-1} if they are destined to be placed on the market after total removal of hepatopancreas, soft tissues or any other toxic contaminated part.

On the basis of this Decision, a restricted harvesting regime of scallops with DA concentration (whole body) >20 μg g^{-1} and intended to be processed, can be initiated if the DA concentration in whole scallop (whole body) is <250 μg g^{-1} and if the DA concentration of the anatomical parts intended to be placed on the market and analyzed separately (edible parts), is <4.6 μg g^{-1}. The latter condition was introduced to take into account the large inter-animal variability, which exists in the concentration of DA in scallop organs. It is not a lowering of the regulatory limit. It assists in ensuring that the chance of the DA concentration in the scallop edible part exceeding 20 μg g^{-1} is less than 1 in 1000 upon harvesting (probability "p" of 0.1%). It is based on the analysis of 10 pooled individual edible parts and a 50 % coefficient of variation (as a measure of inter-animal variability in DA concentrations).

In addition to the conditions above for allowing harvesting, additional requirements concerning sampling, number of individuals to make up the analytical sample, transport conditions, authorized establishments, testing of the end product and traceability measures have also been established in the EU Decision.

Sampling Plans

The issue of sampling plans has also been addressed by a Working Group of the EU-NRL network and the conclusions, listed below, may be included in the near future in the EU regulations:

1) Depending on different factors such as the size, type of resource, type of production, etc., production areas as defined in article 2.7 of Council Directive 91/492/EEC [1] (European Commission, 1991a) may be divided into smaller areas (regulatory areas) for which decisions on allowance or prohibition of harvesting are made. For this purpose, a sampling plan taking account of space and time should be designed.

2) The number and location of sampling points should take into account the spatial variability in the toxin content for all the bivalve mollusc species.

3) Sampling frequency for toxin analysis in the molluscs should be at least weekly during the time periods for which harvesting is allowed. This frequency may occasionally be reduced in specific areas for which robust historical data on toxins or phytoplankton occurrence suggest very low risk of toxic episodes. Nevertheless, this should be periodically reviewed in order to assess the risk of toxins occurring in the shellfish from these regulatory areas.

4) When knowledge of toxin accumulation rates is available for a group of species growing in the same area, a species with the highest rate may be used as an indicator species. This will allow the exploitation of all species in the group if toxin levels in the indicator species are below the regulatory limits. When toxin levels in the indicator species are above the regulatory limits, harvesting of the other species should only be allowed if further analysis on the other species shows toxin levels below the limits.

5) The absence of toxic cells in the water does not imply the absence of toxins in the molluscs. It is important to test shellfish for toxin for the purpose of consumer protection. Notwithstanding, monitoring of toxic phytoplankton is considered to be extremely useful to predict the accumulation of toxins in molluscs, especially when molluscs sampling frequency is less than once per week. Toxic phytoplankton monitoring should be carried out by weekly sampling during harvesting periods. The samples should be representative of the water column and should provide information on the presence of toxic species as well as on population trends. If any changes in toxic populations that may lead to toxin accumulation are detected, the sampling frequency of molluscs shall be increased or precautionary closures of the areas will be established until results of toxin analysis are obtained.

6) The size and the number of shellfish from a given sampling point should be decided on the basis of species, toxin type and knowledge of toxin variability.

7) Once an area has been closed, two consecutive results below the regulatory limit separated at least 48 hours are necessary to re-open it. Information on phytoplankton trends may be included in this decision. In those cases when there are robust data on the dynamics of the toxicity for a given area, and provided that recent data on decreasing trends of toxicity are available, the competent authority may decide to re-open the area with results below the regulatory limit obtained from one single sampling.

8) *Pectinidae* from natural beds are toxin vectors and therefore they should be subject to monitoring phytoplankton and toxins with the same requirements that apply to *Pectinidae* from aquaculture areas. However, in the case of open-sea beds, the usefulness of phytoplankton monitoring as a prediction tool is questionable and therefore not currently considered necessary until further scientific evidence is available.

9) In order to monitor open-sea beds, fishing areas should be clearly defined. Monitoring may be carried out on shellfish collected at sea or on shellfish collected at landing. Measures should be applied to guarantee traceability.

10) Where possible, the frequency of monitoring for toxin analysis in *Pectinidae* should be at least once a week when the areas are open for exploitation.

Other current activities of the EU-NRL network include the preparation and distribution of toxin standards and reference materials, organization of proficiency tests, inter-laboratory studies and validation of analytical methods. The latter also includes cooperation with Institutions outside the EU. An example is the participation of four (EU) NRLs in an inter-laboratory exercise on LC/MS methodology for ASP/DSP toxins, organized by the Cawthron Institute, New Zealand [17]. The other four laboratories in this study originated from APEC countries. This inter APEC-EU cooperation on method development and validation might become more significant in the coming years, also because the EC's 6th Framework programme (FP6) in principle offers possibilities for various non-EU countries to participate in EC-funded research projects, although the EC will not financially sponsor non-EU participants.

The European Standardization Committee (CEN)

In addition to the EU-NRLs activities, the work of the European Standardization Committee (CEN) is to be mentioned, a committee integrated by members of national standardization bodies from approximately 25 European countries. CEN produces standard methods to enable food manufacturers to determine with reasonable certainty whether a production batch may be put on the market and to enable regulatory authorities to determine whether foodstuffs on the market comply with legal limits. Within CEN, Working Group 5 (biotoxins) of Technical Committee 275 (Food analysis-Horizontal Methods) works on the selection and elaboration of methods that are to become EU standards. CEN standards are not necessarily official EU methods but the European Commission may refer to CEN standards for substances subject to legal limits. The CEN WG 5 works closely with the EU-NRLs network and jointly they are currently elaborating a report on performance criteria for analytical methods for phycotoxins. The criteria will be based on the results of validation studies and published data and will include scope and definitions, tables with precision data (RSD_r and RSD_R), recovery characteristics for various phycotoxins, information about extraction solvents, and applicability to different types of seafood. At the time of writing CEN had the following methods in the last phase of the standardization process: two for PSP toxins, one for DSP toxins and one for ASP toxins. [18, 19, 20, 21] (CEN, 2002a; 2002b; 2004a; 2004b). For the near future the CEN working group biotoxins may also start standardizing LC/MS methodology as well as functional assays. CEN does not standardize *in vivo* assays, such as the mouse bioassays for PSP and DSP toxins.

Other European Developments

The increasing demand for analytical quality assurance has led several organizations to undertake proficiency testing. In these tests, participants receive similar samples from a coordinator to be analyzed under the same conditions as participants would carry out their routine analysis. In addition to relevant activities already organized by the CRL and some of the NRLs (see section 'The European National Reference Laboratories Network") there are now two European organizations starting to undertake proficiency tests for shellfish toxins on a regular scale: [21] FAPAS® and Quasimeme®. Both organizations exist already for a longer period, but their activities in the marine biotoxin area are just starting for PSP [22] (FAPAS®) (Food Analysis Performance Assessment Scheme, 2003) and ASP (Quasimeme®; www.quasimeme.marlab.ac.uk/scheme04version1.pdf). It is expected that these programmes will expand their activities in the coming years.

In many European countries there is increasing resistance towards the use of *in vivo* assays for the detection of marine biotoxins [23]. Also European legislation [24] stipulates that "an experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonable and practicably available." This has led researchers to explore the possibilities for alternatives. A European Organization active in this respect is ECVAM (European Centre for the Validation of Alternative Methods). In an attempt to stimulate the development of accepted alternatives to the *in vivo* assays for PSP and DSP toxins currently practiced in Europe, a recommendation with supportive and convincing documentation has recently been issued by representatives of governmental institutions of Germany, the UK and the Netherlands [25] to the members of the ECVAM Scientific Advisory Committee (ESAC). In reaction to this letter, ECVAM has decided to install a Task Force on Shellfish Toxin Testing. This task force is expected to come up with proposals on the next steps.

CONCLUSION

The European Union has established and updated detailed regulations for various marine biotoxins of European concern: paralytic shellfish poisoning toxins, okadaic acid and dinophysis toxins, pectenotoxins, yessotoxins, azaspiracids and amnesic shellfish poisoning toxins. Groups of scientists, organized through the network of National Reference Laboratories on Marine Biotoxins and the CEN Working Group on Biotoxins are active to provide a scientific basis for the regulations and to develop and validate analytical methodology to make enforcement of the regulations possible. The increasing resistance towards the use of *in vivo* assays focuses the major analytical challenges to (bio-) chemical methods that can be used in regulatory analysis as successful alternatives to the current official

rodent tests. Very helpful for the analyst are the proficiency testing schemes, organized by various European organisations and their expansion in the coming years should be stimulated.

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INTERNATIONAL STANDARD SETTING ON MARINE BIOTOXINS IN SHELLFISH

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ABSTRACT

World Health Organization (WHO), jointly with Food and Agriculture Organization of the United Nations (FAO) provides scientific advice to the Codex Alimentarius Commission and Member States to improve global food safety through establishing international food standards. Currently the Codex Committee on Fish and Fishery Products (CCFFP) which has a responsibility to elaborate world-wide standards for fresh, frozen (including quick-frozen) or otherwise processed fish, crustaceans and molluscs is discussing the Proposed Draft Standard for Live and Raw Bivalve Molluscs in which maximum levels for different toxin groups will be included. To facilitate the progress of the elaboration of the standards, FAO, the Intergovernmental Oceanographic Commission of UNESCO (IOC) and the WHO are planning to hold a joint expert consultation in September in 2004. Based on the advice from the consultation, the standards and testing methods of marine biotoxins would be harmonized internationally for protecting consumer health and ensuring fair practice of food trade.

FOOD SAFETY IN THE WORLD HEALTH ORGANIZATION (WHO)

Although governments all over the world are intensifying efforts to improve food safety, the occurrence of foodborne diseases continues to be a prominent health issue. An estimated one in three people worldwide suffer annually from a disease caused by microorganisms in food, and 1.8 million die from severe food and waterborne diarrhoea. In addition, chemical contamination (including marine biotoxins) of food causes a significant, but less well defined, number of different types of disease.

A wide range of diseases is transmitted through contaminated food and water, and foodborne diarrhoea is a major cause of infant and child mortality. In the poorest countries, unsafe food compounds the health consequences of malnutrition. Vulnerable groups, notably the very young, the elderly, pregnant women and the immuno-compromised, are particularly susceptible to the adverse effects of unsafe food.

In 2000 and 2003, the World Health Assembly (the governing body of WHO, comprising 192 Member States) recognized the significance of food safety for global public health. Hence the Organization's activities in this area have been reinforced, and food safety is one of WHO's 12 main priorities for its current General Programme of Work.

Both chemical and microbial contaminants in food can cause severe acute and chronic disease. Food may become unsafe at its animal or plant origin, or it may be rendered unsafe later in the food chain. Therefore, strategies for improving food safety should consider the entire food production chain, in a holistic approach "from farm to fork".

Providing scientific advice on food safety issues to the Codex Alimentarius Commission and Member States is the principal activity of the Food and Agriculture Organization of the United Nations (FAO)/WHO leading to the establishment of international standards for food trade. However, scientific advice is also requested and used by developing countries to create national food safety programs needed for sustainable development and to support public heath. Codex standards are based on scientific evidence supplied by WHO/FAO expert bodies and consequently, the World Trade Organization has formally recognized Codex standards for food products moving in international trade.

As the risk assessment body for food additives, contaminants, veterinary drug residues, and pesticide residues, the Joint Expert Committee on Food Additives (JECFA) and the Joint Meeting on Pesticide Residues (JMPR) were established in the 1956 and 1963, respectively. They have been meeting ever since and to date JECFA has evaluated over 1500 food additives, approximately 40 contaminants and naturally occurring toxicants, and residues of approximately 90 veterinary drugs. JMPR to date has evaluated over 230 pesticides [1]. The Joint Expert Consultation on Microbiological Hazards in Food (JEMRA) was initiated in 2000 to assess microbial hazards in foods and has evaluated 5 pathogen-commodity combinations to date.

THE ROLE OF CODEX

The Codex Alimentarius Commission (CAC) is an intergovernmental body comprised of 170 member states organized to implement the Joint FAO/WHO Food Standards Programme which was established by an FAO Conference resolution in 1961 and a World Health Assembly resolution (WHA 16.42) in 1963. Its primary objective is to protect the health of consumers and to ensure fair practice in the food trade by setting international standards on foods (i.e., Codex Standards) and other texts that can be recommended to governments for acceptance [2].

The standards are compiled in the Codex Alimentarius, an international food code, recognized by the World Trade Organization in 1995 as the global point of reference for food standards. As a result, attention has focused on the ability of food authorities in all countries, and more particularly developing ones, to participate effectively in discussions that lead to the adoption of these international standards.

The food standards, guidelines and other recommendations of Codex Alimentarius shall be based

on the principle of sound scientific analysis and evidence, involving a thorough review of all relevant information, in order that the standards assure the quality and safety of the food supply [2].

Within the framework of the Codex Alimentarius Commission and its procedures, the responsibility for providing advice on risk management lies with the Commission and its subsidiary bodies (risk managers), while the responsibility for risk assessment lies primarily with the joint FAO/WHO expert bodies and consultations (risk assessors). The risk analysis used in Codex should be:

- applied consistently;
- open, transparent and documented;
- conducted in accordance with both the *Statements of Principle Concerning the Role of Science in the Codex Decision-Making Process and the Extent to Which Other Factors are Taken into Account* and the *Statements of Principle Relating to the Role of Food Safety Risk Assessment*; and
- evaluated and reviewed as appropriate in the light of newly generated scientific data.

The risk analysis should follow a structured approach comprising the three distinct but closely linked components of risk analysis (risk assessment, risk management and risk communication) as defined by the Codex Alimentarius Commission, each component being integral to the overall risk analysis. The three components of risk analysis should be documented fully and systematically in a transparent manner. While respecting legitimate concerns to preserve confidentiality, documentation should be accessible to all interested parties.

Effective communication and consultation with all interested parties should be ensured throughout the risk analysis. The three components of risk analysis should be applied within an overarching framework for management of food related risks to human health [2].

Statements of Principle Relating to the Role of Food Safety Risk Assessment (decision of the 22nd CAC 1997) articulated that health and safety aspects of Codex decisions and recommendations should be based on a risk assessment, as appropriate to the circumstances. Food safety risk assessment should be soundly based on science, should incorporate the four steps of the risk assessment process, and should be documented in a transparent manner.

CODEX ACTIVITIES ON MARINE BIOTOXINS

Commodity Committees, which prepare draft standards for submission to the Commission, are classed as either General Subject Committees or Commodity Committees. Commodity Committees have the responsibility of developing standards for specific foods or classes of food. For example, the Codex Committee on Fish and Fishery Products (CCFFP) elaborate world-wide standards for fresh, frozen (including quick-frozen) or otherwise processed fish, crustaceans and molluscs [2]. Currently, CCFFP is discussing the Proposed Draft Standard for Live and Raw Bivalve Molluscs [3]. In the hygiene and handling section of the standards, there are different proposals of maximum levels for different toxin groups. To provide the basis for further Committee's actions in this regard, the CCFFP requested scientific advice from FAO/WHO Expert Consultations.

General Subject Committees are so called because their work has relevance for all Commodity Committees. Since this work applies across the board to all commodity standards, General Subject Committees are sometimes referred to as "horizontal committees." For example, the Codex Committee on Food Additives and Contaminants elaborate world-wide standards for food additives and contaminants. However, to date they do not elaborate any standards on marine biotoxins.

REGIONAL AND NATIONAL REGULATION

Most countries have a regulatory system to prevent foodborne diseases associated with the consumption of shellfish. In this section, I would like to introduce two major regional regulatory systems because these regulations require at least an equivalent system for those countries which export their shellfish into these areas; consequently, a lot of exporting countries, including developing countries, shall implement equivalent control systems.

European Community

The European Community (EC) Directive 91/492/EEC "Shellfish harvesting areas are monitored for the suitability of the shellfish for human consumption under the European Community (EC) Shellfish Hygiene Directive" is the main legislation concerning live bivalves mollusks. It defines conditions for placing those live animals on the market for immediate consumption. It also sets criteria concerning production area, harvesting and transportation, relaying and purification. Public health controls on the commercial production of shellfish for human consumption within the European Union (EU) are determined by the EC Shellfish Hygiene Directive 91/492/EEC. The Directive stipulates microbiological and biotoxin end product standards [4]. This Directive stipulates regulatory limits for paralytic shellfish poisoning (PSP) toxins and diarrhetic shellfish poisoning (DSP) toxins.

Council Directive 97/61/EC was issued in 1997. This Directive states a regulatory limit for the biotoxins responsible for amnesic shellfish poisoning (ASP). An amendment to 97/61/EC was issued in 2002 (Commission Decision 2002/226), which provides more specific detail on regulatory requirements for ASP toxins in scallops [5].

Commission Decision 2002/225 was issued in 2002 and supersedes the Shellfish Hygiene Directive 91/492/EEC in relation to DSP toxins. It provides regulatory limits for lipophilic marine biotoxins, which are divided into four groups [6]:

- Okadaic acid (OA) and *Dinophysis* toxins (DTXs)
- Yessotoxins (YTXs)
- Azaspiracids (AZAs)
- Pectenotoxins (PTXs)

In addition, the Directive permits biological testing for these toxins to be replaced by chemical testing, but only after validation following protocols that are subject to international agreement.

Live and processed bivalve mollusks can be imported into EC countries from "approved" countries. "Approved" countries mean that their legislation requirements, power of competent authorities, and inspection services are at least equivalent to those governing the EU domestic product.

Interstate Shellfish Sanitation Conference

The Interstate Shellfish Sanitation Conference (ISSC) is a tripartite cooperative program formed by the US Food and Drug Administration (FDA), US state regulators, and shellfish industry representatives who develop policies for the safe harvesting, processing and distribution of fresh and frozen shellfish. The US FDA must concur with the ISSC's proposed policy changes before they are incorporated into the National Shellfish Sanitation Program (NSSP) catalogues of safety procedures, refereed to as the model ordinance. When new policies are adopted into the model ordinance, the participating states and foreign countries incorporate them into their own program requirements and are responsible for enforcing them. There are 31 states (plus the District of Columbia) participating in the ISSC, all of which have been certified to harvest, process, and/or ship shellfish commercially. In addition, five foreign countries, Canada, Chile, South Korea, Mexico and New Zealand have memoranda of understanding with the US FDA in which they agree to abide by NSSP shellfish safety policy [7].

HARMONIZATION OF BIOTOXIN STANDARDS

At its 25th session, the Codex Committee on Fish and Fishery Products (CCFFP) asked FAO and WHO to provide scientific advice on biotoxins in conjunction with its work on Proposed Draft Standards for Live and Processed Bivalve Molluscs. The CCFFP, at its 26th session, made the following specific requests to be covered through this advice [3]:

- Provide scientific advice to the CCFFP to enable the establishment of maximum levels in shellfish for shellfish toxins (PSP-, DSP-, ASP-, AZP- and NSP-toxins, and YTXs and PTXs);
- Provide guidance on methods of analysis for each toxin group;
- Provide guidance on monitoring of biotoxin-forming phytoplankton and bivalve molluscs (including sampling methodology);
- Provide information on geographical distribution of biotoxin-forming marine phytoplankton.

In order to respond to their requests and to provide scientific advice to the next session of CCFFP in February 2005, the FAO, the Intergovernmental Oceanographic Commission of UNESCO (IOC) and the WHO are planning to hold a joint expert consultation from 27 September to 1 October 2004.

To implement this programme of work, FAO, IOC and WHO are establishing ad hoc expert working groups on specific shellfish toxins in bivalve molluscs to evaluate the available information (toxicological studies, completed national risk assessments, relevant epidemiological data, prevalence data, control measures, etc.) and to prepare technical working papers for review during the joint FAO/IOC/WHO expert consultation.

The quality of scientific advice from FAO/WHO heavily relies on the quality and amount of data. It is my hope that the expert consultation will provide high quality scientific advice to the CCFFP for further elaboration of the standards. Thus, the maximum levels and testing methods of marine biotoxins would be harmonized internationally for protecting consumer health and ensuring fair practice of food trade.

CONCLUSIONS

By utilizing data relevant to biotoxin contaminations in bivalve mollusks and related foodborne diseases, WHO together with relevant international organizations, provides scientific advice for Codex and member states to elaborate international standards of marine toxins in order to protect consumer health and ensure fair practice of food trade.

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SEAFOOD PRODUCTION AND TRADE IN VARIOUS APEC ECONOMIES: THE NEED FOR HARMFUL BIOTOXIN REGULATORY MECHANISMS

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ABSTRACT

In 1999, the world's seafood (fish, crustaceans, molluscs) production reached 106.8 million mt, with 92.9 million mt derived from capture fisheries and 13.9 million mt from marine/brackish water aquaculture. Out of the total seafood production, molluscs comprised 13.6 million mt, of which 3.4 million mt (25.2%) were from capture fisheries. Seafood is one of the most highly traded commodities in the world market, and has experienced a doubling of trade volume between 1984 and 1994. For 1999, the world's total value of seafood imports was US$57,492,816,000 with the international exports recording a value of US$52,882,533,000. Internationally, Japan ranked first in seafood imports, while Thailand was first in seafood exports in 1999. With globalisation, seafood trade will be subjected to more stringent regulation and control. Presently, most countries are free to impose their own regulations concerning seafood imports for food safety reasons. Regulations imposed on Harmful Algal Bloom (HAB) toxin contamination of seafood have always been contentious issues. Even though an HAB occurrence frequently places molluscs at greater risk of HAB toxin contamination, very often, importing countries choose the safer option by imposing a total ban on all seafood products from the HAB affected country. This action is needless and irrational, and has adverse economic impacts. The need for standardisation of regulatory mechanism in relation to seafood trade and training for regulatory authorities is also discussed.

INTRODUCTION

Fishing dates back to the existence of man; some, however, thought that its origin may stretch back to the existence of pre-hominids whose remains were often found together with prehistoric fish bones [1]. In the beginning, men probably caught enough fish just for their own daily needs. After they learned to preserve fish by drying, smoking and salting, they also started trading fish as a means of exchange for things to meet their other needs. In Europe, a large-scale fishery was already established by the Middle Ages to supply the markets with salted cod and herring. In Malaysia, barter trade between coastal and inland forest settlers was already evident during the Mesolithic period (8000 BCE), with this practice becoming increasingly more important in the Neolithic period (3000 BCE) [2]. Fisheries products traded included dried fish, trepang (dried sea cucumber) and mother of pearl.

The Chinese were the first to undertake aquacultural practices, which date back to the time when they experimented with the culture of the common carp in 1100 BCE. During the last two decades, growth in fisheries production worldwide is mainly from the aquaculture sector since capture fisheries has been exploited at maximal limits throughout the world. Although aquaculture has a long history, intensification dates back only to the 1970s. Aquaculture accounts for about 25% of all fish directly consumed by humans [3] and Asia currently leads in aquaculture production. The main aquaculture products traded internationally include shrimps, prawns, salmon, scallops, clams, seabass and seabream.

With globalisation, seafood trade will be subject to increasingly greater regulation and control, and issues related to environmentally sustainable practices and seafood safety will receive greater scrutiny. In the future, the need for eco-labeling and Hazard Analysis Critical Control Point (HACCP) certification may be made mandatory for all seafood products, and testing laboratories may have to meet the requirement of the International Organisation for Standardisation (ISO) for accreditation. Contaminants frequently monitored in seafood include bacteria, heavy metals, drugs/chemicals and harmful algal bloom (HAB) toxins.

HAB occurrences have been reported since antiquity and their frequencies are believed to be increasing all over the world. The first reference on HABs dates back to biblical times in 1000 BCE, and one of the first recorded incidences of human fatality was reported in 1793 [4]. Paleontological data suggested that dinoflagellates were the cause of animal mortality thousands of years ago [5].

This paper examines seafood (with emphasis placed on molluscs, which are often at greatest risk of HAB toxin contamination) production and trade from various APEC economies located in Asia (Brunei Darusalam, China, Hong Kong-China, Taiwan-China, Indonesia, Japan, Republic of Korea, Malaysia, Philippines, Singapore, Thailand and Vietnam). The need for standardisation of regulatory mechanism in relation to seafood trade and training for regulatory authorities are also discussed.

SEAFOOD PRODUCTION

World seafood production has grown rapidly over the last four decades. Aquaculture production, growing at 11% annually over the past decade, is the fastest growing sector of the world food economy, and is poised to overtake cattle ranching as a food source by the end of

this decade [6]. Production from capture fisheries has, however, tapered off at around 90 million mt.

Capture Fisheries Production

Wild fisheries stocks from all over the world have been heavily exploited. However, overfishing is not a recent issue and was formally recognised internationally in the early 1900s and was the subject of the London Conference on Overfishing in 1947 [7]. An FAO study [8] showed that world fisheries stocks are in a state of crisis. Out of 200 fished stocks in all parts of the world, more than 25% were over-exploited, depleted or recovering and would produce greater catches only if returned to a healthier state; 38% were fully exploited and could not produce more catch without depleting the base stock. In 1999, out of a total of 92,866,600 mt of seafood (fish, crustaceans and molluscs) landed, brackish/marine water molluscs contributed 3,421,703 mt or 3.7% of the production [9].

Asians are heavily dependent on the Pacific Ocean for food, and fish is the main source of protein for an estimated one billion Asians. Over half the world's fish catch is taken in Asian waters. In 1999, four out of the top ten fishing nations in the world were in East Asia, with China producing the highest catch (17,240,032 mt). Japan ranked third with a production of 8,429,290 mt., Indonesia was sixth with 4,149,420 mt and Thailand was ninth with 3,004,900 mt [9]. The depletion of fish species is a major concern in the North-west Pacific, which provides nearly one-third of the world's marine harvest. Fish yields in the Yellow, South and East China Sea fell significantly in the 1990s [10, 11].

Aquaculture Production

In 1999 the world aquaculture production of fish, crustaceans and molluscs from marine-, brackish- and fresh-water totaled 33,310,349 mt valued at US$47,874,991,000 [12]. Out of the top ten aquaculture producers in the world, six were from APEC economies located in East Asia, namely:

1) China: 22,789,887 mt valued at US$22,631,659,000
2) Japan: 759,262 mt valued at US$3,365,565,000
3) Indonesia: 647,640 mt valued at US$1,851,012,000
4) Thailand: 602,807 mt valued at US$1,706,312,000
5) Vietnam: 594,910 mt valued at US$1,396,065,000
6) Philippines: 328,375 mt valued at US$630,384,000

In 1999, aquaculture production from the marine/brackish water environment totaled 13,920,298 mt, with molluscs ranking the highest in terms of production. In 1999, a total of 10,150,558 mt of molluscs were produced from brackish/marine water aquaculture contributing to 72.9% of the total seafood (fish, crustaceans and mollusks) aquaculture production. Oyster production at 3,711,606 mt ranked the highest, followed by clams, cockles and arkshells, which had a production of 2,744,846 mt, mussels at 1,451,032 mt and scallops and pectens at 951,866 mt.

SEAFOOD TRADE

Fisheries commodities rank as one of the most highly traded food products in the world, and recorded a doubling in international trade between 1984 and 1994. Developed countries account for 85% of world fish imports, with Japan as the leading fish-importing nation. In 1999, Japan accounted for 27.4% of world fish imports valued at US$ 15,539,507,000 [13]. Thailand is the leading seafood exporting country contributing to an average of 7.9% (derived from three consecutive years from 1997-1999) of the total exports.

Most of the APEC economies located in the ASEAN region are actively involved in the trading of fisheries commodities, which is comprised largely of seafood products. Many of these economies are listed among the world's top 50 importing (Table 1) and exporting (Table 2) countries for fisheries commodities.

Compared to the output, international trade in molluscs is relatively limited, amounting to less than 10% of the total production, with major species traded comprised of fresh and frozen scallops and clams [14]. Principal importers of molluscs are Japan, United States of America and France, and major exporters are China and the Republic of Korea. Table 3 lists the amount and value of the major mollusc species traded by the principal importing and exporting APEC economies in Asia.

HAB OCCURRENCES AND REGULATORY MECHANISMS

Presently, it is not mandatory for the various APEC economies in Asia to inform their trading partners of a HAB outbreak. Very often information on such outbreaks is obtained from the local newspapers, television, radio or other news media from the affected economy. Other economies may then seek clarification from the affected economy by writing officially to the government departments such as the health or fisheries agencies or the department for international trade in the country affected by the outbreak. Some of these requests may not even receive a response. The affected economy prefers to deal with the problem internally and may not want to release any additional information except for what has already been reported in the media for fear of panicking the consumers and causing adverse effects on trade. Several APEC economies belonging to the Association of South East Asian Nations (ASEAN) attempted to facilitate exchange of information on HAB outbreaks through a Network initiated by the ASEAN-Canada Cooperative Programme on Marine Science (ACCPMS-II) implemented from 1992-1999. However, responses from most participating countries were lukewarm and many chose to remain silent and not to update the Network with fresh HAB outbreaks. After the

termination of the ACCPMS-II Programme, this Network has become even more ineffective. Meanwhile, importing countries, fearing for the safety of their consumers, may take the drastic and irrational action of banning all fisheries products from the affected country. This situation calls for better awareness and understanding between the countries so that regulatory procedures can be discussed and standardised. The affected country should give accurate and up-to-date information to their trading partners, who in turn should implement rational contingency plans to ban only the affected seafood.

Some of these developing economies do not yet have a legal regulatory standards, which are enforceable under their Food Act, and meanwhile are adopting the more commonly acceptable standards used in some developed countries. There is also a need to standardise testing procedures and test animals. Some countries even have difficulties procuring consistent supplies of mice of the recommended strain and size, and some have resorted to using other species. Some officers in charge of seafood regulations may also lack awareness on HAB issues. Very often, in cases of emergency, they have to refer to the more knowledgeable scientists on basic issues, leading to the loss of valuable time in the implementation of contingency plans and decision-making. There is therefore a need in some cases to upgrade the knowledge of regulatory authorities to enable them to carry out their function more effectively.

Many developing countries may lack trained manpower to implement more sophisticated analytical methods for regulatory use. Presently, most of the developing countries only have the capability to monitor HAB toxins using the mouse-bioassay method, and the use of analytical instruments like the HPLC for monitoring purposes are either too costly or too sophisticated for many of their technicians. The need to choose standard methods over performance criteria, and the need for trained personnel to ensure quality assurance and control of test results are some of the problems that these economies may need to discuss and standardise. Since many of these developing economies are important mollusc exporting countries where mollusc exports contribute substantially to their foreign exchange, it is imperative that regulatory mechanisms of these countries and technical knowledge of the regulatory authorities be upgraded. The APEC Task Team on Algal Biotoxin Regulations during its fifth meeting in Haikou, Peoples Republic of China in April 2000 recommended the creation of a group of regulatory authorities on seafood safety with regard to HABs. Their terms of reference should be formulated and training should be initiated so that they will understand better the duties and responsibilities delegated to them.

The Second International Conference on Harmful Algae Management and Mitigation held in Qingdao, China from 12-16 November 2001 has therefore come at an opportune time to enable all APEC economies to come together and formalise standardised regulatory procedures and agree on how best to disseminate information regarding HAB outbreaks. It is imperative that all economies work together, with the more competent members helping the less competent, since most of the seafood produced by the developing economies will be exported to the more developed nations where food safety is of paramount importance.

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Table 1. Imports from APEC Economies actively involved in international trade in fisheries commodities [13].

Country	1997 (US$000)	1998 (US$000)	1999 (US$000)
Japan	15,539,507	12,826,543	14,748,712
China, Hong Kong	2,096,894	1,611,747	1,593,661
Korea, Rep.	1,019,276	563,180	1,140,022
China	1,183,283	991,463	1,127,412
Thailand	866,250	832,366	840,679
China, Taiwan	659,786	481,645	556,873
Singapore	626,876	416,498	475,224
Malaysia	347,018	226,474	258,747
Philippines	135,303	94,046	121,492
Indonesia	106,143	45,688	86,555
Total	22,580,336	18,089,650	20,949,377
% World Total	39.8	32.8	36.4
World Total	56,661,188	55,084,330	57,492,816

Table 2. Exports from APEC economies actively involved in international trade in fishery commodities [13].

Country	1997 (US$000)	1998 (US$000)	1999 (US$000)
Thailand	4,329,541	4,031,279	4,109,860
China	2,937,281	2,656,117	2,959,530
China, Taiwan	1,779,800	1,579,836	1,763,572
Indonesia	1,620,628	1,628,494	1,527,092
Korea Rep.	1,376,152	1,245,858	1,393,428
Vietnam	763,257	820,588	940,473
Japan	889,409	718,163	719,839
Singapore	494,246	394,848	390,062
China, H. Kong	635,930	417,325	383,398
Philippines	435,262	445,045	372,274
Malaysia	337,046	310,465	299,437
Total	15,598,552	14,248,018	14,818,965
% World Total	29.1	27.8	28.0
World Total	53,420,234	51,188,041	52,882,533

Table 3. Major amounts (mt) and values (US$) of mollusc imports and exports (in brackets) by principal APEC economies in Asia [13].

Species	Country	1997	1998	1999
Abalones (fresh, chilled, frozen)	China-Hong Kong	963 mt (30 mt) $32,536 ($773)	1661 mt (14 mt) $46,405 ($335)	1477 mt (96 mt) $42,049($1,119)
	Japan	787 mt (-) $30,571 (-)	680 mt (-) $22,233 (-)	760 mt (-) $25,609 (-)
	China-Taiwan	112 mt (4 mt) $773 ($18)	133 mt (3 mt) $1,085 ($15)	195 mt (0) $1,409 (0)
	Total	1862 mt (34 mt) $63,880 ($791)	2,474 mt (17 mt) $69,723 ($350)	2,432 mt (96 mt) $69,067 ($1,119)
	Grand total (plus those from other countries	2,029 mt (1,153 mt) $68,684 ($33,099)	2,659 mt (1,388 mt) $74,704 ($30,953)	2,678 mt (1,542 mt) $74,723 ($23,209)
Oysters (fresh, chilled and frozen)	Japan	6,535 mt (2,342 mt) $44,372 ($7,323)	8,985 mt (447 mt) $47,297 ($5,708)	12,485mt (422 mt) $74,655 ($5,342)
	Korea Rep.	2,141 mt (6,849 mt) $4,211 ($45,236)	42 mt (9,287 mt) $123 ($49,609)	121 mt (12,148mt) $252 ($70,348)
	Singapore	829 mt (144 mt) $6,987 ($1,949)	811 mt (174 mt) $4,471 ($1,013)	1255 mt (169 mt) $5,175 ($1,501)
	China	23 mt (1,449 mt) $78 ($3,946)	184 mt (1,684 mt) $255 ($2,837)	189 mt (2,151 mt) $715 ($4,125)
	China- Taiwan	622 mt (-) $1,018 (-)	1,424 mt (-) $1,992 (-)	2,743 mt (4 mt) $3,650 ($23)
	Total	10,150 mt (10,784mt) $56,666 ($58,454)	11,446mt (11,592 mt) $54,138 ($59,167)	16,793mt (14,894mt) $84,447 ($81,339)
	Grand total (plus those from other countries)	26,581 mt (25,481mt) $109,697 ($101,353)	30,957mt (27,026 mt) $110,209 ($105,792)	37,786mt (33,752mt) $144,407 ($134,703)
Mussels (fresh, chilled, frozen)	China	881 mt (10,485 mt) $837 ($13,440)	393 mt (8,395 mt) $377 ($9,883)	380 mt (12,719mt) $380 ($14,681)
	Grand total (plus those from other countries	162,529mt (197,485mt) $206,228 ($241,752)	185,032 mt (189,286 mt) $226,172 ($220,794)	192,482 mt (201,809 mt) $235,119 ($238,872)
Scallops	China	1044 mt (28443 mt) $6656 ($107,908)	796 mt (17,079 mt) $989 ($67,471)	2,232mt (25,235mt) $3,897 ($62,423)
	Japan	420 mt (6784 mt) $1,772 ($86,581)	455 mt (6,466 mt) $2,074 ($80,272)	535 mt (3,729 mt) $3,216 ($58,720)
	China-Hong Kong	2,497 mt (543 mt) $39,396 ($5,989)	1,922 mt (288 mt) $21,167 ($2,887)	1,672 mt (321 mt) $20,547 ($1,518)
	Total	3,961mt (35,770 mt) $47,824 ($200,478)	3,173 mt (23,833 mt) $24,230 ($150,630)	4,439 mt (29,285 mt) $27,660 ($122,661)
	Grand total (plus those from other countries)	69,368mt (76,244 mt) $564,651 ($555,539)	63,435mt (66,677mt) $529,362 ($518,588)	66,498mt (78,452 mt) $506,727 ($481,623)

Table 3. (continued).

Species	Country	1997	1998	1999
Clams	Japan	119,970 mt (-) $187,666 (-)	132,760 mt (-) $177,765 (-)	125,237 (-) $193,450 (-)
	China	- (75,771 mt) - ($86,440)	- (79,854 mt) - ($88,031)	- (82,048 mt) - ($96,641)
	Korea Rep.	4,037 mt (15,700 mt) $20,888 ($93,937)	1365 mt (25,638 mt) $6,699 ($89,706)	5049 mt (18,653 mt) $11,634 ($79,874)
	Thailand	19,729 mt (1,004 mt) $2,947 ($1,048)	16,270 mt (1,183 mt) $1,794 ($1,423)	16,260 mt (3,651 mt) $1,905 ($3,923)
	Philippines	- (522 mt) - ($1966)	- (-) - (-)	- (139 mt) - ($495)
	China-Taiwan	90 mt (-) $226 (-)	41 mt (-) $66 (-)	140 mt (-) $253 (-)
	Total	143,826mt (92,997mt) $211,727 ($183,391)	150436mt(106675mt) $186,324 ($179,160)	146,686mt(104,49mt) $207,242 ($180,933)
	Grand total (plus those from other countries)	154,927mt (139,404mt) $253,754 ($311,719)	162158mt(155256mt) $238,667 ($292423)	15864mt(161273mt) $264,732 ($324,463)

INFORMATION RESOURCES AND COMMUNICATIONS

INTERNET-BASED COMMUNICATION TOOLS FOR DISSEMINATION OF INFORMATION ON HARMFUL ALGAL BLOOMS (HABS)

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ABSTRACT

An important aspect of harmful algal bloom (HAB) management is the dissemination of information among managers, between managers and the public, or among managers, the public and researchers. The Internet can be an important tool in this process. There are several different types of websites and Internet resources available to the HAB community, with different communication objectives. This paper describes these Internet-based communication tools and highlights their benefits as well as the difficulties, both technical and political, involved in establishing and maintaining them.

WEBSITES

There are a wide variety of websites devoted to HAB research, monitoring and outreach. In designing a website, managers should consider their key audience(s) and tailor the site contents to these groups. As maintenance and update can take a significant amount of time and resources, it is best to start small and build from there as time permits. It is also advisable to periodically evaluate the site's effectiveness – this can easily be done by inviting comments from site visitors through an automatic e-mail form. In this section, we provide examples of three different types of websites – all applicable to the dissemination of information on HABs.

Harmful Algae Website

The Harmful Algae website (http://www.whoi.edu/redtide) is maintained by the U.S. National Office for Harmful Algae and Marine Biotoxins. The primary objective of this site is to serve as a comprehensive resource for a broad range of user groups, including scientists, managers, the general public, journalists, and students. Although there are many HAB-related websites in the U.S., this is the only one that deals with the entire range of U.S. HAB problems and provides access to information on national HAB research programs. This is an example of a comprehensive, national website that could be used as a template for other national or regional websites.

The Harmful Algae page provides details on such topics as:

- U.S. HAB species and their impacts - links to additional information and images;
- human health problems associated with HABs - including information on their diagnosis and treatment;
- maps depicting the distribution of HABs in the U.S.;
- news articles of HAB events in the U.S. and foreign waters;
- funding and conference announcements;
- useful references;
- links to electronic versions of several science plans, reports, and other resource materials; and
- links to many related websites.

The webpage's primary focus is on HABs in the U.S., but information about HABs in foreign waters is included as well – for example – posting of events in the news media, and links to the Intergovernmental Oceanographic Commission (IOC) world maps depicting the distribution of HABs.

Researchers and managers view the Harmful Algae webpage as a central repository for information about HABs in the U.S. [1]. Many reports and national plans are posted here (in html and PDF format) such as: the *U.S. National Plan for Marine Biotoxins and Harmful Algae* [2]; *The Ecology and Oceanography of Harmful Algal Blooms: A National Research Agenda* [3]; and *Estimated Annual Economic Impacts from Harmful Algal Blooms (HABs) in the United States* [4]. Many questions are answered from individuals accessing this site – students seeking help with their science projects or reports, journalists looking for HAB images they can use in their articles, and concerned individuals when a toxic event occurs.

The Harmful Algae webpage is updated and revised as new information is obtained and as feedback is received from those who utilize the website. One such area worth discussing relates to the news articles posted on HAB events in the U.S. and foreign waters. These articles present a useful picture of how particular events are presented in the media and thus viewed by the general public. Ideally, news articles would be reviewed before being posted to decide if the science being reported was sound and the reporter was correct; however, the breadth of scientific expertise required for this oversight exceeds the capabilities and time commitments of most webmasters. Although a disclaimer is included on the front page of the news articles section of the site, some readers thought that posting of articles conveyed the message that the National Office approved and endorsed those articles. The *Pfiesteria* Working Group (see below) had a constructive discussion about this issue and recommended that the disclaimer be strengthened and that it include a suggestion that readers seek information from other sources. The statement reads as follows: "The purpose of this section of our website is to provide access to HAB-related articles as they are being presented in the media. Posting these articles on this site does not mean, in any sense, that we endorse them.

Please use judgment in quoting or referring to any of the information posted here and seek out other sources (scientists, journalists) to get a more complete picture." The disclaimer is also posted on each of the individual articles. It reads: "Disclaimer: These postings were sent to us from a variety of media sources over the Internet. The content has not been reviewed for scientific accuracy or edited in any manner."

Despite this effort to be objective, not all articles on HABs are posted. News articles viewed as "personal attacks", sensationalistic or unprofessional are rejected.

Pfiesteria Technical Website

A second type of website is a technical website used to exchange information on a particular subject, among scientists and managers, rather than the general public. An example of such a website, is the *Pfiesteria* Technical Website (http://www.whoi.edu/redtide/pfiesteria). This website was used for several years by U.S. federal and state agencies and academic researchers involved in monitoring and/or research on *Pfiesteria* and *Pfiesteria*-like organisms (PLO's), but is no longer updated or maintained due to a decrease in *Pfiesteria* monitoring activities and reduced funding. A 'working group' communicated regularly via monthly conference calls to share information about ongoing monitoring efforts for *Pfiesteria* [5, 6, 7]. This webpage was initially created for use by the *Pfiesteria* Working Group and all information on the site had to meet the group's approval in advance of posting. The site was used as a repository for analytical and monitoring protocols, a glossary of *Pfiesteria*-related terms, molecular information on PLO's, workshop reports, reference lists, fact sheets, etc. This was not a public webpage, but it was not password-protected or restricted in any way, although that certainly would have been possible.

The *Pfiesteria* Technical website served as a central location for individuals directly involved in *Pfiesteria* research and/or monitoring to find the information described above. This provides an example of a technical webpage that is relatively easy to update and maintain. In short, it served the needs of a specific working group and did not need to address the issues of interest to a wider and more diverse audience.

Project-Based Websites

Two examples of project-based websites are the ECOHAB-Gulf of Maine site (http://www.whoi.edu/ecohab) and the ECOHAB-Florida site (http://www.floridamarine.org/features/category_sub.asp?id=1918). These sites provide information on the status of each research program, the ECOHAB – Gulf of Maine and ECOHAB - Florida. The sites are designed primarily for use by the researchers and managers involved in the ECOHAB (The Ecology and Oceanography of Harmful Algal Blooms) program. Project data are posted on each page once they become available so all investigators can have easy and prompt access. The status of data analysis and model development is provided as well as workshop announcements, reports, etc.

The ECOHAB-GOM page has a password protected ftp site for exchanging files among the researchers and funding agencies. Understandably, project researchers are concerned about losing control of their data before they are able to publish it – the restricted section of the site addresses this potential problem.

These examples illustrate the use of webpages by research groups - however, once those programs are completed, the webpages may not remain active or be updated.

Other HAB-Related Websites

Table 1 provides information on the key elements of several relevant HAB websites. There is a wealth of information on the Internet; however, it is often scattered and it can be difficult to locate reliable sites. Although not a complete list, those sites included in the table are updated regularly and should serve as a useful resource to individuals, managers and researchers working on HABs.

The Bigelow Laboratory site provides useful information on many aspects of HABs and toxin syndromes, but presents it in a format that should prove especially helpful for students. The site includes many images and graphics, and utilizes mouse 'rollovers' to link to additional sites and information. The Florida Fish and Wildlife Conservation Commission site is extensive and provides up-to-date information on red tides in Florida as well as current HAB events in the news. A useful HAB glossary is also included on this site. The IOC website provides access to several databases, including an International Directory of HAB experts; a bibliographic database; decadal maps of HAB events in the North Atlantic; and access to HAEDAT (Harmful Algae Event Database). These selected sites were highlighted since they are provide good examples of HAB-related webpages that "work" – in that they provide useful, updated information in a user-friendly manner. Those intending to set up their own webpages for HAB information might benefit by closely examining these pages. Several other sites in Table 1 are also useful, informative sites; however, they could not all be described in detail here.

INTERNET LISTSERVERS

A very useful communication tool is an Internet listserver, allowing rapid communication among subscribers about a particular topic. Announcements, information, and questions can be sent quickly to a large list. Listservers may be public (e.g., the Phycotoxins list), open to members (e.g., International Society for the Study of Harmful Algae (ISSHA) list), or restricted (e.g., the *Pfiesteria* listserver). Before being added to the *Pfiesteria* listserver, approval must be obtained by an appropriate federal or state agency. Listservers may also have a webpage associated with them, whereby all messages are archived, allowing for searches by topic.

Table 1. Selected websites with HAB-related information (in alphabetical order):

URL	Sponsored by/site name	Key elements
http://www.red-tide.org/new_site/html_index.html	Association on Harmful Algal Blooms in the South China Sea (AoHABSCS)	HAB database; images of red tide causative organisms in the South China Sea
http://cgca.rncan.gc.ca/pubprod/dinoflaj/index_e.php	Bedford Institute of Oceanography/ DINOFLAJ, Dinoflagellate Classification Database	Database system containing a current classification of fossil and living dinoflagellates down to generic rank, and an index of fossil dinoflagellates at generic, specific, and infraspecific ranks.
http://www.bigelow.org/hab	The Bigelow Laboratory for Ocean Sciences, Maine, US/Toxic and Harmful Algal Blooms	Impacts and human health problems associated with HABs; food web paths; maps of affected areas in the U.S.; toxin structures
http://www.cawthron.org.nz/harmful_algal_blooms.htm	Cawthron Institute, New Zealand	General background information on phytoplankton and harmful algal blooms; use of molecular probes for toxin detection; ongoing research
http://www.china-hab.ac.cn/english/	China Harmful Algal Bloom Page	Frequently asked questions; occurrences in China; ongoing HAB research programs in China; information on HAB organisms found in China
http://www.floridamarine.org/features/default.asp?id=1018	Florida Fish and Wildlife Conservation Commission/Florida Marine Research Institute	Florida HAB information and status; historical red tide information in Florida
http://www.cbr.nrc.ca/issha	The International Society for the Study of Harmful Algae (ISSHA)	Numerous links to other sites with HAB information; journals that publish HAB articles
http://www.issc.org/	Interstate Shellfish Sanitation Conference	U.S. organization - resource with shellfish sanitation information, including marine biotoxins
http://www.oceanportal.org	IOC/Ocean Portal	Search tool for ocean data and information – access to over 3000 websites and indexed files
http://www.ioc.unesco.org/hab/	IOC Harmful Algal Bloom Programme	Introduction to HABs and their impacts; causative organisms; International Directory of Experts on Harmful Algae; Harmful Algae Event Database; Decadal maps of HAB occurrences in the North Atlantic; Information on training courses on harmful algae; Harmful Algae News
http://www.marine.ie	Marine Institute – Ireland	Information on the Irish monitoring program for shellfish toxins; instructions for collecting samples; causative organisms – locations, images, maps;
http://www.mote.org/~mhenry/WREDTIDE.phtml	Mote Marine Laboratory Red Tide Information page	Information on red tide in Florida including latest updates on outbreaks
http://www.nwfsc.noaa.gov/hab/	Northwest Fisheries Science Center/Marine Biotoxins and Harmful Algal Blooms	U.S. West Coast HAB problems; bibliographies; background information on phytoplankton and harmful algae; detection of toxins in seawater; methods of analysis; current research
http://www.pfiesteria.org/	North Carolina State University Aquatic Botany Laboratory Website	Information on *Pfiesteria:* life cycle information; human health impacts; ongoing research; bibliography; abstracts; image archive

http://megasun.BCH.UMontreal.CA/protists/	Protist Image Data Website	Pictures and information on selected genera of algae and protozoa; links to several protist databases
http://ccmp.bigelow.org/	Provasoli – Guillard National Center for Culture of Marine Phytoplankton (CCMP)	Information on culturing of marine phytoplankton; database of phytoplankton cultures
http://www.start1.com/	START (Solutions to Avoid Red Tide)	Non-profit citizen's organization - information on red tide conditions, impacts in Florida and current research programs
http://www.tpwd.state.tx.us/hab/	Texas Parks and Wildlife Red Tide page	Red tide FAQs and updates on conditions in Texas; information on red tide and "golden algae" blooms – caused by *Prymnesium parvum*
http://www.geo.ucalgary.ca/~macrae/palynology/dinoflagellates/dinoflagellates.html	University of Calgary/ Dinoflagellate Page	Information and images of dinoflagellates, including: basic anatomy, biology and life cycle, classification, cyst structure and fossil record
http://www.rsmas.miami.edu/groups/niehs/science/toxins.jsp	University of Miami, Marine and Freshwater Biomedical Sciences Center	Background on marine biotoxins including: clinical presentation; diagnosis; management and treatment; chemical structure; molecular mechanism of action; references and sites
http://vm.cfsan.fda.gov/~mow/intro.html	U.S. Food and Drug Administration/Bad Bug Book	Information on shellfish toxins and ciguatera fish poisoning
http://www.whoi.edu/redtide	U.S. National Office for Marine Biotoxins and Harmful Algal Blooms/Harmful Algae Page	HAB information including photographs of various HAB organisms, blooms, and impacts; human health problems and information on diagnosis and treatment; national program documents and reports; many links to other HAB resources; maps depicting the distribution of HABs

The Phycotoxins listserver is used for reporting significant HAB events and for discussions of bloom dynamics, taxonomy, laboratory techniques. It is also used extensively for announcing relevant conferences and workshops. Individuals wishing to subscribe to the Phycotoxins listserver may find the relevant information at this website: http://www.agr.gc.ca/archives/phycotoxins.html.

ISSHA's mandate is to "promote and foster research and training programs on harmful algae, and to co-sponsor meetings on this topic at the national, regional, and international level." New members of ISSHA are automatically added to the mail server. The following website provides the information necessary to join ISSHA: http://www.cbr.nrc.ca/issha/New_ISSHA/Member_info.html.

One area deserving careful consideration when setting up a listserver is the handling of replies. When the *Pfiesteria* listserver was initially set up, replies were automatically sent to every subscriber on the list. In principle, this was a good idea, since everyone would have the opportunity to see the replies and would benefit from discussions involving others. Problems arose, however, with individuals who were away or on vacation and had set up an automatic reply on their e-mail account. These e-mails were automatically sent to the whole list, generating a continuous loop of e-mail replies from the vacationing subscriber that filled everyone's mailboxes. Filters can be used to identify and isolate these type of automatic messages – using key words such as "vacation", "away", "automatic", etc., but since each message is unique, it is impossible to filter them all. Another option is to have all e-mails go through a moderator before posting to the list, but that requires constant monitoring. The final option is to set up the listserver so that replies go to the sender only. This means that the replier has to consciously address the e-mail to the whole list if he or she wishes broader communication. This method is currently used on the *Pfiesteria* listserver as well as the Phycotoxins listserver.

SUMMARY

Several types of Internet communication tools are available for disseminating information on HABs, ranging from basic websites providing general information to sophisticated, interactive sites. Depending on the need and desired audience, Internet websites and listservers may be completely open and unrestricted, partially restricted or password-protected, or completely restricted. Since no resource can serve all needs,

managers should give careful consideration to their target audience and objective before setting up a website or listserver. Additionally, they should periodically review these objectives to determine if they are still being met and to make any necessary revisions or updates to their communications resources. Individuals accessing these resources should seek information from multiple sources as the depth and focus of web sites can differ significantly.

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HAB MONITORING AND DATBASES: THE FLORIDA *KARENIA BREVIS* EXAMPLE

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ABSTRACT

Blooms of the toxic dinoflagellate *Karenia brevis* are a natural feature of the eastern Gulf of Mexico that occur almost annually on the west Florida shelf. These blooms significantly affect both the ecology and economics of the region. Detrimental human health effects associated with these blooms include neurotoxic shellfish poisoning (NSP), resulting from humans consuming molluscan shellfish contaminated with brevetoxins, and respiratory irritation from exposure to aerosolized brevetoxins. The more obvious environmental effects of these blooms include effects on natural resources, including surface-water discoloration; fish kills; and mortalities of marine mammals, birds and sea turtles.

To protect public health and the environment from harmful bloom effects, the Florida Fish & Wildlife Conservation Commission's Fish and Wildlife Research Institute (FWRI, formerly Florida Marine Research Institute) has developed a red tide monitoring program in cooperation with another state agency, which consists of both microalgal and shellfish monitoring components. As part of this monitoring program, FWRI created and maintains a historical (1953–2004) database of *K. brevis* cell concentrations and associated data (e.g., collection time, date, location, associated water-quality parameters). It is updated daily by FWRI staff as new data are available. Currently (2004), the database includes more than 63,000 records. Public and scientific access to the database is provided by 1) a CD-ROM that includes a subset of the database, Geographic Information System tools to view the data, Java-based query tools to ask summary questions of the data, and remote sensing images for the identification and potential prediction of red tide blooms; and 2) web access to the data, which will eventually incorporate additional meteorological and physical oceanography (i.e., current) data. The establishment and maintenance of this type of dataset is essential for future prediction and management of harmful algal bloom events for addressing hypotheses concerning the ecophysiology of blooms, and for providing information to the public on current bloom status and locations.

RED TIDES IN FLORIDA

Blooms of the toxic dinoflagellate *Karenia brevis* in the eastern Gulf of Mexico significantly affect both the ecology and economy of Florida [1]. *Karenia brevis* produces a suite of brevetoxins, potent neurotoxins that bind to sodium channels in cell membranes and result in persistent activation of neuronal, skeletal-muscle, and cardiac cells [2, 3].

Brevetoxins associated with *K. brevis* blooms can affect human health via consumption of molluscan shellfish contaminated with brevetoxins or by inhalation of aerosolized brevetoxin. The aerosol results from disruption of *K. brevis* cells in breaking waves, surf, or onshore winds [4]. Brevetoxins are also responsible for annual, widespread mass mortalities of fish and for more infrequent mass mortalities of marine mammals, birds, and sea turtles [5-10] in Florida waters.

Karenia blooms occur in the Gulf of Mexico almost yearly, generally in the late summer or early fall, and can account for >20% of annual primary productivity in the eastern gulf [11, 12]. They are most common off the central and southwestern coasts of Florida between Clearwater and Sanibel Island, but they may occur anywhere in the gulf. Most blooms last three to six months and may affect hundreds of square miles. Occasionally, however, blooms continue sporadically for up to 18 months and may affect thousands of square miles.

Karenia brevis blooms have a long history in the Gulf of Mexico. Anecdotal records of bloom effects (i.e., fish kills, red water) date to the 1530s, when the first Spanish explorers in the region reported them [1]. Fish kills and 'poisoned water', which killed fish in the live-bait wells of boats along Florida's coastal and shelf regions have been reported since the early 1800s [13], but the causative dinoflagellate responsible for these fish kills was not identified and named until the 1946-1947 red tide outbreak, when Davis [14] identified a dinoflagellate and named it *Gymnodinium brevis*. This dinoflagellate has been subsequently reclassified as *Ptychodiscus brevis*, and, currently as *Karenia brevis* [15]. The first report of respiratory irritation caused by a Florida red tide dates from 1917 [16]. Although NSP outbreaks have been known since the 1880s [17], a linkage between *K. brevis* and human poisonings associated with bivalve shellfish consumption (i.e., neurotoxic shellfish poisoning, NSP) in Florida waters was only identified in 1960 [18, 19].

During the 1980s, the State of Florida formalized a federally approved biotoxin control plan that regulates shellfish harvesting during *Karenia brevis* blooms, to reduce the public health risks associated with red tides. Under this plan, guidelines were established for monitoring cell concentrations and closing shellfish beds when *K. brevis* was detected at concentrations above background levels in bay and lagoon passes or inlets. Harvesting bivalve (filter-feeding) shellfish is prohibited in an approved or conditionally approved shellfish harvesting area when concentrations in the area reach 5 x 10^3 *K. brevis* cells L^{-1}. When the bloom terminates and the *K. brevis* population drops below that level, shellfish usually purge the toxins from their systems in two to six

weeks [19]. The shellfish meats are tested for toxicity during that period, and the beds are reopened when mouse bioassay results demonstrate that shellfish meats are <20 Mouse Units (MU) per 100 grams of shellfish meat [19]. Harvesting bans do not apply to crabs, shrimp, lobsters, or fish, which are safe to eat even during red tide blooms because brevetoxins do not accumulate in the parts of those organisms that are consumed by humans.

Karenia brevis blooms can have large economic effects. Coastal communities that rely on the tourism industry lose millions of dollars when dead fish wash up on beaches or beachgoers experience eye and respiratory irritation. The seafood industry loses income when shellfish beds must be closed because of *K. brevis* blooms. Even tourism, recreational activities, and other businesses not actually at the bloom site may be adversely affected. Although the number of dollars lost is difficult to calculate, estimated losses from *Karenia* red tides that occurred in the 1970s and 1980s have been estimated at up to US$25 million [20, 21].

RED TIDE MONITORING: DATA MANAGEMENT NEEDS AT THE STATE LEVEL

Currently, the State of Florida continuously monitors for *K. brevis* and other potentially toxic, harmful, or nuisance microalgal bloom species in state waters and, working with the Florida Department of Agriculture and Consumer Services' Division of Aquaculture, successfully manage shellfish resources to protect the public from NSP. Monitoring is necessary for a variety of reasons, including protection of the public from consumption of toxic shellfish and the provision of red tide data for coastal businesses that rely on tourism and are adversely affected by red tides. Development of an effective red tide monitoring program for Florida has involved four major components: 1) establishment of a network of individuals ranging from state employees to volunteers to individuals associated with marine-related industries affected by red tide, who provide water samples collected from diverse regions; 2) training staff to routinely identify and enumerate toxic phytoplankton within these samples to the species level; 3) development of a database suitable to the specific program needs; and 4) identification of methods and means to provide this data to the public on a timely basis.

The occurrence, distribution, and abundance of *K. brevis* had been documented by biologists for more than 40 years prior to the establishment of a formal monitoring program. Although there were a few targeted research programs during this period, most sampling occurred after a bloom had reached maintenance stages in nearshore environments, as evidenced by reports of dead fish, discolored water, or respiratory irritation. Data collected from such response-oriented sampling is often incomplete and limited, because sampling occurred too late in the bloom to study the initiation and growth phases and because of the logistical difficulties in mobilizing resources quickly enough to document the event adequately.

In the 1970s, new technology was employed to detect blooms -- satellite technology with color imagery. The validity of this approach was verified in 1978 by comparison of a color image of chlorophyll concentrations on the southwestern Florida shelf with concurrent *in situ K. brevis* concentrations. The use of satellite technology to monitor red tides remains limited, however, because such images track only chlorophyll *a* concentrations in surface waters and require the collection of *in situ K. brevis* concentration data to validate the images. After validation, satellites can be used to track surface blooms as they move, but they cannot yet detect the low chlorophyll *a* concentrations associated with bloom initiation and development stages or subsurface blooms. Therefore, the collection of field data for *K. brevis* counts remains integral to a harmful algal bloom (HAB) monitoring program in Florida.

Several current research programs initiated specifically to study the possibility of mitigating the effects of red tides through prediction or advanced warning have also provided data that have been incorporated into the historical dataset. Investigators involved in the Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) program in Florida collected data from research cruises and from moored buoys to study the biology of *K. brevis* and its movement in response to environmental variables (such as temperature, salinity, and currents) on the continental shelf. Physical, chemical, and biological data are used to model and predict bloom initiation, growth, maintenance, and dissipation/termination; to evaluate life-cycle processes; and to study the transport and eventual fate of the brevetoxins. A pilot project called the Harmful Algal BloomS Observing System (HABSOS), a National Oceanographic and Atmospheric Administration (NOAA) funded program in the Gulf of Mexico, has collected available data on red tide events from federal, state, and academic laboratories for compilation in a central, accessible repository. Such a system will give investigators the ability to study events as they occur and perhaps forecast the movement and probable effects of a bloom throughout the gulf.

Other efforts to understand and predict red tides include the creation of a task force to recommend future research avenues and the creation and development of a volunteer program for statewide harmful algal bloom sampling. The Florida Harmful Algal Bloom Task Force was established in 1997 to identify gaps in the data that have been collected and to recommend additional research and monitoring needed on Florida red tides, other HABs, and their associated effects. The task force consists of representatives from federal agencies, state and local governments, water management districts, universities, private laboratories, and a citizen's organization. The FWRI volunteer program was established in 2000 to help monitor the extensive region over which Florida red tides may occur. Volunteers (e.g., other state-agency staff, recreational and professional fishermen, public volunteers) coordinate with FWRI staff and collect water samples from established onshore-offshore transects in a network extending from Pensacola to the Florida Keys, and samples are then sent to FWRI

for analysis. As this sampling network has expanded, additional volunteers have been located to assist with HAB-event response sampling involving unusual HAB blooms or associated events (e.g., marine-mammal mortalities).

HAB monitoring in Florida waters has thus evolved over a 50-year period from a collection of regionalized, largely event-driven responses and decentralized scientific studies of opportunity to an organized program that incorporates state monitoring networks, dedicated FWRI staff, a volunteer program, and links with established academic research programs that focus on HAB research. Integral to the development of this program has been the creation of a functional database that has incorporated both historical data and current monitoring information, as outlined below.

DEVELOPMENT OF A RED TIDE DATABASE: THE FLORIDA EXAMPLE

The HAB Historical Database Project began because scientists, modelers, and environmental managers needed to understand more about *K. brevis* bloom initiation and movement. To predict such events, it is imperative to have central access to data for a long period of time in a particular area. Unfortunately, funding and projects that focused on Florida red tides were localized within many separate institutions and government agencies, with individual datasets maintained at a variety of locations.

Additionally, early data collected by state agencies were recorded and maintained on paper, making it difficult to both access and analyze this information. In July 1998, FWRI staff, with support and assistance from the NOAA and Mote Marine Laboratory, began locating, accumulating, and deciphering HAB data in Florida. This initial phase of the database-development project involved two scientists, required a year and a half to complete, and resulted in identifying over 15,000 records spanning 50 years from 54 agencies, organizations and volunteers. Once historical hardcopy data were centralized and analyzed, and the entities and attributes were identified, a relational database design was outlined, relevant database parameters were identified (Fig. 1, Table 1), and the database and application were created with Microsoft Access 97 software. This second phase of database development was critical because the data was transformed from hardcopy datasheets and spreadsheets into a digital format, where data was readily accessible and the database could be queried in real time. Three data-entry staff and one database administrator/application programmer worked for more than a year entering and verifying each data field, a task that was complicated by the various parameters, equipment, units, and location descriptions used by different researchers, institutions, and projects. It was particularly difficult to determine sample locations, because the datasheets contained generic place names and the database required a latitude and longitude position. Emphasis was placed on data accuracy at this stage, with assistance from HAB scientists when needed. Modifications to the database continued throughout this phase as new data, including light measurements and drift-bottle cruise data became available.

Concurrent with this phase of database development, an effort was made to create metadata files to accompany the database. Metadata, the documentation of associated sample collection and analysis methodology, are essential because they provide information on the "who, what, why, where, and when" questions that are asked about the data. Metadata collection involved a dedicated effort in which each project's principal investigator was contacted directly to collect and record as much information as possible. Often, documentation within metadata files was limited due to the age of the data.

As the historical data was incorporated into the database, the need to incorporate data from FWRI's on-going red tide monitoring program and other concurrent research programs that provided *K. brevis* concentration data to the state (e.g., the ECOHAB: Florida program and Mote Marine Laboratory's survey cruises) into the database became a priority. Because this more recent data existed in electronic format, the database-entry application was modified again to import and convert these new datasets. The record count grew to more than 28,000.

In July 2000, the next phase began, adding a geographical component to the dataset. This effort offered additional monitoring and management tools to aid in determining how blooms start, where they occur, and where they move. This database-development phase was complex and involved specialized input from a variety of staff members. One Geographic Information System (GIS) analyst created advanced tools with ESRI ArcView® (version 3.2) software while another analyst created a limited version with the freeware product ArcExplorer® (version 2.0). These applications displayed information on location and cell concentrations for a selected date range. Digitized NOAA nautical charts were also added to cell concentration, region, and county layers to show the nature and form of the coastline, water depth, tidal range, and locations of manmade aids to navigation in specific areas of interest. Concurrent with this effort, a JavaScript™ expert was contracted to build applications to create bar and scatter-plot graphs for cell counts, salinities and temperatures for selected date ranges and locations. Additionally, a staff member, with expertise in remote sensing, located and obtained permission to include satellite imagery that had been used to identify and track harmful algal blooms. Another program was written to display the images in a time loop series to show selected historical bloom movement. These diverse database-interpretation and visualization tools were combined into a user-friendly Adobe Acrobat (version 4.05) application that incorporated all GIS layers, Java applications, satellite images, HAB database, HAB organism/ bloom images, freeware software, and support documentation together. All files were subsequently included on a CD-ROM entitled "Red Tides in Florida, 1954-1998: Harmful Algal Bloom Historical Database", which has been distributed to more than 150 scientists and managers throughout the world.

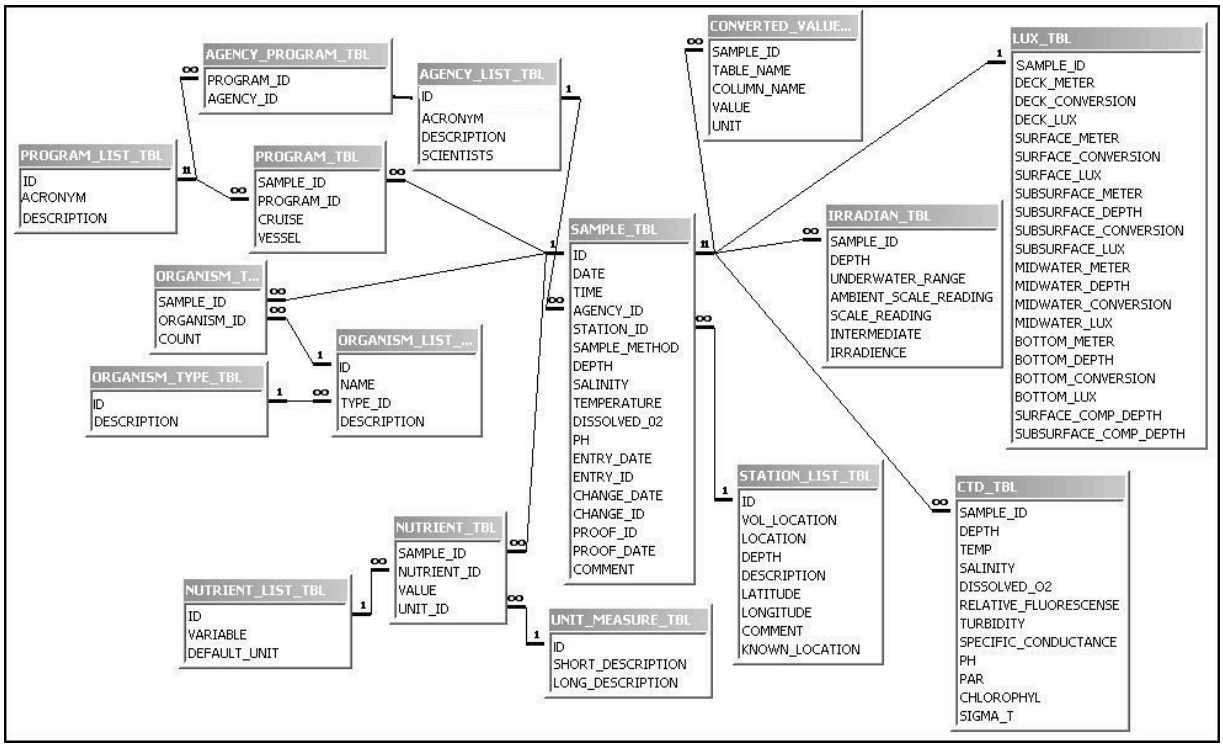

Figure 1. Entity relationship diagram for database released on 'Red tides in Florida: Harmful Algal Bloom Historical Database' version 2.0 (1954-2002) CD-ROM.

Table 1. Explanation of principal database entry fields. Other fields in Figure 1 are support or ancillary data.

Table	Parameter	Explanation
AGENCY_LIST_TBL		Reference table for research agencies
	ID	Auto primary key
	ACRONYM	Unique Agency Acronym
	DESCRIPTION	Expanded Agency Name
	SCIENTISTS	Agency Staff
CTD_TBL		CTD records for individual depths at 'referenced' locations
	SAMPLE_ID	Sample table ID
	DEPTH	Depth of water where sample was taken (meters)
	TEMP	Temperature (°C)
	SALINITY	Salinity ($^{o}/_{oo}$)
	DISSOLVED_O2	Dissolved oxygen (mg per liter)
	RELATIVE FLUORESCENCE	Relative fluorescence
	TURBIDITY	Turbidity (NTU)
	SPECIFIC_CONDUCTANCE	Specific conductance
	PH	pH
	PAR	Photosynthetically active radiation
	CHLOROPHYL	Chlorophyll *a* (μg per liter)
	SIGMA_T	Water density
SAMPLE_TBL		Base table, with sample water depth (meters)
	ID	Auto primary key
	SAMPLE_DATE	Date sample was collected
	SAMPLE_TIME	Time sample was collected
	SAMPLE_METHOD	Nisken bottle, surface bucket or none
	DEPTH	Depth of water sample (meters)
	ENTRY_DATE	Date record was entered in database
	ENTRY_ID	Network ID of person entering the record in the database
	CHANGE_DATE	Date the record was updated in the database
	CHANGE_ID	Network ID of person updating the record in the database
	PROOF_ID	Network ID of person verifying the record in the database
	PROOF_DATE	Date the record was verified in the database
	COMMENT	Any further information pertinent to this sample's record
STATION_LIST_TBL		reference table for known station names
	ID	Auto primary key
	VOL_LOCATION	Identifies the station as one sampled by a volunteer
	LOCATION	Station name
	DEPTHG	Station sampling depth
	DESCRIPTION	Written description of where sample was collected
	LATITUDE	latitude coordinate of station location in decimal degrees
	LONGITUDE	longitude coordinate of station location in decimal degrees
	COMMENT	Any further information pertinent to this station

CURRENT APPLICATION DEVELOPMENT

In 2002, the HABSOS Pilot Project used some of the ideas, data, data categories, and GIS applications from FWRI's CD-ROM for that project. In response to this increased interest in the FWRI database, FWRI staff decided on a two-phased approach to help fulfill these new information requests. First, in early 2003, the "Red Tides in Florida: Harmful Algal Bloom Historical Database" version 2.0, containing all original features with updated data through the end of 2002 was released. Second, in May 2004, with funding from NOAA's Environmental Services Data and Information Management program, FWRI created and began hosting the first Internet Map Service (IMS) application for historical and recent Florida HAB data. It is available at http://ocean.floridamarine.org/mrgis/viewer.htm. An in-house, experienced GIS analyst created the site by using ESRI's ArcGIS and ArcIMS software products to display HAB sampling distributions, HAB regions, nearshore and offshore bathymetry, and scale-dependent versions of Florida's county boundaries and shorelines. Additionally, all of FWRI's Marine Resource GIS-IMS data layers became available for display with the HAB data. This data is available on the web via three separate shapefiles: "recent", "past", and "historic." The "recent" file contains data for the most currently available week. This file contains date, location description, latitude, longitude, and the corresponding category for the cell count range. The "past" file has the same structure as the "recent" file but contains data for the previous two months. The "historic" file contains all data more than two months old. The format for this file is: date, collecting agency, station description, latitude, longitude, cell count, depth, temperature, salinity, and cell count category. All files are refreshed on a biweekly basis because data must pass quality-assurance checks prior to being posted on the Internet. Further details about the data are maintained in the metadata records online.

INTERPRETATION AND USES OF THE FLORIDA HAB HISTORICAL DATA BASE

The ready accessibility of the Florida HAB historical database has allowed a variety of uses: evaluation of the extent and duration of current HAB events in comparison with previous blooms, assessment of relationships between *K. brevis* concentration and physical and chemical parameters over time, and descriptions of regional bloom movements. From a monitoring viewpoint, the database allows access to information concerning the timing and location of previous blooms. Information is readily available on the histories of previous blooms in different areas, from the regional to county to site-specific level. Data on current HAB events is also available in a format that readily allows evaluation of bloom development and movements. Other databases, including mouse-bioassay-result and toxin, can also be linked with this historical database.

The database has also allowed an indirect examination of historical red tide sampling efforts within the state. Sampling effort is presented in Figure 2 as the number of microscopic counts made for *K. brevis*, regardless of the presence or absence of *K. brevis* in samples. Initial sampling efforts were made in the late 1950s in response to one of the largest harmful *K. brevis* red tides in Florida history (1953-1957), 10 years after the identification of the dinoflagellate responsible for the bloom [10]. Sampling efforts during this period included ground-truthing for the evaluation of the application of copper sulfate as a mitigation strategy and aerial over flights as a potential HAB-monitoring tool. Subsequent sampling efforts varied from year to year through the 1960s and 1970s depending upon bloom occurrence and state funding levels. The intensification of sampling efforts during the 1990s is also apparent, as is the incorporation of data provided by the ECOHAB: Florida research program from 1999 on and the creation of the FWRI volunteer monitoring program.

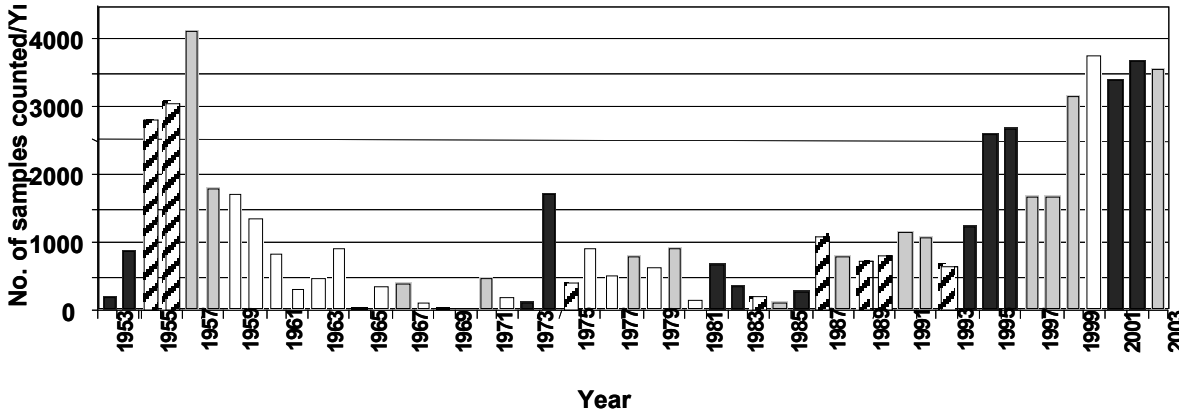

Figure 2. Historical red tide sampling effort and yearly red tide duration in Florida. All data was derived from the FWRI Florida Historical Red Tide database. Shading of the bars indicate the duration of the *K. brevis* bloom during that year: black indicates >8 months duration, grey indicates 4-8 months duration, white indicates 1-3 months duration and hatched bars indicate no red tide that year. Note that number of samples counted per year for red tide is the total number of counts made, regardless of the presence or absence of *K. brevis* in the sample.

ACKNOWLEDGMENTS

Principal funding for the creation and development of the Florida HAB Historical Database was provided by the State of Florida. The core data for this database was provided by cruises and incidental sampling conducted by the Fish and Wildlife Research Institute from 1953 onward. The database was supplemented by contributions from collaborators, both major (including Mote Marine Laboratory, NOAA, and the University of South Florida College of Marine Science), and minor (more than 150 individuals contributed during the 50 years of data collection). Additional satellite images available on the CD were provided by the NOAA CoastWatch Program and the USF College of Marine Science. Advanced Very High Resolution Radiometer (AVHRR) data for the CD was provided by USF, with subsequent processing by the Information Science and Management Program within FWRI.

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APPLICATION OF GIS IN RED TIDE MONITORING IN HONG KONG

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ABSTRACT

Following the recommendation of the consultancy study on red tide monitoring and management in Hong Kong, a project to establish a database system with a Geographic Information System to enable efficient compilation of relevant data and information on red tides and harmful algal blooms in Hong Kong was completed recently. This system not only streamlines recording of all data on red tide monitoring and investigation, it provides near real-time visualization of spatial and temporal distributions of red tides in Hong Kong. It therefore enables faster and more accurate risk assessment, provides early warning capabilities to mariculturists, and facilitates communication amongst concerned departments during red tide outbreaks. In addition, the system also enables faster response to enquiries and allows for retrospective analysis of trends and relationships of red tide outbreaks with environmental changes, which are important in further improving the effectiveness of red tide management.

INTRODUCTION

Geographic Information System (GIS) is a system of computer comprising hardware, software, and procedures, designed to support the capture, management, analysis, modeling and display of spatially referenced data for solving complex planning and management problems. In the recent two decades, GIS has become an invaluable tool for monitoring water quality [1] and pollution effects [2], assessing habitats and management of marine resources [3, 4], which involve a diverse mix of physical, chemical and biological factors and huge load of spatially referenced data. Recently such systems have facilitated the development of interactive impact assessment and evaluation of management options [5] as well as predictive spatial modeling of impacts [6, 7]. The fish killing red tide incident caused by *Karenia digitata* in 1998 in Hong Kong was unprecedented in scale and effect. During the later stage of the incident when the bloom was affecting several fish culture zones around Hong Kong from Kat O in the northeast, Tai Tau Chau in Rocky Harbour to Lo Tik Wan and Sok Kwu Wan in Lamma Island and even Ma Wan in the west, staff were deployed to investigate and assess the impact of the bloom. Although information was coming in from different sites and numerous types of data were collected, it was impossible to conduct immediate analysis of the data to get a better picture of what had happened and estimate how the bloom would move or change in the following few days. Using the conventional method with spreadsheets and static maps to handle and compile the collected data, particularly those involving spatial and temporal domain would require significant staff resources and would take a long time in terms of days or even weeks. The consultancy study on red tide monitoring and management in Hong Kong therefore recommended the establishment of a database system with GIS to facilitate efficient compilation of relevant data and information on red tide occurrences and immediate visualization of spatial and temporal red tide distributions in Hong Kong.

ESTABLISHMENT OF RED TIDE MONITORING SYSTEM (RTMS)

In 2000 the Agriculture, Fisheries and Conservation Department in collaboration with the Information Technology Services Department started a project to set up a database system with GIS for red tide monitoring. A review of all the tasks involved with red tide reporting (e.g., monitoring and management, the parameters taken and recorded, the parties involved, and associated issues during red tide outbreaks) was conducted to determine the scale and logic of the system. A computerized system named the Red Tide Monitoring System (RTMS) was designed to facilitate the following major tasks:

- Monitoring red tides and assessing their risks,
- Providing early warning and advice to
- mariculturists,
- Advising other departments,
- Providing information to the public,
- Understanding red tide development.

The RTMS consists of a centralized database/application server with a local area network of 6 PC workstations, 4 at Cheung Sha Wan Government Office (CSWGO) where red tide identification and related management are conducted and 2 at Aberdeen Fisheries Offices (AFO) where water quality analysis is done. The two locations are connected by a 256K lease line (Fig. 1). Major software required includes a network operating system and a relational database management system (RDBMS) with suitable application development tools, GIS software, spatial analysis tools, statistical software and documentation software.

FUNCTION OF RTMS

Monitoring Red Tides and Assessing Their Risks

Primarily the RTMS provides a secure centralized database for the phytoplankton monitoring programme and investigations of red tide incidents. The phytoplankton monitoring involves weekly sampling from 6 core stations and fortnightly from 5 offshore stations as well as 5 seasonal stations during red tide peak season (January to June and December). The stations were strategically selected to represent major regions of Hong Kong waters. Sampling frequency will be stepped up to twice a week or even daily as

appropriate when harmful algal species are detected. Apart from phytoplankton data, water quality data including temperature, salinity, pH, dissolved oxygen, BOD, chlorophyll, turbidity, suspended solids, and nutrients are taken fortnightly from the core and offshore stations throughout the year.

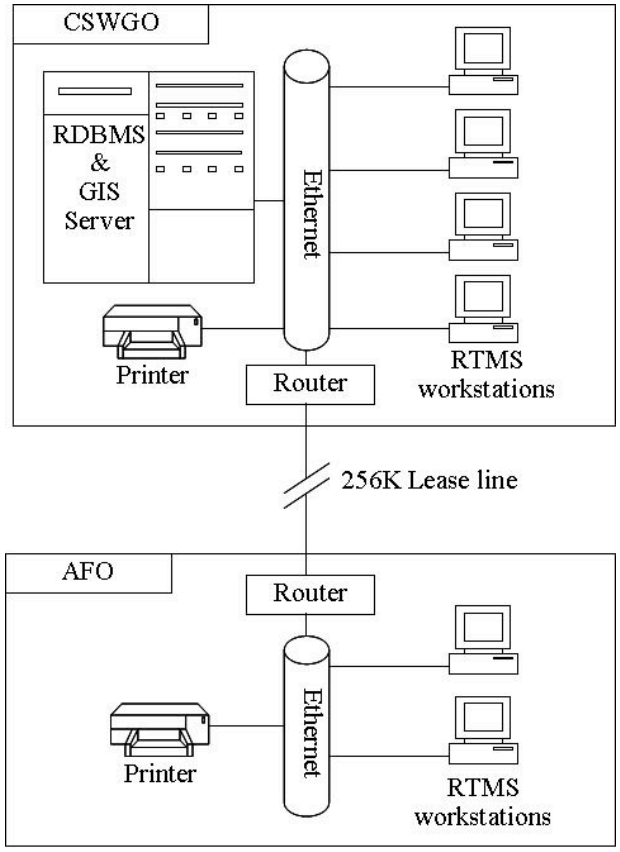

Figure 1. The system configuration of the RTMS (See text above for abbreviations).

The RTMS generates sampling schedules highlighting the outstanding trips for the monitoring programme and keeps all the data systematically. When a red tide is reported, it records details of the sightings such as time, location, affected area, colour, any incidents of fish mortality, informant's name and contact and generates the case number automatically in chronological order to facilitate search and retrieval of data. It also enables direct data input from digitized monitoring and analyzing equipments (e.g., Conductivity, Temperature & Depth Sensors, Nutrient Auto-analyser etc.). With the GIS, location of red tide outbreaks can be recorded accurately either by direct plotting on to the base map in the computer or direct data input from a Global Positioning System data logger or mapping of aerial photos taken by helicopters (Fig. 2). Information of all known algal species in Hong Kong, particularly their likely impacts on ecosystem, fish, shellfish or human health, are installed and updated as soon as new information becomes available. During phytoplankton data input the system highlights any known harmful species and enables quick access to details of harmful algal species, thereby enhancing risk assessment capabilities during red tide outbreaks.

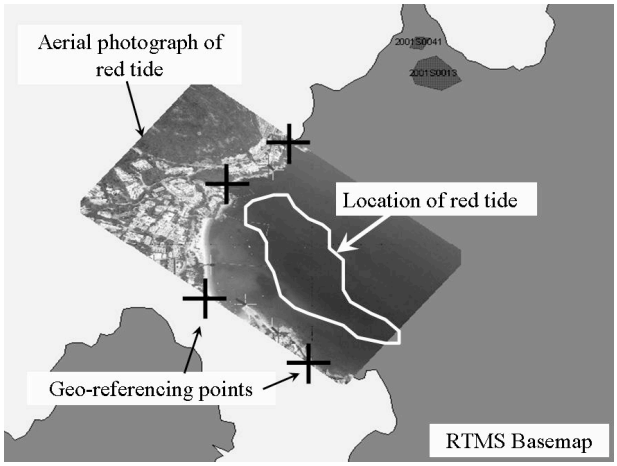

Figure 2. Mapping of the location and coverage of a red tide from an aerial photograph onto the RTMS.

Providing Early Warning and Advice to Mariculturists

One of the major tasks of red tide management in Hong Kong is to provide early warning and advice to mariculturists to reduce the likely impact of red tides. The RTMS includes data of all fish culture zones as well as names and phone numbers of the representatives which allows for quick identification of fish culture zones within given distances and quick access to contacts of these fish culture zones. When raft relocation is necessary, ecologically sensitive areas or navigation channels near to the fish culture zones being alerted can be checked from the no-go area map and advice on where not to relocate the fish rafts can be provided to mariculturists. In addition, with the RTMS having an interface with the real-time meteorological data from the Hong Kong Observatory, the near real-time as well as predicted information of wind direction, wind speed and tidal flow can be obtained from the Hong Kong Observatory through modem dial-up. Spatial query on likely direction and speed of transport of the red tide can be performed (Fig. 3). All of these facilitate prompt warning and advice to mariculturists.

Advising Other Departments

To minimize potential public health risks, when algal species that are known to cause toxic seafood are detected, the Food and Environmental Hygiene Department will step up seafood surveillance and when appropriate the Department of Health will advise medical practitioners to look for possible biotoxin food poisoning cases. When toxic algal species occur in beach water, the Leisure and Cultural Services Department in consultation with the Environmental Protection Department may raise red flags to warn bathers not to go into the water. The RTMS enables the generation of reports on the detection

of potentially toxic algal species detected in water or red tide occurrence for forwarding to the above concerned departments. Currently the reports are faxed to these departments but in the future the reports can be forwarded through e-mails, which will require simply pressing on an icon.

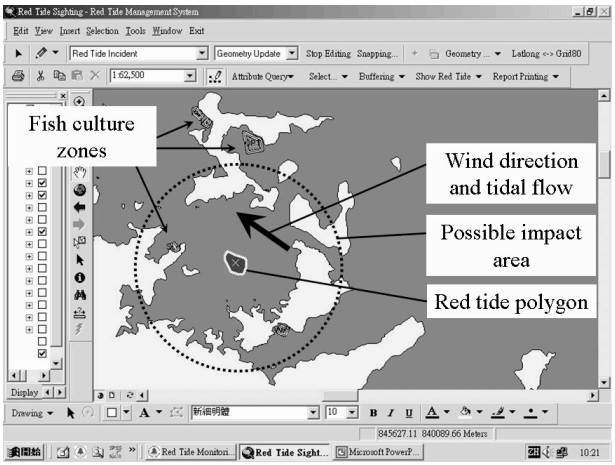

Figure 3. Prediction of the likely direction of a red tide movement.

Providing Information to the Public

To help the general public in understanding the distinction between red tides and harmful algal blooms to prevent unnecessary overreaction during red tide outbreaks, information on red tide outbreaks are issued to the public through weekly press releases and updates on a specific webpage. The RTMS enables fast compilation of the press release and easy updating of the webpage (http://www.hkredtide.org). This webpage is advertised through distribution of posters and leaflets and as a hyperlink in the government website. In addition, the system allows easy retrieval of information on red tide occurrence for rapid and accurate response to public enquiries. The number of enquiries is also logged (about 50 enquiries a year). The system also helps in the compilation of red tide data of the year for preparation of the annual red tide report providing spatial and temporal distribution patterns of red tides.

Understanding Red Tide Development

Detailed records of red tides in Hong Kong date back to 1983 while the phytoplankton monitoring with biweekly water quality data started in 1998. Additional water quality data throughout Hong Kong waters can also be incorporated through downloading from the website of the Environmental Protection Department. These data are invaluable for understanding the development of red tides in Hong Kong. With the RTMS, detailed analyses of the trends and relationships of blooms of different algal species with water quality, weather changes, etc. are made possible. Spatial analysis of the dominant algal density will help to elucidate the movement of the bloom with current and wind. Software for statistical analysis incorporated in the system facilitates analyses of algal species cell growth with water quality changes. Also the time series analysis (e.g., on the occurrence of red tides through the years) will provide a trend of red tide occurrence for the following year.

CONCLUSIONS

The RTMS has streamlined the recording of data and information of red tide monitoring reports and investigations. It enables fast and accurate assessment of the risks of the red tide incidents. Spatial analysis of changes of red tide density with wind and currents helps to predict movement of the red tide and ensure prompt and detailed information is provided to mariculturists. It also increases efficiency of communication between the different government departments and affected sectors as well as the public. Rapid and accurate response can be provided at any time even during emergencies. In the longer term, with analysis of trends and relationships of red tide outbreaks with water quality and weather patterns, etc. by the RTMS, it is anticipated that the dynamics of red tide development can be further elucidated and models may be developed to forecast red tide occurrence enabling better control and prevention of impacts from harmful red tides.

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ADAPTIVE TRAINING AND INFORMATION DISSEMINATION STRATEGY TO COMMUNITIES AFFECTED BY PSP OUTBREAKS IN SOUTHERN CHILE

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ABSTRACT

Impacts of Paralytic Shellfish Poison (PSP) outbreaks to coastal communities are diverse depending on the importance and type of fisheries, aquaculture and tourism activities, geographic features, available infrastructures, trained personnel, the cultural background of key groups, and the community in general. The strategy applied in southern Chile to strengthen prevention considers the monitoring of phytoplankton and PSP levels, but also includes specific activities involving the public: 1) training workshops oriented to community organisations and key groups; 2) a communication strategy to disseminate key information about PSP levels, local harmful algal blooms (HABs), their effects, and management oriented to the community and selected groups; 3) introduction of HAB and PSP key concepts, including social and economical impacts, into basic and middle education, involving teachers and students. All activities are assessed and sequential programmed actions are tailored according to obtained results. This contribution is focused on the activities oriented to the general public.

INTRODUCTION

The South Eastern Pacific area along the coast of Chile between 43º-55ºS (Figure 1) was sporadically affected since the seventies by PSP outbreaks caused by *Alexandrium catenella,* though recurrent events have occurred since the nineties. It appears that *A. catenella* is expanding its distribution from South to North [1]. The affected area has an intricate geography of fjords, channels and hundreds of islands reflecting the last glaciations. The southernmost portion is the Magellan region (48º30'-56º00') with approximately 132,000 km^2. The population is about 150,000 inhabitants, and about 92% is localised in three cities, suggesting that fishing areas within fjords and channels exhibit scarce human presence. Independent fishermen represent about 2500 persons and 760 small fishing boats. The Magellan region has been affected by PSP outbreaks in 1972, 1981 and 1989. Since 1991 a series of seasonal PSP outbreaks have occurred yearly affecting broad areas, and to date toxic shellfish have been continuously present in many areas.

A specific regional programme to reduce impacts was initiated in 1997, and its principal goals are to diminish risks to Public Health and to minimise disturbances to the fisheries sector and other economic activities, including actions to attain behavioural changes of particular groups and the community in general. The strategy adopted to reduce PSP effects considers local management based on PSP levels and *A. catenella* abundance and specific activities oriented to the public (or community). These activities include: 1) a dissemination information strategy to inform and sensitise the community in general; 2) training of selected groups to achieve specific skills; and 3) the incorporation of HAB matters and their effects into formal education, encompassing basic and middle education. The capacitating strategy analyses consider technical elements to contribute to the design and re-design of these strategies oriented to attain capabilities or abilities [2] according to programme's objectives, which at the same time are based on the experiences and necessities of the objective groups.

The programme is conducted as a whole, through four lines of action, each having interrelated specific objectives: 1) Phytoplankton and PSP Monitoring; 2) Information Dissemination; 3) Training actions and 4) Educational activities. Gained experiences and comments in relation to the strategy adopted are presented, considering exclusively activities orientated to the public (or community).

METHODS

The team in charge of conducting different activities was structured by diverse professionals and technicians, including marine biologists, physicians, chemists, veterinarians, biochemists, teachers and journalists, as well as technicians related to mouse bioassay. The working team was trained to achieve comprehension by using a holistic approach and introducing knowledge about HAB key concepts and its relationships to social-economical and natural systems as well as abilities to work with transversal objectives. The activity lasted eight hours, and the tools employed are those which are mentioned in the Training line of action.

- *Information dissemination strategy* (Diffusion line of action). Two groups have been considered: the community in general and selected groups according to their specific activities (e.g., fishermen, students). During the first two years, 1997 and 1998, specific messages were distributed through TV commercial spots and radio capsules, but also printed material as adhesives, triptychs, diptychs and panels were utilized. In 1999, a video and a WEB page were produced and oriented preferentially to young people between 9 and 12 years old. Focus groups

and quantitative enquiries to assess community information impact and messages recall have been made annually since 1999. Since 1998, every year a slogan orients each publicity campaign. In general the basic orientation was to strengthen prevention, including shellfish safety consumption and related matters, local management, and the importance of consuming certified shellfish by the Health Department.

- *Non-formal training to selected groups and community organisations* (Training line of action). Annual activities started in 1997. Groups have been chosen according to professions, occupations and liaisons to profitable or public health activities, taking into consideration relationships to PSP potential impacts. Community organisations have been incorporated since 1999, including neighbours formal organisations, boy scouts and women centres for personal development. Training is performed through workshops, using techniques such as brain-storming, Philips 66 technique, socialized discussions and opinion enquiries. Workshops are conducted through a facilitator, privileging horizontal relationships and sharing experiences. Each workshop is divided in two parts, lasting at least three hours. The following aspects are considered: HAB concept and its relation to Red Tide, PSP and other toxins: DSP (Diarrhetic Shellfish Poison) and ASP (Amnesic Shellfish Poison), transvectors concept and identification of local shellfish species affected; mouse bioassay; spatial and temporal distribution of PSP and *A. catenella* along the Magellan region, relationships of PSP outbreaks with fisheries, aquaculture and tourism activities and its economical and social impacts; finally consumption of certified shellfish and Health Department responsibility is emphasized, including the responsible behaviour of the public. Emphasis and orientations will depend on the previous knowledge and interests of each trained group. Each group is assessed through a pre-test and a post-test, to evaluate previous knowledge and changes resulting from the training intervention.
- *Introduction of the subject into formal education (basic and middle school)* (Educational line of action). The first two years, 1997 and 1998, in each of 82 regional schools, two monitors were trained to incorporate HAB concepts and effects, considering the subject through transversal objectives (i.e., objectives which are attained when different themes included in diverse disciplines are considered in parallel, as biological, social and economic topics related to HAB and effects). The academic range covered by this activity was from fifth level in basic education up to last level in the middle education (fourth level). During this period the first video was produced and some didactic material was provided to each monitor. In 1999 the students' level of knowledge and their attitudes were measured, and teachers' necessities and weakness were assessed. These were performed by using tests for students and enquiries for teachers. During 2000 and 2001, didactic material was produced as a need for school teachers. During the first year four manuals on environmental education were distributed considering differential texts for students and teachers. Also educational exploitation manuals for the two available videos and an interactive luminous panel were made. In the second year, complementary pedagogic modules in Biology, Language and Communication, Study and Comprehension of Nature, and Study and Comprehension of Society were printed. This material includes activities for laboratory, field or classroom, and it is a complement to printed manuals which provided contents about HABs, PSP and its effects. Pilot schools were selected in each city of the region to produce these ideas including teachers as participant agents and to select the appropriate way to incorporate the subject into formal activities.

RESULTS

A representation of the structure and activities included in each line of action since 1997 are presented in Table 1 and Figure 1. These have been focused with increasing complexity, although using an annual framework. In general, the three lines of action are similar in their structure, but each are following a sequential assemblage of different contents, messages and activities, among others.

The results obtained from the assessment of previous stages orients the plans for the next stages. Although each line of action is driven independently, there is a specific connection between each other. Take notice on the sequence given to the slogan that orients the information dissemination strategy since 1998, reflecting clearly community perception and the needs associated to information and activities that should be included in each stage.

DISCUSSION

In non-formal education and utilising participative techniques as mentioned in this contribution, it is propitiated the encounter and articulation of diverse knowledge, which synergistically and utilizing adequate communication models, will give origin to a new knowledge [2]. This new knowledge will be more adequate, to attain the objectives of the capacitating strategies.

There are no pre-established criteria to structure and organize the operational activities related to PSP outbreaks or other types of harmful blooms. All are oriented to strengthen prevention and to minimise impacts. These can be oriented to disseminate proper information to protect public health, and to manage areas according to toxins levels and/or harmful phytoplankton. Also other activities can be incorporated to strengthen prevention, but should be oriented to persons who are the target to be protected. Depending on the responsible behaviour of individuals, measures adopted by the authority will have an expected success, which could fail

if the community or specific groups show an irresponsible behaviour (i.e., if there is no concordance between authority measures and the expected individual responses). This lack of concordance may emerge from an incomprehension of the grounds on which the authority has based its decisions, assuming that information on the subject has been properly distributed. Among the operational activities that should be included in areas affected by HABs, an obligated matter is shellfish toxicity monitoring, some times associated to phytoplankton surveillance. Other activities, as those included in this paper such as information dissemination, non-formal training and formal educational topics, should be considered to improve prevention and minimise impacts. These are oriented to the public or individuals, which finally are the target of all activities considered. The efficiency of adopted protection norms is highly dependent on knowledge and attitudes of specific groups and community behaviour and how the groups relate this new information with their own experiences.

In areas as the Magellan region, since the continuous presence of PSP only began in the nineties, no previous reflex education on the subject was available and a responsible behaviour in relation to safety shellfish consumption associated to marine toxins was almost absent in most segments of the community. In this sense, processing plants and fresh consumption to local population are more easily certified and controlled, but when we are dealing with complex geographical settings such as the Magellan region, with remote areas almost depopulated, a strong responsible behaviour and credibility are needed, principally from independent fishermen, since shellfish extraction, transportation and distribution is under their responsibility. Consequently, non-certified shellfish consumption is possible yet highly risky.

In this respect, the generation of positive responses by fishermen is a slow process. Additionally, it has not been free of uncertainties since changes into the core of these groups are perceived as conflicts due to the structural alterations that determine quotidian order. Any changes in the cultural or symbolic dimensions are not unperceived, since a modification into the existent cultural normality, is produced [3].

The effects of the operational activities started in 1997 are reflected by no fatal cases recorded to date and decreased intoxicated numbers. Today intoxication is an exception, but always there is the risk that any irresponsible behaviour, principally from independent fishermen, may produce an undesirable intoxication by non-certified shellfish consumption in remote fishing areas or due to the distribution of a small pack of non-controlled shellfish. This is an open possibility as a consequence of groups and specific persons that are continuously challenging norms and rules imposed by the authority. No programme or specific activity or information given to risk groups to inhibit such type of human behaviour is available. Therefore, training of specific groups constitutes an obligated matter (e.g., independent fishermen).

The strategy of each line of action is independent if there is good, timely and properly distributed information provided by the PSP and phytoplankton monitoring. In this sense, the cultural background of the local community, the geographical setting and particularities of local economy are key elements. In the short and long term these complementary operational activities, as shown in Table 1, are oriented to decrease social and economical impacts, including health protection.

The selection of these operational activities and its structure and organisation, depend on a variety of local characteristics, which determine the diversity of selected activities and its coordination, assuming that financial support is not a crucial limiting factor. Consequently, each region should adopt a particular strategy in agreement with its local features.

Independently of adopted strategy, this should consider an interdisciplinary (or at least multidisciplinary) and an inter-institutional approach. A scheme considering different institutions is dependent on facilities available in the affected region. This represents a challenge to afford different tasks by a team integrated by diverse professionals and technicians with different training abilities and conceptual approaches. On the other hand, difficulties arise to achieve cooperative relationships among different institutions, when a disciplinary approach has been historically the chosen criterion, since traditionally, institutions are organised considering a specific knowledge area (e.g., fisheries, public health, education, among others). Then in the initial stage of these operational activities, it is very important that an introductory workshop orient each team with a clear comprehensive holistic approach, knowledge about HAB key concepts and the relationships to social-economical and natural ecosystems, as well as abilities to work with transversal objectives.

These complementary activities are particularly useful when HABs have appeared very recently, changed frequency, geographic cover or have not been considered previously. This not only would encourage individuals to live in harmony with this type of phenomenon and the effects, but it also would facilitate the application of public health regulations, safety of local economy and an easier integration to international markets.

The particularity of local strategies emerge from many factors, including type of HAB, cultural background of the local community in general and special objective groups in particular, geographical setting, local economy features, facilities available in terms of professionals, technicians and infrastructure, among others.

On the other hand, the gained experience has shown that strategies adopted should be flexible and periodically adapted according to obtained results in each programmed stage. Therefore, each stage should have very clear indicators which can be assessed through quantitative and qualitative techniques. Also training or educational activities as well as the type of information given to the community in general and particular groups, should consider differences between groups, not only on the breadth of specific subjects but also in the depth of

information given and the type of didactic instruments utilized.

Table 1. Main activities sequence within each line of action.

INFORMATION DISSEMINATION	
1997	No slogan. HAB telephone. Radial programme: 15; 3 TV and 3 radio spots. Triptych: 13,000; Poster: 600; Adhesive: 1,000. Daily infogramme in local newspaper. Bulletin: in local newspaper, 6,500. Focus group: Fishermen
1998	Slogan: *Learning to live in harmony with Red Tide*. HAB telephone. 3 TV and 3 radio spots. Diptychs: 20,000. Posters: 2,000 (2 designs). Adhesives: 2,000 Focus groups: Coastal rural inhabitants, 125 families
1999	Slogan: *Assist us to help, share this information*. HAB telephone. 2 TV and 2 radio spots. Diptych: 2,000 (bilingual). Diptychs: 7.000; Triptychs: 1,000; Poster: 2,500; Adhesive: 4,000. Web page and Video. Quantitative enquiry: 300 family homes. Focus group: 7 Fishermen families.
2000	Slogan: *Consume Shellfish with safety*. HAB telephone. 2 TV and 2 radio spots Diptychs: 12,000; Poster; 2,500; Workshops: 50 adult men and women, and fishermen. Focus groups: 31 fishermen and women in charge of their own homes.
2001	Slogan: *Attention! Red tide is still present.* HAB telephone. 2 TV and 2 radios spots Diptychs: 10,000; Posters: 2,000. Workshops: Educational schools, 185 persons; Neighbours junta associates, 79 persons; State officials, police and coastguards personnel, 304 persons; home workers; managers, 8 persons. Focus groups in three localities: main inhabited centres (Punta Arenas, Puerto Natales and Porvenir).
TRAINING ACTIVITIES	
1997	Workshop with programme's team, Fisheries National Service, Policemen, Coastguards and Special Country Programme. Workshops: fishermen 152 persons, 90 minutes; Tourism guides, 32 persons, 12 hours; Health personnel, 34 persons, 12 hours.
1998	Fishermen monitor formation (sampling, labelling, complementary co-operation) 40 – 130 persons. Workshops: Communication media personnel, 21 persons; Tourism guides, 38 persons; Health personnel, 70 persons.
1999	Experimental fishermen incorporation to complementary monitoring sampling, success: 21.2%. Workshops to tourism students, total 142 persons and an experimental control of 32 persons.
2000	Workshops: tourism students 42 persons, social working students 102 persons, and infirmary students 188 persons; risk prevention technicians and health personnel, 35 persons; fishermen, 86 persons; 10 visits to laboratories linked to HAB and PSP monitoring.
2001	Workshops to 55 persons: Health personnel, Social working and infirmary students. 1500 Comic for fishermen. 1500 Playing cards with messages. 1500, "2002 allusive calendar."
EDUCATIONAL ACTIVITIES	
1997	Introduction of HAB into formal education: monitor formation; 2 teachers per school. 60 hours. Students Workshop: 50 selected students. Didactic material on HAB and its effects.
1998	Monitor formation reinforcement, 85 teachers, using: Dynamic group techniques and Conceptual maps. Reinforcement in three stages. Total time: 66 classroom hours and 40 practical hours. Construction of didactic material on HAB.
1999	Students' knowledge level and attitudes measurement in 72 schools. At least 95 Teachers were assisted in necessities assessment on didactic material, training and others. Schools assessment on effected activities and work with at least 504 parents and school directives.
2000	Experimental introduction of inter-disciplinary contents into 2 selected schools from Punta Arenas. 42 teachers trained on transversal objectives. Interactive luminous panel. Video exploitation manual. 2 texts for teachers; 2 texts for students: basic and middle levels.
2001	Experimental introduction of inter-disciplinary contents into 16 selected schools of 4 cities. Complementary didactic modules: Biology, Spanish Language & Communication, Natural Sciences, and Social Sciences. 42 teachers involved.

CONCLUSIONS

Operational activities should be addressed to monitor temporal and spatial patterns of toxins and harmful species distribution and abundance. There are also other operational activities that should be considered, besides monitoring programmes, in order to increase prevention and minimise impacts. These activities are oriented to individuals, including key information, training and educational subjects.

The is no pre-established formulae to structure and organise operational activities oriented to individuals, and the orientation is determined by local factors, including cultural background, geographical setting, local economy features, and facilities available in terms of professionals, technicians and infrastructure.

Operational activities oriented to persons should consider an interdisciplinary approach (or at least

multidisciplinary) and if it is necessary an inter-institutional co-operation agreement.

The strategy should be flexible and adapted periodically according to obtained results, so a sequential of logical steps will be characteristic. Well-identified indicators are needed to assess activities progress.

Responsible teams, besides being integrated by professionals and technicians of diverse disciplines, should be trained to achieve a clear comprehension of a holistic approach, knowledge about HAB key concepts and relationships to social-economical and natural systems, as well as abilities to work with transversal objectives.

Training and educational activities should consider differences between objective groups, not only in the level of information given, but also in the type of didactic instruments utilised.

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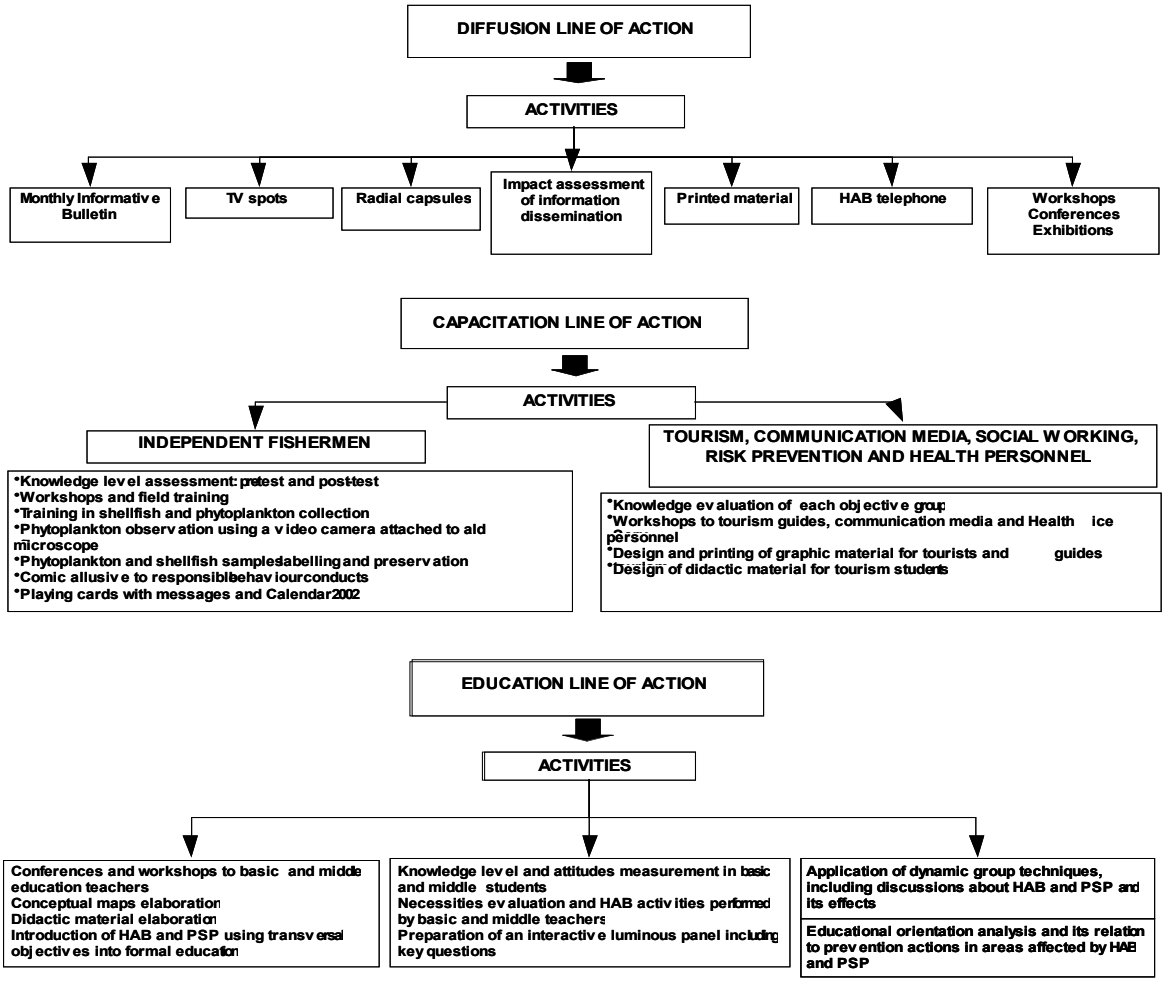

Fig. 1. Schematic distribution of activities within each line of action.

MANAGEMENT STRATEGIES

PREVENTION, CONTROL AND MITIGATION OF HARMFUL ALGAL BLOOMS: MULTIPLE APPROACHES TO HAB MANAGEMENT

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ABSTRACT

The diversity of harmful algal blooms (HABs) and their impacts presents a significant challenge to those responsible for the management of threatened coastal resources. A recent review highlights the many different strategies adopted by countries and commercial enterprises worldwide to monitor and manage HABs in coastal waters. Here the objective is to provide a current perspective on some of these strategies, emphasizing the distinctions between management actions that fall into the categories of *mitigation, prevention,* and *control*.

Many of the management actions taken to respond to HABs can be termed mitigation – i.e., dealing with an existing or ongoing bloom, and taking whatever steps are necessary or possible to reduce negative impacts. Examples of this type of activity include the routine monitoring programs for toxins in shellfish, the towing of fish net pens away from the sites of intense HABs, and processing of shellfish in such a way as to reduce toxicity to an acceptable level. Prevention refers to actions taken to keep HABs from happening or from directly impacting a particular resource. There are three general categories of activities that can lead to bloom prevention. Examples are given of the effect on HABs from: 1) controls on the flow of materials into the coastal region (mainly nutrients and fresh water); 2) modifications of physical conditions (e.g., freshwater flow, tidal exchange); and 3) restrictions on activities, which might result in the accidental transfer of harmful algal species into environments where they do not naturally occur. Control is perhaps the most challenging and controversial aspect of HAB management. The concept refers to actions taken to suppress or destroy HABs - to directly intervene in the bloom process. This is one area where HAB science is rudimentary and slow moving. Five categories or strategies that can be used to combat HABs include: mechanical control, biological control, chemical control, genetic control and environmental control.

Overall, the topic of prevention, control and mitigation of HABs is broad and encompasses many different approaches and strategies. Some are well established, at least in the laboratory, and others are only at the theoretical or conceptual stage. Many require considerable research and testing before they can be fully evaluated.

INTRODUCTION

Algal blooms are diverse in many ways. Some are massive in scale, covering thousands of km^2 [e.g., 1], while others are small and localized [e.g., 2, 3]. Many are non-toxic, but some produce potent toxins that can poison human consumers of shellfish, kill wild and farmed fish in large numbers, and disrupt ecosystems at all levels, affecting even top predators such as sea lions and whales. Non-toxic blooms can also be harmful, typically when the blooms decay, leading to anoxia and large-scale mortalities of seaweeds, fish, and many other organisms. Many different species of algae can cause harm in this way, whereas only a few dozen species are known to produce toxins. Harm can also result from the smell or noxious character of algal blooms, driving tourists and residents from beaches and coastal waters, fouling fishermen's nets, or causing a general reduction in the enjoyment or commercial utilization of nearshore waters.

This diversity in blooms and their impacts presents a significant challenge to those responsible for the management of coastal resources threatened by harmful algal blooms (HABs). The strategies needed to protect fisheries, minimize economic and ecosystem losses, and protect public health vary considerably among locations and among HAB types. A recent review [4] highlights the many different strategies adopted by countries and commercial enterprises worldwide to monitor and manage HABs in coastal waters. Here the objective is to provide a current perspective on some of these strategies, emphasizing the distinctions between management actions that fall into the categories of *mitigation, prevention,* and *control*, as defined by Boesch et al. [5].

MITIGATION

Many of the management actions taken to respond to HABs can be termed mitigation – i.e., dealing with an existing or ongoing bloom, and taking whatever steps are necessary or possible to reduce negative impacts. Obvious examples of this type of activity are the routine monitoring programs for toxins in shellfish, as currently conducted in more than 50 countries [6]. The detection of dangerous levels of any of the HAB toxins in shellfish will lead to harvesting restrictions to keep contaminated product off the market. Another common mitigation strategy is the towing of fish net pens away from the sites of intense HABs. Though expensive and occasionally costly with respect to lost or damaged fish, this remains one of the primary tools used by fish farmers to combat HABs [7].

Another strategy to mitigate the impact of HAB toxins in shellfish is to process those shellfish in such a way as to reduce toxicity to an acceptable level. A clear example is the removal of scallop viscera and the marketing of only the adductor muscle, which generally contains little or no HAB toxins. Another example is provided by the Commission of European Communities (CEC) decision (91/492/EEC) which establishes

conditions for harvesting and processing the cockle *Acanthocardia tuberculatum* from areas where PSP levels exceed the 80 µg STX eq 100 g^{-1} safety limit. If the cockles are processed and canned in the manner specified (which involves washing, heat treatment, and removal of certain tissues), toxicity can be reduced to levels below the detection limit, as determined by mouse bioassay [8]. These and other details on seafood processing to reduce toxin levels are reviewed in Anderson et al. [4], including a new chemical method for decontamination of PSP toxins in shellfish developed by Lagos et al. [9]. This procedure involves immersion of contaminated product (e.g., live animals or shucked meats) in an alkaline solution, followed by boiling. Washing steps are required to remove toxins released into solution. This method is reported to yield 99% decontamination of mussels that had an initial toxicity of 6800 µg STX eq 100 g^{-1}.

These are but a few examples of many different mitigation strategies. In effect, we use these strategies to live with HABs and to manage around them. The question often arises, however, as to whether we can be more pro-active. Can we do something about these blooms before they happen or can we do something to destroy or suppress them while they are occurring? These questions highlight the "prevention" and "control" aspects of HAB management that will be the focus of the remainder of this discussion. The intent is not to provide a thorough overview of these topics, but rather to highlight important issues by providing examples of successful or promising strategies.

PREVENTION

Prevention refers to actions taken to keep HABs from happening or from directly impacting a particular resource. Several problems are immediately apparent in this regard. For one, we do not have all of the knowledge we need about why HABs form in many areas, so it is obviously difficult to regulate or control those factors. This argues for substantial and sustained research on all aspects of HABs, including their ecology, physiology and oceanography. All too often managers and agency officials view these topics as fundamental or basic science issues that have little direct practical utility, but in reality, such knowledge is essential for the design and implementation of effective prevention and control strategies.

Another problem that arises with regard to HAB prevention is that even if we know that certain environmental factors are influencing the population dynamics of a specific HAB organism, there are limitations on what we can feasibly do to modify or control those factors. For example, we might know that a particular HAB is strongly influenced by the outflow of a river system – that it is associated with a buoyant coastal current, for example, but we are unlikely to be able to justify the alteration of that river flow solely on the basis of HAB prevention. As discussed below, it is nevertheless important to factor the possible impacts on HABs into large-scale policy decisions on such topics as pollution reductions or alterations in freshwater flows due to agricultural and drinking water demands.

There are three general categories of activities that can lead to bloom prevention. These include:

1. Controls on the flow of materials into the coastal region (mainly nutrients and fresh water).
2. Modifications of physical conditions (e.g., freshwater flow, tidal exchange).
3. Restrictions on activities, which might result in the accidental transfer of harmful algal species into environments where they do not naturally occur.

Alteration of Nutrient Inputs

The rapid increase in the input of plant nutrients, particularly nitrogen compounds, into coastal waters throughout the world reflects the growing disposal of sewage from expanding populations, increased use of chemical fertilizers in agriculture, and increased fossil fuel combustion. Of considerable concern, particularly for coastal resource managers, is the potential relationship between the apparent increase in HABs and the accelerated eutrophication of coastal waters due to human activities [10, 11]. Linkages between HABs and eutrophication have been noted within the past several decades, though the linkages remain circumstantial and thus are not universally accepted. For example, enhancement of red tides and HABs by pollution has been inferred from data showing that both parameters increase in parallel. A number of examples of increases in blooms or primary production in coastal waters in parallel with nutrient increases are provided by Smayda [10].

Those requiring more proof of this causality can now consider newer data demonstrating that HABs tend to decrease when nutrient loadings are reduced [11]. In other words, reversals in the increasing trends in HAB incidence that occur when government policies reduce pollution loading strengthen the case considerably that those nutrients were responsible for the increases in the first place.

The earliest example of this relationship was seen in the Seto Inland Sea in Japan, where the number of visible red tides (high biomass blooms) increased seven-fold between 1960 and the mid 1970's. This increase paralleled the increase in industrial production and chemical oxygen demand (COD) from domestic and industrial wastes. In 1973, Japanese authorities instituted the Seto Inland Sea Law to reduce COD loadings to half of the 1974 levels over a three-year period. As a result, the number of red tides began to decrease in 1977, dropping to levels approximately one-third of the peak frequency. This lower level of bloom incidence has been maintained to the present [12]. These data demonstrate a general increase in phytoplankton abundance due to over-enrichment of coastal waters, followed by a proportional decrease in blooms when that loading was reduced. Interestingly, toxic blooms (in this instance, those that caused fish mortalities or other fisheries damage) also decreased after the loadings were reduced.

These toxic blooms have always been a small fraction of the total bloom number, so the decreasing trend was less obvious, but still apparent [11].

A second example is from Tolo Harbor, Hong Kong, where increasing population growth in the watershed in the late 1970s and early '80s coincided with a dramatic increase in the number of visible red tides. The implication was that the increased pollution loadings from those population increases simulated red tide occurrence [13]. Sewage inputs to Tolo Harbor were later diverted from Tolo Harbor and discharged elsewhere. Although red tides still occur at a relatively high frequency, data presented by Yung et al. [14] suggest that the community composition may have shifted after these diversions – from dinoflagellate to diatom dominance. It is tempting to link the increase in blooms to pollution and the decrease (or at least the change in bloom-type) to the diversion of sewage. As pointed out by Kueh [15], however, there are other possible explanations for the trends in bloom incidence, including large-scale biological or climatological phenomena that operate in approximate ten-year cycles.

Repeated incidence of high-biomass blooms such as those described above for the Seto Inland Sea and Tolo Harbor provide evidence for a broadly based stimulatory effect of anthropogenic nutrients on phytoplankton in coastal waters. The legislative or policy changes implemented in the Seto Inland Sea and Tolo Harbor [14] demonstrate that control of sewage discharges has the potential to prevent certain types of HABs. Many countries are implementing sewage reduction strategies, and this trend should be encouraged. Nevertheless, there are other important sources of nutrients to coastal waters, and these are proving much more difficult to control, given the increased population pressures and the need to feed a growing world population. In particular, the steady expansion in the use of fertilizers for agricultural production represents a significant and worrisome source of plant nutrients to coastal waters.

A striking example of the impact of fertilizer usage on HAB incidence is seen with the northwestern Black Sea, which experienced heavy pollution loading in the 1970s and '80s from the eight countries within that watershed. This was reflected in significant increases in inorganic and organic nutrients over that 20-year interval: NO_3^- was 2.5-8 times higher, and PO_4^{3-} was up to 20-fold higher [16]. A consequence of this enrichment was an increase in the frequency and magnitude of algal blooms. In the 1960s, high biomass blooms were rare**,** but during the two decades of intense eutrophication pressure, blooms became recurrent, with cell densities greatly exceeding past abundance levels [16]. During the 1980s, 49 major blooms were reported, of which 15 had >10 million cells L^{-1} [17]. Anoxia, fish mortalities, and other impacts were frequent. A noteworthy characteristic of this interval was the decreased abundance of diatoms and larger algae and their replacement by flagellates and nanoplankton. In a striking reversal, algal blooms began to decrease in 1991, both in number and in size, and this trend has continued to the present. Diatoms became more dominant, and nanoplankton and flagellates decreased. From 1991-1996, there were only three blooms with cell concentrations in excess of 10 million cells L^{-1}. This reduction in blooms coincided with significantly decreased nutrient loading to the Black Sea due to reduced fertilizer usage as a result of reduced economic subsidies that accompanied the breakup of the former Soviet Union [17]. This raises the question of whether we will see a recurrence of the planktonic ecosystem changes in the coming years, leading to the high biomass blooms and fish mortality events that characterize the early 1980's. The extent to which the system reverts to the deteriorated conditions of the past will be determined by the pace of economic recovery, as well as the willingness of governments and farmers to make efforts to control non-point source pollution.

Some countries are making good strides towards more efficient fertilizer application methods or are instituting other controls that help to capture the nitrogen and phosphorus before they enter rivers and streams. However, many other countries continue to expand their use of fertilizers at a rapid rate, a trend that is facilitated in part by the relatively low cost of nitrogen fertilizers. There are also trends towards the production of more beef and other meat products that are inefficient in nitrogen assimilation, resulting in extremely high nitrogen usage per kilogram of food produced, compared to production of vegetables and grains. As pointed out in Smil [18], the net result is that the magnitude of human alteration of the global nitrogen cycle is huge - much larger than the changes we are making to the atmospheric CO_2 and greenhouse gas problem. There are obviously many benefits to the world population that derive from our ability to turn atmospheric nitrogen in to fertilizer salts. One wonders, however, how long it will take for policy makers throughout the world to recognize the negative aspects of our increasing reliance on nitrogen to produce the crops needed to feed the growing world population. In effect, we are feeding the world, but in doing so, are over-enriching the coastal ocean.

Freshwater Flows

Another topic that falls under the Prevention category of HAB management involves modification of freshwater flows. Human activities can profoundly affect the amount of fresh water entering the coastal zone, and this can affect HABs. In addition to the obvious role fresh water plays in diluting pollution loads as they enter marine systems, it also affects the stratification of coastal waters, which has always been an important determinant of phytoplankton community composition. Buoyant coastal currents can be critical in the development and transport of certain types of HABs [e.g., 19], and as a generalization, stratified waters are often thought to favor the growth of dinoflagellates and other motile groups that can access the higher nutrients typically found below the nutrient-deplete surface layer.

Another area where changes in freshwater flow may be affecting the patterns of HAB incidence is in the Bohai Sea of China. Due to droughts and water diversions for drinking water and agriculture, several of the rivers that used to flow freely into the Bohai are now dry for many days every year. This affects the dilution of

pollution loads in nearshore waters, and reduces stratification. The Bohai is one of several regions in China where the number of HABs has increased dramatically in recent years. It is not known how the changes in freshwater inputs have contributed to this trend, but it seems probable that they have. It is of note that China has plans to divert the flow of several rivers that presently discharge in the south to deliver water to the Bohai region. These types of large-scale fresh water diversion projects could have a major affect on HAB events. One hopes that comprehensive plankton and water quality monitoring programs will be sustained in that region so that the effects of these public works projects can be documented and lessons learned.

Dams can also affect HABs, again through effects on fresh water. Dams can decrease the availability of silicate to downstream waters due to sediment trapping within the impounded waters. A decrease in the amount of silicate reaching coastal waters, concurrent with increases in nitrogen and phosphorus due to domestic and agricultural pollution could lead to dramatic shifts in the important nutrient ratios that regulate phytoplankton community composition [e.g., 10].

CONTROL

Control is perhaps the most challenging and controversial aspect of HAB management. The concept refers to actions taken to suppress or destroy HABs - to directly intervene in the bloom process. This is one area where HAB science is rudimentary and slow moving. Five years ago, Anderson [20] wrote a commentary highlighting the virtual lack of research activity on bloom control, in contrast to aggressive policies to control tests and nuisance species in terrestrial agriculture. A number of reasons were listed for this "reticence" or reluctance to explore control strategies. For each of the points commonly used to argue against bloom control, there are reasonable counterpoints. These opposing viewpoints can be summarized as follows:

Argument #1: *HABs are complex phenomena in highly dynamic environments. Many are large, covering thousands of km^2.*

Counter argument: This may be true for some HABs, but is not a valid generalization across all types. Some blooms are highly localized, and even some of those that are large may have originated from much smaller populations at an earlier time, such as those linked to specific cyst seedbeds. Furthermore, no one is arguing that all HABs can or should be controlled – just those that are feasible given reasonable expenditures of money and effort relative to potential benefits and impacts.

Argument #2: *HAB phenomena remain poorly understood – "We can't control what we don't understand".*

Counter argument: Some HAB phenomena in certain regions have been studied for more than three decades. While it is true that not everything is known about these outbreaks, a great deal is understood, and one might argue that it is time to begin discussing control or suppression strategies. There will always be scientific uncertainty; the challenge is to determine when sufficient knowledge exists to support the formulation and evaluation of treatment strategies.

Argument #3: *The solutions may be worse than the HAB problem being treated.*

Counter argument: This is an argument that is frequently invoked in discussions of control possibilities, but those who use it often fail to acknowledge the damage cost of the HABs. For example, if it were possible to treat a Florida red tide or New York brown tide at an early stage when those blooms are relatively small and localized, might the potential impact of the bloom event be much lower than would be the case with the widespread, long-lasting blooms that might derive from these initial populations? In other words, what impacts are we willing to accept on a small scale in order to prevent bloom impacts that might be much more widespread and long-lasting?

Argument #4: *It's simply too risky and too difficult to control a HAB.*

Counter argument: This statement reflects the view that it's always easier to study the problem than it is to pursue direct solutions, since scientists are more comfortable pursuing basic research results than they are attempting difficult, controversial, and highly visible studies such as those on bloom control that may lead to failure. The counter argument here is that this is not the approach taken by other scientific disciplines such as agricultural pest control, where there have been significant successes.

Overall, there seems to be a general concession by the HAB community that blooms cannot be controlled, that the problems are too difficult and complex. Given this view, progress in this area will be slow unless steps are taken in the following areas:

- We need to change the "mindset" of HAB scientists to make them more willing to undertake risky studies that may include failures. To do this, the community must be more supportive of those who attempt these types of studies. This support could take the form of more tolerance to proposals submitted seeking funding for this type of work, offers of assistance and advice to make the research team

stronger, and a willingness to keep an open mind on the topic. The simple fact is that there has not been sufficient research on any of the possible bloom control strategies to support a conclusion that they are not feasible, yet many still cling to this preconception.

- We have to set practical goals for bloom control. This means that one needs to start small, with mesocosm and pilot-scale treatments rather than moving immediately to large-scale bloom control. A series of small successes under relatively controlled conditions will do much to alter the receptivity of scientists and managers to the concept of bloom control.

- We need to provide targeted funding for control research. This could best be done through a program on prevention, control and mitigation, for example, which would be distinct from other programs such as those on the ecology and oceanography of HABs. It is important that funding for PCM research be kept separate, in that it also should not come at the expense of funding intended for other HAB disciplines.

- We need to enlist the help of those experienced in mitigating the impacts of terrestrial or aquatic pests. There is a great deal of knowledge and technology that can be of great benefit to those working on HAB control. A joint workshop or working group might be an excellent way to foster this type of interaction.

The challenges and inertia associated with bloom control are significant, yet with these types of approaches, it should be possible to focus the energies and expertise of a subset of the HAB community on control research. Only after that research is completed should we then conclude that it is or is not possible to control HABs.

Experience From Other Disciplines

Harmful algae are not the only "pests" that threaten marine resources. It can therefore be instructive to examine the actions taken by other disciplines in response to outbreaks of potentially damaging organisms. In particular, those concerned with introduced or invasive species have long considered the concept of control. Workers in that field recognize five categories or strategies that can be used to combat an invasive species [21]. These include: mechanical control, biological control, chemical control, genetic control and environmental control. Several of these have already been applied to HAB species. For example, one form of mechanical control is the removal of HAB cells from the water using clay flocculation. This strategy has been employed with good success in Korean waters threatened by red tides of *Cocchlodinium polykrikoides* [22]. For invasive invertebrate species, mechanical control sometimes involves the harvesting of water hyacinths and other destructive plants from waterways. One advantage of this approach is that the target plant is physically removed from the water, minimizing environmental impacts. Clay flocculation of HABs, on the other hand, relies on the sedimentation of clay/cell flocs to bottom sediments, where negative impacts or eventual escape of the flocculated cells are possible. Direct removal of HAB cells through filtration, air sparging, or other approaches has not yet been attempted.

There are a variety of organisms that could theoretically be used to control HABs, but in reality, biological control has many logistical problems and is far from the application stage. Introduction of non-indigenous species or strains poses unknown risks and may be irreversible. Biocontrol is used extensively in agriculture, such as in the release of sterile males or the use of pheromones to control insect pests [23], but there is still considerable opposition to the concept of releasing one organism to control another. This concern is likely to be greatly magnified if the marine environment is to be the site of the release, as there is little precedence for such activities. Despite frequently cited examples where such an approach has had negative long-term consequences on land (such as with the introduction of the mongoose to oceanic islands or the giant toad to Australia [24], there are cases where the approach has been both effective and environmentally benign [23, 25]. The concept deserves some consideration in marine systems.

Zooplankton that graze on HAB species have been proposed as biological control agents [26, 27], but this has never been attempted because of the logistical impracticality of growing and maintaining zooplankton predators in sufficient quantity. Viruses, parasites, or bacteria are more promising control agents. Viruses are abundant in marine systems, replicate rapidly, and tend to be host-specific, suggesting that a single algal species such as *Heterosigma akashiwo* [28, 29] or *Aureococcus anophagefferens* [30] could be targeted. In reality, however, viruses are sometimes so host-specific that they are often unable to infect different genetic strains of the same host species. If HABs are genetically heterogeneous, only part of a population will be affected. Parasites [31] and bacteria [32] also have potential to control HAB species, but specificity is again an issue. There are numerous examples of bacterial strains which exhibit strong and apparently specific algicidal activity, but no field applications have yet been attempted. Clearly, the environmental impacts of the release of non-indigenous organisms will need to be carefully considered and discussed before a biological control strategy could be fully implemented for HABs.

Chemical control relies on toxic chemical release, including the potential development of species specific chemical control agents. Chemical control was attempted in 1957 against the Florida red tide organism now called *Karenia brevis* using copper sulfate delivered with crop dusting airplanes [33]. Although successful in destroying several large patches of red tide cells, this treatment was not considered a success because the red tide re-appeared several weeks later, following the transport of offshore populations to the treated areas. In addition, there was the unquantified but probable collateral mortality of co-

occurring organisms due to the broad lethality of copper. This single effort at chemical control of HABs has not been followed by other attempts, presumably because of the general feeling in the HAB community that it will be difficult and perhaps impossible to find an environmentally acceptable chemical that would target a particular HAB species but not cause widespread mortality of other organisms.

Experience with several invasive invertebrate or plant species suggest that under certain circumstances, the concern over collateral mortality might be outweighed by the benefits of chemical control, at least for some situations. One such example occurred in the summer of 2000, when the Mediterranean green seaweed, *Caulerpa taxifolia*, was discovered in a lagoon in Southern California, USA. This was considered an extraordinarily worrisome situation, since this same species has overgrown huge expanses of the Mediterranean coast since its first accidental introduction about ten years ago [34]. Recognizing that they had to act decisively and quickly, authorities treated the algal beds with liquid chlorine [21]. Although high mortality of the *Caulerpa* followed, individual plants did survive this treatment. The infestation, however, is considerably smaller, and additional efforts were needed to eradicate survivors. It remains to be seen whether this introduction or invasion of *Caulerpa* was prevented.

A similar emergency control effort was mounted in March 1999 against the Asian fouling mussel (*Mytilopsis sallei*) when it was discovered in large densities in three marinas in Darwin, Australia [35]. These marinas were treated with liquid chlorine and copper sulfate. The treatments killed all of the targeted mussels, and a considerable amount of other marine life. This is the same type of "collateral" mortality that many HAB scientists cite as an argument against chemical control. Were these *Mytilopsi* treatments successful? One might argue that the complete destruction of the mussel was worth the effort, despite the mortality of other organisms, since the invasive species was totally eliminated. Even in the case of *Caulerpa*, where there were survivors, the chemical treatment might also be considered partially successful, since it at least slowed the invasion process, and presumably the next treatment might be on a smaller scale.

These two efforts highlight an important issue – namely that drastic control measures might be deemed environmentally acceptable if the situation is considered an emergency. In the context of HABs, this might not be the case for recurrent blooms of toxic or harmful species that are manageable in some way, such as with harvesting quarantines for shellfish. On the other hand, we can wonder what steps should be taken if a particularly toxic or dangerous algal species is detected in a bloom for the first time near a major aquaculture center, for example. Quick action to destroy most of the blooming organisms might prevent not only the immediate impacts, but impacts in future years if that species is not allowed to colonize the area, such as with cysts. Likewise, one wonders what might be considered if large numbers of the endangered Florida manatee were once again dying due to toxic algae, as occurred several times in the past [36; Jan Landsberg, pers. comm.]. If this were in a localized estuarine site, it might be possible to treat that bloom without adverse impacts on the manatees or the ecosystem. Right now, decisions like these are very difficult, in large part because there is insufficient information about the effectiveness and the impacts of different forms of bloom treatment. In the future, however, it may be much easier to decide whether the treatment is acceptable or not if there is a database from laboratory, mesocosm and field studies of different control strategies. The issues surrounding this type of treatment have not been thoroughly debated among the HAB community and the affected resource managers, so it is premature to attempt such drastic treatments at this time. However, it does seem appropriate to keep the concept alive, and to recognize that certain types of events might be sufficiently worrisome and dangerous that drastic preventive actions might be justified, as they were with the invasive mussel and seaweed species.

Another strategy for control of introduced or exotic species is genetic control – the genetic engineering of species that are purposely introduced to alter the environmental tolerances, reproduction or other processes in the undesirable species. The issues surrounding this type of control strategy are similar in many ways to those associated with biological control – concerns about the possible negative impacts of introducing a non-indigenous organism to an area. There are numerous examples where genetic approaches have been used successfully in terrestrial agriculture, such as the engineering of plant crops so that they are capable of producing their own insecticides. Similar genetic manipulations might be used on marine pests such as HABs. It might be possible, for example, to engineer a HAB species so that it no longer produced toxin. Likewise, one can envision genetic manipulations which might make a particular bacterial strain more pathogenic, or more specific in its activity towards HAB cells. However, society's concerns loom large for these types of strategies, and one can expect that it will be exceedingly difficult to obtain approval for such approaches in the near future. Nevertheless, we should not rule out these strategies on the basis of "gut feelings" or speculation, but rather should pursue the research and testing needed to obtain the data on which to base such decisions.

The last of the five control strategies is environmental manipulation – physical or chemical modifications of the environment so that either the target species is affected and/or a natural or introduced biocontrolled species is enhanced. For HABs, this might involve the large-scale manipulation of nutrient levels in coastal waters through pollution control policies. The affects of these policies will likely take years or even decades to become apparent, but are likely to lead to changes in the coastal phytoplankton community in heavily polluted areas, and potentially in a reduction in HABs. On shorter time scales, environmental manipulation becomes more difficult to envision but might include efforts to alter water circulation or residence time such as through dredging or opening of channels. Another approach might be aeration or other

methods to disrupt stratification, again leading to changes in the phytoplankton community composition.

SUMMARY

The topic of prevention, control and mitigation of HABs is a broad one that encompasses many different approaches and strategies. Some are well established, at least in the laboratory, and others are only at the theoretical or conceptual stage. All require considerable research and testing before they can be fully evaluated. With appropriate research progress will be made and our ability to manage HABs will greatly improve.

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THE USE OF CITIZEN-BASED ENVIRONMENTAL MONITORING PROGRAMS FOR MAKING REAL-TIME OBSERVATIONS ABOUT POTENTIALLY TOXIGENIC PHYTOPLANKTON

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ABSTRACT

The integration of citizen-based environmental monitoring with monitoring efforts of regulatory agencies has provided for more robust data sets, improved trust of agency activities and enhanced public education on the issues that surround environmental regulation. In the state of Maine, environmental monitoring has involved citizen volunteers for many years in assessment of water quality in fresh and saltwater environments. These programs have enabled community-based decision making to be effective and have helped to create collaborative relationships between citizens and government. In recent years (since 1996) the citizen-based environmental monitoring has added potentially toxigenic phytoplankton to its monitoring needs.

The Maine State Department of Marine Resources (DMR) is responsible for monitoring biotoxin levels in shellfish in order to ensure that marketed shellfish are safe for consumption. Until recently, this program was based solely on the levels of toxin found in the tissues of shellfish samples from the harvest areas and analyzed in the laboratory. In 1996 a partnership of organizations created the Maine Phytoplankton Monitoring Program which utilizes trained citizen volunteers to collect samples of phytoplankton and provide relative abundance data of potentially toxigenic phytoplankton to the DMR. There are currently 40 stations along Maine's coast that are being sampled at least weekly by over 65 volunteers who collect water temperature, other meteorological information and phytoplankton samples. Samples are analyzed in the field with light microscopes by volunteers who identify potentially toxic phytoplankton genera and document the presence of additional genera. Sampling is conducted between April and November each year. Results are sent to the DMR by fax and DMR is able to determine whether it is necessary to sample shellfish in the area for biotoxins. Although the volunteer information is not used for regulatory purposes, there have been repeated instances over the past five years of the program when volunteer observers have documented the presence of *Alexandrium spp.* in the water shortly before the DMR found elevated PSP toxin in shellfish in that area.

The information gathered by the volunteers is also being examined by researchers studying the ecology of harmful algal blooms in the Gulf of Maine, and is generally adding to the knowledge base by providing observations at geographic and temporal scales not otherwise possible. In addition to observations of *Alexandrium spp.*, volunteers are also documenting the occurrence of other harmful algal events in this region to better understand their potential impacts on aquaculture and general ecology. This paper will present these findings and general information about this community-based approach to environmental monitoring.

INTRODUCTION

The protection of public safety from seafood-borne marine biotoxins in Maine is accomplished principally through aggressive monitoring of post-harvest shellfish and analysis of shellfish tissues using the mouse bioassay. With a coastline of approximately 4500 miles (~8000 kilometers), much of which is harvested for clams (*Mya arenaria*), blue mussels (*Mytilus edulis*), quahogs (*Mercenaria mercenaria*), ocean quahogs (*Arctica islandia*) and oysters (*Crassostrea virginica*), this requires significant resources by the regulatory agency. The Maine Department of Marine Resources (ME-DMR) is responsible for the monitoring of molluscan bivalves and the growing areas where they are harvested. At present the ME-DMR has two laboratories (West Boothbay Harbor and Lamoine) where shellfish are analyzed for marine biotoxins. The sampling of shellfish is accomplished by agency staff, either by collecting animals directly from the harvesting areas, or from shellfish dealers. Samples are delivered to the respective laboratory and analyzed using the accepted methods prescribed by the most current version of the National Shellfish Sanitation Program (NSSP) Model Ordinance as developed by the Interstate Shellfish Sanitation Conference (ISSC). Historically the biotoxin of principal concern along Maine's coast has been paralytic shellfish poison (PSP) associated with blooms of *Alexandrium spp*. [1]. In recent years there have been growing concerns because of reports from near-by maritime Canada that other potentially toxigenic phytoplankton may be present in Maine [3]. Therefore the ME-DMR has been adopting other extraction and analysis methods in order to detect other marine biotoxins including diarrhetic shellfish poison (DSP) toxins and domoic acid.

Since *Alexandrium spp.* blooms principally between the months of April and October along Maine's coast, the ME-DMR shellfish sampling program has typically been limited to this 7-month period. A tiered approach using primary, secondary, and tertiary stations is used and the various species of shellfish are analyzed with blue mussels being tested first at primary sampling stations. If toxin is found, closures are implemented accordingly and secondary stations are sampled for both blue mussels and soft shell clams. In this manner the ME-DMR is able to maintain safe shellfish areas in an open status and is able to close discreet areas for specific species of shellfish [2]. This monitoring strategy requires a staff of approximately four full-time scientists and technicians

and an additional two individuals in the summer to assist with sampling and laboratory support. A significant cost for the laboratory analysis is the mice that are used for the bioassay. ME-DMR estimates that they use >6000 mice per year at a total cost of $30,000 US per year [Hurst, pers. comm.].

Although this monitoring strategy has worked effectively for over 50 years in Maine with very few reported marine biotoxin-related illnesses and no deaths [Hurst, pers. comm.], this system is expensive and lacks the flexibility and sophistication that may be necessary if other marine biotoxins become a concern in this region. For several years scientists in the region have attempted to develop predictive models using improved knowledge of the life history of *Alexandrium spp.* and a greater understanding of the oceanography of the Gulf of Maine. In spite of these advances, there is no proven predictive capability for harmful algal blooms in the region. The regulatory agencies and industry members are in need of predictive tools for HABs, rapid detection methods for marine biotoxins, and early warning monitoring programs for potentially toxigenic phytoplankton. In 1996, the ME-DMR in partnership with the US Food and Drug Administration (US FDA), the University of Maine Cooperative Extension (UMCE), and the Maine Sea Grant Program initiated a citizen-volunteer phytoplankton monitoring program. Equipped with training and field sampling gear this group of citizens is providing information to the regulatory agencies about the presence of various phytoplankton species along Maine's coast [2].

APPROACH

The Maine Phytoplankton Monitoring Program is a coordinated citizen-based effort composed of approximately 40 monitoring locations and upwards of 65 volunteers. Beginning in late spring, after ice has safely retreated from Maine's harbors, the volunteers are trained, or retrained, and begin sampling at least once per week at selected sampling stations. Using phytoplankton nets and field microscopes they make observations of the phytoplankton and report relative abundance for each of the key species to the ME-DMR by fax, email, or by telephone. ME-DMR manages the information with a database and is able to target areas for subsequent laboratory analysis with the standard bioassay-based biotoxin monitoring program.

Volunteer Recruitment and Training

The volunteers in the network include teachers, students, retirees, conservationists, industry-members and community leaders who are interested in this opportunity to learn more about their coastal ecosystems while helping to provide a service to those responsible for monitoring and maintaining ecosystem health and seafood safety. Since 1997 more than 100 individuals have participated in this program. Many of the original members remain active today. Participants have been recruited from other coastal monitoring and stewardship programs and through contacts with educators and community conservation groups throughout Maine. Recently several representatives of the aquaculture industry in Maine have become involved, as they too see a value in knowing more about the microscopic organisms in the vicinity of their culture operations.

All volunteers are provided with written materials including standardized operating procedures for conducting the sampling and photomicrographs and diagrams to be used for identification of the organisms. In addition there are two events held each year for volunteers. The spring event is principally an all-day training event to ensure that the participants have reviewed protocols, practiced identifications with the field microscopes and to confirm that their equipment is in sound shape. Site visits are made to provide follow-up for those volunteers that request additional assistance and for new volunteers. At the end of the sampling season a second event is held to debrief on the results for that season, identify improvements to the program and to celebrate the contribution of these volunteers to this program. Many of the training events have included participation by researchers in phytoplankton ecology and other related experts that help the volunteers to better understand how their participation integrates with the greater scientific understanding of HABs. A critical component of the training is the time spent using the microscopes to identify known and unknown samples of phytoplankton. The Provasoli-Guillard National Center for Culture of Marine Phytoplankton collection at Bigelow Laboratory for Ocean Sciences has provided cultured organisms for these training exercises and has also participated in the identification of unknown samples that are occasionally delivered to their laboratory by the volunteers. It is quite difficult for even the trained eye to identify some of the phytoplankton species of interest through a light microscope and repeated training and quizzing helps to minimize confusion and erroneous information.

Coordination, Advisors, Technical Support, and Funding

The phytoplankton monitoring program has been coordinated from the beginning by a professional from the University of Maine Cooperative Extension Program. This coordinator is a critical component of the program and provides support in recruitment, training both with workshops and in the field, providing on-going feedback to volunteers, coordination of data management and tracking equipment and technical assistance needs for the volunteers. The ME-DMR also provides critical technical support to the program, assists with the training, and manages the data. A steering committee provides advice to the program through quarterly meetings. The steering committee has included staff from the ME DMR, UMCE, Maine Sea Grant, the University of Maine, and Bigelow Laboratory for Ocean Sciences. This team approach allows the program to evaluate itself and make incremental improvements while also making modifications in response to new discoveries about phytoplankton in the Gulf of Maine.

Funding for this program has involved several different sources over the years. An initial seed grant from the US FDA allowed the program to get started and a two-year grant from the Maine Outdoor Heritage Fund allowed the program to grow rapidly to 22 programs in 1998. In subsequent years the ME-DMR has provided much of the funding for materials and training while the funds for the coordinator have come from the Maine Sea Grant Program and the University of Maine Cooperative Extension Program.

Sampling, Identification and Reporting Procedures

The methods used for collecting phytoplankton samples are described in the Standard Operating Procedures and are part of the training protocols for the volunteer observers. Collection bottles are attached to the cod-end of a 20-μm plankton net with an elasticized cuff and rubber band. The rope used for "swimming" the net is marked at one-meter intervals. The net is submerged and is "fished" in the water for three minutes gathering an integrated sample from the entire column of water. This swimming pattern is repeated for three minutes to standardize sampling time. The sample bottle is removed from the net by releasing the rubber band, being careful to retain the contents of the bottle. The collected sample is examined immediately with a field light microscope, or removed from sunlight and warmth by placing it in a cooler for transport, if it is to be examined at a later time. A flat glass capillary tube is filled with the material from the sample and the tube clipped to the microscope for examination. Observers are instructed to look at three fields of view in two capillary tubes (a total of six fields of view) in order to standardize observations. They look at three different locations along the tube (3 fields) at 10X. In order to identify what is being viewed, a higher objective is used to examine the capillary tube. Three fields are then viewed on each capillary tube and used to evaluate the number of cells present. Observers then record the species that are seen on the field sheet. Field sheets are faxed to the ME-DMR as soon as possible for review and data entry.

RESULTS

Predictive Capacity

The use of volunteers provides for a monitoring program with spatial and temporal scales that cannot be achieved through agency-based or research-based programs. It is simply too expensive to pay for enough professional technicians, scientists, or even graduate students to be able to cover the coast at the level that this network of citizen volunteers can. On several occasions since this program began, the volunteers have reported observations of potentially toxigenic species several days before the ME-DMR detected the associated toxin in shellfish from those sample areas. Figure 1 indicates the number of samples taken and observations made by volunteers each year between 1997 and 2001. Participation in the program continues to grow with nearly 1000 observations recorded in 2001.

Figure 1: Number of Samples Taken By Volunteers Annually

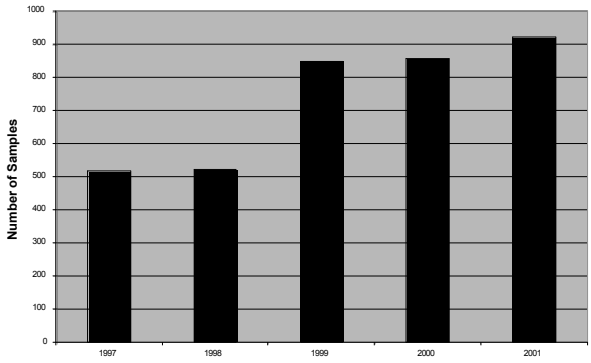


Figure 2 shows the observations of *Alexandrium* during 2001 along with the closures made by ME-DMR in response to elevated PSP levels in shellfish using the mouse bioassay. The white arrows identify those cases in which high levels of *Alexandrium* were observed by volunteers, and within seven days, a local shellfish harvesting area was closed. There were no cases of false positives (a volunteer thinking that he/she was seeing *Alexandrium* and that not being the case) or false negatives (a volunteer not seeing *Alexandrium* but a closure occurring) during the 2001 season. In subsequent years there have been instances when volunteers had trouble identifying *Alexandrium spp.* and program organizers have had to double-check and confirm or discount positive observations. This is a limitation of this type of program, which is being addressed through training and experience.

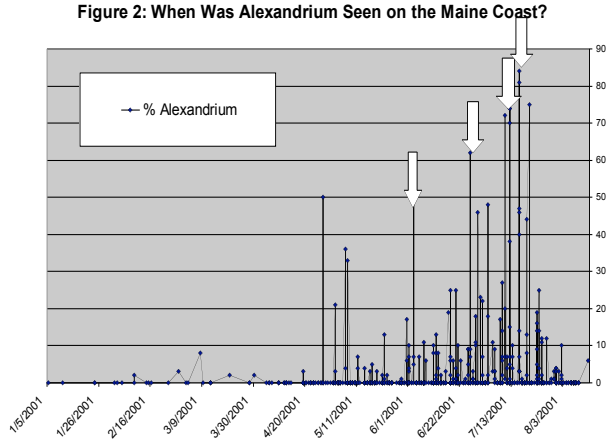
It is not possible to present all of the data collected in recent years, but the above example indicates that this program provides some predictive value to the regulatory agency and the shellfish industry regarding the relative abundance of these organisms in Maine waters. Observer data is still being analyzed for other trends, comparing presence of the organism and the results of biotoxin analysis for the other species of marine phytoplankton that have been observed. Other notable program achievements include:

- 2800 recorded observations of phytoplankton species in database
- Citizen participants range in age and background from high school students to retired scientists
- In the fall of 1999, a methodology for counting phytoplankton cells was developed and one of the participating groups has tested the new procedure for feasibility of deployment throughout the network
- Using information on the large *Dinophysis* populations from the volunteer monitoring effort, a NOAA biotoxin team was assembled to determine if diarrhetic shellfish poisoning (DSP) occurs along the coast of Maine. This is the first study demonstrating the possibility of DSP on the Maine coast. Since okadaic acid (*Dinophysis* toxin-1) was detected in the dinoflagellate *Prorocentrum lima*, a protocol has

been developed for volunteer monitors to identify *P. lima* and a pilot program to test this protocol will be in place in 2002.

- Currently 6 aquaculturists (finfish and shellfish) monitor with the program providing data not only to the ME-DMR as part of the first-alert system but also using the data for their own management purposes to understand the food source available to their crop and potential bloom conditions. The data generated by these individuals may be used to develop management decisions for their business.

Figure 2: When Was Alexandrium Seen on the Maine Coast?



DISCUSSION

The results presented here indicate that the Maine Phytoplankton Monitoring Program has value in helping the state biotoxin monitoring program to know when *Alexandrium spp.* are present in the water column thereby allowing better planning and targeting of the sampling and analysis of shellfish. However, it is recognized that blooms of *Alexandrium spp.* are likely originating off-shore and the factors dictating the development and movement of a bloom of this phytoplankton species are not fully understood. Therefore it is not possible for this type of observer network to be used as a definitive predictive tool, but rather as a warning signal that can be used as part of the management for shellfish safety. Indeed, the ME-DMR has included the phytoplankton monitoring program in their biotoxin monitoring plan that is periodically filed with the FDA. Beyond the situation with PSP in the Gulf of Maine, there are other toxic phytoplankton species that could present a public health risk in the region. There is evidence of the presence of both DSP and ASP in the Canadian maritimes and it is important that the State of Maine be able to identify these organisms and be prepared to monitor for, and manage the threat of these other toxins. The phytoplankton species known to create these other toxins (*Pseudo-nitzschia* and *Dinophysis*) are included in the training and identification schemes for the phytoplankton monitoring volunteers, who are therefore able to alert the ME-DMR about the relative abundance of these other organisms when they occur.

Research Opportunities and Other Future Concerns

Several researchers in the Gulf of Maine have integrated the Maine Phytoplankton Monitoring Program into their studies to provide valuable sampling support, to test methods, and to corroborate their findings. Dr. Stephen Morton conducted studies on the toxicology of the *Dinophysis spp.* in the Gulf of Maine utilizing observations of this network to show that, although the phytoplankton was extremely abundant that year, the toxin (DSP) was not found at levels of concern [3]. This discovery led other researchers to suggest that a low level of DSP that was identified in blue mussels may be associated with another phytoplankton species, *Prorocentrum lima*. Dr. Lucie Miranda conducted studies on the incidence of *P. lima* and incorporated some members of the monitoring network to participate in this specialized sampling protocol [4]. As a result, some of the groups in the program are now equipped to sample this epiphytic species of phytoplankton.

The recruitment of members of the aquaculture industry was an important step in the development of the observer network. Growers of both shellfish and finfish have ample reason to be interested and concerned about the microscopic organisms that are present in the vicinity of their culture facilities. These interests include the need to know when toxigenic species are in the vicinity for the human health concerns, nuisance species that can cause harm to finfish, and other phytoplankton that represent natural food for shellfish.

There is a growing interest and effort in developing new analytical tools for the identification of potentially toxigenic phytoplankton in field samples, and for determination of toxicity in shellfish. As these research activities proceed, there is a need to compare new methods with standard laboratory analytical procedures for biotoxin and to compare these results with the occurrence of these species of phytoplankton in the environment. This network of volunteer observers is uniquely capable of providing a general sense of the relative abundance of these organisms on a temporal and spatial scale that can be useful to the scientists that are developing these new technologies. For further information about the Maine Phytoplankton Monitoring Program, contact Ms. Sarah Gladu at PO Box 309, Waldoboro, Maine, USA 04572 or by telephone: (207) 832-0343.

General information is available on the world wide web at: http://www.ume.maine.edu/ssteward/phyto.htm.

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PHYTOPLANKTON MONITORING AS A MANAGEMENT TOOL: TIMING OF ALEXANDRIUM AND PSEUDO-NITZSCHIA BLOOMS IN THE BAY OF FUNDY, EASTERN CANADA

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ABSTRACT

Water samples have been collected since 1988 at 4-18 stations (inshore and offshore) in the western Bay of Fundy, eastern Canada. Shellfish in the area have been monitored for PSP toxins since 1943. Water samples were settled in counting chambers for enumeration of phytoplankton species and abundance. Harmful algal bloom (HAB) species that have had the most impacts on shellfish or finfish resources are *Alexandrium* spp. and *Pseudo-nitzschia* spp.

The 15-year (1988-2002) phytoplankton dataset represents more than 70,000 records. It has been examined from several perspectives, including the annual timing of blooms. When cumulative cell counts within each year were calculated, beginning in January, the cumulative curves indicated that the increase in diatom abundance began around day 110 (mid April), reached 50% of its annual total between days 150 and 250 (late May to early September) and returned to background levels around day 275 (October 1). Cumulative curves for total dinoflagellate abundance showed the annual increase was around day 150 (late May); about 40 days later than for diatoms. The range in median day for the dinoflagellates was narrower than for diatoms – between days 210 and 230 (late July – mid August).

Alexandrium spp. blooms occur annually between late May and August, with highest concentrations observed during most years in July. As few as 200 *Alexandrium fundyense* cells L^{-1} can result in detectable levels of toxins in shellfish in the Bay of Fundy. During the 1997-2000 period, cell concentrations were lower than normal with less than 1.0×10^4 cells L^{-1} observed during each of the years with shellfish toxicity levels also below average. The highest *A. fundyense* concentrations during this period were observed in 1989 with 1.62×10^5 cells L^{-1}.

Domoic acid production has been associated with *Pseudo-nitzschia pseudodelicatissima* in the Bay of Fundy and generally requires more than one million chains of cells L^{-1} to be in the water in order for shellfish to become toxic (to the consumer) above the regulatory level (20 µg g^{-1}). Blooms tend to occur during late spring/summer but the highest observed concentrations occurred during late August to early September in both 1988 and 1995.

INTRODUCTION

The Bay of Fundy is a physically [1] and biologically diverse and dynamic system that supports commercial fisheries, aquaculture and ecotourism industries, among others. The bivalve fisheries in the Bay of Fundy are based on harvests of soft-shell clams (*Mya arenaria*), sea scallops (*Placopecten magellanicus*), and blue mussels (*Mytilus edulis*). These have a long history of being impacted by the harmful alga, *Alexandrium fundyense,* and its resulting paralytic shellfish poisoning (PSP) toxins. The accumulation and retention of PSP toxins by these benthic, filter-feeding molluscs and the subsequent effects on warm-blooded vertebrates in Atlantic Canada have been well documented [2]. During the summers of 1976 and 1979, hundreds of tons of Atlantic herring (*Clupea harengus harengus*) were killed as a result of the transfer of PSP toxins through planktonic herbivores [3, 4]. In 1988, domoic acid was detected for the first time in shellfish from the Bay of Fundy and *Pseudo-nitzschia pseudodelicatissima* was implicated [5]. Shellfish areas were subsequently closed to harvesting in the summers of 1988 and 1995 as a result of unsafe levels of domoic acid.

A salmonid aquaculture industry was introduced to the western Bay of Fundy in 1978 and experienced a period of rapid growth in the years immediately following. Faced with an increasing number of aquaculture sites and increased public awareness of coastal systems being impacted by human activities throughout the world, a phytoplankton monitoring program was initiated in 1988 in the western Isles region of the Bay of Fundy to document species composition and abundance of the whole phytoplankton community, including HABs. Some objectives of the study were to: establish baseline data; determine temporal and spatial variations and trends in the phytoplankton community as a whole as well as in the abundance of specific species; act as an early warning of HABs to both the salmon aquaculture industry and the Canadian Food Inspection Agency (CFIA) (who are responsible for measuring the levels of toxins in shellfish tissues and for alerting the surveillance agencies as to shellfish closures); provide data for hindcasting events; and determine linkages with the physical and chemical oceanographic characteristics of the area.

Toxicity levels in shellfish have been monitored at various locations in the Bay of Fundy since the 1940s resulting in the closure and opening of shellfish harvesting areas. The CFIA oversees the collection and analysis of all regulatory shellfish samples. When shellfish toxin levels exceed the regulatory levels set by Health Canada, the CFIA notifies Fisheries and Oceans Canada (DFO) with a recommendation of closure. The DFO is in turn responsible for the legal closure of these harvest areas, the posting of warning signs and the routine enforcement of area closures. In some regions of Canada, such as the Bay of Fundy, the ongoing phytoplankton monitoring programs have acted as an

early warning for the CFIA and have complemented ongoing toxin analyses. When the numbers of a particular phytoplankton species increase to levels of concern, the CFIA is notified resulting in modifications to sampling schedules as deemed appropriate.

In this article we present information about the phytoplankton monitoring program in general, time series of the concentrations of *Alexandrium* spp. and *Pseudo-nitzschia* spp. and of shellfish toxicity as well as preliminary results on spatial and temporal variability in the phytoplankton community.

MATERIALS AND METHODS

Samples were collected from 4-18 stations in the western Isles region of the Bay of Fundy, with the number of sampling sites decreasing to the 4 that have been monitored and studied to this day (Fig. 1) [5]. Measured parameters include the abundance of each phytoplankton species, the concentration of dissolved oxygen, nutrients (nitrate, phosphate, silicate and ammonia), chlorophyll *a*, as well as water temperature and salinity.

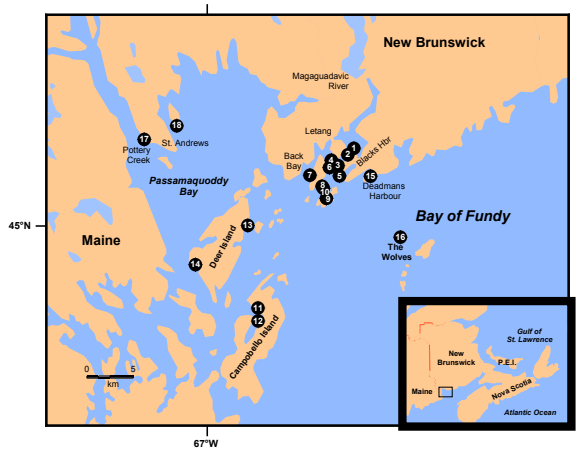

Figure 1. Sampling locations in the western Bay of Fundy.

Surface phytoplankton and nutrient samples are collected by bucket from the research vessel, PANDALUS III, from all four stations (Stations 3, 15, 16 and 17) and with a Niskin bottle at depths of 10 m, 25 m, and 50 m at Station 16. Weekly samples are collected from early May to the end of September. Biweekly sampling is conducted in October and samples are collected monthly from November to April. Additional water samples are collected when HAB organisms are detected or relevant problems are reported with the fisheries or at aquaculture farms. Ships of opportunity, the CFIA, the salmon aquaculture industry and volunteers may collect these additional samples. More than 260 samples are collected each year. During the summer months a vertical plankton haul is made with a 20-µm mesh net, 0.3 m in diameter. A subsample is preserved with formalin:acetic acid (1:1 by volume) for further identification and scanning electron microscopy (SEM). In addition, a live subsample is taken for further identification and culture of selected organisms. Live phytoplankton samples are immediately iced for the return trip to the laboratory. Water samples (250 ml) are immediately preserved with 5 ml formalin:acetic acid solution. Later, 50-ml subsamples are settled for 16 hours in Zeiss counting chambers. All phytoplankton greater than 5 µm are identified and enumerated (as cells L^{-1}) using a Nikon inverted microscope. Further identification is achieved using either a JEOL JSM-5600 or a Hitachi S-2400 SEM. For species identification purposes, the *P. delicatissima* group included both *P. pseudodelicatissima* and *P. delicatissima*.

Vertical profiles of water temperature, salinity, dissolved oxygen and fluorescence (with a SeaBird 25 CTD profiler) were also determined. Water samples were collected with Niskin bottles for laboratory nutrient analyses (nitrate, phosphate, silicate and ammonia). The results from these sampling efforts are available but are not provided in this text. The phytoplankton data from 1988-2002 have been entered into an ACCESS database and EXCEL spreadsheets and includes more than 70,000 records to date. These data have been analyzed using PRIMER (Plymouth Routines in Multivariate Ecological Research) software that consists of a range of univariate, graphical and multivariate routines for analyzing matrices of species by sample abundances from studies such as community ecology. For generalized descriptions of the phytoplankton community, identifications were grouped into diatoms, dinoflagellates and "other" (which included smaller zooplankton, silicoflagellates and ciliates).

Both PSP and domoic acid were analyzed at various CFIA laboratories including Black's Harbour, Moncton, Halifax and Dartmouth. Shellfish toxicity data have been collected in the Bay of Fundy since 1943 and entered into EXCEL spreadsheets.

RESULTS AND DISCUSSION

The number of stations and depths sampled as part of the phytoplankton monitoring program has varied over the years since the sampling was initiated. In early 1988, samples were collected from 12 stations in the western Isles region of the Bay of Fundy (Fig. 1). Ten of these sites were in the Letang region, all in close proximity to Station 3, an area where the majority of the salmon aquaculture industry was located. In the late spring of 1988, the number of sites was expanded to 18 and Stations 15 and 16 were among those added. Sampling of these 18 sites continued for 2 years but in 1990, five locations were discontinued in the Letang area due to the similarity of results between the sites as well as to reduce costs. In November, 1991, the number of sites was further decreased to the four that continue to be monitored today (Fig. 1) – Station 17 (a brackish location influenced by the St. Croix River); Station 3 (in the Letang Estuary where a number of aquaculture sites are located); Station 15 (an open bay with offshore

influence); and Station 16 (a site to provide an indication of offshore populations) (Fig. 1).

One hundred and ninety-one species of phytoplankton were observed throughout the sampling period, with the following breakdown: 140 diatoms, 36 dinoflagellates and 15 "other" (includes ciliates, silicoflagellates, flagellates and micro-zooplankton) [6,8]. The majority of these species are not reported to cause harm. Typically, few species were present during the winter months and many species were present in the spring, summer and early fall months. Cumulative frequency plots of total organisms calculated within each year, beginning in January, indicated that phytoplankton numbers tend to increase around mid-June and peak in early September. The annual increase in diatom abundance tended to occur around day 110 (April 20) with 50% of the total cells observed between days 150 (May 29) and 250 (Sept 7) (Fig. 2). Dinoflagellates tended to increase in the water column around day 150 (40 days after the diatom increase) (Fig. 3). The annual timing for dinoflagellate appearance or peak concentration is more variable than for diatoms.

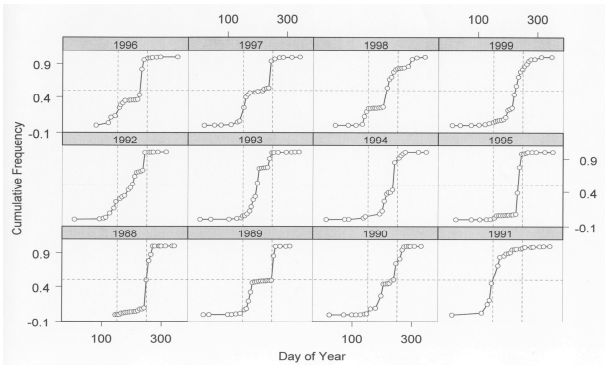

Figure 2. Occurrence of diatoms at Station 16 (1988-1999).

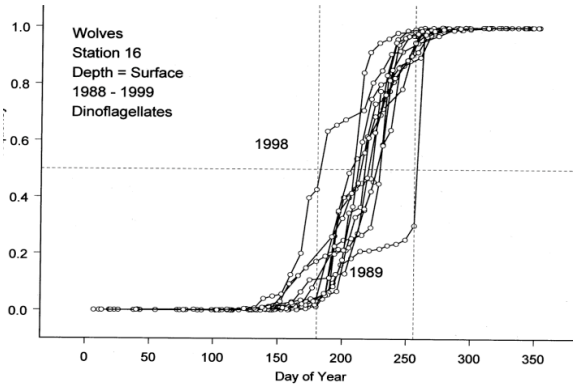

Figure 3. Occurrence of dinoflagellates at Station 16 (1988-99).

Multi-dimensional scaling (MDS) analyses of the total phytoplankton from 12 sampling stations during 1991 indicated that there was spatial variation among sampling locations. Stations located in estuarine (1 and 17) and offshore (16) areas separated from the remaining stations suggesting inshore and offshore source areas for cells. The remaining stations were located in between the bounding stations and indicated a mixture of populations that depended on location [7].

Harmful species that have resulted in shellfish toxicity during the 15-year period are *Alexandrium fundyense* (PSP) and *Pseudo-nitzschia pseudodelicatissima* (domoic acid or amnesic shellfish poison (ASP)) [6,8]. Although the two species bloom every year, shellfish harvesting areas are closed to harvesting annually (generally during the summer) only as a result of unacceptable levels of PSP toxins. Only twice, in 1988 and 1995, have shellfish areas been closed to harvesting as a result of unacceptable levels of domoic acid.

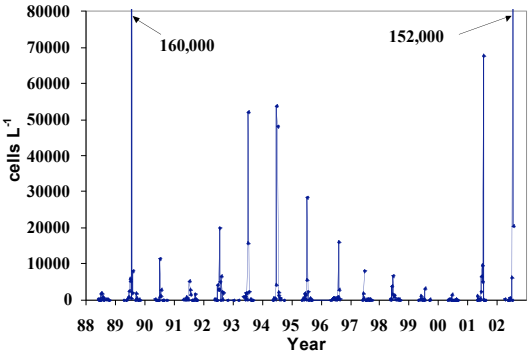

Figure 4. *A. fundyense* concentrations in cells L^{-1} at Station 15 (1988-2002).

Figure 4 shows *Alexandrium* concentrations at Station 15 from 1988-2002 where *Alexandrium* blooms are an annual occurrence but with considerable inter-annual variability in the maximum observed concentrations of cells. Highest concentrations (in cells L^{-1}) during each of the years were: 1.88×10^3 (1988), 1.62×10^5 (1989), 1.16×10^4 (1990), 5.34×10^3 (1991), 2.01×10^5 (1992), 5.22×10^4 (1993), 5.38×10^4 (1994), 2.86×10^4 (1995), 1.62×10^4 (1996), 8.2×10^3 (1997), 6.72×10^3 (1998), 3.20×10^3 (1999), 1.52×10^3 (2000), 6.78×10^4 (2001) and 1.52×10^4 (2002). Since at least 200 *Alexandrium* cells L^{-1} can result in detectable levels of PSP toxins in shellfish from the Bay of Fundy, regular phytoplankton monitoring can act as an early warning of increasing shellfish toxicity. An increase in PSP shellfish toxicity tends to occur within a few days to a week of increasing cell concentrations. For example, Figure 5 shows shellfish toxicities near Station 3 and *A. fundyense* concentrations during late June to early July, 1994. During some years, there are two *A. fundyense* blooms, one in late May-early June and a later one in July. Very occasionally the later bloom can extend to late summer. The sampling results also suggest that Stations 3, 15 and 16 (outside Passamaquoddy Bay) act in a similar manner with respect to temporal variation in the abundance of *Alexandrium* although higher cell concentrations are observed at stations 15 and 16. Station 16 appears to act as an indicator site for offshore blooms that are advected into the inshore shellfish areas. This is consistent with earlier surveys for *Alexandrium* cyst beds [9, 10] and

summer vegetative cell levels showing the highest concentrations tended to be observed in the offshore Bay of Fundy waters [11].

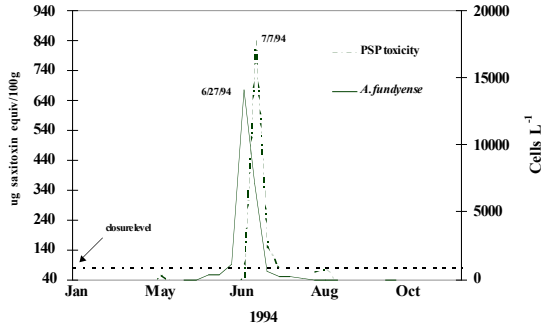

Figure 5. *A. fundyense* concentrations (——) and PSP shellfish toxicity (·····) at Station 3 during 1994. Level of 80 µg 100 g^{-1} is indicated by dotted straight line.

The PSP shellfish toxicity data indicate that some years experience higher toxicity values than others. For example, it appears that during the mid 1940s, early 1960s, and late 1970s to early 1980s higher levels of toxins were measured in soft-shell clams (*Mya arenaria*) than in the intervening years (Fig. 6). Detailed analysis and interpretation of these data need to be conducted with caution since during some years more samples were collected and on occasion samples were only collected until levels exceeded regulatory levels and not resumed until later in the summer or fall.

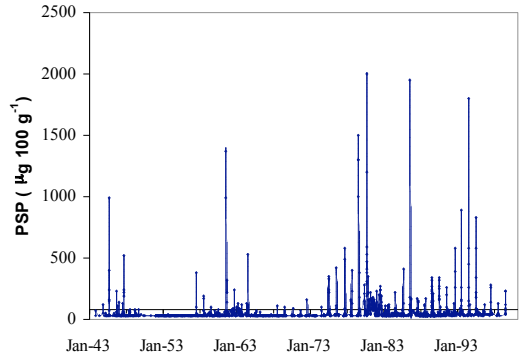

Figure 6. PSP shellfish toxicity results from soft-shell clams (*Mya arenaria*) at Lepreau Harbour (1943-1999).

Pseudo-nitzschia pseudodelicatissima was determined to be the source of domoic acid in Bay of Fundy shellfish in 1988 [5]. Although the *P. delicatissima* group has been observed each year since the monitoring program was initiated (Fig. 7) and earlier records [12] indicate that it is not new to the region, domoic acid levels exceeding the regulatory limit of 20 µg g^{-1} have only been encountered in 1988 and 1995. Concentrations of *P. pseudodelicatissima* exceeded 1 million chains of cells L^{-1} (the average concentration required for shellfish to surpass the 20 µg g^{-1} regulatory level) at certain locations during these two years. During 1988, *P. pseudodelicatissima* comprised more than 95% of the total cell count of phytoplankton organisms present whereas in 1995, it comprised a considerably lower percentage. Domoic acid content in shellfish closely follows the *P. pseudodelicatissima* bloom. Figure 8 shows cell concentration at Deadmans Harbour (Station 15) during 1995 and the corresponding domoic acid level measured in soft-shell clams from a nearby clam harvesting area. The domoic acid toxicity lagged behind the increased cell concentrations by a few days indicating that here too, phytoplankton monitoring can provide an early warning of increasing toxicity.

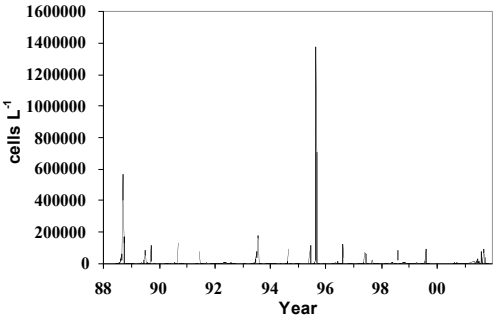

Figure 7. *P. pseudodelicatissima* concentrations in cells L^{-1} at Station 16 (1988-2001).

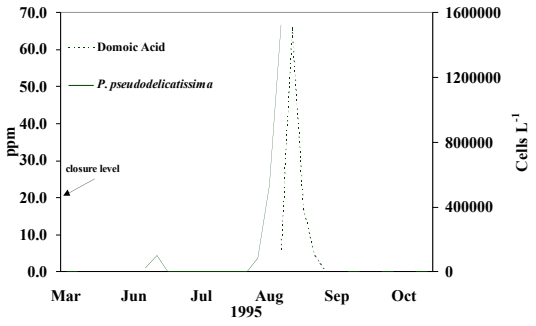

Figure 8. Concentrations of *P. pseudodelicatissima* and domoic acid content in soft-shell clams at Station 15 (1995).

Although we are not able to predict *Alexandrium* or *Pseudo-nitzschia* cell densities from one year to the next, we are able to provide an early warning to industry and regulatory agencies of the progress of a particular bloom. The monitoring program indicates wide inter-annual variation in the density of populations and the necessity to monitor weekly, at the very least, in order to provide an early warning. Future plans include continued monitoring; further refinement and quality control of the data; statistical exploration of temporal and spatial

variability in the indices of the community structure and abundance of specific HAB species; estimation of the biomass/volume ratio for dominant species; investigation of potential linkages between the physical and chemical parameters of the environment; and the possibility of modeling the HABs.

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NORTH COAST WATER QUALITY & BIOTOXIN PROGRAM

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ABSTRACT

The North Coast Water Quality & Biotoxin Program coordinates biotoxin and water quality monitoring and testing services on the north coast of British Columbia, Canada. It is a non-governmental organization that consists of representatives from local First Nations and non-First Nations communities, shellfish farmers, scientists, economic development groups, and local, provincial and federal government. Coastal British Columbia has endemic Paralytic Shellfish Poisoning (PSP) blooms that are of particular concern to local First Nations who harvest shellfish regularly as part of their traditional heritage. Monitoring stations tend to be very remote, so the North Coast Water Quality & Biotoxin Program faces a complex set of challenges.

INTRODUCTION

The North Coast Water Quality & Biotoxin Program is a unique collaboration. It started out as a community driven pilot project that was initiated in 1998 and expanded in 1999. The goal was to re-establish an official biotoxin sampling and testing program for the North Coast of British Columbia. Participants include two shellfish lease sites (Humpback Bay and Kagan Bay) and the First Nations communities of Metlakatla, Kitkatla, Kitimaat, Lax Kw'alaams, Hartley Bay, and Haida Gwaii. The North Coast Water Quality & Biotoxin Program is based in Prince Rupert.

British Columbia has approximately 26,000 kilometers of coastline. Its complex shoreline consists of rugged mountains, inlets, fjords and islands. Paralytic Shellfish Poisoning (PSP) blooms are endemic in coastal British Columbia. The North Coast is very isolated, with only one major highway connecting the coast to the interior. There is no road along the coastal mainland north of the Vancouver area. On the North Coast, there is a history of domoic acid outbreaks as well as *Alexandrium* blooms. PSP illnesses have been recorded in British Columbia through out the last century, and the coastal First Nations have traditionally avoided harvesting shellfish during the spring and summer months as that is when toxic *Alexandrium* blooms are known to occur.

The North Coast has not had a water quality and biotoxin testing program since the federal government ceased monitoring biotoxins on a regular basis in this area in 1964. The absence of such a program is a serious barrier to aquaculture and new fisheries development, as access to a testing program is a requirement for an aquaculture license. The lack of biotoxin monitoring also puts the health and safety of residents and visitors at risk. Furthermore, although First Nations residents are guaranteed access to shellfish for food, social and ceremonial purposes under Section 35 of the Canadian Constitution, they are officially prohibited by Fisheries and Oceans Canada from harvesting shellfish on the North Coast due to the lack of biotoxin monitoring.

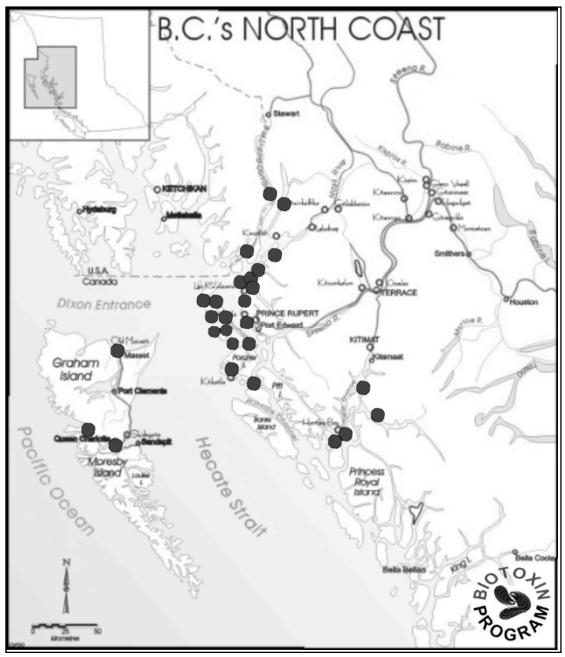

Figure 1. Map of monitoring station locations.

In December 1999, a Coordinator was hired to manage and expand the program. In January 2000, a Steering Committee was established. Its mandate was "to contribute to the design and implementation of a north coast water quality and biotoxin program which will ensure priority access for First Nation food, social and ceremonial requirements, and facilitate commercial aquaculture development." In addition to conducting regular sampling, the program's objectives included developing local laboratory ability for approved water quality and biotoxin testing, and investigating alternative technologies such as the MIST ALERT test and phytoplankton monitoring.

The Steering Committee consists of community representatives, biotoxin station managers, shellfish farmers, government representatives from the Canadian Food Inspection Agency (CFIA), Environment Canada, Fisheries & Oceans Canada (DFO), Indian & Northern Affairs Canada, Health Canada, and the BC Ministry of Agriculture, Food and Fisheries, economic development representatives, and other interested parties. This committee meets regularly to share information and direct the expansion of the program.

MONITORING AND TESTING

Biotoxin and phytoplankton sampling occur weekly from 1 May - 31 October and every two weeks from 1 November - 30 April. Currently there are 13 biotoxin monitoring stations; six of these are also phytoplankton monitoring sites. Up to 16 more biotoxin monitoring stations may be established in the near future. Stations are maintained by trained volunteers. California mussels (*Mytilus californianus*) are the indicator species used for monitoring.



Figure 2. California mussels used for biotoxin monitoring.

Biotoxins associated with three illnesses are monitored: Paralytic Shellfish Poisoning (PSP), Amnesic Shellfish Poisoning (ASP) and Diarrhetic Shellfish Poisoning (DSP). Biotoxin results are posted on the program's website. This regular biotoxin monitoring fulfills the Canadian Food Inspection Agency's regulatory requirements.

Water testing for shellfish growing areas is the mandate of Environment Canada, and is conducted on a three year rotational basis for the North Coast mainland area, the Queen Charlotte Islands, and the Central Coast. In June 2001, Environment Canada conducted shoreline surveys and fecal coliform testing for areas on the North Coast near Lax Kw'alaams, Metlakatla, Kitkatla, Humpback Bay, Oona River, Hartley Bay and Kitimaat. These areas were classified by the Pacific Shellfish Classification Committee on 10 October 2001, and are now eligible to be officially opened for shellfish harvesting by Fisheries and Oceans Canada. The Steering Committee is currently working very closely with Fisheries and Oceans Canada to assist them in fulfilling their obligations, including stock assessment, harvest plans, product handling and inspection plans, enforcement plans, funding arrangements and business plans, so that areas can be officially *Opened* for First Nations intertidal shellfish harvesting on the North Coast of British Columbia.

On 27 April 2001, the shellfish farm on Porcher Island, Humpback Bay Oysters, was classified as *Open* for the harvesting of oysters by Fisheries and Oceans Canada. It is the first area to be classified as *Open* under the North Coast Water Quality & Biotoxin Program. Humpback Bay was surveyed by Environment Canada in 1998, and has been conducting regular biotoxin and phytoplankton monitoring since 1999.

Northern Laboratories Ltd., based in Prince Rupert, is the only lab in Canada which has been approved to both: 1) prepare PSP and ASP extracts for the Canadian Food Inspection Agency, and 2) process fecal coliform tests for Environment Canada. Biotoxin samples must reach the lab within 24 hours of collection, and water samples must reach the lab within six hours of collection. Given the remote location of most of the North Coast communities participating in the North Coast Water Quality & Biotoxin Program, this local laboratory capacity is vital.

The North Coast Water Quality & Biotoxin Program has been involved in investigating a number of alternative testing and monitoring technologies for PSP, ASP and DSP. These include Jellett Rapid Testing Ltd.'s MIST Quanti and MIST ALERT tests for PSP, satellite algal bloom data, and phytoplankton monitoring. Over the past three years, a series of studies have been conducted in conjunction with Northern Laboratories Ltd., Jellett Rapid Testing Ltd., Ocean Ecology, the Shellfish Monitoring Project, the BC Shellfish Growers Association and the Canadian Food Inspection Agency.

Currently Dr. Barb Shaw, Ocean Ecology, continues to analyze regular phytoplankton samples and provide reports on the prevalence of *Alexandrium*, *Pseudonitzschia* and *Dinophysis* species at each of the six monitoring stations. The *Alexandrium* and *Pseudonitzschia* results are correlated with the Canadian Food Inspection Agency's PSP and ASP results. Phytoplankton monitoring is used as an early warning system for biotoxin blooms. The North Coast Water Quality & Biotoxin Program monitors for DSP this way, as the Canadian Food Inspection Agency does not test for DSP unless there is an illness reported.

In 2000, there were toxic PSP blooms recorded in the mussels at five of the monitoring stations: Birnie Island (max. 120 μg), Hogan Island (max. 120 μg), Trail Bay (max. 200 μg), Metlakatla Bay (max. 190 μg) and Humpback Bay (max. 350 μg). The action level for PSP in Canada is 80 μg per 100 g of tissue. These toxic PSP blooms lasted approximately five weeks. There were no detectable levels of domoic acid at any of the stations in 2000.

In 2001, there were toxic PSP blooms at five stations: Metlakatla Bay (max. 2900 μg), Humpback Bay (max. 700 μg), Freeman Pass (1300 μg), Bully Island (1800 μg) and Masset (1800 μg). Unfortunately, results were not available for stations in Work Channel during the summer months. Work Channel, in Lax Kw'alaams Territory, is the area where the highest levels of PSP (approximately 38,000 μg) have been recorded in Canada. In 2001, the PSP blooms lasted approximately eleven weeks.

CONCLUSION

Due to the hard work and cooperative efforts of participants in the North Coast Water Quality & Biotoxin Program, the food safety monitoring infrastructure necessary for commercial mariculture development and First Nations traditional shellfish harvesting is now in place on the North Coast of British Columbia, Canada. The first steps are underway to expand the shellfish industry on the North Coast. Over the next few years, the North Coast Water Quality & Biotoxin Program will continue to work with all stakeholders to build a

comprehensive monitoring, testing, research and educational program for the area.

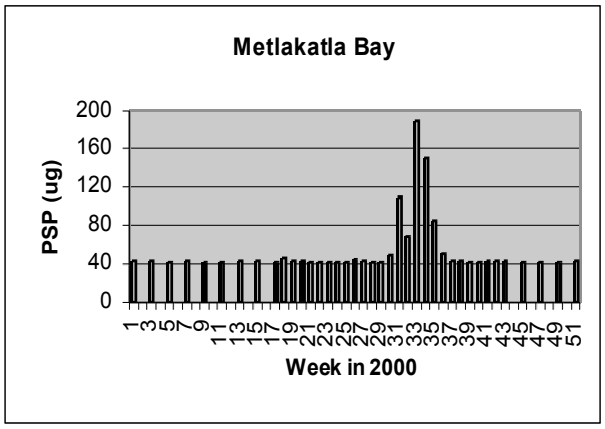

Figure 3. PSP levels at Metlakatla Bay monitoring station during 2000.

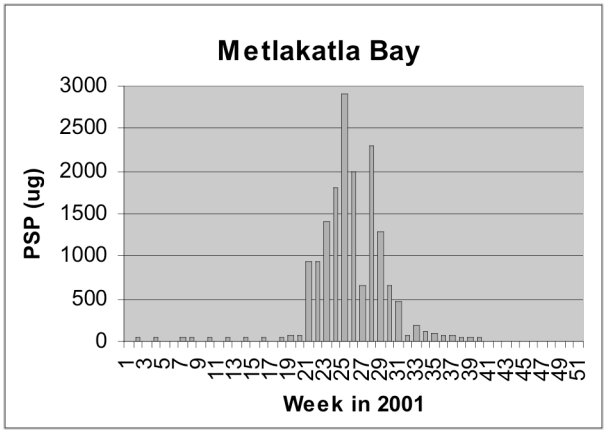

Figure 4. PSP levels at Metlakatla Bay monitoring station during 2001.

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HARMFUL ALGAL MONITORING PROGRAM IN URUGUAY

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ABSTRACT

The National Direction of Aquatic Resources established the harmful and shellfish toxicity monitoring program after the first paralytic shellfish poisoning (PSP) intoxication in 1980 where 60 persons were affected with neurotoxic symtomatology. Since then no human poisoning has been reported. The monitoring consists of harmful algae quantitative analysis and mouse bioassay for PSP, diarrhetic shellfish poisoning (DSP) and amnesic shellfish poisoning (ASP) in molluscs. Five fixed stations located along the Atlantic coast is monitoring with a frequency of 7-15 days. In case a discoloration is detected at any other places, training personnel from other institutions collaborate with the sampling.

The frequency of harmful algal bloom (HAB) events has increased since 1991. PSP, DSP, ASP and microcystin are registered so far in the Uruguayan coast. The species associated with toxic PSP events were *Alexandrium tamarense* and *Gymnodinium catenatum*. DSP events were associated with *Dinophysis acuminata* and *Dinophysis caudata*. Although several species of *Pseudo-nitzschia* genus are frequent in the plankton, the first report of ASP was in 2001 associated with *Pseudo-nitzschia multiseries*.

There are many reports of water discoloration produced by algal blooms, but most of them are harmless. Cyanobacteria blooms of *Microcystis aeroginosa* are frequent in the summer. This phenomenon caused public impacts, fish mortality and problems in drinking water. The authorities of the City Hall of Montevideo closed the beaches affected by the algae and warned about harmful effects. The National Direction of Aquatic Resources authorities recommended not to consume fish in affected areas.

In Uruguay, the algal blooms impacted the commercialisation of bivalve molluscs (*Mytilus edulis, Donax hanleyanus* and *Mesodesma mactroides*), public health and tourism, especially during summer time.

MANAGEMENT AND MITIGATION

The first intoxication report of people becoming sick by a toxic phytoplankton outbreak dates back to 1980 [1]. Since that time a program to monitor plankton and toxicity in molluscs has been carried out by National Direction of Aquatic Resources [2, 3], and it has so far prevented any intoxication. This program has allowed detection of toxic blooms and toxicity in shellfish and as a consequence the closure of harvesting and commercialisation of bivalve molluscs [4].

The monitoring consists of harmful algae quantitative analysis and mouse bioassay with wild molluscs from five fixed stations located along the coast. Samples for qualitative purposes are collected with a 25-µm mesh plankton net and those for quantitative analysis of phytoplankton are counted following the Utermöhl method [5]. PSP in molluscs is determined according to the AOAC method [6], DSP according to a modified Yasumoto method [7] and ASP toxins by the standard high performance liquid chromatography (HPLC) method [6]. Species monitored include mussels (*Mytilus edulis*), clams (*Mesodesma mactroides* and *Pitar rostrata*), cockles (*Donax hanleyanus*), scallops (*Zigochlamys patagonica*) and gastropods (*Zidona dufresnei*). Monitoring stations (Piriápolis, Punta del Este, La Paloma, Arachania and Punta del Diablo) are sampled with a frequency of 7-15 days. Besides, three offshore areas are sampled depending on the enterprise requirement (Fig. 1). Likewise, temperature, salinity and water color are registered.

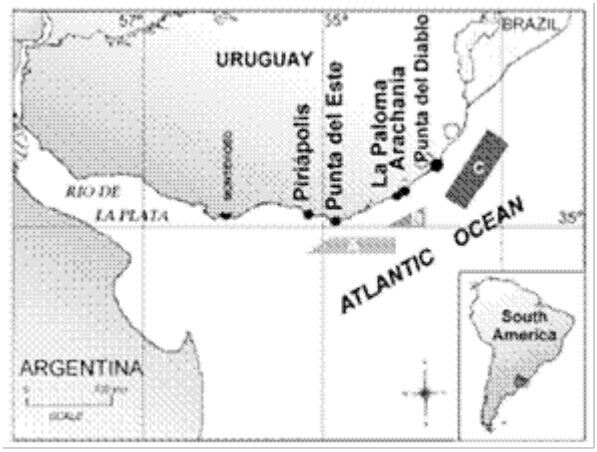

Figure 1. Map of Uruguay showing the sampling stations and offshore clams recollection areas (A, B, and C).

HOW THE HAB CONTROL SYSTEM WORKS

Figure 2 shows how the HAB control system works in Uruguay. The National Direction of Aquatic Resources (DINARA) is responsible for both plankton and toxicity monitoring, and the Air-Naval Military Base, Local Prefecture (Marine Police), Punta del Este and La Paloma DINARA Bases and artesanal fishermen collaborate when necessary in the detection and sampling of water discolorations.

When PSP levels are up to 80 µg STX eq 100 g^{-1} tissue or DSP assays give positive results, a mollusc ban is implemented. Public Health Authorities, the Toxicity Centre and Local Prefectures are immediately informed, and a press release is made.

During summer 2001 another ban was imposed only with a bloom of *G. catenatum,* although PSP levels were incipient. This is an example of foreseen capacity of the plankton monitoring in order to prevent public heath problems [8].

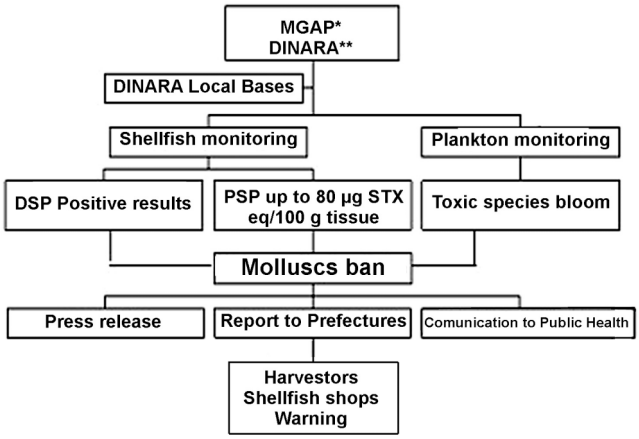

Figure 2. How the HAB monitoring program works in Uruguay. *MGAP- Cattle, Agriculture and Fishing Ministry. **DINARA National Direction of Aquatic Resources.

MONITORING RESULTS

Although the monitoring system has operated since 1980, the frequency of HAB events has increased since 1991. The poisons registered are PSP, DSP, and ASP toxins and microcystin. *Gymnodonium catenatum* and *Alexandrium tamarense* are the PSP causative dinoflagellates, while *Dinophysis acuminata* and *D. caudata* are the species associated with DSP events. ASP events were linked to the presence of *Pseudo-nitzschia multiseries*. As a consequence, several ban periods were imposed and the commercialisation of molluscs was prohibited [8].

SPECIES PRODUCING PSP

Chronology of toxic outbreaks from 1980 to 1998 is shown in Table 1, including information of maximum toxicity during toxic events, the associated species, and maximum bloom density. *Gymnodinium catenatum* is associated with PSP in summer (from January to April) while *Alexandrium tamarense* blooms often occur in early spring (from August to October); their toxin production has been confirmed by HPLC analysis [9].

The highest PSP report was associated with an *A. tamarense* bloom with 8285 µg STX eq 100 g^{-1} tissue during spring 1991 [2, 3]. The phytoplankton community composition and the environmental factors of this event were described by Brazeiro *et al.* [10]. This *A. tamarense* outbreak in 1991 coincided with the occurrence of a stratified water column, a high atmospheric pressure system with high air temperature and calm winds. On this occasion the fishing and commercialisation of molluscs were prohibited for two months. A bloom of *A. tamarense* is frequently reported in early spring in this area.

Table 1. Toxic PSP events off the Uruguayan coast from 1980 to 2001. n/d = not determined; * indicates PSP detection without ban.

Locality	**Toxic period (d/m/y)**	**Max. Toxicity µg STX eq 100 g^{-1} tissue**		**Sp. associated**	**Max. cells L^{-1}**
		M. Edulis	*D. hanleyanus*		
La Paloma	16/02/80 15/08/80	1250	n/d	*Gonyaulax* sp.	n/d
Pta. del Este	27/08/91 15/10/91	8285	n/d	*A.tamarense*	31000
P. del Este La Paloma	17/02/92 16/03/92	387 n/d	n/d 1478	*A. fraterculus* G. catenatum	40000 93000
La Paloma	03/10/92 27/10/92	n/d	97.6	*A. tamarense*	1800
Pta. del Este La Paloma	12/03/93 13/04/93	290 n/d	n/d 150	*G. catenatum*	10400 26000
Pta. del Este	05/08/93 24/09/93	157.6	n/d	*A. tamarense*	34500
Pta. del Este	25/02/94 17/03/94	125	n/d	*G. catenatum*	3600
La Paloma	6/12/95	n/d	73*	*A.tamarense*	440
Pta. del Este	14/02/96	67.3*	n/d	*G. catenatum*	24000
La Paloma	23/04/96	60*	n/d	*G. catenatum*	4800
Pta. del Este La Paloma	14/08/96 23/10/96	473.5 n/d	n/d 940	*A. tamarense*	16360 80325
Pta. del Diablo	17/3/98 23/4/98	167	n/d	G. catenatum	221000
Pta. del Este	23/02/01 05/03/01			*G. catenatum*	69000

Distribution and abundance of this species in this area during the spring periods of 1991-1994 were described by Méndez *et al.* [11]. This study suggested that the coastal influence of the Malvinas Current front displacement to the north with high frontal strength and the permanence of

the frontal position at the beginning of spring regulate the development of this species. The river discharge retrenchment during dry periods flows salty waters into the Río de la Plata estuary. Blooms of *A. tamarense* have been associated with the presence of a frontal system also in Argentina [12].

PSP outbreaks in Uruguay were associated with *Gymnodinium catenatum* during summer time (January to April). A bloom of *Gymnodinium catenatum* reached a maximum density of 2.0 x 10^5 cells L^{-1} in the east station, but the maximum toxicity reached was 1500 µg STX eq 100 g^{-1} at an abundance of 93000 cells L^{-1} [13]. In coastal sediments high cyst densities of this species were also reported [14]. These cysts showed vitality and quick germination. The hypothesis regarding the initiation of toxic blooms in the Río de la Plata of *Gymnodinium catenatum* might be an endemic problem and its outbreak initiation is due to cyst resuspension from the bottom, unlike *A. tamarense* which is likely to be transported to the area by southern currents.

SPECIES PRODUCING DSP

Several species potentially produce DSP such as *D. acuminata, D. caudata*, *D. sacculus*, *D. acuta*, *D. rotundata* and they have been reported in the area during the whole year. However, DSP in molluscs was only reported associated with *D. acuminata* and *D. caudata* blooms [15]. The highest cell concentration of *D. acuminata* occurred during summer 1996 with a maximum of 15,000 cells L^{-1}, but no DSP bioassay was done during this bloom. However, positive results in mouse bioassay were obtained when cell concentrations exceeded 80 cells L^{-1} (Table 2).

Mass mortality of yellow clams, *Mesodesma mactroides* and clockles *Donax hanleyanus* occurred in the eastern coast (Chuy; Fig. 1) consistently with DSP positive results associated with *D. acuminata* and *D. caudata* [15]. The same massive mortality happened in Brazilian southern beaches and in Argentina [16, 17]. It is not clear the relation between the toxicity and the mortality.

SPECIES PRODUCING ASP

Several ASP producing species of *Pseudo-nitzschia* group (*P. australis*, *P. delicatissima, P. pungens* and *P. seriata*) have been previously reported in the Uruguayan waters [19, 20, 21]. But the first report of ASP was in December of 2001 associated with the presence of *P. multiseries* [22].

CYANOBACTERIA SPECIES

Cyanobacteria blooms of *Microcystis aeroginosa* are frequent in summer time. The bloom is formed in fresh water and overcomes the whole Río de la Plata estuary. In summer 2001, a very extensive *Microcystis aeruginosa* bloom in different aquatic systems and in the coast of Río de la Plata was reported [23]. The microcystin-containing bloom of the cyanobacterium *Microcystis aeruginosa* was analysed, showing low levels of 155 µg g^{-1} in the sample [24]. This episode was accompanied by other cyanobacteria species: *M. novacekii*, *M. flos-aquae*, *M. wessenbergii*, *Anabaena circinalis* and *A. spiroides*. This phenomenon caused public impacts, fish mortality and problems in drinking water. Many people had skin problems, however none were reported to the Public Health authorities or to the Toxicity centre. The authorities of the City Hall of Montevideo closed the beaches affected by the algae and warned about harmful effects. The National Direction of Aquatic Resources authorities recommended not to consume fish in affected areas [23].

Blooms of the cyanobacterium *Trichodesmium erythraeum* were frequent in summertime in the Uruguayan coast, but no specific toxicity test was performed [25].

Table 2. DSP positive results off the Uruguayan coast from 1992 to 1996.

Locality	Date (d/m/y)	Mollusc	Species associated	Density (cells L^{-1})
Piriápolis	7/02/92	*Mytilus edulis*	*D. acuminata*	1500
P. del Este	7/02/92	*M. edulis*	*D. acuminata* *D. caudata*	4500 40
La Paloma	7/02/92	*M. edulis*	*D. acuminata*	2000
P. del Diablo	21/01/92 07/02/92	*M. edulis*	*D.acuminata*	4480
Chuy	13/12/94	*M. mactroides*	*D. acuminata* *D. caudata*	3560 4600
La Paloma	16/02/96 08/03/96	*Donax hanleyanus*	*D. acuminata*	80
La Paloma	18/02/96	*Donax hanleyanus*	*D. acuminata*	80

NON-HARMFUL SPECIES

There are many reports of water discoloration produced by algal blooms, but most of these blooms are harmless. *Gymnodinium sanguineum, Noctiluca scintillans, Polykrikos kofoidii, P. schwartzii, Prorocentrum minimum, P. scutellum, Scrippsiella trochoidea, Asterionellopsis glacialis, Skeletonema costatum, Trichodesmium erythraeum* and *Mesodinium rubrum* are frequent along the Río de La Plata fluvio-marin system and the ocean coast and often form blooms that discolour the water [25].

ECONOMIC IMPACTS

In Uruguay, the algal blooms economically impact the commercialisation of bivalve molluscs and fish, public health, and tourism. Additionally, there is a cost associated

with monitoring and management. The annual cost of monitoring and management is set at US$ 36,300.00, including the transport, scientific and technical staff, sampling materials and laboratory expenses (Table 3). The economic value of the non-commercialised mussels during bans is about US$ 128,800.00, equivalent to 94 ton yr^{-1}. The economic loss in national fish commercialisation due to the social impact and consumption decrease during HABs is difficult to estimate but could reach 40% of loss in fish commercialisation during the molluscs bans. Public health economic impacts were not evaluated because no health problems occurred since the first time in 1980. Quick economic evaluations of the decrease in tourists at beaches due to *Microcystis* blooms indicated a loss of US$ 600,000.00 during a summer proliferation [26].

Table 3. Annual average economic impacts of HABs in Uruguay.

HAB Impacts	**Annual Costs (US$)**
Monitoring & Management	36,300
Shellfish Commercialisation	128,000
Tourism	765,100
Total	936,300

In Uruguay, the are small artisanal fishing communities established along the coast, whose yearly economical support depends on their harvest of mussels, cockles and clams in summer and the great economic direct impact is on the fisherman. In the export clams zone offshore, phycotoxins were not detected and the enterprise that exports to the European Union has not had problems in the export authorization.

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POSSIBILITIES FOR ENVIRONMENTAL AND BIOLOGICAL MONITORING OF HARMFUL ALGAL BLOOMS (HABS) IN THE PACIFIC COAST OF CENTRAL AND NORTH AMERICA

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ABSTRACT

The Pacific coast of Central and North America has been and still is impacted by the flourishing of microalgal populations known as harmful algal blooms (HABs). The organisms that have caused recent HAB episodes in the region include *Gymnodinium catenatum*, *Pyrodinium bahamense* var. *compressum*, and recently *Cochlodinium* cf. *catenatum*. In spite of the piling of effects on the human health, the economic activities and the environment, there is still a lack of information available on the subject. The augmented use of coastal zones for human activities is also paralleled by increased awareness of global climate changes. Thus, discriminating whether anthropogenic or natural phenomena, or both, are the major driving forces is not an easy task. The long-term data sets available for reduced regions, as well as some sporadic observations during notorious blooms, allowed us to heed major changes in the biodiversity and biogeography of HAB organisms in the region. The number of events, the area covered, the duration and frequency, the number of species blooming and the appearance of not previously reported harmful taxa are among the factors affected. The variables more clearly related to these dynamic phenomena seem to be sea surface temperature and wind force; however, to date it is not possible to weight its contribution. The effect of rain has not yet been fully evaluated. The collaborative communication among monitoring groups in the region resulted in the wide spread knowledge of peaking concentrations of HAB organisms and toxins, thereby diminishing the risk of poisoning.

INTRODUCTION

Even though harmful algal blooms (HABs) have occurred long before humans started to heavily modify the coastal ecosystem, information compiled in recent decades on the regions affected by human events has shown there has been an increase in the effects of HABs [7, 8]. The problem is far from being delimited and is serious. The impact of HABs goes farther than economic losses and human health problems. When HABs contaminate or destroy coastal resources, the survival of local residents is threatened, and sustainability of biological populations, including humans, is heavily compromised. Clearly, there is a need for developing effective answers to cope with HABs through management and mitigation. These require the knowledge of the factors that control distribution and population dynamics of microalgae species reputed to be harmful, and their interactions with other components of the ecosystem.

Tuna, anchovy, sardines, and herring, shrimp and associated fauna, squid and bivalve mollusks, and sharks are among the most profitable fisheries along the Pacific Central American Coastal Large Marine Ecosystem (PCAC-LME) [1]. The high demand on shellfish has forced price rises in the market. These conditions are ideal for the development of profitable aquaculture activities. Profitable is not the same than sustainable, and currently aquaculture and fisheries are jeopardized by "natural" events that have highlighted the risk of collapse. Two HAB related phenomena have seriously impacted fisheries and aquaculture globally; mass mortalities of organisms (both in culture and under natural conditions) caused by red tides, and the accumulation of toxins and other by-products when the organisms feed on toxic microalgae, making the products unsuitable for food.

Scientific interests on HABs have increased recently. Health problems and economic losses in the coastal zones are important issues. New interests have arisen related to the apparent increase on the number, frequency, and intensity of HABs in the coastal zones worldwide [2]. The effects of global change, interannual (ENSO) or interdecadal (DPO), affecting distribution and composition of marine populations have also influenced HABs [2, 3]. Several countries have used this approach to understand the problem, incorporating biological end environmental monitoring to study the relationships between HABs and environmental variability. Developing predictive capabilities will allow the establishment of adequate preventive and corrective measures.

The first documented report of a shellfish poisoning event in the PCAC LME dates from 1976 [4], with seven cases and two casualties. Ochoa et al. [5] and Sierra-Beltrán et al. [6] reviewed the field and emphasized the need for wide and permanent monitoring to avoid and reduce the impact of such events. Worldwide there is no evaluation of the impacts caused by such phenomena that is trusted, and consequently, funds are scarce. Part of the problem is because of limited systematic monitoring and part to the lack of experts and trained personnel to develop such activities. Because HABs are a constituent part of the ecosystem, there is the need for an interdisciplinary approach to cope with these issues: SCOR-IOC, 1998 [7], GEOHAB Science Plan, 2001[8]. The aim of this study is to evaluate the possible relationship among environmental variables and HAB occurrences as an aid to develop predictive capabilities in the PCAC-LME region.

METHODS, RESULTS AND DISCUSSION

Though the northern boundary of the PCAC LME is in Cabo Corrientes (about 20°N latitude), in this paper we discuss events at slightly higher latitudes because of their importance and proposed participation in the introduction of species into new regions. The intensity of human activities developed within the BACs allowed for some degree of record or observation when evident events occurred. In the countries along the Pacific Central American Coastal LME (PCAC-LME), most of the activities related to HAB monitoring are traced back to human poisoning events, then, US Food and Drug Administration (FDA) recommended the countries to establish activities similar to those of the National Shellfish Sanitary Program (NSSP). It is evident that those activities are far from efficient because they remain only at few locations, where bivalve mollusks are cultured for export to northern markets making the monitoring a profitable investment. Most of the coastal zones are unattended in a region where "the inhabitants belong to the most impoverished segment of the population" [1]. Within the PCAC-LME, the participation and importance of the Biological Activity Centers (BACs) on the population dynamics of HABs is largely unknown. The absence of knowledge on the subject in the region is huge. This is not because of the absence of events, but due to the little attention focused on them. The data were obtained from point observations and a few permanent monitoring activities; In Mexico in Guaymas, Son. and Mazatlán, Sin. for 26 and 18 years [9] and in Acapulco, Gro. intermittently for ~25 years (Health Ministry, unpublished results), and in Costa Rica, in the Gulf of Nicoya, [10]. To our knowledge, the vast studies in Golfo Dulce, Nicaragua, with the aid of European countries, did not consider monitoring HABs as part of their ecological studies.

Between July 1927 and April 1999, on the Pacific coasts, from southern USA to Panama in Central America, at least 45 reports deal with HAB-related toxicity [11]. These events can be roughly classified in three categories; PSP, ASP, and Ciguatera. Additionally, there have been HAB events not related to mass poisoning but representing odd observations in the region (blooms of some cyanobacteria, *Gyrodinium impudicum*, *Dinophysis caudata*, *Cochlodinium sp*., etc.). Amnesic shellfish poisoning (ASP) caused by the toxic diatom *Pseudonitzchia australis* on the Pacific coasts of USA and northern México, including the Gulf of California (5 events), affected mainly animals (fish, birds, and marine mammals). Ciguatera events, poisoning of humans by consumption of carnivorous fish, occurred well above the described limits of the disease always after ENSO anomalies (4 events). Paralytic shellfish poisoning (PSP) of humans on the Pacific coasts (35 events) accounted for at least 500 cases. PSP is used here to exemplify the model proposed by us.

In southern Mexico (Guerrero, Oaxaca, and Chiapas), spring blooms were caused by *G. catenatum* (or *A. catenella* on one occasion) for 10 years since 1976. After the summer 1987, when the *Pyrodinium bahamense* var. *compressum* bloom occurred in Guatemala, recurrent events in autumn-winter developed along the coasts of Central America, up to the state of Guerrero, Mexico (18°N latitude) (Reviewed by Ochoa et al., [12-13] and Sierra-Beltrán et al., [6]). In spring 1999, with events starting in Manzanillo (19°N latitude) [14] conditions were optimal for the proliferation of *G. catenatum* in Acapulco displacing *P. bahamense* that did not bloom that year. In 2001, *P. bahamense var. compressum* occurred along the coasts from Costa Rica to Mexico, poisoning humans and causing fish mortalities. Even though both phytoplankton species are PSP producers, there are important biological and public health implications related to the presence of one or another as the causative species of a specific event. The general clinical image of PSP poisoning is the same as related to the neurothropism of the toxins, going from a mild poisoning to a severe one, even causing the death of the affected individual by respiratory arrest [4, 15–17]. The main difference is the time of the onset of the symptoms with an average of 4 hours for *G. catenatum* toxins, but only 1-2 hours for *P. bahamense* var. *compressum* toxins [4, 15–18]. This is explained by the specific toxic chemical mixture produced by each species showing clearly that *P. bahamense* var. *compressum* is more dangerous because of the reduced time for toxicity thus reducing the possibility of providing adequate assistance to the affected individual.

We attempted to relate HAB episodes of *G. catenatum*, *P. bahamense var. compressum* and *C. cf. catenatum* to coastal climate variability. For this, we used monthly means of sea surface temperature, zonal winds and meridional winds as provided by the NCEP/NCAR Reanalysis project [19-21], available from the WWW site of the NOAA-CIRES Climate Diagnostics Center (http://www.cdc.noaa.gov). From this set, series of monthly means were extracted for 25, 2.5º x 2.5º selected areas shown in Figure 1. Each monthly series was converted to anomalies and standardized, resulting in 25 monthly-standardized anomalies series spanning for 1975-2002. Figure 2 shows the anomaly series plotted as an evolutive diagram (i.e., contours for time vs. area.). The analysis of the available

environmental information together with HABs suggests some possible relationships:

a) The variability patterns in the region. For this region (6°S - 36°N), it is possible to recognize a warm band or pond between 13.5°N and 17.25°N, off Acapulco, Gro. During summer, it expands from northern Colombia up to Pto. Vallarta, Jal., Mexico (7.5°N - 22.5°N). In this region, looking directly to SST, it is possible to note the subtle effects of atmospheric phenomena (El Niño - La Niña), but it is clearer when the anomalies are analyzed (Figure 2). Positive anomaly in SST is stronger to the south of 17°N latitude (area-box 11th). Northward movement of the warm plume is weaker, net variability being evident only during the summer 1997 (ENSO effect). The events characterized by a decrease in sea surface temperature (negative anomaly) are clearly evident in both directions from the 17°N latitude (La Niña effect). In 1985 the warm water pond completely vanished, even off Acapulco. During 1988-1989 it drastically decreased in both directions, showing the same effect in 1996-1997. If anomalies are analyzed, El Niño and La Niña effects are evident in both directions from 17°N latitude.

b) The species alternance in the region. In the region of the BAC of Tehuantepec, on the south of the Mexican Pacific coast (area-boxes 9th & 10th), red tides have been infrequent phenomena. Acapulco has been inhabited for 500 years with no report of poisoning events there before 1976. It is possible to argue that the events in the region (Guerrero, Oaxaca, and Chiapas) started near 1976, when *Gymnodinium catenatum*, a temperate organism, produced several poisoning cases. More recent reports were associated with *Pyrodinium bahamense* var. *compressum*, a tropical organism. These changes in the phytoplankton community have important implications as reported for several other regions where, after the introduction of a species to a new ecological niche, it is able to adapt and survive in the new location even though the conditions that allowed for its arrival disappear as occurred for the introduction of *G. catenatum* in Australia and recently in New Zealand [3]. Also, *Prorocentrum lima,* a tropical organism, is well adapted to temperate conditions in Nova Scotia, Canada [22]. Another example in Mexico is the recent introduction of *Cochlodinium cf. polykricoides (=catenatum)* [23], which deposited cysts and started blooming again during 2001. This apparent pause in the occurrence in Acapulco, Oaxaca, Chiapas and Costa Rica (area-boxes 5th to 10th) of *P. bahamense* var. *compressum* blooms (a tropical species) and the resurgence of *G. catenatum* (a temperate species) during 1999, might be related to climate change effects like El Niño-La Niña.

c) The introduction of alien *P. bahamense* var. *compressum* in the region. In 1942, there appeared the only report of *P. bahamense* along Mexican Pacific coasts [24]; there is no later evidence of its presence on the Pacific coasts of America. The description in 1942 [24] does not report the presence of a very important conspicuous characteristic of *P. bahamense* var. *compressum,* the formation of long chains [25]. We suggest an error in classification of the sample, being instead, the non-chain forming species known now as *P. bahamense* var. *bahamense*. The first occurrence of the species in the PCAC-LME (Guatemala coast, July 1987 [16]) was immediately preceded by a strong ENSO (1983-1984) and a strong La Niña (1984-1985).

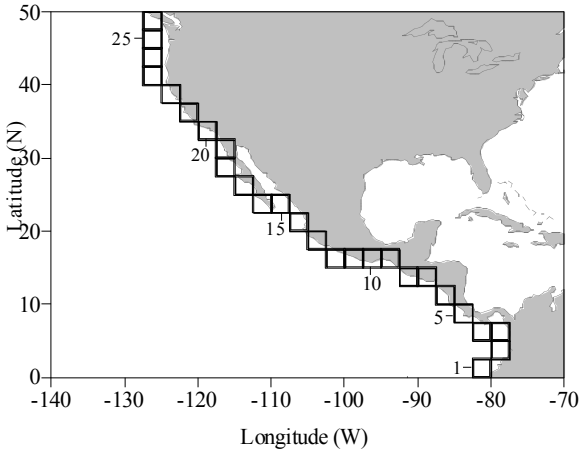

Figure 1. The coastal areas used for the analysis of sea surface temperature and the zonal and meridional components of surface wind speed. Areas are 2.5º x 2.5º, and were numbered poleward along the coast.

From the above information (a, b, and c), we propose that the toxicity events caused by *P. bahamense* var. c*ompressum* on the Pacific coast of America [4-6, 9, 16-18, 26-32] always occurred in the zone or band considered a warm pond, never in an anomalous zone produced by ENSO and, apparently, always preceded by a cold period immediately before its occurrence. Thus, the occurrence of the species *P. bahamense* var. *compressum* on the American coasts is the result of a recent and active transport processes. Likewise those in Southeast Asia, we suggested oceanic transport by water mass movements under the pressure of ENSO. The relaxation of the 1997-1998 ENSO allowed for the appearance of *G. catenatum* in the region displacing *P. bahamense* var. *compressum* from the north, whereas after the cold phase of 1999-2000, this "alien species" occurred again, blooming since winter 2000 in Costa Rica and spreading north when the current changes in spring 2001. Furthermore, its displacement northward to the Costa Rica oceanic gyre occurred when the ITCZ movement allowed the Costa Rica current to flow northwards along the coast. Because the data reported is based mainly on toxicity events instead of an active monitoring of phytoplankton, it is necessary to recognize that it is a minimum estimate of the presence of the species. In the absence of broad sets of data between events it is not possible to talk about the absence of the species. Indeed, cysts of *P. bahamense* were observed in Acapulco during 1999 and 2000, though no bloom of the species occurred during the *G. catenatum* bloom. But, we were not successful in trying to germinate those cysts in the laboratory and their viability is yet unknown.

When analyzing the data on wind stress (both sets Zonal and Meridional) very interesting observations can

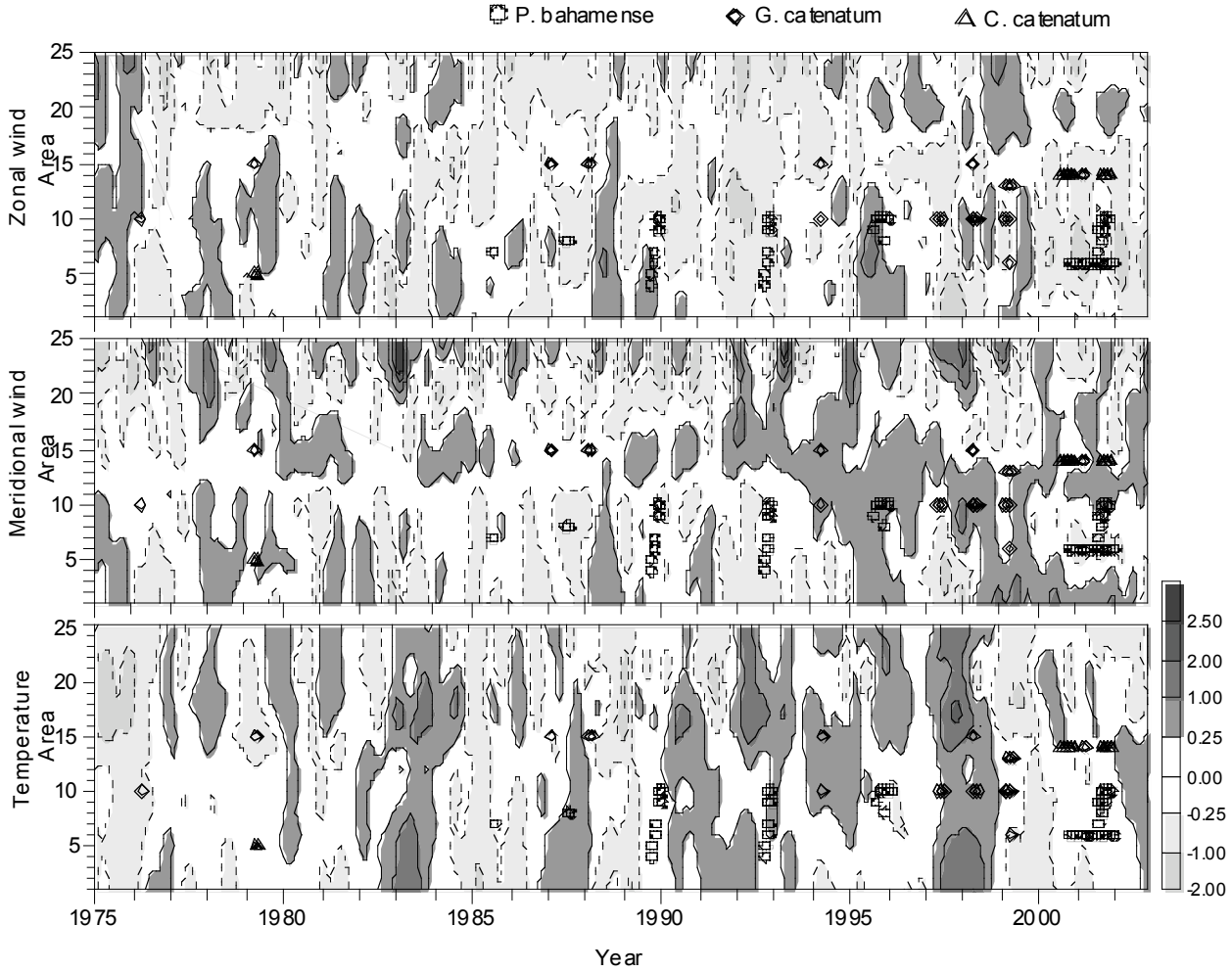

Figure 2. Presence of HABs (symbols and species shown on top) and the evolutive diagrams of the zonal winds (upper panel), meridional winds (middle panel) and sea surface temperature (lower panel) standardized anomalies. All negative contours are plotted with interrupted lines, and the zero-countour line was omitted. In all plots, the y-axis labels are the areas shown in Figure 1.

Table 1. Specific toxin composition determined by HPLC in the toxins accumulated in mollusks. Samples were obtained during major outbreaks in the Pacific coasts of América.

Place & year of event	**Toxin profile by HPLC & method used**	**Organism detected & possible producer of toxins**	**Reference**
Guatemala, 1987	GTX5, neoSAX, SAX. Sullivan´s method.	*Pyrodinium bahamense* var. *compressum*	Rosales-Loessener et al., 1989.
México & Guatemala, 1995	GTX5, GTX6, neo-SAX, SAX. Sullivan´s method. Lawrence´s method.	*Pyrodinium bahamense* var. *compressum*	Washington Seafood Laboratory, FDA, 1995. Sierra-Beltrán & Flores, 1999, (unpublished).
México, 1999	C1, C2, GTX6, GTX5, dcGTX2+3, GTX3, GTX2, neoSTX, dcSTX.	*Gymnodinium catenatum*	Sierra- Beltrán & Flores, 1999, (unpublished), Sierra-Beltrán & Franco, 2001, (unpublished).
Costa Rica, Guatemala & México, 2001	GTX6, GTX5, neoSTX, dcSTX, STX. Lawrence´s method & Franco´s method	*Pyrodinium bahamense* var. *compressum*	

Table 2. Latitudinal distribution of net toxicity monitoring by mouse bioassay during the recent *P. bahamense* var. *compressum* bloom, 2000-2001. X= result above 80 µg/100g limit. 0= negative.

Locality from South to North		**Jan-Feb**	**Mar-Apr**	**May-Jun**	**Jul-Aug**	**Sep-Oct**	**Nov-Dec**
Costa Rica	Golfo de Nicoya	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
Guatemala	Las Lisas				X	XX	
	Tecojate				X	X	
	Pto. San José				X	X	
	Champerico				X	X	
México	Chiapas, P. Madero				X	XX	
	Chiapas, Pijijiapan					XX	
	Oaxaca, sur					XXXX	XX
	Oaxaca, centro					XX	XX
	Oaxaca, norte					XX	XX
	Guerrero, sur						XXX
	Guerrero, centro						XXX
	Guerrero, norte						0000000
	Michoacán						0000000

Table 3. Events in Mazatlán, changes in duration, number of events and number of causative species along the last 22 years.

	1979-1989		1990-2000		TOTAL 22 years	
	average	total	average	total	average	total
Number of events	3.72	41	5.45	60	4.59	101
Length of event in days	7	186	14	357	10	543
Number of species	7		11		13	

be drawn. The regions where the displacements of the toxic species *G. catenatum* by *P. bahamense* var. *compressum* is more evident are characterized by the presence of very low energy in the winds (doldrums), both zonal and meridional as observed on the raw data (data not shown). When standardized anomalies (deviations from average) are observed, it is interesting to note that in both data sets (Zonal and Meridional) negative anomalies frequently preceded the occurrences of *P. bahamense* var. *compressum* blooms and they occur under low energy wind stress (Fig. 2). On the contrary, *G. catenatum* prefers lower temperatures associated with a slightly higher wind stress, mostly during negative anomalies (Fig. 2). It seems that this characteristic behavior of the species is closely related with the influence of the displacement of the Intertropical Convergence Zone (ITCZ) along the Pacific coast reaching its northward position during Summer- Autumn and its most southward displacement during Winter-Spring [1]. Toxin profiles of the contaminated mollusks (Table 1) as well as net toxicity results obtained during toxin monitoring along the Pacific coast (Table 2) provide data that strongly support the periodical changes on the dominant species as well as the limits for *P. bahamense* var. *compressum* colonization (Figs. 1 & 2).

Mazatlán and Guaymas [9, 32-35] deserve special attention. Located in the mouth of the Gulf of California, slightly to the north of Cabo Corrientes (the northern boundary of the PCAC LME), they also are influenced by the California Current. Red tides in the Gulf of California are common, mainly formed by the ciliate *Mesodinium rubrum*. Also common are *Noctiluca scintillans* and, recently, the cyanophyte *Oscillatoria erythraea*, a clear indicator of intrusion of tropical waters in the region [36]. Graham in 1943 described *Gymnodinium catenatum* as a consequence of a big bloom and it is considered the main toxic species since [37]. The changing conditions have allowed for the presence of other toxic species, either forming blooms or at densities considered harmful. Among them, in Bahía Concepción, there is *Dinophysis caudata* [38], *Alexandrium catenella*, *Prorocentrum minimum* [39], *Alexandrium tamiyavanichi* [6], *Pseudonitzchia australis* in the upper Gulf of California [6, 31, 40], and in Mazatlán *G. catenatum*, *P. minimum* and

Gymnodinium sanguineum [33, 41]. What is clear in Mazatlan, after more than 20 years of monitoring, is the rising number of events, frequency, duration, and number of species causing the blooms [34]. The data collected on 101 blooms during the last two decades showed that the increase observed on the last 7 years was not related to the years in which strong ENSOs occurred (Table 3). During the 1980s, the main season for HABs was the spring, whereas during the 1990s, big events also were evident in summer-autumn (Fig. 4). The average number of events per year increased from 3.72 to 5.45 while the duration increased from 7 to 14 days in 20 years. There were 7 harmful species in the 1980s, 11 species formed blooms during the 1990s (Table 3). We suggest the data demonstrated that the conditions favoring HABs have

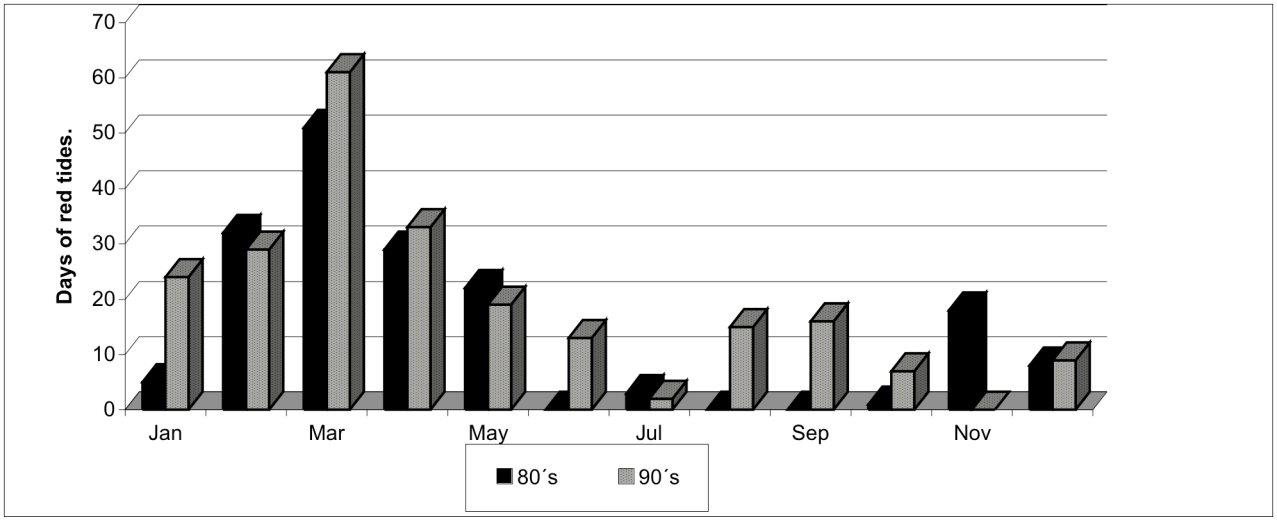

Figure 3. The events in Mazatlán in the last two decades (1980-2000) were dominated by dinoflagellates (1 cilliate vs 12 dinoflagellate species). Observations allowed for evidence of a change from a classic spring bloom (unimodal) to a biphasic Spring and Autumn blooms (bimodal).

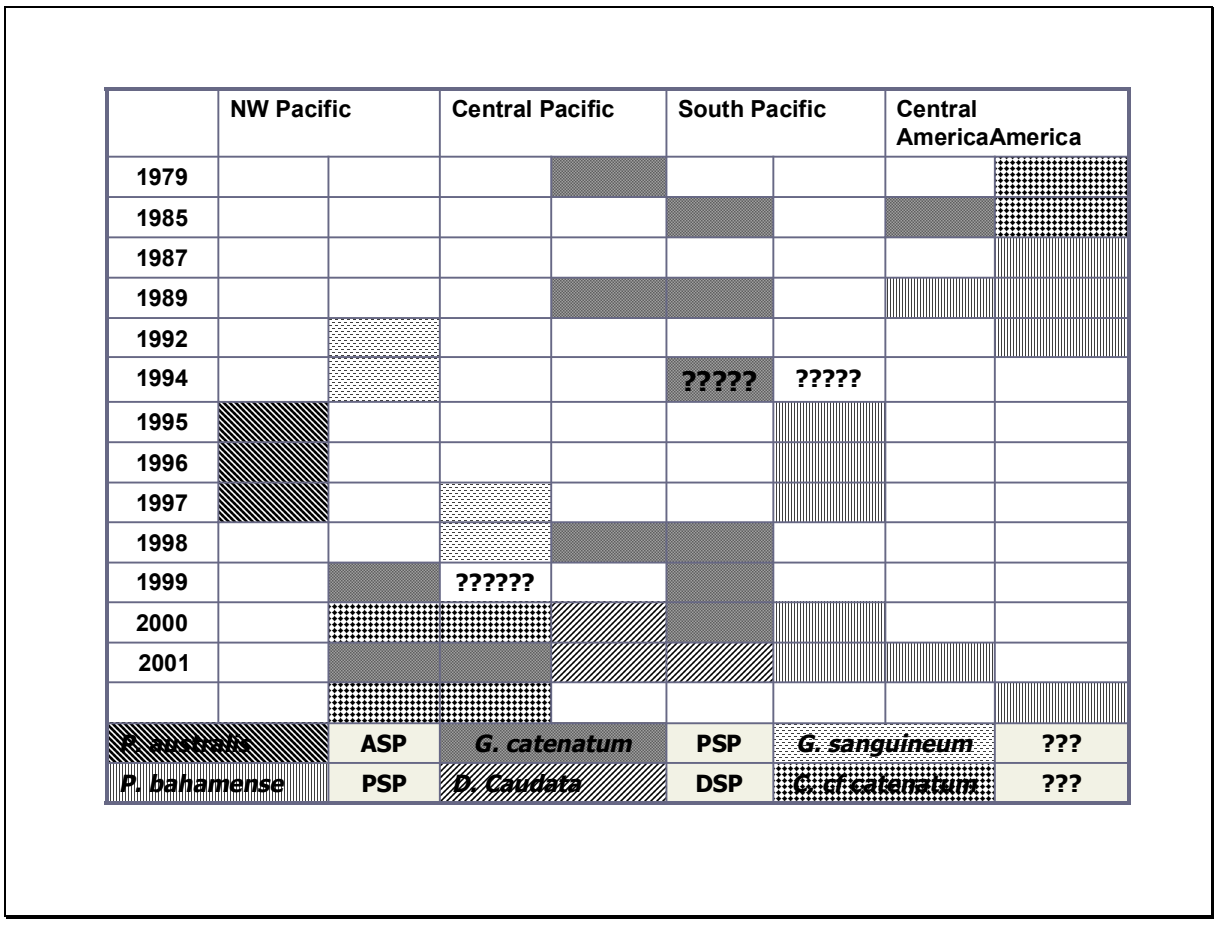

Figure 4. Square map of major events allowed for the identification of patterns of distribution: neighboring colonization like in *P. bahamense* var *compressum* or hierarchical spreading like in *C. cf. catenatum* species.

improved, thereby allowing for the establishment of new species, their development, and permanence in the region.

A similar pattern of displacement or colonization caused by warm water intrusion into temperate waters was recently identified for the species *Cochlodinium cf. polykrikoides (=catenatum).* The species, reported as *Cochlodinium catenatum,* bloomed in the Gulf of Nicoya, Costa Rica, at least since the spring of 1979 [42]. During 2000, it occurred for the first time on northern coasts in Pto. Vallarta (20°40´N latitude) in early August, two weeks after it bloomed in Mazatlan (23°N latitude) and again, two weeks later it reached La Paz (24°N latitude) across the Gulf of California [23]. During October-November, 2000 it bloomed again (intermittently) in Puerto Vallarta and in August 2001 was evident the resurging of the populations in La Paz. After the first bloom in La Paz, we checked for the presence of cysts in the bay during winter 2000 and spring 2001 and found live cysts as well as germinated ones. The severe September-October 2001 blooms along the Pacific coasts (20°-22°N) produced huge mass mortalities of fish on small bays and reefs.

The displacement of this species northward apparently does not follow the path of a discrete colonization of neighboring niches suggesting the participation of a strong anomalous event forcing the leap of the species to its new location in the form of a hierarchical colonization. This anomalous driving force seems to be the recent El Niño event of 1998 that forced huge masses of hot water northwards from the Equator. The analysis of the SST images provides a strong support to this idea (Fig. 2). Nevertheless, some other critical factors (increased nutrient loads, coastal modifications, etc.) may also play some role that deserves close follow up.

From the observed data and the literature, it is possible to speculate that a certain amount of forecast, regarding *P. bahamense* var. *compressum* blooms is possible. Furthermore, we can also speculate that this species has reached its northern limit in relation to spreading and colonization, since above 19°N, the wind driven energy in the water column is too intense to allow for the permanence of the species. It should be also noted that during the relapses of *P. bahamense* var. *compressum,* the niche will be rapidly occupied by *G. catenatum* being necessary the permanence of monitoring efforts in the region while, their frequency, can be modulated during the year with the aid of forecasting.

Unfortunately, the information obtained to date, even though it supports the model proposed, did not allow for the weighting of the variables because of the low density of observations (Fig. 3). Also, the influence of smaller scale oceanographic phenomena such as salinity plumes, oceanic gyres and upwelling sites deserves ample studies. In the absence of a permanent monitoring of biological, environmental, and oceanographic data, it will not be possible to improve our forecasting abilities.

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DESIGN AND IMPLEMENTATION OF MONITORING PROGRAMME ON HARMFUL ALGAL BLOOMS

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ABSTRACT

Harmful algal blooms (HABs) are an important environmental problem in coastal areas and may do great harm to society and economy. There are no unified planning and implementing methods for surveying and monitoring harmful algal blooms, especially in China. Here is a proposal for the task.

INTRODUCTION

Harmful algal blooms (HABs) can be defined as an ecological abnormal phenomenon, where the concentration of one or several algae or zooplankton reach elevated levels, causing changes in the color of seawater, environmental abnormalities, causing harm or death to other marine organisms. Fish, shrimp or shellfish may accumulate algal toxins and become toxic to human consumers. Harmful algal blooms may cause economic loss to mariculture, fisheries, and tourism, in addition to the environment and human health.

Blooms of algae are natural phenomena that occur in upwelling areas and estuaries, where a combination of enrichment (Nitrogen and Phosphorus) and physical conditions can result in high concentrations of algae. They also occur in freshwater and oceanic waters and, depending on the algae and the location, different factors are responsible for bloom initiation.

There are more than 80 genus of marine red-tide organisms, most of them are phytoplanktons, others include few germs and protozoa. More than 5000 species of phytoplankton are present in oceans, among them 83 species have the latent toxins, most of them belong to Pyrrophyta. There are more than 30 genus HAB species about 100 species and 10 toxic in China [1-5].

PRINCIPLE OF DESIGN

The design of a monitoring programme on red tide should start by defining the kind of information that is needed to protect the special resource. This should be done in close cooperation between the users of the results of the monitoring and the authorities, institutions, and aquaculture companies involved in the monitoring and evaluation of monitoring results [6, 7] so that the evolution up-trend should be forecasted and evaluated as well as some management steps be carried out to decrease the damages of harmful algal blooms. Also one of the first steps is to define clearly what the problems in the region are. What species form blooms in the area? During what time of year do they occur? What are the impacts of those blooms? What appears to cause/lead to bloom initiation?

There are some differences of HAB monitoring programmes between various monitoring systems. For example, the programme designed for mariculture would ensure an early warning system, identification of the species of blooms and the harmful effects to be expected. The monitoring of HABs should be continued until the risk of any harm passed and some management steps must be carried out to decrease the damages. However, we should not only monitor during blooms. The background conditions must be prepared adequately in order to forecast and know when a bloom is starting. For the marine ecosystem, the programme could assist in preventing marine ecosystem damage. Again it would provide an early warning system, indication of species and the harmful effects to be expected. In addition, the quarantine concept of ballast water monitoring should be introduced to prevent non-indigenous species damaging marine ecosystems. And for fisheries, a monitoring program for shellfish or fish would be a warning that certain toxic algal species could introduce toxins to seafood. The programme may show the need to close some certain fisheries for fish or shellfish to prevent toxic fish or shellfish entering the market. We also need to confirm is there nothing in place at all in this area currently to close shellfish beds when they are toxic? A little information about how things currently operate would be useful.

The monitoring system should allow for exchange of information between agencies and ensure that the effects by further HAB's outbreaks are reduced.

MONITORING SYSTEM AND PLAN FRAME

See Figure 1.

THE CONTENTS INTO PRACTICE *IN SITU*

Once the programme has been designed the challenge is to implement it. The monitoring plan should include monitoring data of remote sensing, which can supply large scale information, field information on environmental conditions (hydrology, water quality) and species isolated. The bloom scale and its development may be explained by salted career man. In China, satellite remote sensing data can be gained from SeaWifs, FengYun, SPOT and NOAA.

HAB monitoring goals means monitoring the occurrence of harmful algae to prevent algal toxins from reaching the human consumers to minimize damage to living resources such as shellfish and fish, as well as economic loss [6].

The Station and Item of Monitoring

A. Principle

The number and site selection of monitoring stations should be based on where problems are known to occur, regions where blooms pose the largest threat, and regions that would provide more information about how/why blooms develop. This means a monitoring progamme involves a plan for sampling in time and space (i.e., definition of sampling areas or stations and sampling frequency, algal sampling, identification and quantification of algal species).

B. Methods

Considering synthetically the scope of harmful algal blooms, excursion state, ocean current, tide and wind, the monitoring stations would be setup equally and one or more comparison stations should be setup outside bloom area.

The estimation of harmful algal blooms scope (sub-area statistics when gathered)

A. Aviation remote sensing

Calculate according to the aviation photographs and flight parameters by marine monitoring planes of State Oceanic Administration (SOA).

B. Satellite remote sensing

Satellite images would give more global insight into distribution and movement of water mass and biomass of algae in the surface water [6]. We can calculate according to satellite photographs from SeaWiFs, FengYun and HaiYang-1 satellites.

C. Ships cruises

Estimate according to the track and orientation of ships from SOA or others.

D. Fixed-point observation

Estimate according to the symbols.

Table 1. Suggested parameters for harmful algal blooms monitoring plan.

Item	Parameter [8]
Observation *in situ*	Scope, Photographs, Sensory observations
Meteorology	Atmospheric pressure, Wind speed, Wind direction, Air temperature, Precipitation rain fall, Sunlight, Light attenuation
Hydrology	Salinity, Water temperature, Water color, Transparency, Current speed and direction, Wave amplitude, Run-off
Phytoplankton	Species composition, Toxic species, Abundance, Chlorophyll a, *Primary productivity
Zooplankton	Species composition, Abundance, Biomass
***Cysts**	Species composition, Abundance, Bourgeon test
Toxins	Phycotoxin, Shellfish poison (PSP, DSP, ASP, NSP, CFP)
Other Organism	Protozoaplankton, Pelagic bacteria, *Fish, *Benthos, *Birds
Chemical parameter	pH value, Salt concentration, Dissolved oxygen, *Chemistry oxygen demand, Nitrate, Nitrite, Ammonia, Phosphorus, *Silicate, *Fe, *Mn

***** indicates not necessary

TRANSFER AND MANAGEMENT OF HARMFUL ALGAL BLOOM INFORMATION

Information Transfer and Feedback

Once harmful algae blooms develop somewhere, the in situ circumstances should be reported to SOA and NMEMC by communication, network, etc. as soon as possible. The primitive data must be assembled (paragraphs, documents and monitoring data report forms) for inquiring. In addition, disaster evaluation and economic loss estimates should be referred. At last, monitoring results and the treating scheme of HABs must feedback to all or different levers of management departments along with other departments as aquaculture products, AQSIQ and sanitation epidemic prevention institution along coast.

Standard of Judgment and Evaluation of Harmful Algal Blooms

There is no general rule to define harmful concentrations of cells in an algae bloom; the concentration in a HAB is species-specific [6]. In general, we often use some indexes as standards of judgment and evaluation of harmful algal blooms. For example, **Sense Index:** the color of seawater, smell, flavor and transparency, etc. **Biomass Index:** species, size and density of harmful algae in plankton community. **Nourishment quantity Index**: single and synthesize index used as evaluate guideline for eutrophication. **Toxin Index**: HABs toxins and poison.

Disaster Evaluation Caused by Harmful Algal Blooms

We can evaluate the disaster caused by harmful algae blooms from economic losses of fishery, mariculture, tourism, human health, etc.

Prevention and Cure Countermeasures of Harmful Algal Blooms

The purpose for HAB monitoring is to prevent and cure their endangerment. Several techniques may be used as follows: **Biological methods:** Restrain the evolution of harmful algal blooms by the choosing certain microorganisms or zooplankton which may be inhibiting the growth of algae. **Chemical methods:** Some chemical reagents are chosen which may be very useful to restrain algae but they have no social effects of pollution to the environment. **Physical methods:** Such as broadcast sowing kaolinite clay/Guard fence methods/Oxygen increasing methods. **Evitable:** Transferred cultural instruments in harmful algal blooms to safe regions. **Toxins removing:** Adopt depurate breeding or ozone treatment to cleanup the toxins concentrated in molluscs rapidly.

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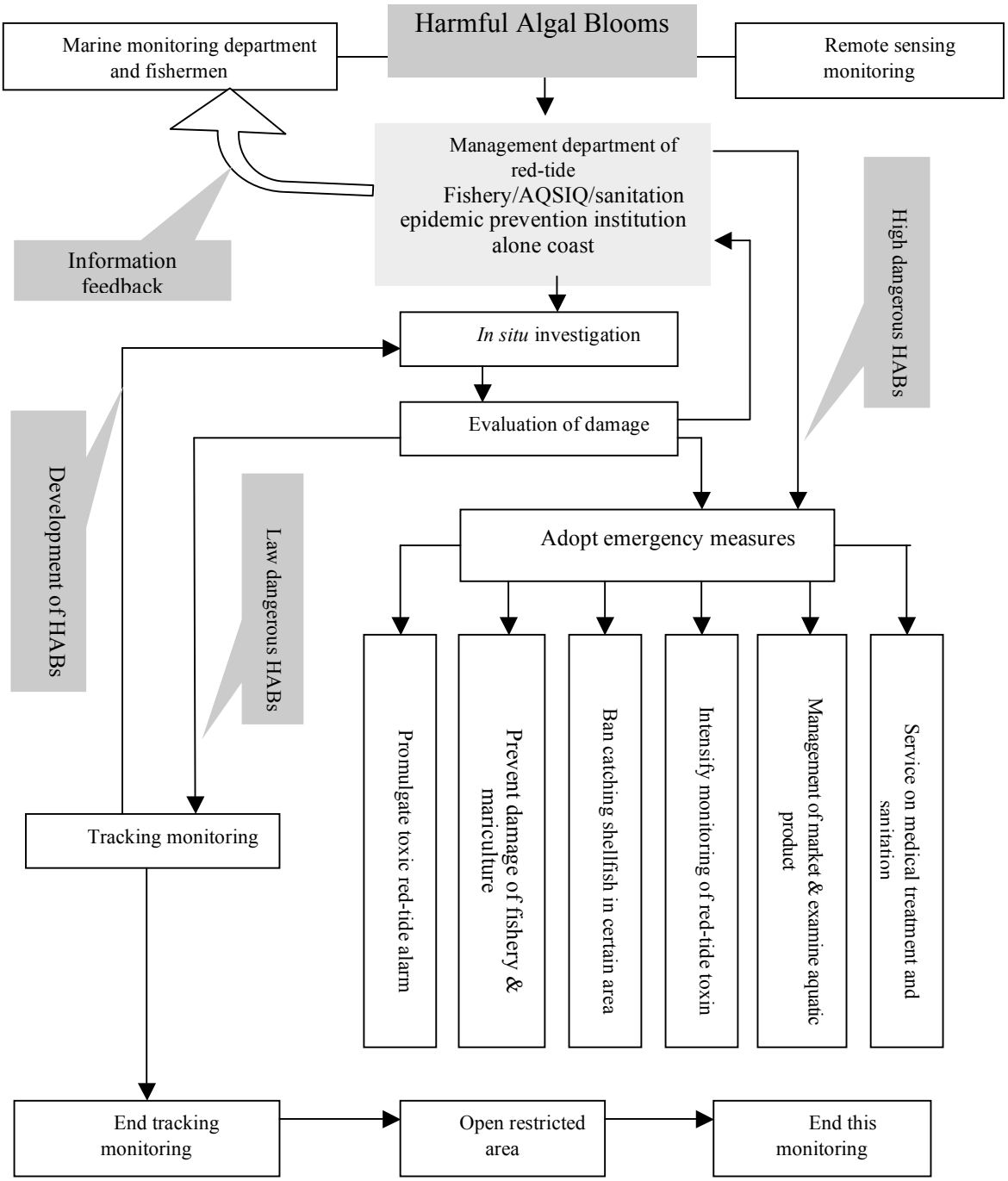

Figure 1. Proposed monitoring system for harmful algal blooms.

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Asia-Pacific Economic Coooperation (Singapore): APEC Publication #204-MR-04.2

TOXIN DETECTION

MARINE BIOTOXIN MONITORING OF NEW ZEALAND SHELLFISH - A NEW MANAGEMENT PROGRAMME BASED ON LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY (LC-MS)

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ABSTRACT

Marine biotoxin monitoring in New Zealand combines regular shellfish flesh testing and phytoplankton monitoring to manage shellfish harvesting and mitigate the risks associated with harmful algal blooms. A new programme is being developed to provide data that is more accurate, available in a shorter time and obtained without the use of mouse bioassays. Progress has been made towards implementation of this programme using test methods based on LC-MS to provide chemical analytical data for individual toxins in place of bioassay screen test results. Cawthron has developed and validated two new LC-MS methods for toxins in shellfish. One method determines ASPs (domoic acid and isomers) while the other provides for highly sensitive and specific detection of ASP, DSP and other lipophilic toxins (17 toxins and metabolites). Issues are discussed relating to the development and implementation of new test methods including method validation, regulatory limits, availability of reference materials, comparisons to existing tests, type of instrumentation and international cooperation.

INTRODUCTION

Current regulation in New Zealand of marine biotoxins in shellfish harvested commercially or in recreational shellfish stocks is mainly by assessment of toxicity through mouse bioassays. These tests are complemented where possible by phytoplankton monitoring in water samples to provide early warning of potential toxic events. These monitoring programmes are fully funded by the shellfish industry and Ministry of Health, respectively. All the principal toxin groups have been associated with shellfish toxicities in New Zealand over the past decade including amnesic (ASP), diarrhetic (DSP) paralytic (PSP) and neurotoxic (NSP) shellfish poisoning plus pectenotoxins and yessotoxins. NSPs have only been implicated in one incident and ASPs have rarely approached regulatory limits. Currently, the Cawthron Institute is responsible for more then 95% of the analyses associated with routine monitoring of shellfish and water samples in New Zealand, amounting to some 6000 and 3500 tests per annum, respectively.

The dependence on mouse bioassays for marine biotoxin detection has a number of drawbacks: 1) little information is provided on toxin composition; 2) the toxicity from intra-peritoneal dosing may bear little relation to oral toxicity; 3) they can be difficult to implement for high-throughput, fast turnaround testing; 4) the use of animals for routine testing of food is increasingly unacceptable; 5) results are variable and affected by strain of mice, non-biotoxin components in shellfish extracts and other factors; 6) the sensitivity can be marginal and therefore information is often lacking on the build-up towards toxic events or on subsequent depuration.

The international trend is towards use of *in-vitro* assays or target analyte chemical analyses based on immunoassay or chromatographic techniques [1]. Research and development in these fields has provided a number of suitable technologies for marine biotoxins. ASP toxins are routinely tested by methods based on HPLC with UV detection [2]. Of particular interest has been the development of multi-toxin methods based on LC-MS because of their high sensitivity and specificity.

LC-MS is a well-accepted technology for quantitative determination of pesticides, drugs, environmental contaminants and their metabolites in biological matrices. The advantages of LC-MS are also recognised in the field of marine biotoxins and it has become an essential research tool for advancing knowledge in this field [3]. LC-MS methods for ASP toxins [4, 5] and DSP toxins have been published [6-11, 3]. However, the coherent combination of a range of newer test methods to provide comprehensive and high-throughput results has not been documented for any routine marine biotoxin screening programme. Thus international progress towards elimination of the dependence on animal assays has not been as rapid as desired. In this paper progress with a new programme for management of marine biotoxins in New Zealand based on LC-MS is reviewed.

MATERIALS AND METHODS

ASP Toxins by LC-MS/MS

Shellfish tissue homogenate (4 g) was blended with methanol/water 1+1v/v (16 ml). Following centrifugation an aliquot (0.5 ml) was diluted with acetonitrile/water 1+9v/v (4.5 ml). An aliquot (1 ml) was passed through a C18 SPE column (50 mg, Phenomenex). The Quattro Ultima (Micromass, Manchester) triple stage quadrupole LC-MS was operated under the following conditions: Phenomenex Luna 5 µm C18(2) 150x2 mm column operated at 0.2 ml min^{-1} and 30°C with a water/methanol/buffer eluant (90% of methanol/water 3+7v/v plus 10% 40 mM ammonium hydroxide/500 mM formic acid). Injection volume was 10 µL and run time was 10 minutes. The Z-spray electrospray source was operated at capillary 3.5 kV and using nitrogen gas for nebulisation and desolvation. A multiple reaction monitoring (MRM) window was established for domoic acid (DA) using specific daughters m/z 266 and 161 from m/z 312 (MH^+) at cone voltage 50 V and collision energy 20 eV.

Quantitative data from MRM peak areas were calculated using linear calibrations from six dilutions of a certified standard solution (DACS-1D, NRC, Canada) at 5 to 1000 ng ml^{-1} in 10% acetonitrile.

ASP and DSP Toxins by LC-MS/MS

High purity reference standards were available, for the following five 'Quan' toxins: DA (NRC, Canada), okadaic acid (OA, NRC Canada), pectenotoxin-2 (PTX2, Cawthron/ Suzuki), yessotoxin (YTX, ESR Ltd) and gymnodimine (GYM, AgRes Ltd).

The following six 'Qual' toxins, only available in crude extracts of contaminated shellfish, were partially validated: iso-DA, dinophysistoxin-1 (DTX1), pectenotoxin-1 (PTX1), pectenotoxin-1 seco-acid (PTX1sa), pect-enotoxin-2 seco-acid (PTX2sa), 45-hydroxy yessotoxin (45OH-YTX). The method was also set-up to establish the presence of the following six related toxins where only small amounts of impure reference materials were available: dinophysistoxin-2 (DTX2), azaspiracid (AZA1), desmethyl-azaspiracid (AZA2), methyl-azaspiracid (AZA3), pectenotoxin-6 (PTX6), homo-yessotoxin (homo-YTX).

Tissue homogenate (2 g) was blended with methanol/water 9+1v/v (18 ml). An aliquot of extract (2 ml) was washed with hexane (5 ml), centrifuged and the lower layer transferred to an autosampler vial. DTX3 (acyl esters of OA and DTX1) and diol esters of OA were determined after conversion to OA/DTX1 by alkaline hydrolysis of the crude methanolic extract [11].

The LC-MS was operated with gradient elution and positive /negative ion switching: Phenomenex Luna 5 µm C18(2) 150x2 mm column 0.2 ml min^{-1} with a water /acetonitrile/buffer gradient (13% to 77% acetonitrile at constant buffer composition of 4 mM ammonium hydroxide/50 mM formic acid). Injection volume was 10 µl and run time was 25 minutes. A MRM window was established for each toxin using a specific daughter from the molecular anion or cations: MH^+ for DA, iso-DA, GYM and AZA's; from MNH_4^+ for PTX's and PTXsa's (2 epimers each); and from $[M-H]^-$ for OA, DTX1, DTX2 and YTX's. Optimal cone potentials and collision energies were chosen for each species. The system was calibrated using a set of five Quan standard mixes (5 – 200 ng ml^{-1}) in 80% methanol. Qual toxins were calibrated using response factors (RFs) derived from those for related Quan toxins. A QC sample containing all Quan and Qual toxins was prepared by fortifying a contaminated shellfish extract with additional toxins.

Method Validation

Validation of the two methods followed the principles laid out in guidelines published by the New Food Safety Authority [13] and an expert panel [14]. Whole tissues of Greenshell™ mussel, Pacific oyster and cockle, and roe of scallop were studied for interferences and recovery of toxins following fortification. Certified reference materials (CRM) were available for DA and OA/DTX1 (mussel, MUS-1B and MUS-2, NRC, Canada) and were diluted into blank mussel homogenate to appropriate levels for accuracy/bias and extractability studies. Shellfish contaminated by ASPs or DSPs were also used for extractability studies and for recovery and precision estimates for DSP toxins.

RESULTS AND DISCUSSION

ASP Toxins by LC-MS/MS

This method has been developed and validated as an alternative to the standard method for domoic acid (DA) in shellfish based on LC with UV detection [2]. It uses a similar sample extraction with methanol/water (1:1). A simple SPE clean-up removes lipophilic coextractives. The LC-MS determination of DA and epi-domoic acid (combined peak) in the multiple reaction monitoring (MRM) MS-MS mode of operation provides very high specificity of detection. There were no significant interferences to the determination of DA in the four shellfish species down to very low levels (Fig. 1).

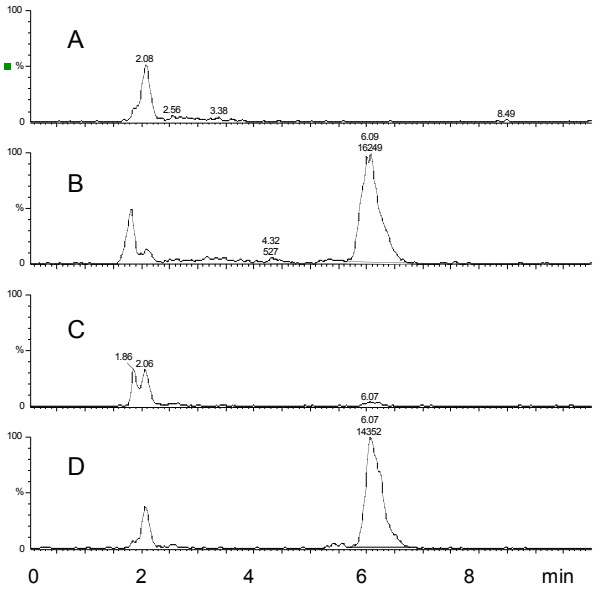


Figure 1. LC-MS/MS MRM chromatograms for domoic acid in shellfish (312 > 266 channel). A) Cockle blank, B) Cockle spike 1 mg kg^{-1}, C) Greenshell™ mussel blank, and D) Greenshell™ mussel spike 1 mg kg^{-1}.

The LC-MS determination had a highly linear response over the working range of DA levels in shellfish of 0.25 – 50 mg kg^{-1}. Recovery and precision were established using fortification studies with replication at DA levels of 1 and 20 mg kg^{-1}. The accuracy of the method was established using the CRM. A single extraction was shown to extract >90% of DA from contaminated shellfish. The following parameters were established for the method:

Recovery:	83 – 112% at 1 mg kg^{-1}
	84 – 105% at 20 mg kg^{-1}

Working Range:	0.25 to 50 mg kg^{-1}
Limit of Detection:	0.15 mg kg^{-1}
Limit of Quantitation:	0.5 mg kg^{-1}
Precision (repeatability) RSDr:	15.5% at 1 mg kg^{-1} 8.2% at 10 mg kg^{-1}
Precision (reproducibility) RSD_R:	16.4% at 1 mg kg^{-1} 8.6% at 10 mg kg^{-1}
Accuracy:	Bias less than 5%

Information was gathered showing the comparability of results for DA between the LC-MS and LC-UVD methods. Robustness testing of a number of method variables established that there were no significant factors beyond routine operator control that could lead to unreliable results. Advantages of this method include speed, an improved signal to noise ratio and a very high degree of specificity. Monitoring of method performance using QC spike samples (Fig. 2) has shown that the longer term reproducibility of the method is better than the preliminary estimate, from the validation data, of 16.4%.

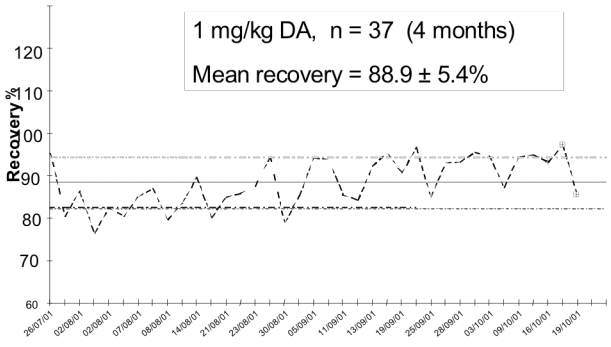


Figure 2. Control chart for spike recovery samples (blank shellfish fortified with DA at 1 mg kg^{-1}) for 37 batches of samples over a 4 month period.

The method is also suitable for determination of an isomer of domoic acid (iso-DA) that eluted after DA in the LC-MS system. This novel isomer has recently been observed at significant levels in some samples of New Zealand shellfish. It has been shown to have non-conjugated double bonds on the basis of MS/MS and UV spectra. Consequently, it may have been under-reported in assays using HPLC with UVD. See Figure 3 for chromatograms of extracts of a contaminated scallop sample and a mussel CRM.

DA and epi-domoic acid (in tail of DA peak) were unresolved and were quantitated as DA. Iso-DA was partially resolved and, on the basis of selected ion recording data for MH^+, was quantitated using an MRM response factor of 0.52 that for DA. The criteria established for confirmation of identity of DA and iso-DA based on the responses for the quantitation (312 > 266) and confirmation (312 >161) MRM channels were area ratios of 3.1 and 7.1 respectively. Other earlier eluting isomers of DA could also be observed but were not quantitated.

ASP and DSP Toxins by LC-MS/MS

A comprehensive in-house validation has been completed using fortified homogenates as well as shellfish naturally contaminated by ASP or DSP events. Data were also gathered on extractability, recovery from CRM tissues and storage stability of standards. The method provided quantitative data for the five key Quan

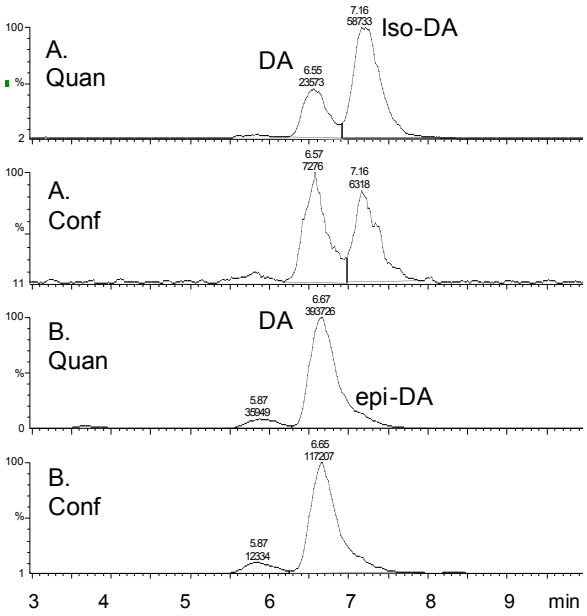


Figure 3. LC-MS/MS chromatograms for quantitation (312>266) and confirmation (312>161) channels, each normalised to largest peak. A) Scallop roe containing 1.4 mg kg^{-1} DA and 7.9 mg kg^{-1} iso-DA, B) Mussel CRM containing 20 mg kg^{-1} DA.

toxins where pure standards and certified reference material were available. Semi-quantitative screening was developed for the seven Qual toxins and metabolites where pure reference standards are currently not available. Crude standards or naturally contaminated shellfish were used to provide LC-MS reference signals and to develop key QA data such as extractability, linearity, recovery and precision. Several other toxins such as azaspiracids were also detected and will be fully incorporated into the method when standards come available. RSD_R (reproducibility under within-lab conditions) for most toxins was not markedly greater than RSDr, similar to the results for the ASP toxin method. The LC-MS determination step seems the most variable aspect of these methods, presumably due to varying effects of coextractives on the electrospray ionisation process. Key method performance parameters from the validation are summarised in Table 1.

The RF approach to calibration for the Qual toxins represents the best that can be accomplished until reference standards are available. For example, the RF for PTX2sa was taken as that for PTX2. Exchange of a contaminated shellfish sample with Queensland Health (G. Eaglesham, pers.comm.) established PTX2sa levels of 31 and 20 µg 100 g^{-1} in the two labs using different LC-MS methods.

The multi-toxin method based on LC-MS/MS of crude methanolic extracts of shellfish has proved very robust and suitable for routine use. Figure 4 illustrates the chromatographic output for a sample of Greenshell™ mussel contaminated by several DSP toxins (non-detect channels not shown). The excellent signal/noise ratios for the low levels of OA and PTX2 are noteworthy. The simple extraction procedure and gradient LC elution enables a through-put of up to 30 samples per day plus calibration and QC samples. The mix of quantitative and semi-quantitative data extends the current analytical capability for marine biotoxins. The method has been used to track depuration of DSP toxins from Greenshell™ mussels following a mixed phytoplankton bloom [15] (Fig. 5).

Table 1. Performance parameters for ASP and DSP toxins by LC-MS/MS. SQ – semi-quantitative analysis.

Toxin and Fortification Level µg 100 g^{-1}	**Rec-overy** %	**RSD$_r$** %	**Bias** % of CRM value	**LOD** µg 100 g^{-1}
Domoic acid 5 - 50	99	17		2
50 - 2000	82	4.6	82	
Okadaic acid 5 - 20	99	15		1.6
20 - 200	96	10	92	
PTX2 5 - 20	101	16		1
20 - 200	98	9		
Yessotoxin 5 - 20	71	14		1.6
20 - 200	75	13		
Gymnodimine 5 - 20	91	10		0.06
20 - 200	94	8		
DTX1 5 – 20	CRM only	9	104 - 129	1.6
DTX3 and diol esters	Detection as OA/DTX1 following hydrolysis.			
PTX1 5 - 20	114	12	SQ	1
PTX6 5 - 20			SQ	1
PTX seco-acids 10 - 100	140 - 160	17 - 8	SQ	2
45OH-YTX 5 – 50	79	7	SQ	1.6
Homo-YTX, DTX2	Detected – semi-quantitative analysis			1.6
Azaspiracids	Detected – qualitative analysis until standards are available			

Homo-YTX has not been found in New Zealand shellfish contaminated by *Protoceratium reticulatum*, although LC-MS/MS has revealed a range of other polar YTX derivatives [15]. The metabolic profile for YTX in Greenshell™ mussel is complex. In addition to the 45OH-YTX monitored by the ASP/DSP method, an earlier peak in this MRM channel (14.96 min, Fig. 4) possibly corresponds to the aldehyde intermediate to carboxy-YTX [16].

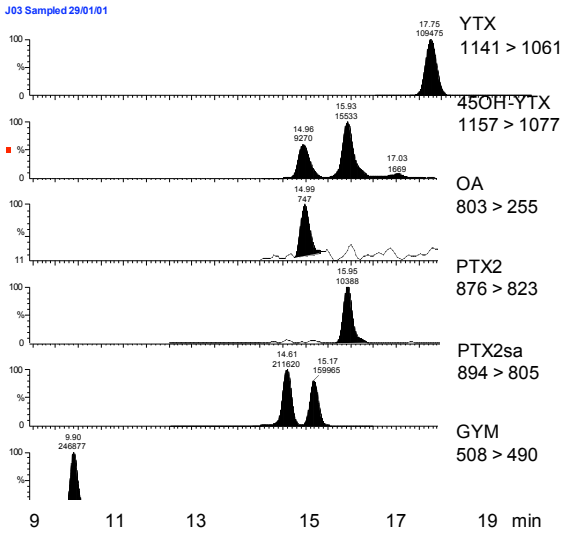


Figure 4. LC-MS MRM chromatograms for Greenshell™ mussel sample contaminated with DSP toxins, each normalised to the largest peak. Levels in µg 100 g^{-1}: GYM 8.5, PTX2sa (sum of epimers) 180, PTX2 11, OA 1.7, 45OH-YTX 38, YTX 169.

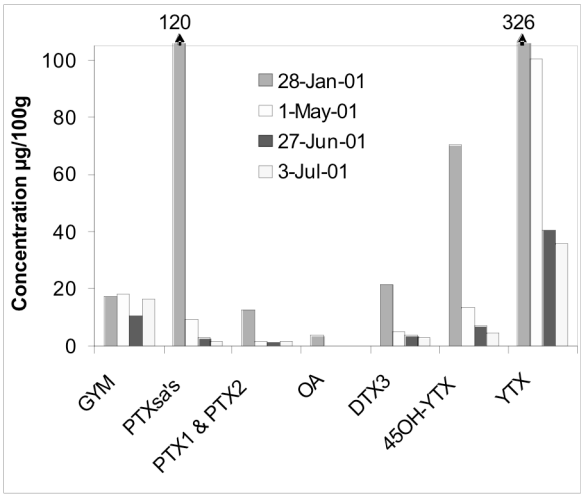


Figure 5. Depuration of toxins from Greenshell™ mussel following contamination by a mixed phytoplankton bloom.

The method provides a rapid and precise alternative to the use of animal bioassays for monitoring of DSP and other lipophilic toxins in shellfish. ASP toxins are also determined. Uncertainties and ambiguities are removed as to the types and concentrations of toxins present, particularly where levels are below, but approaching, regulatory thresholds. This is leading to better identification and management of risks to consumers. The validation document has been made available to the international biotoxin community and will form the basis for collaborative studies.

Development of a New Monitoring Programme

A review of current monitoring practices showed that key changes could only occur with introduction of new test methods that would reduce the dependence on mouse bioassays. Interactions between various disciplines were required in order to develop and implement cost effective, robust methodologies that met a range of regulatory and scientific criteria. Some of these factors are illustrated in Figure 6.

There is a strong emphasis on full and thorough validation of new methods to meet stringent MAF standards [13]. This has required considerable investment by Cawthron Institute with support from the shellfish industry. The two new LC-MS methods described above have full MAF approval for use in regulatory testing and are now being used routinely in monitoring programmes.

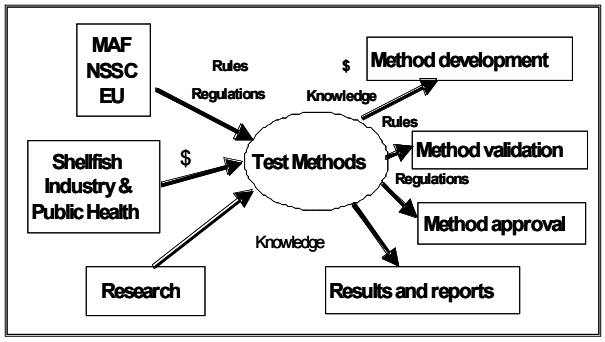
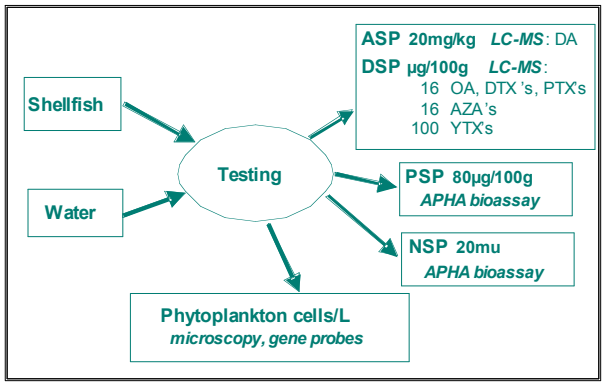


Figure 6. Interacting factors and processes to introduce new test methods to the New Zealand biotoxin monitoring programme.

The test methods currently used in the new biotoxin monitoring programme and the New Zealand regulatory limits are summarised in Figure 7. NSP and PSP toxins are monitored by a combination of phytoplankton testing on water samples and the standard APHA NSP-ether or PSP bioassays on shellfish. The MIST-Alert PSP immunoassay strip kit [17, 18] is approved for use where harvest areas are closed due to PSP. This kit is suitable to determine whether it is likely that toxin levels have fallen enough to pass the regulatory limit by bioassay. LC-MS methodologies for NSP [19] and PSP [3] toxins are currently being investigated for use in the monitoring programmes.

Figure 7. Test procedures in the new programme.



LC-MS MRM methods detect target compounds and little information is obtained for other toxins or potential new toxins. The monitoring programmes therefore place some reliance on phytoplankton monitoring to determine the presence of novel and potentially toxic organisms at non-surf beach sites. However, LC-MS MRM can detect isomeric forms of toxins and this has proved useful for detection of iso-DA and an isomer of PTX1 produced by the dinoflagellate *Dinophysis acuta* [15].

A range of further issues must be addressed when target compound methods are introduced into regulatory programmes. These include:

1) Availability of analytical standards and reference materials. This issue has been clearly identified [20, 21]. The great value of the NRC Canada certified reference materials program to this project and the international biotoxin community is acknowledged. Cawthron Institute, through its phytoplankton culture and field collection capabilities, is working with NRC to improve the availability of key newer toxins such as PTXs and YTXs.

2) Comparability with standard bioassay test procedures. This is probably not achievable. The recoveries of toxins and metabolites through the bioassay extraction protocols have not been established for many toxins. Furthermore the interactions of mixtures of toxins and the IP administration route make results of mouse screening tests for DSP and NSP toxins difficult to interpret in terms of levels of toxins. Further research on the toxicology of orally administered individual toxins and metabolites is required for setting of regulatory limits and interpretation of LC-MS data.

3) International regulatory limits for individual toxins or groups of toxins. Fernandez [21] has reviewed the status of harmonization activities in this area. The EC has recently published draft regulations covering some DSP toxins in shellfish tissues which go part way to meeting needs in this area [22] with proposed limits of 16 µg 100 g^{-1} for total content of OA, DTXs and PTXs; 100 µg 100 g^{-1} for YTX equivalents and 16 µg 100 g^{-1} for AZAs.

4) Cost of LC-MS technology. Considerable capital investment is required for instrumentation and implementation of test methods. Payback times appear reasonable provided high sample throughputs can be achieved. Less sophisticated mass spectrometers (e.g., single stage quadrupole or ion-trap) may prove to be suitable for routine screening down to levels below the regulatory limits for most toxins.

CONCLUSIONS

LC-MS methods have been developed, validated and implemented which show that this powerful analytical tool is suitable for routine use in a marine biotoxin monitoring programme. High sample throughputs have been achieved for primary analysis of both ASP and ASP plus DSP toxins. These methods form the core for a new programme in New Zealand, centred on the laboratories of the Cawthron Institute, which has faster sample turn-around times and greatly reduced the number of mouse bioassays. The greater information content and higher

reliability of LC-MS results is leading to improved management of marine biotoxins in shellfish.

ACKNOWLEDGEMENTS

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LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS): A UNIVERSAL METHOD FOR THE ANALYSIS OF TOXINS?

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ABSTRACT

The utility of the liquid chromatography-mass spectrometry (LC-MS) is first reviewed in terms of its advantages and disadvantages. Then a method is presented for the simultaneous analysis of a wide range of shellfish toxins in a single analysis.

INTRODUCTION

The combination of liquid chromatography and mass spectrometry (LC-MS) has proven to be a powerful tool for the detection and quantitation of toxins in plankton and shellfish at part-per-billion levels, the identification of new toxins, and the investigation of toxin metabolism in shellfish [1-3]. LC-MS methods have been developed for the following toxins: domoic acid and other ASP toxins; okadaic acid and related DSP toxins, including DTX1-5 toxins; pectenotoxins; azaspiracids; yessotoxin; saxitoxin and other PSP toxins; brevetoxins; gymnodimine and spirolides; and ciguatoxins. LC-MS is in fact the only analytical method that has been shown to be suitable for the analysis of all toxins.

LC-MS meets all the needs of laboratories involved in both monitoring and toxin research:

- universal detection capability
- high sensitivity
- high selectivity and specificity
- minimal sample preparation
- ability to deal with the structural diversity and labile nature of toxins
- separation of complex mixtures of toxins
- precise and accurate quantitation
- wide linear range
- automation
- high throughput
- rapid method development
- legal acceptability for confirmation
- structural information for identification of new toxins, analogs and metabolites

The high capital cost of LC-MS systems does present difficulties for many laboratories but the recent introduction of less expensive, easy-to-use "bench-top" instruments should help to reduce these problems. In addition, the actual cost per sample must be considered carefully when comparing different methods. Analyses by LC-MS can be very rapid (as low as 2-3 min in some cases) and can be totally automated, resulting in a very low cost per sample. The cost of LC-MS analyses is also low because minimal sample preparation is required, compared to other analysis methods based on complicated cleanup and derivatization schemes. This feature is also important for sample throughput, as sample preparation is usually the major bottleneck in most laboratories. An additional item of importance to some laboratories is that an LC-MS system can be used for both research and monitoring work, and for a variety of analytes, not just toxins.

An LC-MS instrument certainly does need a steady flow of samples to justify its purchase, unless it is also used for research. Obviously the LC-MS option is best suited to a central laboratory that would have the technical expertise and the workload to justify such an instrument. One of the most appealing features of LC-MS to many laboratories is the possibility that a wide range of methods could be replaced by just one instrument. Caution must be exercised here, as complete reliance on one instrument could present difficulties in that any breakdown could result in delays during a crucial monitoring program. However, our experience is that the relatively inexpensive modern LC-MS systems are very reliable and give a 90%+ up-time; service contracts on such instruments usually allow a 24-48 hr repair. The bottleneck of using just one instrument can also be a problem, but with automation and high-speed analyses, an LC-MS system can deal with many emergencies. A laboratory basing its entire operation on LC-MS, may wish to have 2 instruments in any case, one for routine monitoring and another for research and methods development as well as being a backup to the routine system. Of course this is a difficult proposition for many institutions that cannot even afford an LC system! The combination of rapid assay methods for screening and an LC-MS system in a central laboratory for research and confirmation may be the best route to follow for many economies.

Another cautionary note is that a fair amount of technical expertise is involved in operating an LC-MS system, although the newer bench-top LC-MS systems are becoming very easy to use and maintain. In our laboratory, we have many instrument users, including temporary summer student assistants. Training usually takes no more than 1-2 days for routine use. On the other hand, research analytical chemists generally carry out the method development work. One final concern, which applies to all instrumental methods and many assay methods, is the shortage of calibration standards for marine toxins. The development of readily availalable calibration standards and certified reference materials (CRMs) will be essential for the full implementation of LC-MS methods in regulatory laboratories.

Whatever the opinions about investing in LC-MS systems as a tool for routine toxin analysis, most people will agree that this technique will continue to play a significant role in the future of toxin research. This paper will present an LC-MS method for the simultaneous

analysis of many shellfish toxins, a procedure that could replace several individual methods.

EXPERIMENTAL

Plankton and shellfish samples were extracted with three portions of methanol/water (8:2) with a final volume/sample ratio of 10 (e.g., 1 g extracted into 10 ml solvent). After filtration, the extract was injected directly into the LC-MS system. The LC column was a Keystone Scientific 50 x 2 mm Quicksilver cartridge packed with 3 μm Hypersil-BDS-C8, equipped with a guard column. The mobile phases were as follows: A = water and B= acetonitrile:water (95:5), both containing 2 mM ammonium formate and 50 mM formic acid. Gradient elution from 5% to 100% B was performed over 10 min and then held at 100% B for 10 min, before returning to initial conditions and equilibrating for 7 minutes. The flow rate was 0.2 ml min^{-1} and the injection volume was 5 μl.

The LC system was a HP 1100 with binary pump, variable volume injector and autosampler. The mass spectrometer was a Perkin-Elmer Sciex API165 single quadrupole bench-top system equipped with ion-spray source. Eluent from the LC was split 10:1 with 20-30 μl min^{-1} going to the MS. Selected ion monitoring was used for data acquisition.

RESULTS AND DISCUSSION

My current research has been directed towards finding a single column and mobile phase that would be suitable for a wide range of toxin families, in both plankton and shellfish samples. Using short columns and rapid gradients, it is possible to analyze several toxins in one extract by one LC-MS analysis.

Of course, one limitation of this multi-toxin approach lies not in the LC-MS system, but in sample preparation – i.e., finding a universal extraction solvent and cleanup scheme that will give good recovery for all toxins. In reality, however, it is unlikely that all toxins would have to be monitored at once – most locations only suffer from one to three different types of toxins. We have now developed preliminary extraction and LC-MS methods for domoic acid, gymnodimine, spirolides, the okadaic acid family of toxins, pectenotoxins, azaspiracids, and yessotoxin in one group and the very polar PSP toxins and tetrodotoxin in a second group. Figure 1 illustrates application of the technique to a mussel tissue extract spiked with assorted toxins. The method has proven to be very sensitive and provides a high degree of certainty in toxin detection. Further confirmation of toxin identity or the identification of new toxin analogues can be assisted with the use of tandem mass spectrometry (MS/MS). Accurate quantitation is reliant on the availability of accurate calibration standards. However, estimation of concentrations of toxin analogues can be achieved through the use of estimated relative molar response factors. Further method validation in a regulatory setting is still required of course. Additional research at IMB, under the auspices of the Certified Reference Materials Program, is being directed towards the development of a series of calibration solution CRMs for world-wide distribution.

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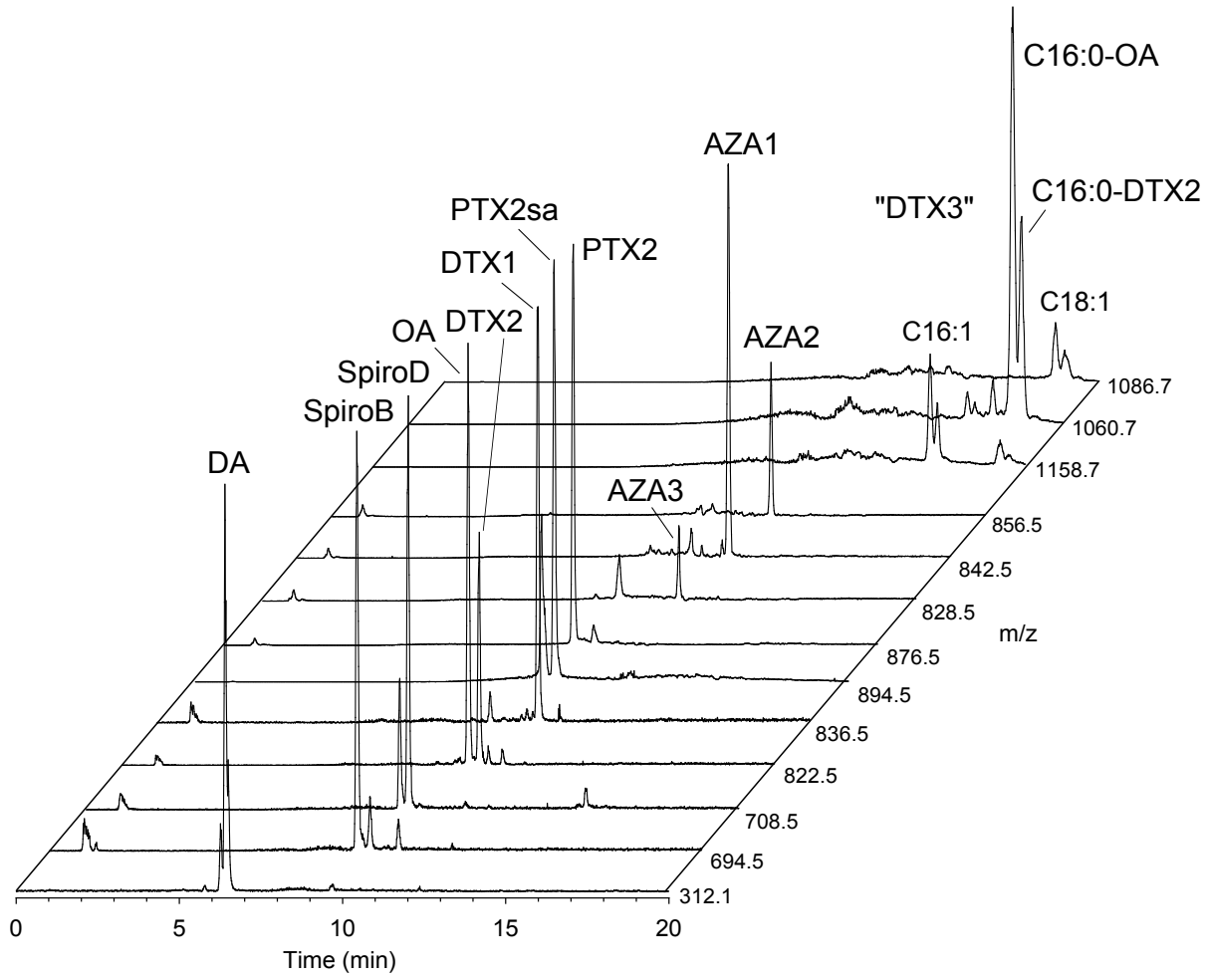

Figure 1. A gradient elution LC-MS analysis of an extract of mussel tissue spiked with assorted toxins: domoic acid (DA), spirolides (B and D), okadaic acid (OA), dinophysistoxin-1 and –2 (DTX1 and DTX3), pectentoxin-2 (PTX2) and its seco acid (PTX2sa) and azaspiracids (AZA).

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EXTERMINATION AND CONTROL

TREATMENT OPTIONS FOR THE CONTROL OF THE ICHTHYOTOXIC DINOFLAGELLATE KARLODINIUM MICRUM IN AN ESTUARINE AQUACULTURE FACILITY: A CASE STUDY

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ABSTRACT

Between 1996-1999, three fish kill events have been associated with blooms of the toxic dinoflagellate *Karlodinium micrum* at HyRock Fish Farm, an estuarine pond production facility raising hybrid striped bass along a tributary of the Chesapeake Bay, Maryland, USA. At HyRock, it was observed that ponds containing blooms of *K. micrum* treated with copper sulfate resulted in greater fish mortality than ponds treated with potassium permanganate. We were able to show that a Chesapeake Bay isolate of *K. micrum* produces several toxic fractions which are released upon cell destruction due to copper sulfate [2 mg L^{-1} $CuSO_4$ caused >70% cell lysis with a significant (p<0.0001) increase in hemolytic activity compared to controls and Cu alone]. Treatment with 4 mg L^{-1} $KMnO_4$ also resulted in >70% cell lysis but hemolytic activity was significantly reduced [(p<0.0001) compared to the 2 mg L^{-1} $CuSO_4$ treatment]. The same results were obtained with purified toxic fractions. Copper had no effect on toxicity while potassium permanganate removed it. At HyRock, the addition of an ozone generation system inline with the main estuarine water supply appeared to reduce the occurrence of fish kills associated with *K. micrum* blooms. We showed that a 0.4 mg L^{-1} ozone treatment also caused cell lysis (>80%) along with the removal of hemolytic activity. It appears that several algicidal treatments will kill *K micrum*, but only strong oxidizing agents such as potassium permanganate and ozone will also reduce mortality due to its associated toxins.

INTRODUCTION

Opened in 1993, HyRock Fish Farm consists of 37 acres of impoundments supplied with water from the Manokin River, a tidally influenced tributary of the Chesapeake Bay located in Princess Anne, Maryland, USA. Average salinity of the incoming Manokin river water is 10 psu (range 4.5 – 18 psu). On 30 July 1996 a large mortality of ca. 15,000, 1-1.25 lb (2.20-2.75 kg) reciprocal cross hybrid striped bass (*Morone saxatilis* male × *Morone chrysops* female) occurred following a copper sulfate treatment (<2 mg L^{-1}) used to arrest a dense bloom containing 6 × 10^4 cells ml^{-1} of the toxic dinoflagellate *Karlodinium micrum*. The bloom had been developing for over one week prior to the events of 30 July, but no mitigating actions were taken due to the misdiagnosis of the deep mahogany water coloration as tannins. Limited fish mortalities had occurred in the days preceding 30 July. To avoid low dissolved oxygen problems, paddle wheel aeration was initiated maintaining dissolved oxygen levels >5 ppm. Alkalinity was 75 ppm and other water quality conditions immediately prior to the main kill were within normal mid-summer ranges for HyRock Fish Farm [1]. Foaming and a "petroleum-like" odor were present in the days preceding the large kill. Treatment of a neighboring pond using potassium permanganate (<4 mg L^{-1}), a strong oxidizing agent, appeared to arrest the bloom without fish mortality. Since that time, two additional fish mortality events have co-occurred with blooms of this organism, 8000 adults on 13 August 1997, and 5000 fingerlings on 15 May 1999. In both circumstances, mortality was stopped following a <4 mg L^{-1} potassium permanganate treatment, with fish returning to feed in 3-5 days. In an attempt to minimize the continued introduction of bloom-forming dinoflagellate species, common in the Manokin River, an ozone generation system was added in-line with the main water supply in the summer of 1998.

As part of the investigation into the causes of these repeated kills at HyRock fish farm, we eventually discovered that cultures of a Chesapeake Bay isolate of *K. micrum* produce an easily released suite of toxins with hemolytic, cytotoxic, and ichthyotoxic properties [2]. Similar compounds were later isolated directly from water samples, containing >4 × 10^4 cells ml^{-1} of *K. micrum*, collected from a South Carolina brackish water retention pond in which a mixed fish kill had recently occurred [3].

The purpose of this report is to chronicle the methods employed at HyRock fish farm to control and/or prevent toxic dinoflagellate blooms, and also to describe a commercial scale ozonation system employed for these same purposes. The events described in this report took place between the years 1993-2000 (the last year fish were commercially raised at the facility). To our knowledge, this is the first report of a commercial scale ozone generation system used to prevent the introduction of bloom-forming dinoflagellates into an estuarine aquaculture facility.

MATERIALS AND METHODS

Facilities

HyRock Fish Farm consists of five 2.2 acre ea^{-1} fingerling ponds and five 5.1 acre ea^{-1} grow-out ponds (average depth for all was 4-5 ft; Fig. 1). Water was supplied from the Manokin River by way of a 25-ft wide × 8-ft deep (tidal average) × 1600-ft long channel. Water

was delivered to the ponds by a Babcock and Wilcox pump (10-inch line in, 8-inch line out) at a maximum rate of 1800 gallons min^{-1}. Individual ponds were filled via a 12-inch main line with 8-inch spurs to each pond. Ponds were constructed above ambient water levels so that ponds could be drained by gravity through 10-inch lines into a ditch which flowed into a man-made salt marsh before draining back into the Manokin River. Approximate time to completely replace the water in a pond was 5 days.

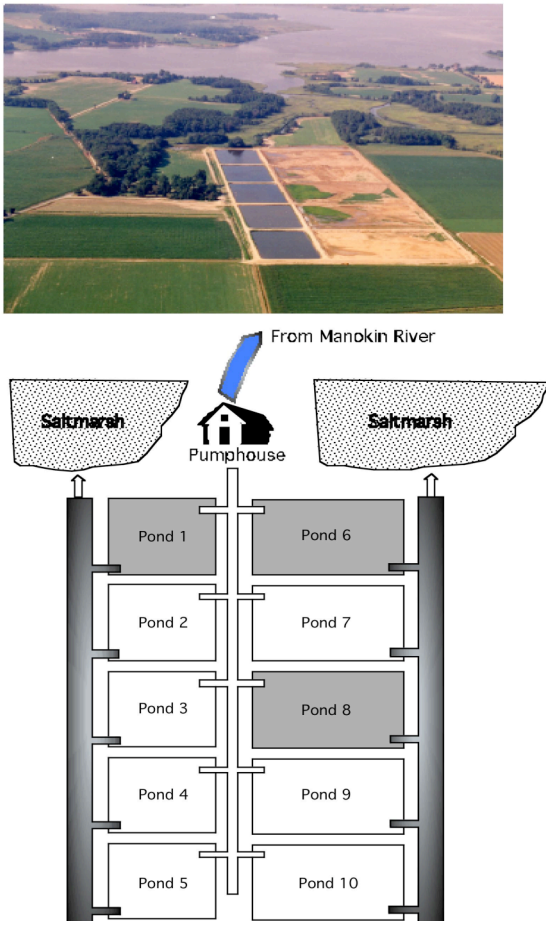

Figure 1. Top: Aerial view of HyRock Fish Farm during construction circa. 1993. Bottom: Schematic diagram of HyRock Fish Farm. Ponds 1-5 are 2.1 acres each. Ponds 6-10 are 5.2 acres each. Average depth for both is 4-5 ft. Shaded ponds indicate sites of fish kills that co-occurred with blooms of *K. micrum*. Pond 6: July 1996, 15,000 adults. Pond 8: August 1997, 8,000 adults. Pond 1 - May 1999, 5,000 fingerlings.

To control green algal densities, copper sulfate was occasionally used (Blue Stone, large crystals). Copper was applied by hanging a sufficient amount to achieve 1-2 mg L^{-1} in porous bags in front of paddle wheel aerators. After the first kill on 30 July 1996, associated with a dinoflagellate bloom and copper treatment, potassium permanganate was used to control dinoflagellate blooms (CAIROX ZM free-flowing grade, Carus Chemical Company, Peru, Illinois). A solution of potassium permanganate was hand distributed to achieve a concentration ca. 4 mg L^{-1}.

In the summer of 1998 an ozone generation system was installed in-line with the main water supply pump. The system was a PCI-WEDECO model GS-40, water-cooled, coronal discharge ozone generator supplied with pure O_2 that was generated on-site. Calculated and measured maximum system capacities were 40 lbs of O_3 day^{-1} and 31 lbs of O_3 day^{-1}, respectively. The system was typically run at 2/3 of capacity giving an ozone output of ca. 20 lbs of O_3 day^{-1} or an initial aqueous concentration of 1 mg L^{-1} O_3. The 12-inch main line to the ponds acted as the ozone contact chamber. Distance from the point of ozone generation to the first and last ponds was ca. 330 ft and 1900 ft, respectively.

Algicidal Treatments

Effect on Dinoflagellate Cell Lysis and Release of Toxic Activity

To assess the effects of algicidal treatments used at HyRock Fish Farm on cell lysis and the subsequent release of toxic activity, a Chesapeake Bay isolate of *K. micrum* (CCMP 1974) (4×10^4 cells ml^{-1}) was exposed to either 2 mg L^{-1} $CuSO_4$ (as $CuSO_4 \cdot 5\ H_2O$) [= 0.8 mg L^{-1} Cu], 4 mg L^{-1} $KMnO_4$, or ozone (approximately 0.2, 0.3, or 0.4 mg L^{-1}). Values of $CuSO_4$ and $KMnO_4$ (2 mg L^{-1} and 4 mg L^{-1}, respectively) approximated dosages typically applied at HyRock Fish Farm. Dissolved ozone concentrations were achieved by directly adding O_3 to 100 ml dinoflagellate cultures using a Pacific Ozone Technologies ozone generator (SGA Series). Ozone was delivered using Teflon lined tubing with a course glass diffuser for either 0.5, 1, or 2 min at a flow rate of 1 SCFH. Ozone dosages (mg L^{-1}) were determined by immediately testing concentrations in culture media controls using a colorimetric ozone test kit (AccuVac, HACH, Loveland, Colorado). Cell lysis was evaluated using a Coulter Multisizer II particle counter with enumeration of the 7–20 μm size fraction using a Coulter Accucomp software package (Coulter Electronics Limited, Miami, Florida). Hemolytic activity remaining in solution was assayed as described in Deeds et al. [2].

Effect on Purified Toxic Fractions

As part of a previous study [2], aliquots of two toxic HPLC fractions (KmTx 1 and *p*KmTx 3, formerly Tox A and Tox B, respectively) isolated from *K. micrum* (CCMP 1974), were diluted back to a cellular equivalent of 3.0×10^4 cells ml^{-1} and mixed with either 2 mg L^{-1} $CuSO_4$ (as $CuSO_4 \cdot 5\ H_2O$), or 4 mg L^{-1} $KMnO_4$. After a one hr incubation, each was tested for hemolytic activity as described in Deeds et al. [2]. EDTA (2 mM) was added after the one hr Cu exposure but prior to hemolytic testing to ensure any hemolytic activity was due to released toxins and not due to exogenous copper. Previous studies had shown that the 2 mM EDTA treatment was sufficient to bind >90% of free Cu [2]. For all of these experiments, dinoflagellate culture media (12

psu artificial sea water, Instant Ocean Brand, alkalinity 75 mg L^{-1} $CaCO_3$, with f/2 nutrient mixture and 1% soil extract, preparation described in Deeds et al. [2]) was used for controls and toxin dilutions.

Analysis of variance with Scheffe's F post-hoc test was used to test for statistically significant (p<0.05) differences among treatments (StatView 4.5, Abacus Concepts Inc., Berkley, California).

RESULTS

Treatment with either copper, potassium permanganate, or ozone caused significant lysis of *K. micrum* cells (>70%; Fig. 2A-C). Toxic activity was enhanced only after treatment with copper and eliminated following treatment with potassium permanganate or ozone (minimum residual ozone concentration 0.40 mg L^{-1}; Fig. 3A,C). This observation in cultures is consistent with observations made at HyRock Fish Farm where significantly higher mortality was observed following treatment of a *K. micrum* bloom with copper sulfate compared to treatment with potassium permanganate.

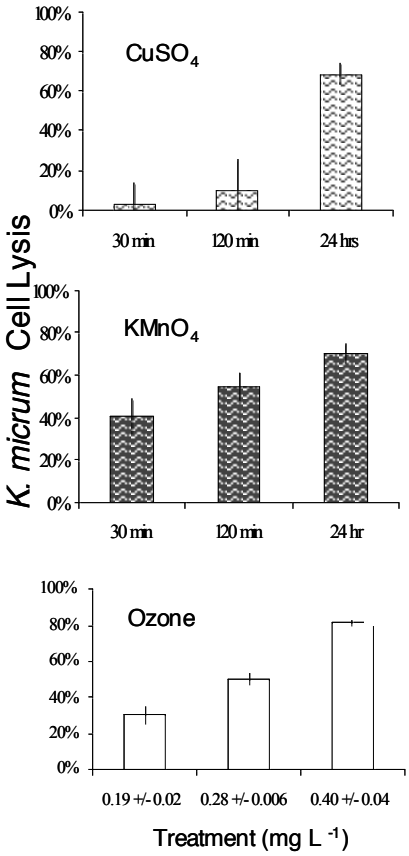

Figure 2. Effect of algicidal treatments on cell lysis of *K. micrum*: A) 2 mg L^{-1} copper sulfate, B) 4 mg L^{-1} potassium permanganate and C) an increasing concentration of ozone. Data for A and B are adapted from Deeds et al. [2]. Bars represent one standard deviation, n=3.

In laboratory trials, 2 mg L^{-1} $CuSO_4$ had no effect on the hemolytic activity of *K. micrum* toxic HPLC fractions KmTx 1 and *p*KmTx 3, while 4 mg L^{-1} $KMnO_4$ caused the complete elimination of hemolytic activity in both fractions (Fig. 3B).

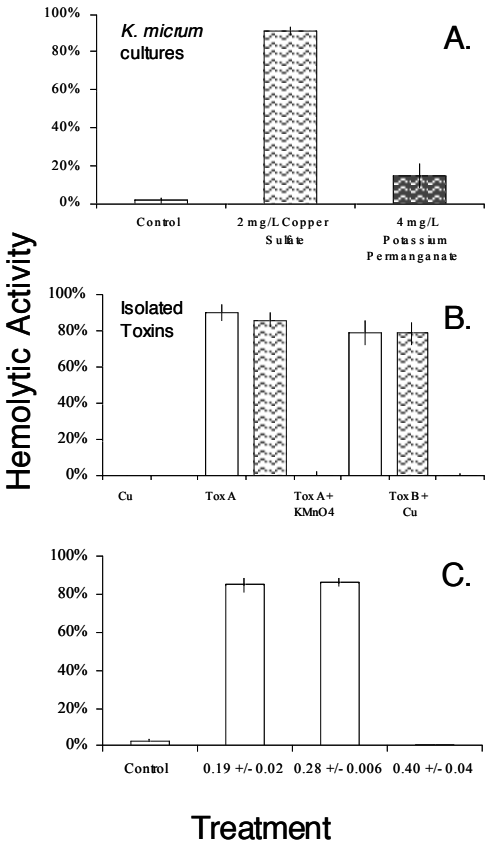

Figure 3. Effect of algicidal treatments on hemolytic activity from both cells and isolated toxins from *K. micrum*: A) effect of copper sulfate and potassium permanganate on release of hemolytic compounds from *K. micrum* cells, B) effect of copper sulfate and potassium permanganate on toxic fractions KmTx 1 and *p*KmTx 3 from *K. micrum* and C) effect of an increasing concentration of ozone on the release of hemolytic compounds from *K. micrum* cells. Data for A and B adapted from Deeds et al. [2]. Bars represent one standard deviation, n=4, *** signifies (p<0.0001).

DISCUSSION

Few algicidal treatments are available to the aquaculture industry, due mainly to concerns over both expense and the regulatory issues involved with the use of harmful chemical substances with food fish. Two of the more commonly utilized treatments for the control of phytoplankton abundance in aquaculture ponds are copper sulfate ($CuSO_4$) and potassium permanganate ($KMnO_4$).

Costing ca. $25.00 USD to treat one 5 acre pond (average depth 5 ft; 1999-2000 pricing), copper sulfate is one of the most commonly used chemicals for the control of both noxious weed species and infectious diseases in

fishponds and hatcheries [4, 5]. Application rates for copper sulfate at HyRock typically ranged from 1-2 mg L^{-1}, well below the experimentally determined 96 hr LC_{50} of ca. 8 mg L^{-1}, determined for striped bass fingerlings at comparable salinities (although the Rearden and Harrell study was performed at higher alkalinities than those typically found at HyRock) [6]. Regardless, prior to the events of 30 July 1996, this application dosage had been used at HyRock, at the same alkalinities present during the 1996 fish kill, to control green algal blooms without difficulty.

Potassium permanganate has been used in aquaculture facilities for various reasons ranging from disease and external parasite treatment to the oxidation of organic and inorganic substances to reduce both biological and chemical oxygen demands [4, 7-10]. An added benefit of algicidal $KMnO_4$ treatment is that during the reduction of potassium permanganate to manganese dioxide (MnO_2), the manganese dioxide forms a colloid with an outer layer of exposed OH groups. These groups are capable of adsorbing both charged and neutral particles from the water column, thereby further promoting the precipitation of microorganisms [11]. The 96 hr LC_{50} for potassium permanganate was shown to range from 4.5 to 17.6 mg L^{-1} for channel catfish fingerlings depending on the amount of dissolved organics in the system [8]. Any confined animal feeding operation, HyRock being no exception [1], tends to possess very high organic loads. One would expect this to push the lethal concentration for potassium permanganate towards the upper end of this range. A simple method for determining the required dosage in such an environment is the 15 min $KMnO_4$ demand in which the concentration of $KMnO_4$ required to color the water for 15 min is multiplied by 2.5 to determine the application rate [9]. At HyRock, the typical application was <4 mg L^{-1}, actually below recommended, due mainly to cost constraints. Treatment of one 5-acre pond (avg. depth 5 ft) costs ca. $500.00 USD (1999-2000 pricing).

Ozone has been used for years as an alternative treatment method in the wastewater and drinking water industries for such varied purposes as bacterial and viral disinfection, oxidation of taste and odor compounds, increased organic biodegradation, and coagulation and filter improvement (for review see United States Environmental Protection Agency Alternative Disinfectants and Oxidants Manual at www.epa.gov/safewater/mdbp/pdf/alter/chapt_3.pdf). More recently, these properties of ozone have begun to be exploited by the aquaculture industry (for review see [12]). Additional benefits to the use of ozone in aquaculture include the production of oxygen as a byproduct and the oxidation of nitrogenous waste products, particularly nitrite, to the less toxic form nitrate [13]. Ozone is a powerful oxidant second only to the hydroxyl free radical among chemicals used in water treatment. Because ozone is very unstable, it must be generated on-site. Aqueous molecular ozone will either react directly with organic or inorganic substrates or will decompose spontaneously generating hydroxyl free radicals which will further react with available substrates. Under certain conditions more stable reactive byproducts can be created. For example, in seawater ozone reacts with bromide ions to produce reactive compounds such as hypobromous acid (HOBr) and hypobromite ions (OBr^-) which are more persistent in solution than the original ozone molecules [12]. More research needs to be done to determine the harmful effects of these byproducts and to find reliable methods for the removal of these compounds under varying environmental conditions. At HyRock, adverse effects were never observed following addition of ozonated water. On the contrary, on several occasions reduced parasite loads were observed in ponds that received ozone treated waters, although these observations were never quantified.

The use of ozone has been suggested for use in both the removal of toxin producing dinoflagellates and for the direct destruction of the toxins they produce [14, 15]. In this study, exposure to an increasing oxidative residual due to ozone resulted in both the destruction of *K. micrum* cells and the removal of released hemolytic activity due to toxic compounds. However, it should be noted that a minimum residual ozone concentration of 0.4 mg L^{-1} was required to remove hemolytic activity while lower amounts were required for cell lysis and release of hemolytic activity. Therefore, there is a definite danger in under-dosing. This observation will apply to any oxidative compound that is utilized as it reacts with dissolved and particulate organic matter.

CONCLUSIONS

Despite the vast difference in treatment cost, based on the data from this study and from Deeds et al. [2], potassium permanganate is recommended over copper sulfate for the treatment of *K. micrum* blooms in aquaculture facilities. Although the ozone generation system at HyRock was only in operation for a few seasons, ozonation of incoming water did appear to improve water quality and reduce the occurrences of fish mortality due to toxic dinoflagellate blooms. The system as described was only useful in treating incoming "replacement" water and could not be used to treat blooms that developed within the ponds *in-situ*. With minimal engineering, it would have been possible to rout the drainage outlets from the individual ponds back through the pumping station, thereby creating isolated loops to each pond. By doing this it would have been possible to treat individual ponds with ozone when needed.

HyRock Fish Farm stands as an example of the successful use of ozone on a commercial scale to reduce the introduction of harmful algal species from source waters into an estuarine aquaculture facility. Still, at present, ozonation systems are expensive to install and maintain, and are relatively complex. But with increasing limitations on the use of chemicals for food fish, both from a food safety and environmental impact perspective, these technologies appear to be well suited for the aquaculture industry. With an ever-increasing demand for quality seafood coupled with the continuing depletion of natural stocks, hopefully, the demand will also

increase for the production of lower-cost and more reliable ozone generation and distribution technologies that will make these treatment options more accessible and economically feasible to the aquaculture industry worldwide.

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EFFECTS OF OILS AND CHEMICAL DISPERSANTS ON THE GROWTH OF THE HARMFUL DINOFLAGELLATE COCHLODINIUM POLYKRIKOIDES

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ABSTRACT

This study was conducted to understand the effects of the oil spill accidents and the use of dispersants on the bloom of *Cochlodinium polykrikoides*. Kuwait oil, the United Arab Emirates oil, Bunker-C, kerosene and diesel oil, and two types of oil spill dispersants, Hi-Clean and Seagreen 805A were added with a series of 10 ppb to 300 ppm in the f/2-Si medium at 20°C under a photon flux from cool white fluorescent tubes of 100 μmol m^{-2} s^{-1} in a 14:10 hr light-dark cycle for the culture of *C. polykrikoides*. In low concentrations of ≤1 ppm of examined oils no impact on the growth of *C. polykrikoides* was recorded, while in high concentration of ≥10 ppm cell density, it was significantly decreased with the range of 10 to 80% in comparison with the control. The growth of *C. polykrikoides* after the addition of the dispersants and the mixtures combined with oils and dispersants of ≥10 ppm appeared to decrease, whereas the growth of *C. polykrikoides* exposed to ≤5,000 ppb showed little serious impact. However, almost all the *C. polykrikoides* cells died regardless of types of dispersants and combined mixtures within a few days after the addition of high concentrations.

INTRODUCTION

Oil pollution has become one of the most important problems we face nowadays. We should find ways to avoid this problem by certain precautions or to discover ways to save polluted places [3]. Much research has already been done in this field, but there is still a need for further work if we are to consider the wide range of species, and the numerous ways used to deal with pollution [3]. Recent massive oil spill accidents in the waters off Kwangyang, Yeosu and Busan in Korean peninsula, have led to the use of a great deal of dispersants [1, 3]. In 1995, the greatest amount of fish deaths caused by *Cochlodinium polykrikoides*, and the massive oil spill caused by the tanker "Sea Prince", attracted great public attention in Korea [3]. In the present study, bioassay and toxicity tests were conducted to investigate the effects of various concentrations of Kuwait oil, the United Arab Emirates oil, Bunker-C, diesel oil, kerosene, oil spill dispersants and mixtures of these oils and dispersants on the growth of *C. polykrikoides*.

MATERIALS AND METHODS

Culture

The dinoflagellate C. polykrikoides was isolated from the waters off Tongyong. After a clonal culture was established, it was maintained in a f/2-Si medium [2] at 20°C under a photon flux from cool white fluorescent tubes of 100 μmol m^{-2} s^{-1} in a 14:10 hr light-dark cycle.

Oils and Dispersants

The test oils used in this study were Kuwait oil, the United Arab Emirates oil Emirates oil, Bunker-C, kerosene and diesel oil obtained from a Korean Oil Refinery Company (Sun Kyung), and two types of oil spill dispersants, Hi-Clean (Daeil Chemical Industry Company) and Seagreen 805A (Korean Chemical Industry Company) were applied. Samples of oils and dispersants were diluted in a series between 10 ppb to 300 ppm with sterilized seawater to provide nominal stock solutions of 1,000,000 ppm for the concentration of test oils and chemical dispersants, and kept in plastic bottles until required. The oils were almost suspended on the surface of mediums, but the dispersants and dispersants/oils mixtures were well mixed with mediums.

Test Organism Conditions

Experiments were done in duplicate, with 10 ml culture medium in 50 ml test tubes. Each tube was inoculated with approximately 300 cells ml^{-1} and grown at 20°C in 12:12 light-dark cycles of white fluorescence illumination, providing a photon flux density of about 70 μmol m^{-2} s^{-1}. Cell numbers were determined by direct cell counts at an interval of 2 days for 20 days, using an inverted Carl Zeiss MC80.

RESULTS AND DISCUSSION

Effect of Oils

Figure 1 shows the effect of the United Arab Emirates oil on the growth of *C. polykrikoides* for 20 days. In low concentrations of ≤1 ppm no impact of growth was recorded. At higher concentrations of ≥10 ppm, cell density significantly decreased, with a range of 10 to 80%. Variations in cell density for those exposed to Kuwait oil, Bunker-C, kerosene and diesel oil were found to be similar as the concentrations of the United Arab Emirates oil. Generally, crude oils are known to be more toxic than Bunker-C, because crude oils contain

potentially toxic compounds such as benzene, toluene and xylene, which are carcinogenic substances. Bunker-C oil contains less acute toxic compounds and contains a great deal of non-dissolved substances which can persist in water for quite a long time compared with crude oils [1]. Additionally, some studies have suggested that concentrations above 100 ppm of diesel oil inhibited protoplast fission and nuclear division for algae and metabolism for marine organisms [4, 6]. This result indicates that high concentrations of tested oils may significantly suppress the growth of *C. polykrikoides*.

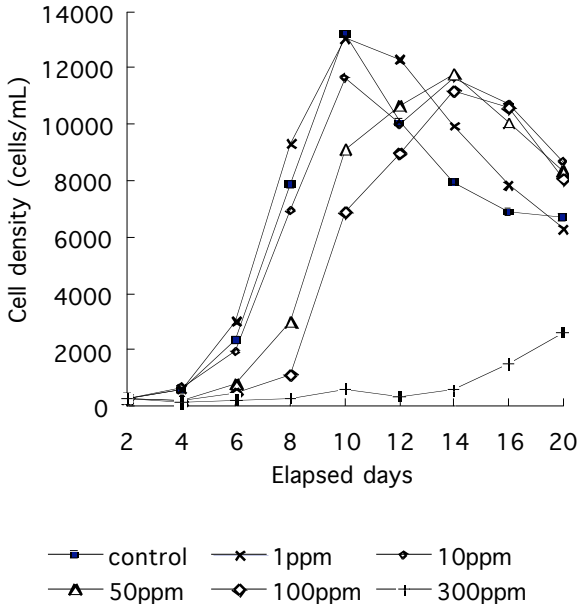

Figure 1. The growth of *C. polykrikoides* for 20 days after treated with a different concentration of the United Arab Emirates oil.

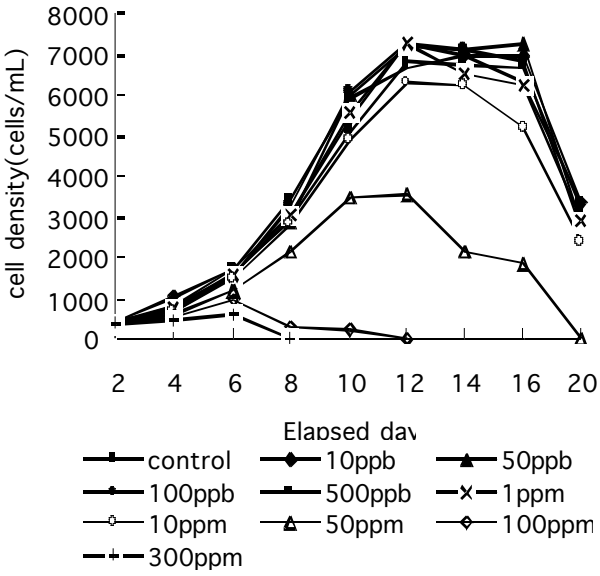

Figure 2. The growth of *C. polykrikoides* for 20 days after treated with a different concentration of oil spill dispersant, Seagreen 850A.

EFFECT OF DISPERSANTS AND MIXTURES

In most cases the dispersant Seagreen 850A appeared to be toxic, extremely decreasing the growth of *C. polykrikoides* after the addition to ≥10 ppm (Fig. 2). Similarly, when the cells were exposed to a higher concentration of the dispersant Hi-Clean, it made a great impact on the growth of *C. polykrikoides*. The combined effect of Kuwait oil and the dispersant Seagreen 850A to *C. polykrikoides* was seen in Figure 3. At concentrations of ≥50 ppm, the cell numbers were decreased by 70% in comparison with the control after 14 days, whereas the growth of *C. polykrikoides* exposed to ≤5,000 ppb showed little serious impact. The toxicity of a mixture of three types of oils and two types of dispersants appeared to have similar variations (Fig. 3). Since the dispersants consist of a mixture of a surfactant and a stabilizing agent, their toxicity may depend on the kind of solvent and surfactant [5]. However, *C. polykrikoides* almost died within a few days regardless of types of dispersants and combined mixture after the addition of high concentrations.

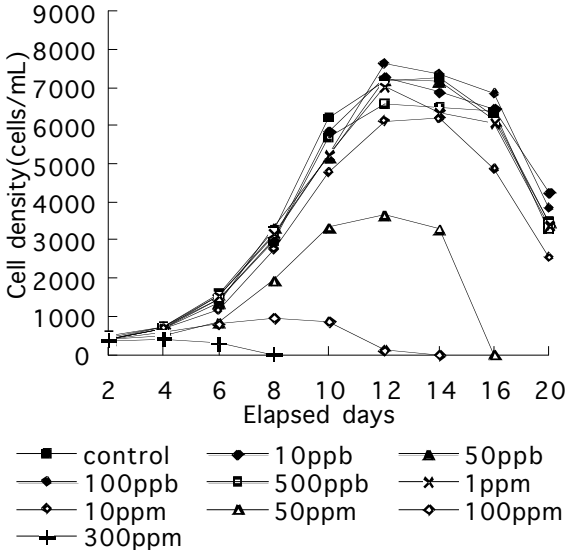

Figure 3. The growth of *C. polykrikoides* for 20 days after treated with a different concentration combined with Kuwait oil and the dispersant Seagreen 850A.

ACKNOWLEDGEMENTS

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A STUDY ON THE EFFECTIVENESS OF USING OZONE TO MITIGATE HARMFUL ALGAL BLOOMS

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ABSTRACT

Experiments showed that ozone was an effective agent in mitigating red tide caused by dinoflagellate blooms. When ozone (optimal injection rate = 1 gm-O_3 m^{-3} seawater) was injected into seawater that contained *Prorocentrum triestinum*, *Scrippsiella trochoidea* and *Karenia digitata* cultures of concentration equivalent to 7200 cells ml^{-1}, all the algal cells died within 15 minutes. The level of dissolved oxygen in the algal culture was also elevated to a level acceptable to the Water Quality Objective of Hong Kong. Furthermore, the levels of ammoniacal nitrogen and total inorganic nitrogen (TIN) in water decreased rapidly due to oxidation process initiated by ozone and residual OH^-. Since ozone was not directly injected into the seawater for fish cultivation (i.e., seawater was pumped into a separated water tank for treatment before refilling into fish tanks) the level of residual ozone could be controlled to a level not affecting the life of cultured fishes. Preliminary economic assessment showed that ozone is more suitable for land-based, tanked cultivation of aquacultural products including fish fries. The potential of using ozone to mitigate HABs is promising and should be further developed.

INTRODUCTION

Harmful algal blooms (HABs), particularly those caused by exaggerated growth of dinoflagellates, cause severe damage to marine ecosystems and the aquaculture industry. For example, the HAB in 1998 in Hong Kong and the South China coast resulted in huge amounts of fish kill in aquaculture zones. The economic loss was reported to be more than 30 million US dollars [1]. Recently, numerous research projects were done to mitigate HABs such that the ecological and economic losses can be minimized. For example, attempts have been made to apply clay or other chemicals to precipitate harmful algae [2, 3, 4]. Some researchers, however, argued that biological control would be more ecologically friendly [5, 6]. Nonetheless, only limited amount of these research products have been found practical for widespread application [7]. The major constraints include: (1) none of these mitigating methods can be universally applicable – different geographic and ecological situations require different mitigating strategies, (2) some of the mitigating methods (e.g., biological control) cannot satisfy immediate rescue needs, (3) the long-term risks of applying artificial chemicals to ecosystem have yet been fully evaluated, and (4) the investment and operation cost can hardly be afforded by the aquaculture industry because the mitigating technologies have yet been maturely developed for industrial-scale manufacturing.

Ozone has been considered one of the agents for mitigating HABs [8, 9]. While none of the available technologies is perfect enough for universal application, ozone as a HAB mitigating agent contains the following advantages: (1) it has been widely used in swimming pools, sewage treatment, drinking water purification, laundry and other bacterial disinfection processes, and so can be easily transformed for aquaculture usage if scientific research proved that it is feasible, (2) it was reported that ozone had a quick reaction rate with microbes and could kill nearly all waterborne microbes in a few minutes, (3) ozone can be generated on site from fresh air (which contains 18-22% oxygen) by means of the recently developed impact-ionization mechanism with simple electrical discharges, and (4) the strong oxidizing power of residual ozone might be able to upgrade water quality. While most of the previous research focused on the effectiveness of ozonation on cyanobacteria [8-10], the present research aims at studying the cost and effectiveness of applying ozone in killing harmful dinoflagellates at an experimental scale.

MATERIALS AND METHODS

Ozone is considered harmful to some of the reared fishes and so cannot be directly injected into fish cages. Therefore, in the present experiment ozone was injected into seawater by means of an indirect diffusion method. That is, water with high concentrations of harmful dinoflagellates was pumped up into a water tank, in which ozone for dinoflagellate bloom mitigation was injected at stable rates of 0.3 - 2.5 gm-O_3 m^{-3} of seawater min^{-1} by adjustment of output of the ozone generator and the use of valves. The levels of ozone were carefully monitored before the treated water was refilled into the fish cultivation tanks. A flow diagram of the experimental device is shown in Figure 1.

Ozone was generated on site by impact ionization and recombination mechanism (model OZM2020U manufactured by Mentor Industrial Co.). When air flows through a narrow annular column, electrical discharges turn flowing air into ozone. Injected ozone was firstly retained in a storage tank that contained seawater. Afterward, water saturated with ozone was pumped into different smaller tanks for treatment and environmental parameters monitoring. Three species of dinoflagellates, namely *Prorocentrum triestinum*, *Scrippsiella trochoidea* and *Karenia digitata*, were tested. They were cultured in K-medium at 20^oC in an environmental chamber with 12 hr light:12 hr dark cycle to about 7200 cells ml^{-1} before ozonation. One population of natural phytoplankton sampled from Tolo Harbour, Hong Kong was also

selected for experiments as control. The concentrations of living algal cells in experimental waters were measured by taking samples at every 2-3 minutes intervals. The number of living cells was counted with Sedgewick-Rafter Chambers under inverted microscopes. Water samples were also taken at every 2-3 min for determining the changes in dissolved oxygen, COD, ammoniacal nitrogen, nitrate nitrogen and orthophosphate. The various chemical analyses followed standard methods recommended by the APAH [11]. Changes of dissolved oxygen were measured by a YSI dissolved oxygen meter.

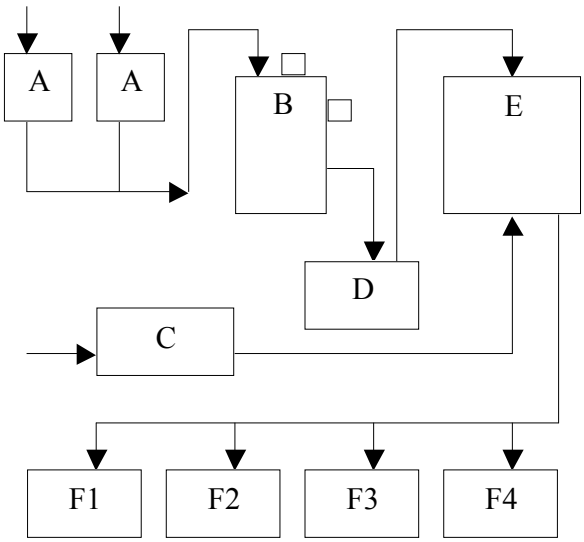


Figure 1. Flow diagram of the ozone injections device. A = ambient air preparation units; B = ozone generator; C = water intake pump; D = injecotr; E = ozonated water tank; F 1-4 = experimental flasks.

RESULTS AND DISCUSSION

Determination of Optimal Ozone Injection Time

To reduce the excessive cost of ozone generation, the optimal ozone concentration for mitigating process must firstly be determined. The dinoflagellate-contained waters were therefore injected with differential concentrations of ozone *viz*. at 0.3, 0.5, 0.8, 1.0, 1.5, 2.0 and 2.5 gm-O_3 m^{-3} of seawater, respectively. The percentages of survival cells at different intervals were counted with results shown in Figure 2.

It was found that 1.0 gm-O_3 m^{-3} seawater was the optimal concentration because below this concentration the mitigation time would be significantly increased and above this concentration different injected ratios had nearly the same effect of killing all dinoflagellates within 15 minutes.

Effects of Ozone on Dinoflagellates

Ozonation of 1 gm-O_3 m^{-3} seawater min^{-1} was considered the optimal treatment concentration. When this concentration of ozone was injected into seawater, it was found that dinoflagellate cells gradually burst and died. Within 10 min, more than 75% of the three species of dinoflagellates were killed (Fig. 3). Within 15 min, all the dinoflagellates died and burst. The present results proved that ozone was as an effective agent in killing harmful dinoflagellates.

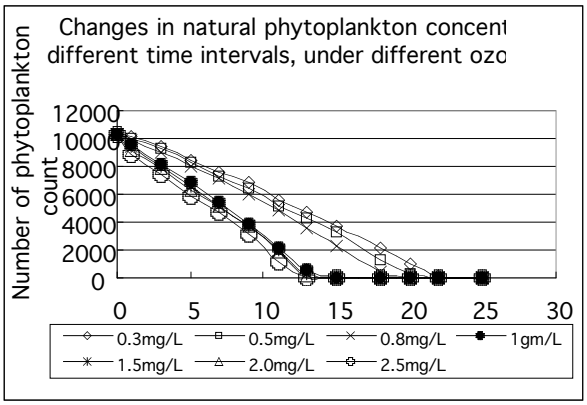


Figure 2. Results for the determination of optimal ozone injection concentration.

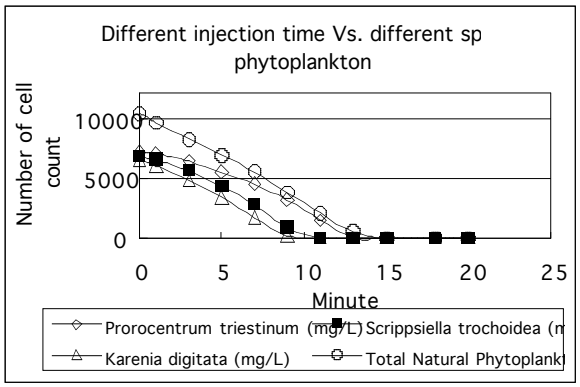


Figure 3. Effectiveness of ozone in killing dinoflagellates.

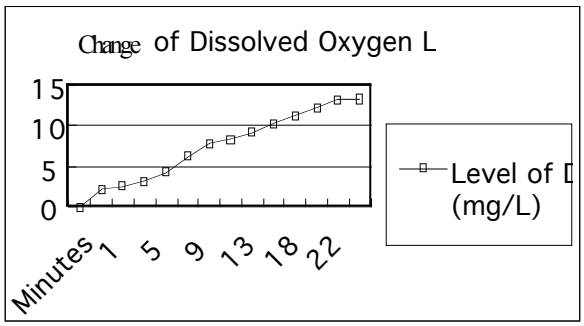


Figure 4. Changes of dissolved oxygen level.

Change in Dissolved Oxygen (DO) Levels

As shown in Figure 4, ozone could not only kill the dinoflagellate cells effectively, but could also increase the level of dissolved oxygen in seawater in a short

period of time. It is not surprising because ozone is very unstable in seawater with most of them changed into dissolved oxygen rapidly. Previous reports showed that deoxgenation was the most common cause of fish kills during red tide [12,13, 14]. Hence, the present increase in dissolved oxygen level after ozonation may be considered a side benefit of HAB mitigation.

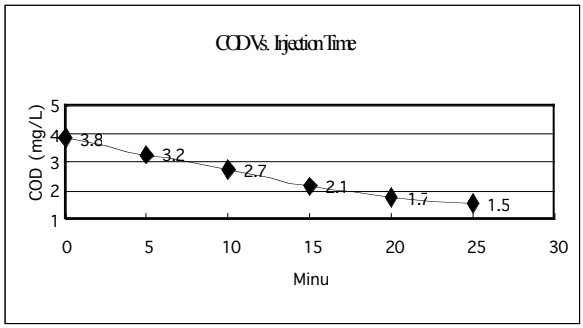
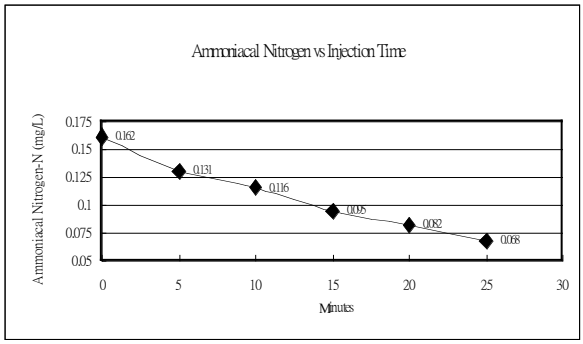


Figure 5. Changes of COD levels.



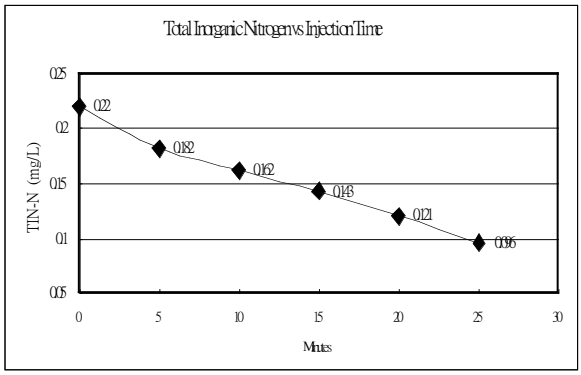


Figure 6. Changes of (a), NH_4-N and (b), TIN levels.

As shown in Figures 5-7, the levels of COD, NH_4-N, TIN and orthophosphate were gradually reduced after ozone injection. This may be due to direct chemical reactions between O_3 and organic matters and the chemical reactions between residual OH^- and the organic nutrients [15, 16]. Although the mechanisms of chemical reactions have yet been clarified, the effectiveness of ozone in improving water quality can be recognized.

While ozone is shown to be effective in killing harmful dinoflagellates as well as improving water quality, the possible risks of ozone application cannot be overlooked. Firstly, direct injection of ozone should be avoided because some fishes and marine organisms might be adversely affected by excessive ozone. With this regard, ozone mitigation should be conducted in primary water tanks where seawater is purified before being used to fill the cultivation tanks/ponds. In consideration of this constraint, ozone injection seems to be more suitable for land-based aquacultural practices such as those that for rearing mollusk and fish fries in tanks or ponds. For fish rafts with enclosed skirts, ozone is also their good choice.

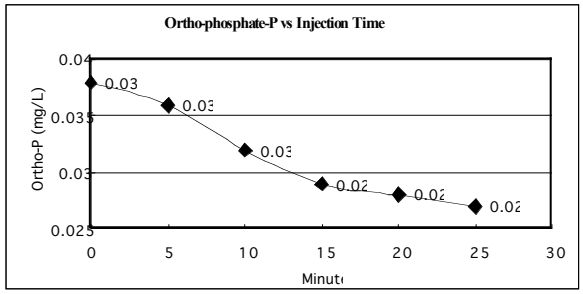


Figure 7. Changes of ortho-phosphate levels.

While excessive ozone and its residual OH^- are considered to be effective in reducing nutrients and organic matters in seawater, the ecological impact of ozone to marine ecosystems has yet been fully evaluated. Therefore, a detailed environmental impact assessment (EIA) must be conducted when this technology is applied at open sea. For land-based aquaculture, a good monitoring strategy must be in place to observe and regulate the excessive ozone and residual OH^-.

As regard to large-scaled application of ozone in mitigating HAB, cost remains a significant factors to be considered. As assessed by local engineers, the capital investment of an ozone machine is 2-5 times higher than an aerator, which fishermen commonly used in increasing DO level during red tides. However, the energy cost of ozone technology is assessed to be 30-60% less than mechanical aerator. Furthermore, ozone generation machine is very handy and is feasible for sharing of use by several fish farms. The annual maintenance and depreciation cost of an ozone generator is much lower than that of a mechanical aerator at least for the first 5 years. Taking into consideration that an ozone generator may have a 3-5 years longer lifespan than a mechanical aerator, the use of ozone generator seems to be more economic than the conventional, mechanical aeration methods, which has been commonly applied by fishermen during HABs. If ozone is going to be commonly applied in aquacultural industry, it is anticipated that a tailor-made generator would be specially designed by manufacturers. With an enthusiastic marketing strategy, the selling price of an ozone generator could be further reduced.

Obviously, ozonation cannot be considered the sole method for HAB mitigation as compared with chemical and clay applications and biological control. However, as discussed above, no single strategy or agent is considered universally applicable in mitigating HABs. In consideration of the advantages of ozonation including speed, effectiveness, improvement of DO and reduction of eutrophication, this technology has a good potential for exploration in HAB mitigation. Further research on its ecological impacts and the most economical design is recommended.

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BIOLOGICAL STUDIES

ECOSYSTEM CHANGES AND HARMFUL ALGAL BLOOMS IN THE BOHAI SEA

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ABSTRACT

Bohai Sea is the largest inland sea in North China with an area of 77,284 km^2 and average depth of 18 m. Many rivers enter here carrying a lot of nutrients. Plankton biomass is high and resources of fish and shrimp are abundant. It is an important fishing ground in North China. Since the 1980's, significant ecosystem changes occurred in Bohai Sea. The results of several investigations showed that eutrophication has accelerated. Dissolved inorganic nitrogen (DIN) increased from 0.03 mg L^{-1} in 1982 to 0.28 mg L^{-1} in 1998. Inorganic phosphate (IP) did not appear to change, resulting in a 7-fold increase in the N/P ratio. There has been a decrease of abundance of phytoplankton and zooplankton biomass. Primary productivity and chlorophyll-a was decreased as well. The occurrence of harmful algal blooms also increased. Since the 1980's the number of causative species has increased. *Noctiluca scintillans* was previously the main causative species; however, several species are currently responsible. For example, *Scrippsiella trochoidea* and *Gymnodinium* sp. formed blooms in summer 1989 and *Ceratium furca, Dinophysis fortii* and *Gonyaulax* sp. formed blooms in summer and autumn of 1998. These two blooms were large in scale and lasted about two months. In August 1998, a HAB appeared in Liaodong Bay and extended southward and in most areas of Bohai Sea until October. In this paper, the ecosystem change and causes of harmful algal blooms are discussed.

NATURAL CHARACTERISTICS OF BOHAI SEA ECOSYSTEM

Bohai Sea is a semi-enclosed inland sea in North China (37°07′~41°N 117°35′~21°10′E), surrounded by Liaoning, Hebei, Shandong provinces and Tianjian municipality. It consists of Liaodong Bay, Bohai Bay and Laizhou Bay and Central Region (Fig. 1). The average depth of Bohai Sea is 18 m and the area is 77,284 km^2. Its seawater temperature averages -1 to 2°C in winter and 24 to 26°C in summer. Salinity of the seawater averages 30 ‰. The mean annual rainfall is 300-400 mm. In winter, the sea ice usually occurs in Liaodong Bay from November to March. Some years, ice can also occur in Bohai Bay and Laizhou Bay.

The seashore is sandy and muddy in Bohai Bay, Yellow River Estuary and the northern part of Liaodong Bay. Fine and coarse sand is present on the west coast of Bohai Sea and the north coast of the Luanhe River mouth. Rocky shores are found along the east coasts of Laizhou Bay and Liaodong Bay. The sediment is coarse silt and fine sand in Liaodong Bay. It is silt mud or muddy silt in Bohai Bay and silt in Laizhou bay.

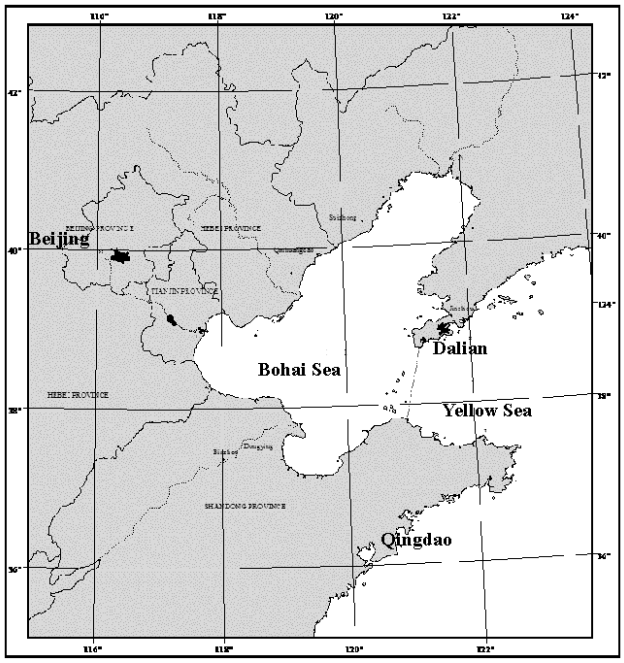

Figure 1. Location of Bohai Sea.

There are more than 40 rivers along the coast of Bohai Sea, such as the Yellow River, Haihe River, Liaohe River, Luanhe River, Shuantaizhi River, and Xiaoqinghe River. The total river run off is about 50 billion cubic meters per year with 1 billion tons of mud and sand. There are three main wetlands, including Liaohe River Estuary, Yellow River Estuary and Haihe River Estuary, which are the spawning and nursery areas for main economical species.

BOHAI SEA ECOSYSTEM CHANGES

Nutrients

From 1982 to 1992, a significant change of nutrients was found in Bohai Sea (Fig. 2) [1-5]. The dissolved inorganic nitrogen (DIN) increased from 0.03 to 0.25 mg L^{-1} while dissolved inorganic phosphorous (DIP) had no clear change. The average ratio of N/P increased from 1.5 to 10.8. The dissolved inorganic silicate also decreased. The decrease of Yellow River runoff may have caused this change. Further changes of nutrients have been observed in recent years. The DIN has increased since 1992 and reached about 0.28 mg L^{-1} in 1998[6-9].

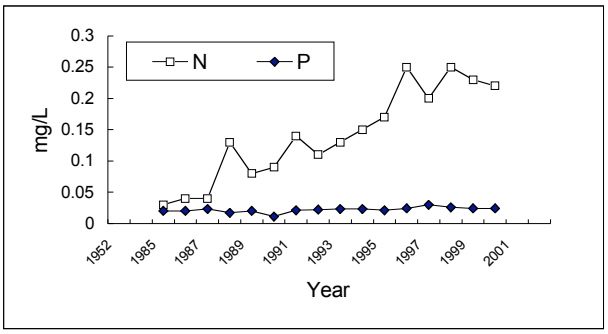

Figure 2. Nutrient changes in Bohai Sea.

Chl-a and Primary Productivity

In Table 1, it can be seen that the mean Chl-a decreased from 1982-1983 to 1992-1993. But the distribution pattern of Chl-a in Bohai Sea seems to have no significant change. Chl-a was highest in Laizhou Bay and lowest in the central region.

Primary productivity in Bohai Sea also decreased from 1982-1983 to 1992-1993 (Table 1). It was highest in Laizhou bay and lowest in the Bohai bay both in the early 1980's and early 1990's. There is a good correlation between the decrease of primary productivity and Chl-a [10].

Table 1. Change of Chl-a (mg m^{-3}) and primary productivity (mg C m^{-2} d^{-1}) in Bohai Sea during 1982-1992.

	Year	Aug	Oct	Feb	June	Mean
Chl-a	82-83	1.15	1.05	1.1	0.64	0.98
	92-93	0.67	0.58	0.73	0.44	0.61
PP	82-83	537	297	207	208	312
	92-93	419	154	127	162	216

Structure of Phytoplankton Community

Thirty-one genus and 70 species of phytoplankton in Bohai Sea have been identified. Most of them are diatoms. The dominant species include *Chaetoceros* spp., *Coscinodiscus* spp. and *Eucampia zoodiacus*. The seasonal change of phytoplankton is very clear (Fig. 3b). In 1982, the highest peak of phytoplankton cell numbers appeared in May. But in 1992 the phytoplankton reached highest density in February. Compared with 1982, the mean cell density of phytoplankton in 1992 declined to 99×10^4 cells m^{-3}, representing ~65% decrease (Fig. 3a). In 1992, *Eucampia zoodiacus* became the most dominant species instead of *Chaetoceros* spp. The distribution of phytoplankton was still similar to that in 1982 with high density in Laizhou Bay [11-13].

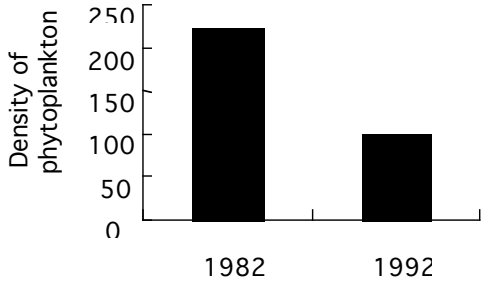

Figure 3a. Mean phytoplankton abundance in Bohai Sea in 1982 and 1992 (cellsx10^4 m^{-3}).

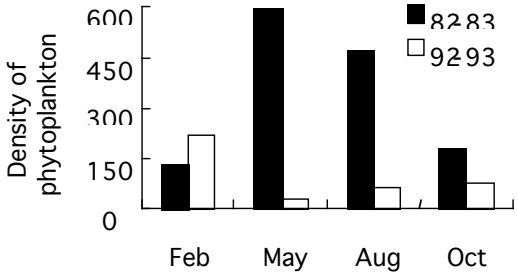

Figure 3b. Phytoplankton abundance in spring, summer, autumn, and winter of 1982 and 1992 in Bohai Sea (cellsx10^4 m^{-3}).

Structure of Zooplankton Community

There were 47 species of zooplankton recorded in Bohai Sea in 1992 and 1993. Among them there were 17 species of copepod and 14 hydromedusae. *Calanus sinicus, Sagita crassa* and *Labidocera euchaeta* were the dominant species. The biomass of zooplankton reached the highest value in May and the lowest value in February (Fig. 4). Compared with 1982, the biomass of zooplankton in 1992 declined about 30% [1].

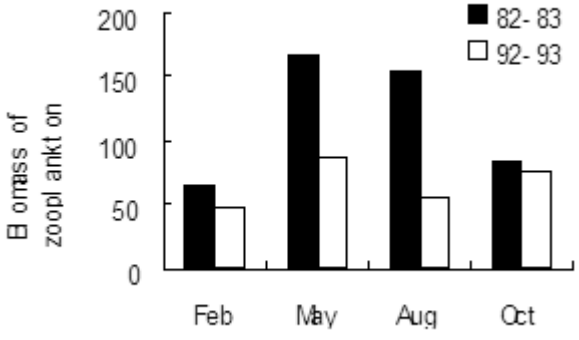

Figure 4. Zooplankton biomass in Bohai Sea (mg m^{-3}).

Changes of Benthic Community

The macro-benthos was sampled by using bottom trawl. Compared with the results in 1982, the number of species representing the benthic community in 1992 did not show a clear change. Crustacea were the most important species and accounted for 47% of total benthic-fauna, molluscs declined to 29% in 1992 (39% in 1982), and echinoderms increased (Fig. 5) [1].

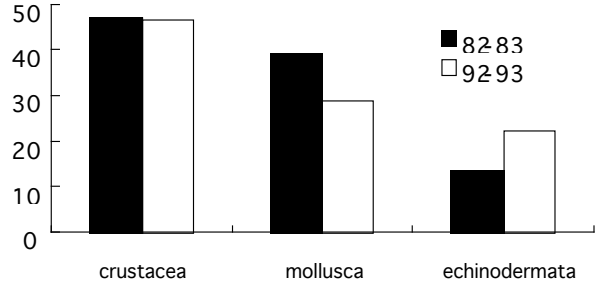

Figure 5. Species composition of macro-benthic community of Bohai Sea.

HARMFUL ALGAL BLOOMS

Occurrence of HABs

The increase of HABs could be a response to the change in the ecosystem. Until 2001, there were 85 HAB events in Bohai. Before 1970, only 2 HABs were recorded. Since the late 1980's, HABs could be found every year and they appear to increase as development increases. In 2001, 18 HAB events were observed (Fig. 7).

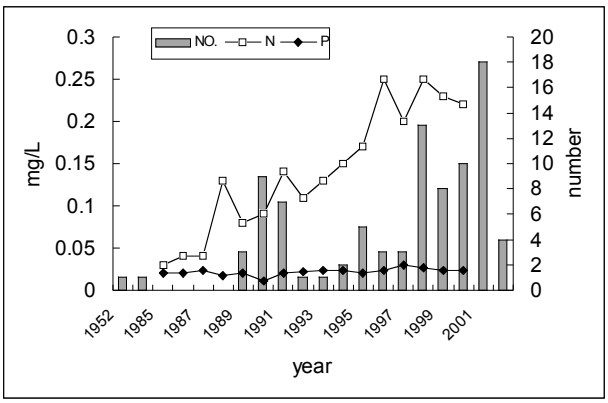

Figure 6. Number of HABs (right axis) and nutrient concentrations (left axis) in Bohai Sea.

Causative HAB Organisms

There are about 13 causative HAB species in this region. HABs caused by *Noctilluca scintillans* have been most common, accounting for 24% of total events. HABs occurred during 1990 to 1997 were all caused by *Noctilluca scintillans,* except for one diatom HAB. In 1998, a large scale HAB of *Ceretium furca* appeared. The list of HAB organisms in Bohai is shown in Table 2. In winter (January & February), only blooms of *Noctilluca scintillans* and *Ceratium furca* were recorded.

Among the HAB species, some are toxic, such as *Prorocentrum minimun* and *Dinophysis fortii*. HABs of *Dinophysis fortii* occurred in 1998 and diarrhetic shellfish poisoning (DSP) was detected in shellfish samples (Table 3) [14-15].

Table 2. List of HAB species that occurred in Bohai Sea in January, February, May, June, July, August, September and October.

Species	Month HAB occurred							
	J	F	M	J	J	A	S	O
Noctilluca scintillans		√	√	√	√	√		
Prorocentrum minimun						√		
Scrippsiella trochoidea					√	√		
Gymnodinium sp.						√		
Coscinodiscus asteromphalus								√
Chaetoceros socialis							√	
Leptocylindrus danicus						√		
Eucampia zoodianus						√		
Lau borealis			√	√				
Bidduplus sinensis							√	
Ceratium furca	√					√	√	√
Gonyaulax sp.							√	√
Dinophysis fortii						√		
Mesodinium rubrum					√	√		
Navicula sp.						√		

Nature of HABs in Bohai Sea

HABs are usually located in estuaries, coastal waters and harbors. The areas with frequent HABs in the Bohai Sea include Liaodong Bay, Bohai Bay, Laizhou Bay, Estuary of Liaohe River, Estuary of Yellow River,

Estuary of Xiaoqing River, Estuary of Hai River, Tianjin Harbor, and Yingkou Harbor.

Some HABs in the Bohai Sea were large in scale and duration. For example, in July 1989 a *Scrippsiella trochoidea* bloom occurred in the coastal water of Huanghua City, then stretched towards the north to Tianjin, towards the south to Southern Bohai Bay and further towards the east to Laizhou Bay in Shandong Province with a total area of 1300 km^2, lasting for about 3 months. From August-September 1998, *Ceratium furca* blooms appeared in Liaodong Bay and then extended into Central Bohai with an area of 5,000 km^2.

Most HABs occurred during June to September. In the Bohai Sea, most HABs occur from April to October. There were 61 HABs recorded from 1990 to 2002. 51 occurred during June to September, accounting for 84% of the total. The highest number occurred in July (Fig. 7).

Table 3. DSP in shellfish samples in Qinghuangdao area in 1995 [15].

Time	Number of Sample	Number of DSP detected
March 95	2	-
April 95	8	2
May 95	11	5
June 95	6	3
July 95	5	1
Aug 95	6	2
Sept 95	7	1
Oct 95	7	-
Nov 95	7	-
Dec 95	5	-

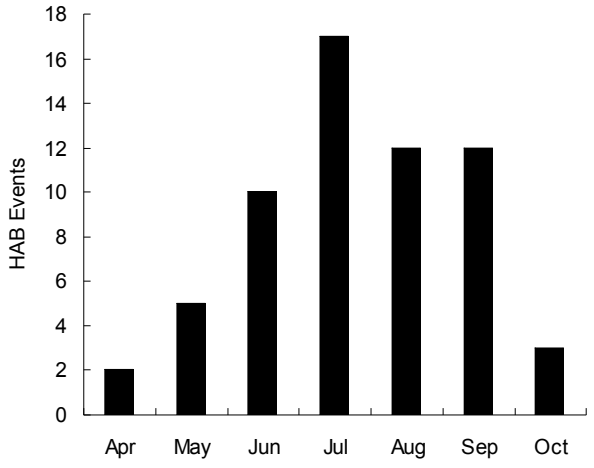

Figure 7. HAB events in different months during 1990-2002.

Causes of HABs

Eutrophication

Clear correlations have been observed between the increase of eutrophication and HAB events [18]. From Figure 6, it can be seen that there was an increase of DIN since the late 1980's, followed by a clear increase in HABs. Table 4 shows the change in nutrients and Chl-a before and after the largest HAB of *Ceratium furca* and *Gonyaulax* sp. in Bohai Sea in 1998. Before the HAB, both DIN and DIP were high. After the HAB, nutrient concentrations decreased sharply. Nitrate and phosphate at some stations were below detection level. During a bloom of *Leptocylindrus danicus* in Yingkou in August 2001, nutrients decreased significantly (Table 5). These results indicate that nutrients were important in the formation of these two HABs.

Table 4. Nutrients and Chl-a before and after a *Ceratium furca* bloom in August 1998.

	DIN μg L^{-1}	DIP μg L^{-1}	Chl-a mg m^{-3}
Before	102.84-328.4	4.4-43.5	<1.0-4.0
After	3.79-5.02	0.3-0.33	12.26-35.61

Table 5. Change in chemical parameters before, during and after a bloom of *Leptocindrus danicus* in Yingkou Habour of Bohai Sea in 2001.

	DIN μg L^{-1}	phosphate μg L^{-1}	Silicate μg L^{-1}
Before	439.32	27.59	487.76
During	210.70	4.96	376.32
After	26.74	5.58	113.12

Change in salinity and precipitation

The reduction of fresh water loading from Yellow River caused the increase in salinity in Bohai. The Yellow River runoff reached a maximum of 973.1×10^8 m^3 in 1964 and the average in the 1960's-1970's was about 400×10^8 m^3. However, in 1997, it was only 18.11×10^8 m^3. The average runoff during 1997-2001 was 57.6×10^8 m^3, 1/7 of that value in 1960's. On the other hand, the days without effluent of Yellow River increased dramatically. In 1997, there were 227 days with no fresh water running into Bohai Sea [16-17]. The salinity in Laizhou Bay was impacted by freshwater input from Yellow River. Compared with 1982, the salinity of this bay in 1992 increased, especially in August and October (Fig. 8). This may be contributed by the decrease of Yellow River runoff. The change in salinity may have impacted the phytoplankton community and HAB species. The causative species *Ceratium furca*, which formed the largest HAB in Bohai in 1998 is adapted to warm water and high salinity. In previous investigations, it usually appeared in August. However, HABs of this species did not occur until the 1990's. The results of XU et al. [16] suggested that less precipitation and higher temperatures may have contributed to HABs in Huanghua in 1989.

Intense rainfall could carry out nutrients into the sea. It was found that if there were more precipitation in a year, HABs of *Noctilluca scintillans* and diatoms would increase as well in Bohai Sea. In 2001, after several heavy rains, the nutrient in seawater was increased and a bloom of *Leptocylindrus danicus* occurred.

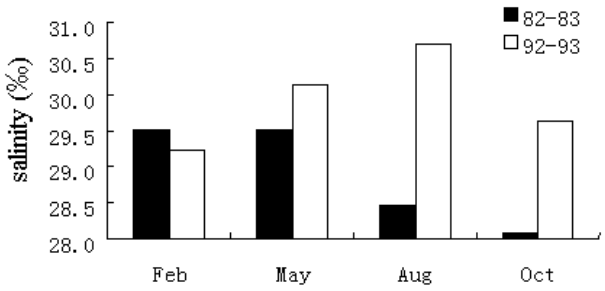

Figure 8. Change in salinity at the surface layer in Laizhou Bay.

SUMMARY

1. Since 1980's, significant ecosystem changes were observed in Bohai Sea, such as increase of DIN and decrease of fresh water loading, abundance of phytoplankton and zooplankton.
2. HABs in Bohai Sea were increasing and the root cause is eutrophication.

ACKNOWLEDGEMENTS

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EUTROPHICATION IN HONG KONG COASTAL WATERS: LONG-TERM CHANGES IN NUTRIENTS, PHYTOPLANKTON AND WATER QUALITY

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ABSTRACT

The coastal waters of Hong Kong are under the influence of nutrient-rich Pearl River, pollution from local sources and the neighboring Shenzhen. Between 1986 and 2000, an increase of inorganic nitrogen and phosphorus was detected in Deep Bay, parts of Southern Water and some urban inshore waters. There has also been a wide spread increase in chlorophyll-*a* values and phytoplankton abundance in the last 10 years, but not when measured against the base year of 1986. While the diatoms remained largely stable, small flagellates, dinoflagellates and other groups showed a steady rise. The phytoplankton densities generally correlated positively with organic matter (BOD_5), total nitrogen, TN/TP ratio and negatively with total and inorganic phosphorus. Relationships between phytoplankton and nutrients were location- and season-specific, Tolo Harbour and Southern Water being more closely related to nutrients and Deep Bay the least. There are indications that Hong Kong coastal waters have experienced two periods of heightened algal activity in the last 20 years, one in the mid to late 80s and another in 90, intercepted by a quiescent period around 1991 and 1993. Longer time series data are needed to confirm the existence of a 10-year phytoplankton cycle and its environmental implications.

INTRODUCTION

The marine coastal waters of Hong Kong cover an area of 1,656 km^2. Situated at the edge of the Pearl River Estuary, the waters are largely oceanic to the east and estuarine to the west, with a transitional zone in between [1]. Tidal currents in the territory are in the general direction of southeast during the ebbing tide; and southwest during the flooding tide. Elevated nutrient levels are found in some waters, in particular Deep Bay bordering Shenzhen, Victoria Harbour which receives about 1.9 million m^3 d^{-1} of sewage effluent and in the Southern Water under the influence of nutrient rich Pearl River flow. Red tides occur regularly, between 20 and 45 incidents per year, mostly in eastern waters. In 1998, a large scale harmful algal bloom occurred in Hong Kong, and caused heavy losses to the mariculture industry and impacted on the recreational use of bathing beaches [2].

To keep track of the eutrophication and long-term water quality changes, the Environmental Protection Department (EPD) implements a comprehensive marine monitoring programme. This comprises monthly monitoring of water quality parameters including nutrients, chlorophyll-*a* and phytoplankton. This paper reports on the current water quality and long-term changes in nutrient levels and phytoplankton species and abundance in the territory - Mirs Bay, Tolo Harbour and Port Shelter in the east, Deep Bay and Northwestern Water in the west, and Victoria Harbour and Southern Water in between. Relationships between phytoplankton and nutrients were also examined to provide an initial understanding of the eutrophication process and relationships to phytoplankton dynamics in these waters.

MATERIALS AND METHODS

The EPD conducted monthly marine monitoring on the vessel *Dr. Catherine Lam* at 75 water quality monitoring stations and 25 phytoplankton monitoring stations (Fig. 1).

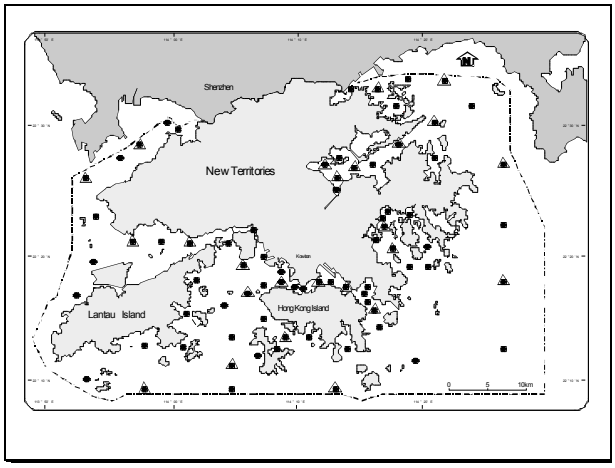

Figure 1. Water quality monitoring stations (•) and phytoplankton monitoring stations (Δ) in marine waters of Hong Kong.

Water samples were collected from 3 water depths (1 m below surface, mid-depth and 1 m above bottom) and profiles of temperature, salinity, pH, turbidity, dissolved oxygen (DO) were measured using a SEACAT 19 CTD profiler. The chemical analyses were carried out by the Government Laboratory and EPD's Environmental Chemistry Laboratory. The parameters analysed included NO_3-N, NO_2-N, NH_4-N Kjeldahl-N, SiO_2, PO_4-P, suspended solids (SS), 5 day-Biochemical Oxygen Demand (BOD_5) and chlorophyll-*a*. Depth averaged water quality data were generally presented in the paper. Phytoplankton samples were collected from 1 m below surface and fixed with Lugol's iodine on site. At EPD's Water Science Laboratory, settled samples were examined under an inverted microscope and all phytoplankton species were identified and enumerated using a Utermohl chamber. Further identification was

done using an SEM when necessary [3]. Details of the water quality analysis methods are found in the annual report *Marine Water Quality of Hong Kong 2000* [4], also available at the website http://www.info.gov.hk/epd.

RESULTS

Current Water Quality

The seawater temperature in 2000 in Hong Kong varied between 14°C in November and 30°C in August (in Deep Bay). Less pronounced temperature variation were observed in other parts of the territory (Fig. 2).

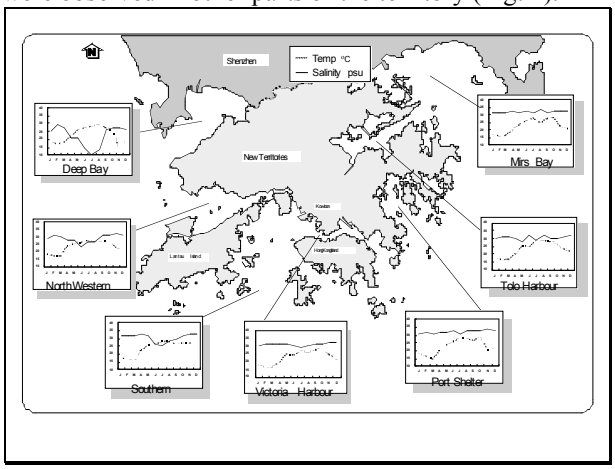

Figure 2. Temperature and salinity in marine waters of Hong Kong, 2000.

Salinity in Deep Bay also showed large seasonal variations (between 4.8 psu in July and 32.0 psu in November). A marked decreasing salinity gradient was observed from eastern to western waters during the summer wet months.

The levels of total inorganic nitrogen (TIN=NO_3-N+NO_2-N+NH_4-N) in Deep Bay were highest in the territory (averaging 1.9 mg L-1), followed by the adjacent Northwestern Water (0.39 mg L^{-1}) and Victoria Harbour (0.37 mg L^{-1}) (Fig. 3). Higher TIN was also found in the Northwestern Water and Southern Water between May and September under the greatest riverine impact. This was not obvious in the eastern waters where a much lower TIN level (mostly below 0.08 mg L^{-1}) was maintained throughout the year. The spatial distribution of PO_4 generally mirrored that of TIN, highest in Deep Bay (averaging 0.19 mg L^{-1}), followed by Victoria Harbour and Northwestern Water (Figure 4).

Other waters generally had a much lower PO_4 concentration of 0.01-0.02 mg L^{-1} and no distinct seasonal pattern was observed.

In contrast with the distribution patterns of TIN and PO_4, chlorophyll-*a* was much higher in Tolo Harbour (averaging 8.5 μg L^{-1}), peaking in summer and autumn (Fig. 5), followed by Victoria Harbour (4.3 μg L^{-1}) and Southern Water (3.9 μg L^{-1}) with distinct peaks in the summer months. Deep Bay, despite its elevated nutrient levels, had a relatively low chlorophyll-a level (2.8 μg L^{-1}). There were a total of 45 red tides in 2000, 39 of which occurred in eastern waters (Tolo Harbour 16, Mirs Bay 12, Port Shelter 11) and no red tide in Deep Bay, Northwestern Water or Victoria Harbour (Figure 6).

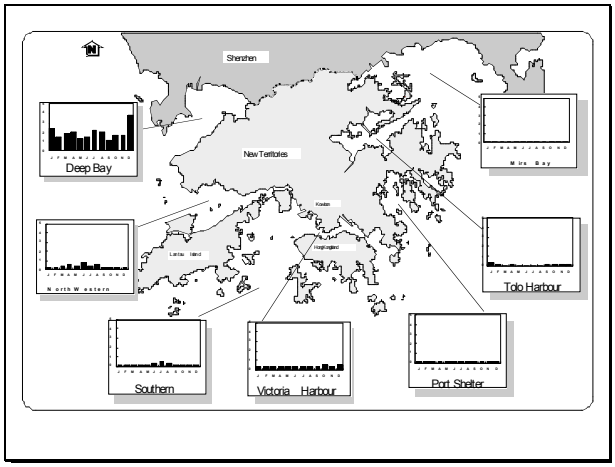

Figure 3. Total inorganic nitrogen (mg/L) in marine waters of Hong Kong, 2000.

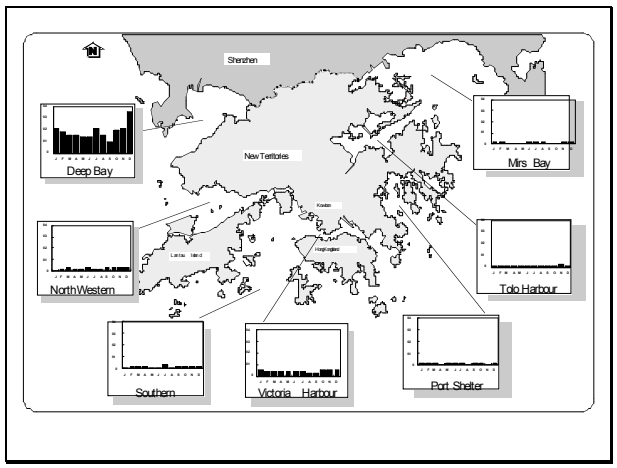

Figure 4. Orthophosphate phosphorus (mg/L) in marine waters of Hong Kong, 2000.

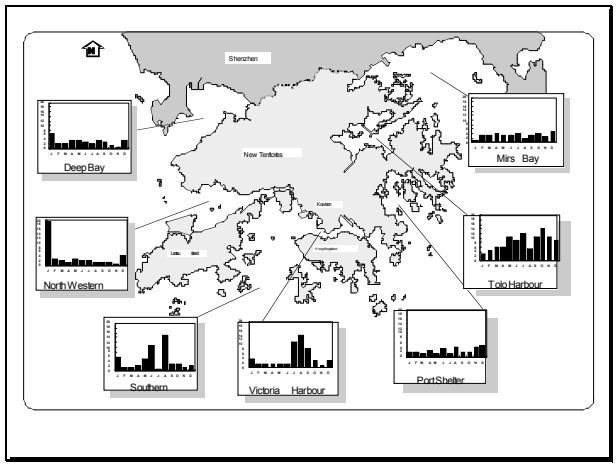

Figure 5. Chlorophyll-*a* (mg/L) in marine waters of Hong Kong, 2000.

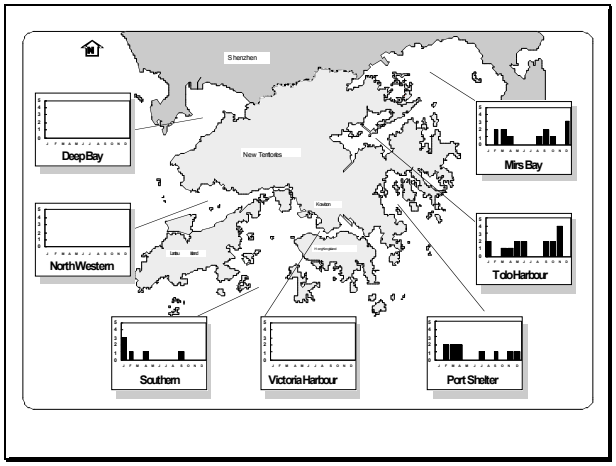

Figure 6. Red tides in marine waters of Hong Kong, 2000

Water Quality Trends in 1986-2000

The Seasonal Kendall Test was used to analyse long-term trends in nutrients and chlorophyll-*a* at each monitoring stations in the last 15 years, and significant increases or decreases (p<0.05) are summarised in Figures 7-10. Increasing trends in TIN were found in Deep Bay, Northwestern Water and Southern Water as well as in the eastern and western parts of Victoria Harbour (Fig. 7) where five major sewage outfalls were located. Similar increases in PO_4 were observed but at fewer stations (Fig. 8). Nutrients in the eastern waters remained largely stable between 1986 and 2000 except in the inner Tolo Harbour, where a reduction of TIN and PO_4 was detected. A more wide spread decrease in BOD_5 was found in Tolo Harbour and Southern Water (Figure 9), reflecting a reduction of organic pollution in these waters. The chlorophyll-*a* levels in the coastal waters, on the other hand, experienced a decrease between 1991 and 1993, and did not show an increase from the 1986 baseline (Fig. 10).

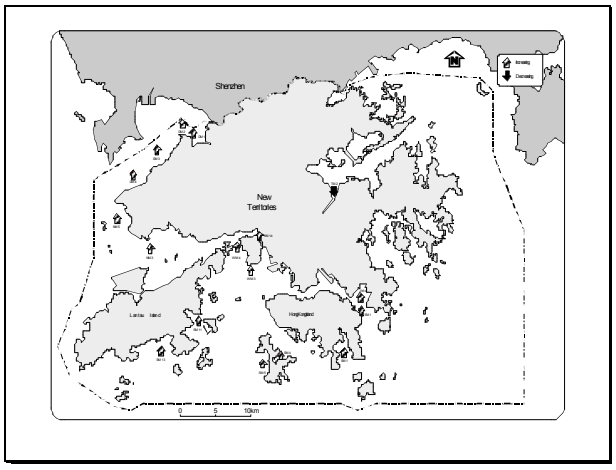

Figure 7. Long-term changes in total inorganic nitrogen in marine waters of Hong Kong, 1986-2000.

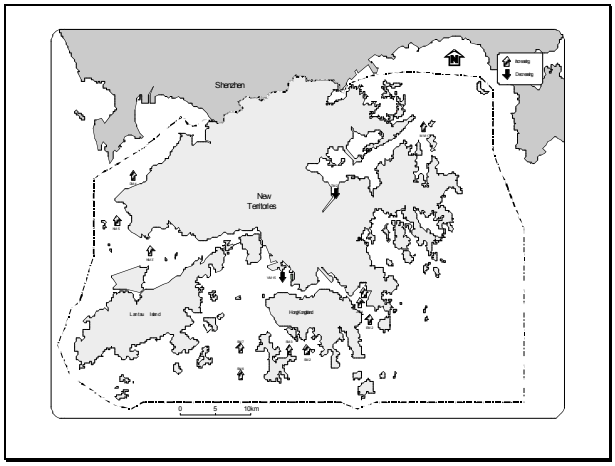

Figure 8. Long-term changes in orthophosphate phosphorus in marine waters of Hong Kong, 1986-2000.

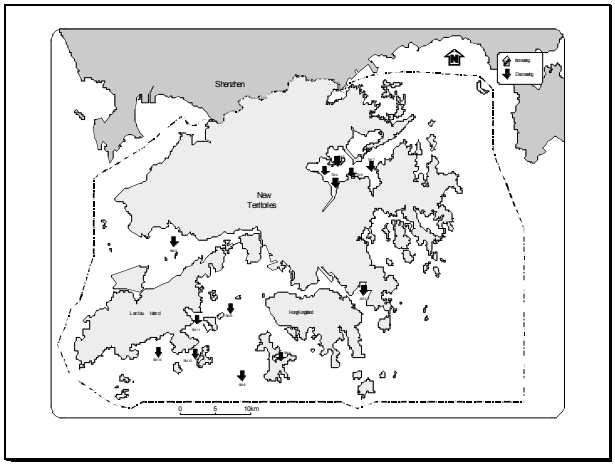

Figure 9. Long-term changes in Biochemical Oxygen Demand in marine waters of Hong Kong,1986-2000.

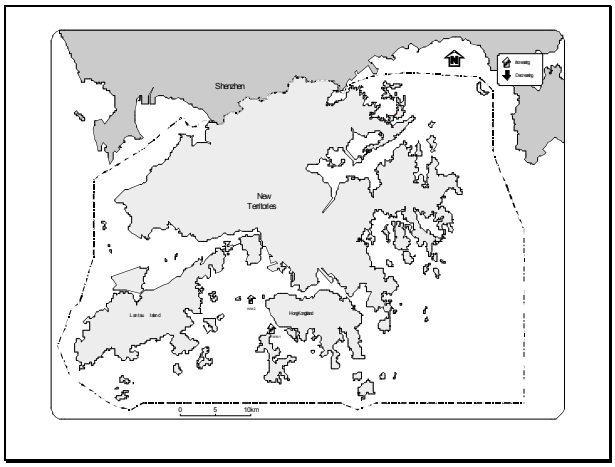

Figure 10. Long-term changes in chlorophyll-*a* in marine waters of Hong Kong, 1986-2000.

Chlorophyll-a and Red Tide Patterns - Last 20 Years

Some chlorophyll-*a* data in Hong Kong dates back to the early 80s. These data were plotted, and the graphs suggest that in the last 20 years, Hong Kong coastal waters may have experienced two periods of heighten algal activities (higher chlorophyll-*a*), one in the mid and late 80s and another in the 90s, intercepted by a relatively quiescent period (lower chlorophyll-*a*) around 1991-1993. This apparent cyclic pattern was observed in many parts of the territory. Figure 11 shows the typical 10-year algal cycles at the monitoring stations in Tolo Harbour, Southern Water and Victoria Harbour. It is interesting to note in Figure 12 that the annual incidents of red tides in the past 20 years also appeared to fall into two distinct periods with maximum occurrences around the year 1988 and the 90s (extending to early 2000). This apparent cyclic pattern seemed to be more common in eastern waters (i.e., Mirs Bay, Port Shelter, Tolo Harbour, and in Southern Water) [4], which were under greater oceanic influence than other areas.

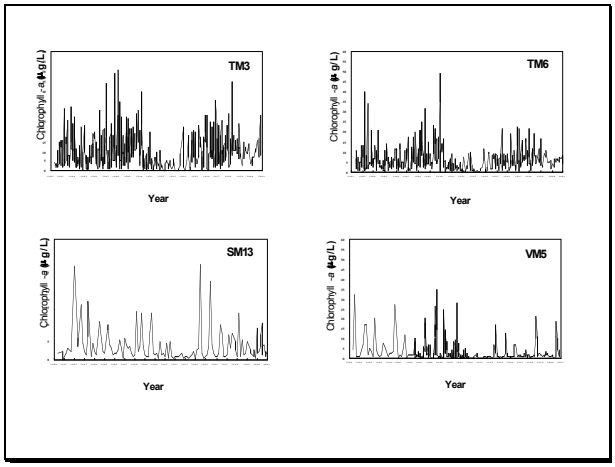

Figure 11. Distribution of chlorophyll-*a* in Tolo Harbour (TM3 and TM6), Southern Water (SM13) and Victoria Harbour (VM5), 1982-2000.

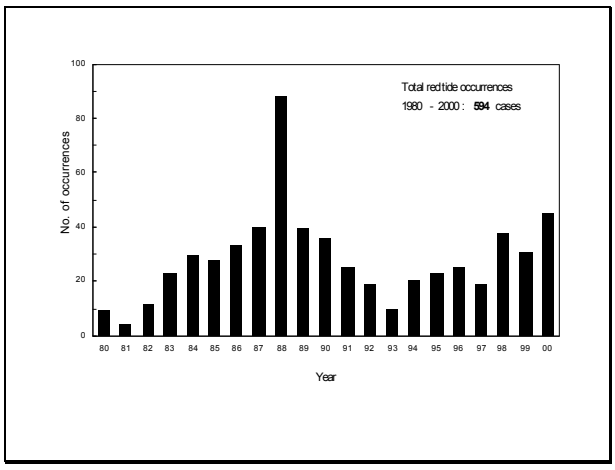

Figure 12. Red tide occurrences in marine waters of Hong Kong, 1980-2000.

Nutrients and Phytoplankton Changes in 1991-2000

Although nutrient values are referred to earlier, it is beneficial to repeat some values here. In the early 1990s, EPD implemented a territory-wide phytoplankton monitoring programme as part of its routine marine monitoring, and more comprehensive phytoplankton data became available. Further analysis was conducted on the water quality and phytoplankton data collected in the last 10 years. The results (Figs. 13 and 14), indicate a significant decrease in nutrients in Tolo Harbour between 1991 and 2000. The averaged TIN has decreased from 0.19 mg L^{-1} in 1991 to 0.09 mg L^{-1} in 2000, whereas PO_4 decreased from 0.05 mg L^{-1} to 0.01 mg L^{-1}. The trend was detected in the 1986-2000 data but became more obvious when the 10-year data set was examined. Victoria Harbour and its western approach have also experienced a decrease in PO_4 (Fig. 14). On the other hand, a significant increase in chlorophyll-*a* was found based on the lower-than-usual 1991 level, with the exception of Deep Bay where no significant trend was observed (Fig. 15). This was consistent with the observation on the 20-year data that the overall algal biomass increased again in the 90s, possibly as part of the 10-year cycle.

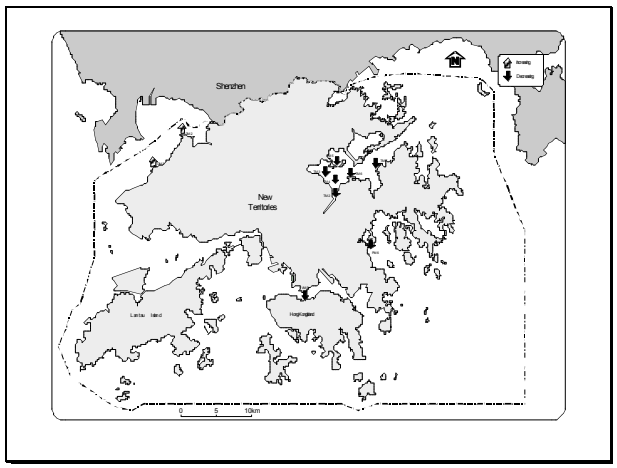

Figure 13. Long-term changes in total inorganic nitrogen in marine waters of Hong Kong, 1991-2000.

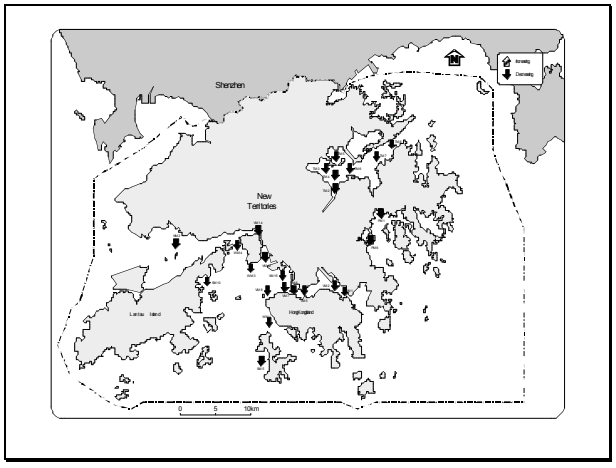

Figure 14. Long-term changes in orthophosphate phosphorus in marine waters of Hong Kong, 1991-2000.

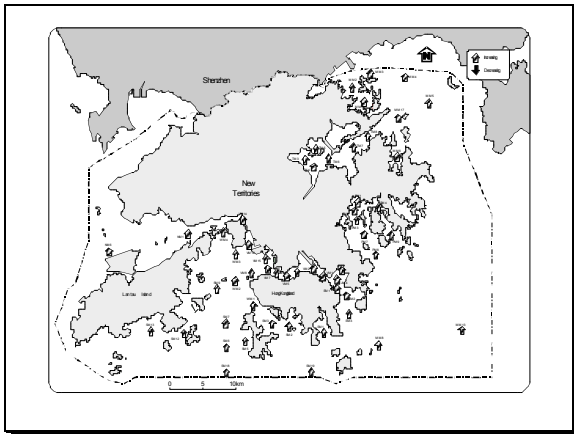

Figure 15. Long-term changes in chlorophyll-*a* in marine waters of Hong Kong, 1991-2000.

The elevation of chlorophyll-*a* in the last decade was also reflected in the increases in phytoplankton cell densities. Table 1 shows significant increases in total phytoplankton in most Hong Kong waters. While the number of diatoms remained largely stable, a marked increase was found with dinoflagellates and other phytoplankton (including small flagellates and *Cryptomonas*). The dinoflagellate counts in Tolo Harbour increased from 9,900 cells ml^{-1} in 1991 to 37,000 cells ml^{-1} in 2000; whereas those in the adjacent Mirs Bay showed a 14 time increase during the same period. An example of the increasing trends in the three phytoplankton groups, as observed in Southern Water, is illustrated in Figure 16.

Table 1. Seasonal Kendall Test for trends of phytoplankton in Hong Kong waters, 1991-2000 (↑ significant increase; - no significant trend; significant at $p<0.05$).

Water Control Zone	Total	Diatoms	Dinos	Others
Mirs Bay	↑	-	↑	↑
Tolo Harbour & Channel	↑	-	↑	↑
Port Shelter	↑	-	↑	↑
Victoria Harbour	↑	-	↑	↑
Southern	↑	↑	↑	↑
North Western	-	-	↑	-
Deep Bay	↑	-	↑	↑

Relationships Between Phytoplankton and Nutrients and Other Parameters

The Spearman correlation test was used to analyse the phytoplankton and nutrient data (1 m below sea surface) from Mirs Bay, Southern Water, Deep Bay and Tolo Harbour. The results, as summarised in Table 2, show that phytoplankton was mostly correlated positively with BOD_5, total N and negatively with PO_4 and total P. Most phytoplankton groups also showed a strong positive correlation with the TN/TP ratio. The responses to TIN and SiO_2 in different waters were more variable. For example, the diatoms in Southern Water were positively correlated with TIN and SiO_2, while those in other waters were mostly negatively correlated with these parameters. The diatom population in the Southern Water was also more highly correlated with nutrient parameters than either dinoflagellates or "other phytoplankton"; whereas in the case of Tolo Harbour, "other phytoplankton" showed more correlations than other groups. Of the waters considered, the phytoplankton in Tolo Harbour and Southern Water was more closely correlated to various nutrients whereas Deep Bay the least.

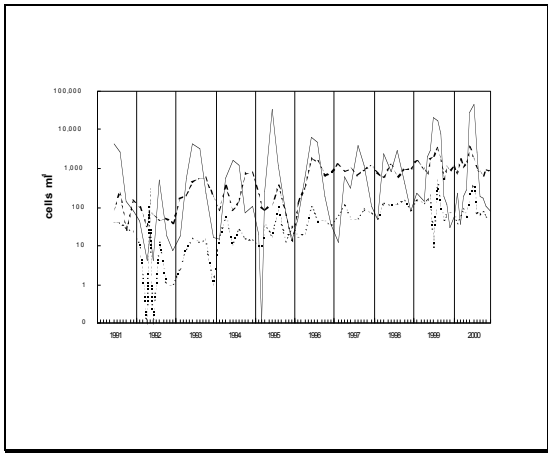

Figure 16. Seasonal variations of diatoms (____), dinoflagellates (……) and other phytoplankton groups (-----) in Southern water, 1991 – 2000.

Analyses were also carried out on the seasonal effects of phytoplankton responses to nutrient and other environmental parameters in Mirs Bay and Deep Bay.

Table 3 shows that phytoplankton population in Mirs Bay generally increased with a decrease of salinity (i.e., greater freshwater input) and an increase of water temperature during spring and winter (a decrease of temperature among the dinoflagellates and other phytoplankton). In the summer, however, the phytoplankton was significantly correlated with nutrients such as PO_4, BOD_5, but unaffected by temperature, salinity and SS. Overall, the phytoplankton in Deep Bay showed low correlation with nutrients, but a negative relationship with SS provided some evidence on the role of suspended solids in limiting phytoplankton growth in Deep Bay.

DISCUSSION

Hong Kong and the Pearl River Delta have experienced rapid economic and population growth in the last 15 years. The population of Hong Kong has increased from 5.5 million in the mid 80s to 6.7 million in 2000, generating some 860 x 10^6 m^3 yr^{-1} of sewage effluent. This, together with 350 x 10^9 m^3 yr^{-1} of highly enriched flow from the Pearl River [5] and discharges

Table 2. Spearman correlations of phytoplankton densities and nutrients in different Hong Kong waters, 1991-2000 (++/-- highly significant at $p<0.01$, +/- significant at $p<0.05$).

	TIN	Total N	PO4	Total P	SiO2	BOD5	TN/TP
Mirs Bay							
Total phytoplankton	- -		- -	- -		++	++
Diatom	- -			- -	- -	++	
Dinoflagellates			-	- -			++
Others			-	- -			++
Southern Water							
Total phytoplankton	++	++	- -	- -	+	++	++
Diatom	++	++	- -	- -	+	++	++
Dinoflagellates			- -	- -	-		++
Others				- -			++
Deep Bay							
Total phytoplankton		+			-	++	+
Diatom					- -	++	
Dinoflagellates				- -		++	++
Others		+				++	++
Tolo Harbour							
Total phytoplankton	- -	++	- -		- -	++	++
Diatom	- -		- -		- -	++	
Dinoflagellates		++	- -			++	++
Others	- -	++	- -	- -	++	++	++

Table 3. Searman correlations of phytoplankton densities and nutrients, environmental parameters in Mirs Bay in different seasons, 1991-2000 (++/-- highly significant at $p<0.01$, +/- significant at $p<0.05$).

	TIN	Total N	PO_4	Total P	SiO_2	BOD_5	Salinity	SS	Temp
Spring (Mar - May)									
Total phytoplankton				- -		++	- -		
Diatom					- -	++	- -	++	++
Dinoflagellates		+		- -		++	- -		-
Others				- -	+		- -		-
Summer (Jun - Sep)									
Total phytoplankton			- -	-		++			
Diatom	- -		- -		- -	++			
Dinoflagellates			-	- -					
Others			- -	- -					
Autumn (Oct - Nov)									
Total phytoplankton				-					+
Diatom				- -					+
Dinoflagellates				- -		+	- -		++
Others									+
Winter (Dec - Feb)									
Total phytoplankton				- -			- -		++
Diatom		- -		- -	-				
Dinoflagellates				- -			-	-	+
Others				- -			- -	-	++

Table 4. Searman correlations of phytoplankton densities and nutrients, environmental parameters in Deep Bay in different seasons, 1991-2000 (++/-- highly significant at $p<0.01$, +/- significant at $p<0.05$).

	TIN	Total N	PO_4	Total P	SiO_2	BOD_5	Salinity	SS	Temp
Spring (Mar - May)									
Total phytoplankton						++			
Diatom						++			
Dinoflagellates						+		- -	
Others						++		-	
Summer (Jun - Sep)									
Total phytoplankton					-	++			
Diatom					- -				
Dinoflagellates						++		-	
Others								- -	
Autumn (Oct - Nov)									
Total phytoplankton		+				+			
Diatom						+			
Dinoflagellates							+		
Others									
Winter (Dec - Feb)									
Total phytoplankton									
Diatom						+	-	-	
Dinoflagellates				-					
Others		+						-	

from Shenzhen are the major contributors to eutrophication in local marine waters.

With the implementation of pollution control measures and sewerage programmes in the last decade, nutrient levels in the coastal waters have maintained largely stable in the last 10 years, with evidence of a decrease of phosphorus around Victoria Harbour, and a decrease of both nitrogen and phosphorus in Tolo Harbour. On the other hand, there has been a wide spread increase in chlorophyll-*a* and phytoplankton density, in particular dinoflagellates and other phytoplankton (including small flagellates and *Cryptomonas*) in the territory. In 2000, based on the monthly data, the "other phytoplankton" was found to be the major group in Northwestern Water and Deep Bay (both constituting 80%) and Mirs Bay (56%); whereas the diatoms were dominant in Victoria Harbour (84%), Southern Water (82%), Tolo Harbour (70%) and Port Shelter (53%) [4]. The number of dinoflagellates was generally low, making up only 1-5% of the total phytoplankton population. The most common diatoms found in Hong Kong waters included: *Skeletonema costatum, Thalassiosira* spp., *Chaetoceros* spp., *Cerataulina pelagica, Cylindrotheca closterium* and the most common dinoflagellates included: *Gymnodinium* spp., *Prorocentrum sigmoides*, *Prorocentrum triestinum*, *Scrippsiella* spp. and *Gyrodinium* species. Small flagellates, *Cryptomonas* spp., *Mesodinium rubrum*, *Dictyocha speculum* and *Ebria tripartita* were the other more frequently encountered phytoplankton.

An increase in "other phytoplankton" in Tolo Harbour was reported by Yung et al. [6] and Kueh et al. [7]. The phenomenon was subsequently found to occur widely in other eastern water as well as Deep Bay, Victoria Harbour and Southern Water. The exact cause for the increase is not known. Unlike the diatoms and dinoflagellates, the "other phytoplankon" generally did not show a regular seasonal growth pattern, indicating their lower sensitivity to factors such as temperature and salinity. This group also includes some of the nutritionally more active and diversified phytoplankton. Small flagellates, because of their small size and high turn over rate, are efficient sequesters of nutrients, including the organic forms. *Cryptomonas* are mixotrophic and able to ingest particles and bacteria cells from the water [8].

Phytoplankton growth in Hong Kong marine waters is highly complex and dynamic, subject to the effects of topography, seasonal and hydrological, physico-chemical and biological processes of the local environment. The phytoplankton population generally correlated positively with BOD_5, total nitrogen, TN/TP ratio and temperature, and negatively with total phosphorus, inorganic phosphorus, salinity and suspended solids. The phytoplankton in the eastern waters (Mirs Bay, Tolo Harbour, Port Shelter) with higher salinity and lower nutrients, suspended solids and current flow were found to be more closely related to changes in nutrient and other physico-chemical parameters. These waters also account for over 85% of the red tide incidents in Hong Kong. On the other hand, algal populations in western waters (Deep Bay, Northwestern Water), with lower salinity and higher nutrients, were least affected by physico-chemical and nutrient parameters, and the red tide incidents were also low. Light limitation may be an important factor in phytoplankton growth in western waters. Deep Bay had a high suspended solid content (averaging 17.8 mg L^{-1}) and turbidity (27.4 NTU), which were 2-8 times those in other waters [4]. The Southern Water in the transitional zone was also sensitive to red tides and its phytoplankton was also closely related to nutrient changes. Victoria Harbour, despite its higher chlorophyll-*a* level, was less prone to red tides. This may be related to its relatively high turbulence and fast flowing current up to 1.0 m s^{-1} [9], which prevents the aggregation of algal cells from forming blooms.

The systematic analysis of long-term monitoring data provides a valuable tool for studying nutrient and phytoplankton dynamics in a complex coastal system such as Hong Kong, and in differentiating anthropogenic impacts from natural background changes. It is apparent that the quantity and quality of the data would affect the accuracy of the observations made and the validity of the conclusions drawn. The preliminary data analysis in this study revealed the possible existence of a 10-year cycle in the phytoplankton population in the last two decades. Biological cycles ranging 3 to 100 years have been observed with the benthic organisms and plankton in the North Sea and Baltic Sea [10]. Spectral analysis of salinity and temperature data from the south coast of Norway between 1920 and 1980 revealed periodic cycles of around 10 years as well as shorter cycles of 3-4 years and 6-8 years. Maximov [11] postulated that while the 6-7 year cycle was a variation in the free fluctuation of the rotation of the pole of the earth, the 10-11 year cycle was that of the sun spot cycle which influenced weather pattern. Longer monitoring data are needed to confirm the existence of regular phytoplankton cycles in this part of the world and their implications on the eutrophication of coastal waters.

ACKNOWLEDGEMENTS

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ECOLOGICAL RESEARCH ON HABS IN THE HONG KONG S.A.R., CHINA

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ABSTRACT

Following a recent red tide consultancy carried out for the Agriculture, Fisheries and Conservation Department of the Government of the Hong Kong Special Administrative Region, China, new management policies were introduced, particularly in relation to monitoring and mitigation measures. As a result, our research in The University of Hong Kong has focused on 4 areas: the biodiversity of HAB species; using telemetry in the prediction of red tides; using proteomics to develop rapid identification methods for HAB species; and nutrient ratios and bloom dynamics. This paper presents a report on the present status of our studies in the first 3 areas, the nutrient ratio studies forming the basis of a separate report.

INTRODUCTION

Harmful algal blooms (HABs) have increased on a global scale and now threaten the coastal areas of many parts of the world [1-4] including China [5]. HABs have been well studied in the Hong Kong SAR since the early 1970s [see, for example, 6-10] and Kong Kong has been described as one of the world's 'hot spots' for HABs.

Since the mid 1970s, the Agriculture, Fisheries and Conservation Department (AFCD) of the Hong Kong SAR Government has maintained a red tide database. Until the 1980s red tides were present in low numbers, but they increased through the 1980s to reach a peak in 1988. After that numbers decreased until the serious blooms of 1998 [11,12], when more than 90% of Hong Kong caged fish were killed in 22 out of 26 coastal fish farms, resulting in a loss of about US$ 32 million.

During the late 1990s the numbers of toxic species increased and both new species of harmful taxa such as *Karenia digitata* [12], and *K. longicanalis* [13], and new records such as *Chattonella ovata*, *C. globosa*, *C. antiqua* [14], *Gambieridiscus toxicus* [15], and *Karenia brevis* [14] have been found during our studies of HAB species diversity and the establishment of a culture collection at The University of Hong Kong.

Many reasons have been put forward for increased algal blooms [1-3], but our research has shown that eutrophication (and particularly the resulting nitrogen to phosphorus ratios) has played a significant role in Hong Kong waters in terms of dinoflagellate induced red tides and HABs [16, 17].

As a result of a recent red tide consultancy for the AFCD, new management policies were introduced, particularly in relation to monitoring and mitigation measures. In order to provide information leading to a better understanding of the mechanisms involved in HAB formation, more rapid identification of the species involved in blooms and better prediction of such blooms, our research at The University of Hong Kong has focused on 4 areas: the biodiversity of HAB species; using telemetry in the prediction of red tides; using proteomics to develop rapid identification methods for HAB species; and N:P ratios in relation to HAB dynamics. This paper deals with the first three areas, since the nutrient ratio studies will form the basis of a separate report.

THE BIODIVERSTIY OF HABS IN HONG KONG

The first bloom recorded in Hong Kong was in 1971 [8], and this was caused by *Noctiluca scintillans*. HABs remained a rare event until the late seventies, but the number of outbreaks increased rapidly in the eighties, with a peak of 89 incidents in 1988, before leveling off to about 20-30 cases each year until the more serious blooms of 1998. A total of 519 HABs have been recorded between 1975 and 1998 in the AFCD database. However, only 1 toxic bloom involving PSP was recorded, and this was in 1989 [18], and the major harmful effects of HABs in Hong Kong have been fish kills [10]. Of these 519 HABs, 239 (or 46%) were recorded in the eastern waters of Hong Kong, including Tolo Harbour and Channel, near Kat O (Crooked Island) and Port Shelter (Fig. 1).

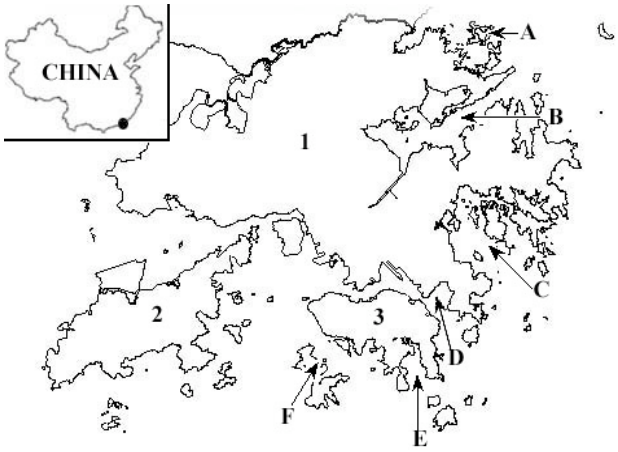

Fig. 1. Map of Hong Kong indicating sampling sites. (Keys: • = Hong Kong, 1 = New Territories, 2 = Lantau Island, 3 = Hong Kong Island, A = Kat O, B = Tolo Harbour, C = Port Shelter, D = Junk Bay, E = Tai Tam Bay, F = Luk Chau Wan, Lamma Island).

A total of 69 HAB inducing species has been recorded (AFCD database) in Hong Kong waters and an increasing number of blooms of new species have been recorded in recent years. Table 1 lists some of the more important species and highlights the newly found species.

Table 1. Some of the harmful species which have caused blooms in Hong Kong waters since 1975.

Alexandrium catenella	*Gyrodinium resplendens*
*Chattonella antiqua**	*Gyrodinium spirale*
*Chattonella globosa**	*Heterocapsa triquetra*
Chattonella marina	*Heterosigma akashiwo**
*Chattonella ovata**	*Kerenia digitata**
Cochlodinium polykrikoides	*Kerenia longicanales**
Cochlodinium sp.	*Noctiluca scintillans*
Gonyaulax polygramma	*Prorocentrum dentatum*
Gymnodinium mikimotoi	*Prorocentrum micans*
Gymnodinium sanguineum	*Prorocentrum minimun*
Gymnodinium simplex	*Prorocentrum sigmoides*
Gymnodinium viridescens	*Prorocentrum triestinum*
Gymnodinium sp.	*Prorocentrum sp.*
Gyrodinium instriatum	*Scrippsiella trochoidea*

* = most recently found species

A total of 8 groups of plankton have been involved in HAB incidents in Hong Kong; dinoflagellates constituted the biggest group in terms of both their percentage (42%) of the 69 bloom forming species (Fig. 2) and the percentage (71%) of the 519 blooms which they caused (Fig. 3). Diatoms were the second most important group (37% of the 69 bloom forming species and 20% of the 519 blooms). The most commonly occurring species was *Noctiluca scintillans*. More recently recorded species include *Heterosigma akashiwo*, *Gambierdiscus toxicus*, *Chattonella globosa* and *C. ovata*.

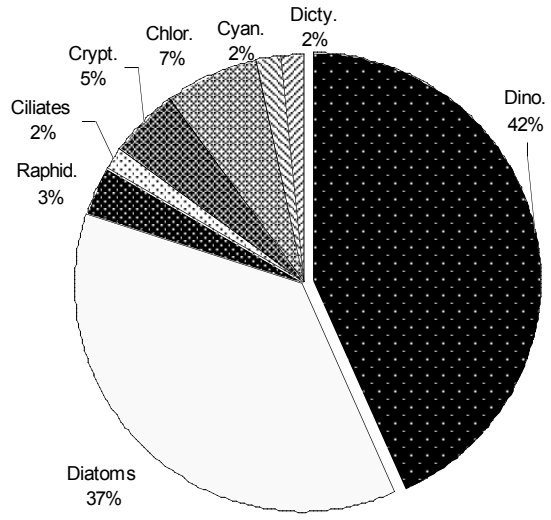

Figure 2. Percentage of the 69 HAB causative species from 1975-1998 belonging to each of the 8 plankton groups.

TELEMETRY AND MARINE MICROALGAL DYNAMICS

These studies form part of a Hong Kong Research Grant Council Group Research Project on the prediction, management and control of HABs, which involved the results of our studies of marine microalgal dynamics and red tide formation mechanics, together with the results of other studies, being used in the production of a model for red tide prediction and early warning.

Our studies on microalgal dynamics and red tide formation mechanisms have involved Hong Kong waters from the North East (Crooked Island or Kat O) through Tolo Harbour, Port Shelter and Junk Bay to Tai Tam Bay and Lamma Island in the South (Fig. 1). This covers oceanic waters to the East, the estuarine waters of the Pearl River to the West and a Southern Transition Zone. HABs are, as pointed out earlier, much more frequent in the Eastern oceanic waters.

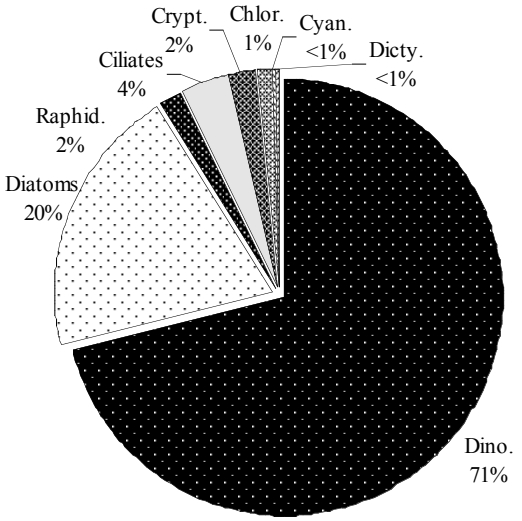

Figure 3. Percentage of the 519 HABs recorded from 1975-1998 caused by each of the 8 plankton groups.

For the telemetric study, two fixed stations (mounted on fish rafts) were developed, one to the North East at Kat O or Crooked Island, and the other to the South at Luk Chau Wan, Lamma Island (Fig. 1). The telemetry system involves a series of probes used to continuously monitor chlorophyll a concentrations, dissolved oxygen, water temperature, solar radiation, wind speed and direction, tidal level and current; a data logger and modem for data collection and transfer; and a receiving computer in The University of Hong Kong for retrieval, storage and analysis of the data. Microalgal field sampling data were used to supplement the telemetry data, since we do not at present have an automatic monitoring system for the microalgae.

The advantage of this system is that it minimizes the constraints of weather conditions on sampling, while providing comprehensive, real-time, continuous data. As a result, it has successfully allowed the short term (3-5 days) prediction of HABs at Kat O in North East Hong Kong waters, on 3 occasions in the past 2 years, based on

changes in dissolved oxygen and chlorophyll a concentrations [19].

TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS (2-D PAGE)

The objectives of this research were twofold: first, to determine whether 2D-PAGE can be used as a simple and fast method for algal identification based on their protein content; and secondly, to document the global protein expressions (proteomes) of laboratory grown pure cultures of diatoms and dinoflagellates using 2D-PAGE so as to produce a reference set of 2D-maps for identification purposes. This is a new application of 2D-PAGE in HAB studies.

The first step involved "First Dimension" Isoelectric Focusing (IEF), where proteins are separated horizontally on gels by their isoelectric point (pI), and the second step involves the "Second Dimension" SDS-PAGE system, whereby proteins are separated vertically on gels by their molecular weight. The resulting chromatograms offer better resolution of single protein species (each spot on the chromatograms), with more information on each protein's isoelectric point, apparent molecular weight and the amount of each protein expressed [20].

Species identification could be achieved in 24 hours by comparing the discriminant species-specific protein patterns of unknown algal species with the computer driven database of known reference sample proteomes. Our studies are currently concentrating on toxin producing species and on the molecular mechanisms involved in the initiation and decline of harmful algal blooms.

SUMMARY

As a result of a recent red tide consultancy, new management policies are being introduced by the Hong Kong SAR Government to limit the effects of HABs. It is hoped that our studies on biodiversity, on telemetric monitoring and modelling, on proteomic identification and on nutrient ratios and HAB dynamics will allow a better understanding of the mechanisms involved in HAB formation; more rapid identification of the species involved in blooms; and better prediction of such blooms. This, in turn, should allow possible control or mitigation measures to be identified and built into HAB management efforts.

ACKNOWLEDGEMENTS

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VIABLE DINOFLAGELLATE CYSTS IN XIAMEN HABOR

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ABSTRACT

To understand dinoflagellate cyst composition and density in Xiamen Harbor, 8 samples were collected and analyzed. Seven species of viable cysts were recorded. They were *Protoperidinium denticulatum*, *Alexandrium tamarense*, *Scrippsiella trochoidea*, *Scrippsiela* sp., *Diplopsalopsis* sp., *Polykrikos schwartzii*, *Gonyaulax spinefera*. The density of viable cysts was high at the Mouth of Jiulongjiang River, up to 12 g^{-1}, and low in Western Harbor and Tongan Bay. Six species of empty cysts were also identified. The cyst density in Xiamen Harbor was too low to initiate a bloom. The results of the cyst survey coincided well with cell survey, except that cysts of G*ymnodinium* spp. Were not observed though they have caused blooms recently.

INTRODUCTION

Total of 109 marine and 44 freshwater species of dinoflagellates are known to produce resting cysts or hypnozygotes [1], which allows them to survive unfavorable conditions. When the environment, mainly temperature and oxygen [2], becomes suitable, cysts will undergo excystment and form vegetative algal cells. The cells then grow by asexual division, and they may transit to a sexual phase later by forming gametes. They eventually fuse, form cysts and settle to the sediment [3]. Little is known of the mechanisms that control the time of encystment, but it is believed that cysts play an important role in bloom initiation [4]. By analyzing the composition and density of viable cysts in marine sediments, it will aid in our understanding of bloom formation.

MATERIALS AND METHODS

Samples were collected in November of 2001 using a grab sampler (Fig. 1). Only surface sediment was collected and stored in the dark at 4°C until further examination.

Approximately 10 g of wet sediment were mixed with filtered seawater to make slurry. The sediment suspension was sonicated for 2 min at 100 watts to separate cysts from sediment. The sample was then screened through a 90 µm sieve and collected on to a 15 µm sieve. The 15 –90 µm fraction was resuspended in 10 ml filtered seawater and concentrated to 0.8 ml by centrifugation. Subsamples of 0.1 ml were counted using an Olympus light microscope at a total magnification of 100X. Dinoflagellate cyst identification was based on morphological criteria [1]. Cysts were also isolated by micropipette and placed in separate wells of a 96 cluster tissue culture plate with each well containing f/2-Si medium [5]. The cysts were incubated at 20°C at an irradiance of 3000 Lux (12:12 hr light-dark photoperiod). Isolated cysts were monitored for germination and the motile cells were used to confirm species identification.

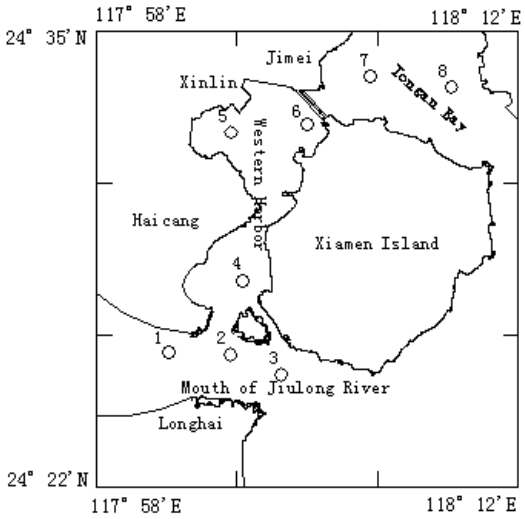

Figure 1. Map of the locations where sediment samples were collected.

RESULTS

Seven species of viable dinoflagellate cysts were identified and described as following:

Scrippsiella sp. - Spherical cyst (20-25 µm diameter) with no ornamentation. The cyst has a thick autophragm and green lipid and starch granules. A prominent red body is visible inside the cyst (Fig. 2a,b).

S. trochoidea - Highly variable ovoid and spherical, dark grayish-colored cyst (20-36 µm long) covered by calcareous spines 2 to 6 µm long. The spine shafts consist of crystalline rods which terminate in blunt or pointed ends. A theropylic archeopyle is present in the epicyst and a bright red accumulation body is often visible (Fig. 2c).

Diplopsalopsis sp. - Spherical cyst (40-45 µm diameter) with a relatively thick (2 µm) and brown autophragm. The cyst has large brown starch and lipid granules. The archeopyle is hexagonal (Fig. 2d,e).

Protoperidinium denticulatum - Brown spherical cyst (40 -55 µm diameter) with a hexagonal, laterally elongated archeopyle formed by the loss of the 2a intercalary paraplate (Fig. 2f).

Polykrikos schwartzii - Elongated, ovoidal, green brown cyst (80-100 µm long, 50-60 µm wide) with an irregular, nearly circular, tremic archeopyle. The processes are fibrous and flare distally to form a reticulate structure. The outer cyst membrane may be microgranulate or microreticulate (Fig. 2g,h).

Gonyaulax spinifera - Spherical cyst (35-45 µm diameter), with membranous processes particularly at the antapical and paracingulum regions. These processes are generally trifurcate with bifid tips. The archeopyle is approximately trapezoidal in outline (Fig. 2i,j).

Alexandrium tamarense - Elongate, ellipsoidal cyst (40-48 µm long) with rounded ends. The cyst wall is thick and covered with an amorphous, transparent, mucilaginous substance, which may incorporate fine detrital particles. The cyst contains pale or colorless starch granules, light brown lipid globules, and one bright orange accumulation body (Fig. 2k,l).

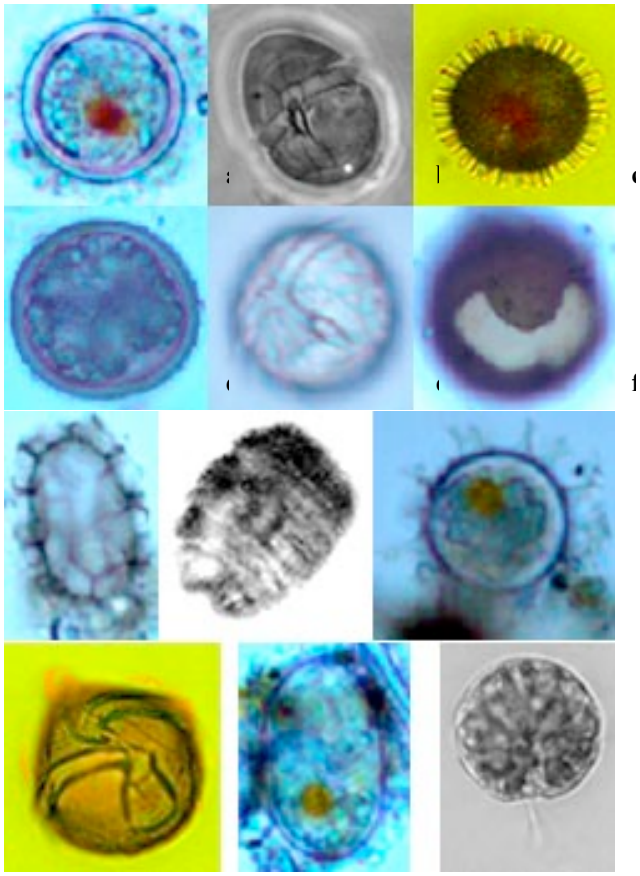

Figure 2. a) *Scrippsiella* sp. (27 µm), b) Motile cell of *Scrippsiella* sp. (30×25 µm), c) *S. trochoidea* (35 µm), d) *Diplopsalopsis* sp. (40 µm), e) Motile cell of *D.* sp. (42 µm), and f) *Protoperidinium denticulatum* (52 µm), g) *Polykrikos schwartzii* (85×43 µm), h) motile cell of *P. schwartzii* (110×70 µm), i) *Gonyaulax spinefera* (35 µm), j) motile cell of *G. spinefera* (32 µm), k) *Alexandrium tamarense* (45×32 µm), and l) motile cell of *A. tamarense* (35 µm).

Besides the seven species of viable cysts, six kinds of empty cysts were also identified. They were *Protoperidinium subinerme*, *P. conicum*, *P. claudicans*, *P. oblongum*, *Pyrophagus stenii* and *Gonyaulax* sp.

Station 3 at the mouth of Jiulongjiang River had a cyst density as high as 12 g^{-1}. Western Harbor and Tongan Bay had low cyst densities, from 3 to 6 g^{-1} (Table 1).

Table 1. Density of viable dinoflagellate cysts in Xiamen Harbor

Stations	1	2	3	4	5	6	7	8
Density (g^{-1})	4	6	12	3	6	4	5	4

DISCUSSION

More than 30 species of dinoflagellate cysts were reported in marine sediments in East and South China Seas [6]. In this study we identified 13 species, including 6 species of empty cysts. The cysts in surface sediment reflected recent dynamics of encystment and excystment. Germination of viable cysts may provide inoculums for the spring bloom [4], but the cyst density in Xiamen Harbor was too low to initiate a bloom. *Scrippsiella trochoidea* was the dominant cyst in Xiamen Harbor, found at half of the stations inspected. It was also distributed widely in the East and South China Sea [5]. This species might produce cysts effectively.

The cyst density in Xiamen Harbor was quite low compared to other regions in Southern East China Sea [7]. The possible reason is that there is a high sedimentation rate in Xiamen Harbor [8] resulting in dilution of cyst density. Station 3 had highest cyst density possibly because it was located at the mouth of Jiulong River. The river brings in nutrients and may result in the high motile dinoflagellage abundance. Stations 1 and 2 are close to 3, but the water depth at Station 3 is much deeper. As cysts tend to concentrate in deeper stations [9], cyst densities at Stations 1 and 2 were lower than 3.

Diatom blooms broke out frequently in Xiamen harbor. The main bloom causing species were *Leptocylindrus danicus*, *Chaetoceros debilis*, *Eucampia zodiacus*, *Pseudo-nitzschia pungens* [10]. Dinoflagellate species were diverse in Xiamen Harbor [11] and most cyst forming species were also discovered in the sediment samples. Unlike diatoms, dinoflagellates rarely caused blooms in Xiamen Harbor [10]. Though in 1986 there was a bloom caused by *Gymnodinium* sp. [12] and in 2003 by the same species together with other *Gymnodinium* species. The cells produced two kinds of cysts in culture, which we had never discovered before. It was also difficult to find these species in the water column during conventional monitoring. *Alexandrium tamarense* caused a bloom in shrimp ponds nearby Xiamen harbor in 1994 [13]. This species appeared frequently in the water column of Xiamen Harbor but no bloom had been recorded. As both cysts and cells were common in Xiamen Harbor, much attention should be paid to this toxic species.

CONCLUSIONS

Cyst density in Xiamen Harbor was low possibly because of the high sedimentation rate in this region. The cyst survey complied well with the cell record but apparently missed some bloom forming species. *Alexandrium tamarense* appeared frequently in the sediment and in the water column and has potential harmful effects.

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GROWTH, PHOTOSYNTHETIC AFFINITY AND TOXICITY OF SINGLE-CELLED AND COLONY-SHAPED *MICROCYSTIS VIRIDIS*

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ABSTRACT

The present study examined the changes in growth and photosynthetic activities in single-celled *Microcystis viridis* as well as the change in toxin contents between single-celled and colony-shaped culture. The toxic cyanobacterium was isolated from the eutrophic Dianchi Lake in Kunming, southwestern China. It produces three microcystins, among which [Dha^7] microcystin RR was the major component representing 70-80% of the total toxins of this strain. During the continuous subculture in the laboratory, this organism gradually became single-celled and it retained the toxin production as mentioned above. It was found that the optimal temperature for the growth of single-celled cultures was ~30°C and the optimal light intensity was ~60 μmol photon m^{-2} s^{-1}. The maximal O_2 evolution rate was 534 μmol O_2 mg^{-1} chl h^{-1} and high photosynthetic affinity to external inorganic carbon was elucidated. By comparison, the duration of exponential growth phase in single-celled culture was shorter than that in colony-shaped culture. Furthermore, the toxin content in single-celled culture decreased by half of that in colony-shaped culture. These findings have shed some lights on the relationship between growth, toxin production and morphology of this strain.

INTRODUCTION

The cyanobacterium *Microcystis* has been widely recognized as the most common toxic bloom-forming organism all around the world [1]. This organism can produce a cyclic peptide hepatotoxin which is composed of 5 amino acids common to all toxin variants and two variable L-amino acids. So for, more than 70 microcystin variants have been detected in *Microcystis* [2, 3].

To date, most studies on the growth and toxin production have been done with *Microcystis aeruginosa* [4-11], whereas little information is available about *M. viridis*, which at times is the major component of the bloom [12, 13]. Our previous report described the relationship between toxin production and morphogenesis in colony-shaped *M. viridis* [14].

In this paper we aimed to understand how light and temperature influence the growth of single-celled *M. viridis* and elucidate the photosynthetic affinity for inorganic carbon. Moreover, we also detected the change in toxin contents between single-celled and colony-shaped cultures. These findings describe the relationship between growth, toxin production and morphology of this strain.

MATERIALS AND METHODS

Organism and Culture Methods

Cyanobacterial *M. viridis* (A.Br.) Lemm. was first isolated from Dianchi Lake in China in early 1996. The organism was then purified and identified as typical colony-shaped *M. viridis*. The colony–shaped culture was kept for about 20 months. It was then gradually disintegrated into single-celled shape. Medium MA [15] was used for batch culture of this strain. For routine culture, it was aerated by air pump at the rate 2 L min^{-1}. Illumination was provided by cool-white fluorescent tubes. Light intensity was adjusted to 60 μmol photon m^{-2} s^{-1} and measured by an OSL-100 (BiosphericalInstruments Inc.) Quantum radiometer/photometer, equipped with a spherical quantum sensor. Different fluorescence rates were accomplished by varying the position of the flask to the light source.

Growth Measurement

Growth of the cultures was measured from increases in optical density at 665 nm ($O.D._{.665}$) over time. The exponential growth rate was denoted as:

$$R_E=(\log_2 X_2-\log_2 X_1)/(t_2-t_1)$$

where X_1 and X_2 are the mean optical density initially (t_1) and after (t_2) days, respectively.

Photosynthesis Analysis

The response of cell photosynthesis to external inorganic carbon was measured using an O_2-electrode (Hansatech, U.K.), 400 μmol photon m^{-2} s^{-1}, 30°C. Exponential-phase cells were harvested by centrifugation (4000 g, 5 min, 20°C), washed and re-suspended in "Ci-free buffer" (25 mM BTP-HCl bubbled with "CO_2-free" air, pH 8.0). Before addition of Ci, the cells were allowed to deplete endogenous carbon sources as measured by the cessation of O_2 evolution. The rate of O_2 evolution was then determined following the addition of varying concentrations of $NaHCO_3$.

Toxin Determination

Freeze-dried samples were extracted with 5% acetic acid stirred in ultrasonication. After centrifugation (4000 rpm, 15 min), the pellets were extracted three times with 100% methanol and the resulting supernatants were dried in a vacuum evaporator. The residuals were dissolved in

5% acetic acid and the supernatants were passed through conditioned (10 ml of 100% methanol, 50 ml of 100% distilled water) Sep-Pak C_{18} cartridges (Waters). The cartridges were then rinsed with 20% methanol and eluted with 90% methanol. After drying, the final residuals were dissolved in 20% methanol. The toxin contents were measured with a Shimadzu LC-10A system with two LC-10A pumps and a UV detector, using a Shimadzu shim-pack (CLO-ODS 4.6×150) in a run with 60% of solution A (100% methanol) and 40% solution B (0.05 M LK_2PO_4, pH 3.0) over 20 minutes. The total flow was 1 ml per minute. Column temperature was maintained at 40°C and the injection volume was 10 µl.

RESULTS

Growth of Single-celled M. viridis *at Different Temperatures*

The optimal temperature for growth in single-celled *M. viridis* was between 25 to 30°C; at 20°C the growth rate was slower compared with the higher temperatures (Fig. 1).

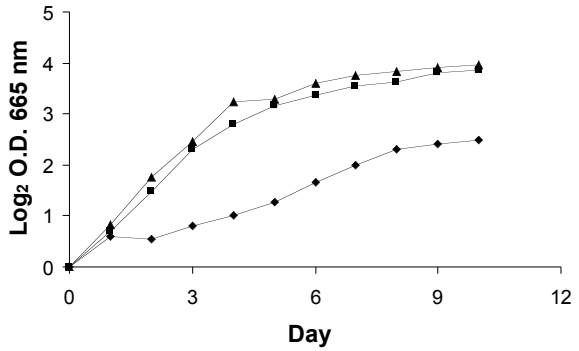

Figure 1. Growth of single-celled *Microcystis viridis* under 20 (♦), 25 (□) and 30 °C (▲).

Growth of Single-celled M. viridis *at Different Light Intensity*

Growth of the single-celled M. viridis *was affected by the light intensity (ranging from 30 µmol photon m^{-2} s^{-1} to 80 µmol photon m^{-2} s^{-1}) at 25 ºC. Figure 2 showed that the optimal light intensity for growth was 60 µmol photon m^{-2} s^{-1}; at higher light intensity of 80 µmol photon m^{-2} s^{-1} the culture kept growing in the initial 3-4 days and then became yellowish in color.*

Photosynthetic Affinity to Inorganic Carbon in Single-celled M. viridis

The responses of photosynthetic O_2 evolution to added external Ci at different temperatures in *M. viridis* are shown in Figure 3. The maximal photosynthetic rate (534.3 µmol O_2 mg^{-1} chl h^{-1}) was observed at 30°C; the lowest value of 335.5 was observed when cultures were grown at 20°C. However, the apparent photosynthetic affinity for inorganic carbon, $K_{1/2}$(DIC), was quite similar in all three temperature treatments. Values fell within the range of 10-20 µmol L^{-1}. The result indicated that this organism had a very high affinity for inorganic carbon and the ability to utilize the inorganic carbon was less influenced by temperature fluctuations.

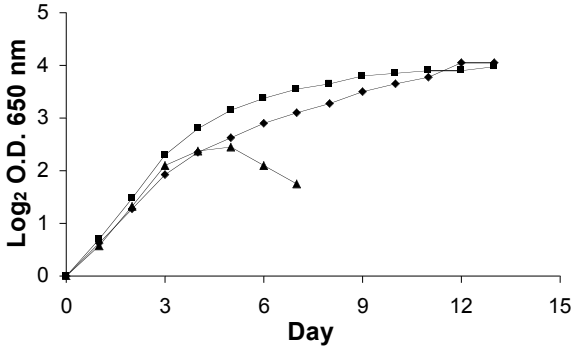

Figure 2. Growth of single-celled *M. viridis* under light intensities of 30 (♦), 60 (□), and 80 (▲) µmol photon m^{-1} s^{-1}.

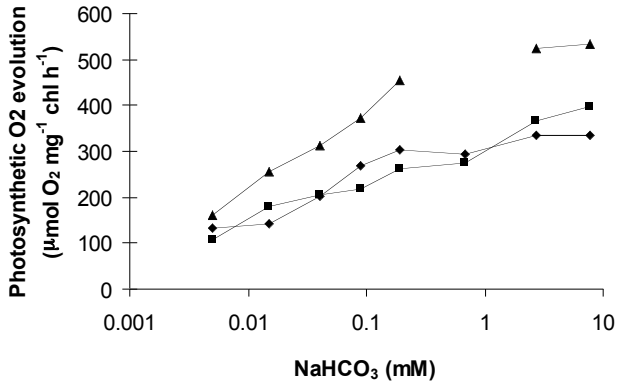

Figure 3. The response of photosynthetic O_2 evolution to added HCO_3 in single-celled *M. viridis* at 20 (♦), 25 (□) and 30 °C (▲).

Comparison of Growth Between Single-celled and Colony-shaped M. viridis

In the previous paper we have measured growth of colony-shaped *M. viridis*. By comparison, the growth in single-celled culture differed both in the duration time of exponential phase and in the response to light intensity. Figure 4 showed that in the single-celled culture the duration of exponential phase lasted for about 3 days, while in colony-shaped culture it lasted for more than 6 days. On the other hand, the tolerance to light intensity in single-celled culture was much less (see Fig. 1) compared to that of the colony-shaped culture (result not shown).

Comparison of Toxin Contents Between Single-celled and Colony- shaped Cultures

Three major microcystin variants, [Dha^7] microcystin-RR, [Dha^7] microcystin-YR and [Dha^7]

microcystin-LR were detected in this strain of single-celled *M. viridis*. The toxin profile was similar to that in colony-shaped culture. However, total microcystin content of the single-celled culture was below 1.2 mg g^{-1}, this amount was less than half of the colony-shaped culture (2.6 mg g^{-1}) growing under the same conditions.

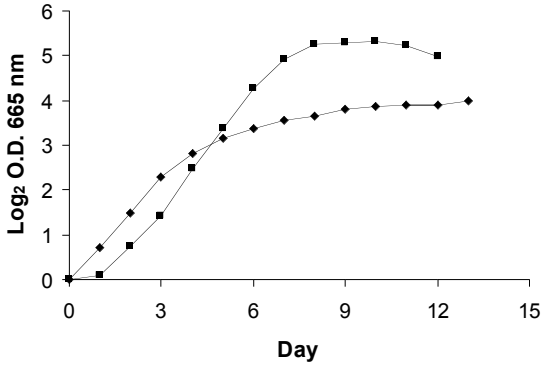

Figure 4. Growth comparison between single-celled (♦) and colony-shaped (□) *M. viridis*.

DISCUSSIONS

In this report we provided evidence that colony-shaped *M. viridis* culture was able to transform into a single-celled culture under continuous subculture conditions and its growth behavior changed during the process. The results of this study suggest that this change in morphology may also influence toxin concentration [see also 14].

There were reports about *M. viridis* toxin production under different growth conditions [11, 12], but little was known about its growth performance and photosynthetic capacity, let alone the difference between colony-shaped and single-celled culture. Our study showed that the optimal growth in single-celled *M. viridis* happened at temperatures between 25 and 30°C and light intensity at 60 μmol photon m^{-2} s^{-1}. In the colony-shaped culture, while the optimal temperature for growth was similar to that of single-celled culture, the ability to tolerate light intensity was higher than that in single-celled culture. One of the reasons for this may lie in the difference of self-shading functions. On the other hand, the single-celled culture expressed an early initiation of exponential growth phase but less duration time. The phenomenon was possibly due to the easy access for nutrients in single-celled culture, which could have initiated rapid growth earlier but may have resulted in the rapid depletion of nutrients in the medium.

Low $K_{1/2}$ (C_i) in single-celled *M. viridis* was obtained which strongly explained photosynthetic affinity for inorganic carbon in this organism was quite high; it thus suggested the strain could make good use of inorganic carbon even when the environmental carbon source is limited. The fact that this affinity remained relatively unchanged in response to three different temperatures illustrated that the ability of utilizing inorganic carbon was less influenced by temperature and quite stable. Other cyanobacteria such as *Synechococcus* and *Anabaena* were less efficient in utilizing inorganic carbon if judged by the $K_{1/2}$ (C_i) data [16-18].

The observation that toxin content declined in the single-celled culture disclosed another interesting phenomenon involved in the control of toxin production in *Microcystis* strains. The result might suggest that morphogenesis of *M. viridis* may influence the regulation of toxin metabolism. The phenomenon was also evidenced previously when increased toxin concentration was coincident with the process of colony formation (the single cells formed into colony in about 3 days after certain amounts of nitrogen were added [see 14]. Our findings were partly supported by the result that the concentration of microcystin was closely correlated with colony size of natural *Microcystis* bloom samples [19, 20].

Most of the blooms of *Microcystis* are colony-shaped in the natural environment [20]. Information directly dealing with colony-shaped cells was scarce, as most authors did not particularly mention the status of the organism they studied. We suggest that further studies examine the controlling factors that govern the transition of the colonies into single-cells and the corresponding changes during this process. This information would aid in our assessments of bloom development and duration as well as the potential for toxicity in particular water bodies.

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STUDY ON THE GROWTH CHARACTERISTICS OF CULTURED HARMFUL ALGAE *ALEXANDRIUM TAMARENSE*

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ABSTRACT

Alexandrium tamarense is one of the main toxic harmful algae that can produce toxins that cause paralytic shellfish poisoning (PSP) and they often form blooms in global oceans. Growth characteristics of this unicellular alga were observed through artificial culturing in the laboratory. In addition, we have confirmed the taxonomic result and have detected toxins of this alga (Mouse Bioassay Method: average value of toxins is 12.88×10^{-6} Mu $cell^{-1}$, in another literature). Results indicate that the optimum growth conditions are: T=21-23°C; S=28-31‰; L=2000-2500 lux and L:D cycle=16:8 hours. The average doubling time for *A. tamarense* population is 85 hours.

INTRODUCTION

Harmful algal blooms (HABs) are serious environmental problems in littoral regions along with increasing pollution and eutrophication [5, 8, 14]. Separation and artificial culturing for toxic algae is very important in most research about HABs for establishing certain species with stable transmissibility in order to find out their physiological characters and ecological behaviors. This may be very useful for helping us increase our abilities on HAB research such as algal physiology, ecology, genetics, toxicity and pathology. At the same time, we also study on standard material and test-kits of toxic algae.

Alexandrium tamarense (Lebour) Balech 1992 belongs to Division Pyrrophyta (Dinophyta), Class Dinophyceae, Order Gonyaulacales, Family Goniodomataceae, and Genus *Alexandrium*. It has many synonyms such as *Alexandrium excavatum* (Braarud) Balech & Tangen 1985, *Gessnerium tamarense* (Lebour) Loeblich, Loeblich 1979, *Gonyaulax tamarensis* var. *excavata* Braarud 1945, *Gonyaulax excavata* (Braarud) Balech 1971, and *Protogonyaulax tamarense* (Lebour) Taylor 1979. This species may still have homonyms that sort out morphologically, biochemically, or geographically with new studies [1, 2, 3].

Alexandrium tamarense is the producer of paralytic shellfish poisoning toxins. PSP is a neurotoxic syndrome resulting primarily from the blockage of neuronal and muscular Na^+ channels. Binding to the Na^+ channel prevents propagation of the action potential, which is essential to the conduction of nerve impulse and muscle contraction. Most strains are toxic but some produce only trace amounts. In a few cases they are reported to cause fish kills [7, 11]. This species is widely distributed in Japan but no red tide has been reported. Paralytic toxins produced by this species have made serious problems for shellfish aquaculture fisheries [4].

This is a widespread coastal species distributed in European waters, including Britain and is also on both coasts of North America, Argentina, Japan, Korea, Gulf of Thailand, Venezuela, Barents Sea, Kamchatka peninsula and south of Taiwan [3]. Some reports indicated that this species is also distributed in China, for example, in Dapeng Bay, East China Sea and Jiaozhou Bay, Bohai Sea. In Taiwan, Xiamen and Hong Kong this species is reported to form red tides [8].

In addition, more than 10 kinds of cysts were collected from these areas using sediment traps and cylindria samples, and only the cysts of *Alexandrium* sp. could germinate into unicellular vegetative cells when they were cultured in the light intensity of 1500-2000 lux and 18°C [4, 12, 15]. But one strain from Japan in the lab grows well below 16°C.

In this paper, we report the growth pattern of *Alexandrium tamarense* in order to find the optimum growth conditions, which may be helpful in harmful algae management and mitigation.

MATERIALS AND METHODS

The *Alexandrium* isolates used in this study were primarily isolated and cultured from benthic cysts that originated from Hong Kong. The strain we have was obtained from the Institute of Oceanology, Chinese Academy of Sciences. The species had been cultured for more than 3 months before this experiment in our laboratory. The test species came from the same strain and was divided into five groups as parallel samples.

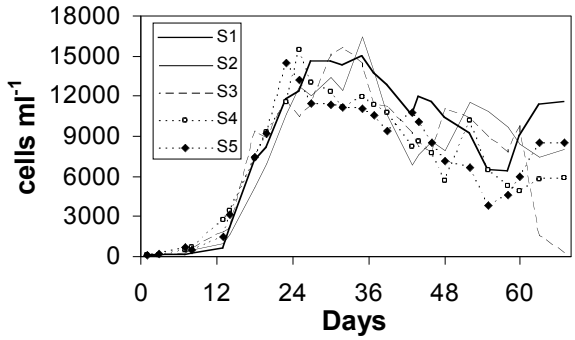
We started this experiment in 125 ml flasks with *A. tamarense,* the original concentration was about 120 cells ml^{-1}. Algae were grown in two types of media: f/2 and L1 [6]. For the purpose of looking for optimum growth conditions, we designed illumination from 300 to 3000 lux using 40 W fluorescence lights and the light time was on from 12 to 18 hours. The temperature and salinity in this study were different from 20-30°C and from 22-34‰.

The optimum growth conditions were determined according to the algae numbers by rude statistics in different cultured conditions. The characteristics of algae growth were analyzed in these selected conditions.

RESULTS

This kind of alga has chloroplasts, becomes an obvious snuff color when they bloom and suspends in the

medium to form columns. Cells were 20-52 μm in length and 17-44 μm in width. They have two flagella and gyrate forward fast in the form of a twist or four cells in good situation. They float on the surface and converge together but seldom were observed on the bottom of containers. In a later period deposition was apparent but most cells floated as usual. This kind of alga is very sensitive to its circumstances and sinks as deposition when discomfort arises such as a big difference in temperature, illumination, or nutrition. Deposition includes the dormancy cysts or downfall of cells. These cysts or temporary depositing cells may excyst to become motile vegetative cells again. Well-grown cells



should not attach to walls or produce velum unless they are exposed to altering circumstances or are cultured for a long time.

Figure 1. Growth of five individual *Alexandrium* isolates.

Figure 1 is the growth of five individual algae of *A. tamarense*. The cells increased slowly in the early 10 days and entered typically exponential increasing stage followed by 3-4 days of a relative decline. The amount of cells remained stable from the 27th-35th days, which was called the stillness stage but some errors caused the temperature to increase quickly and many cells settled and died. Although some cells resumed growth, the entire population was restrained obviously.

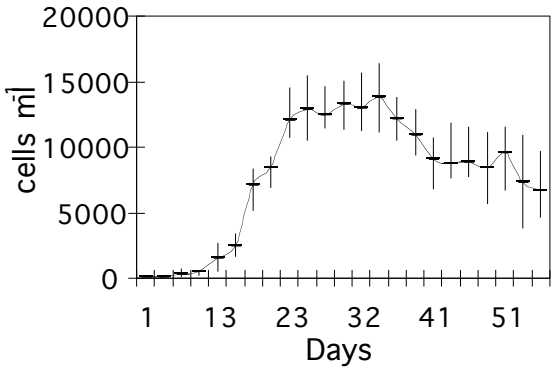


Figure 2. Analysis of growth tendency of *Alexandrium*.

Figure 2 demonstrated the analyses of growth tendencies of *A. tamarense*. The value represented the amount of algal cells and the fluctuation of bars represented the discretion value of statistics data.

The result shows that the population of *Alexandrium* belongs to the typically increasing pattern in accord with the Logistic Equation:

$$N_t = K / (1+e^{\alpha-rt})$$

where N_t is the amount of individuals in the population after t (time), K is the environmental carrying capacity,α represents the linear slope, and r is the linear intercept.

The statistics of population increasing are shown in Table 1. The carrying capacity K was estimated to be 13780.

Table 1. The increasing population of *Alexandrium tamarense.*

Time (d)	Real N (cells ml^{-1})	(K-N)/N	$\log_e$((K-N)/N)	Theoretic N (cells ml^{-1})
1	122	111.95	4.72	54
3	122	111.95	4.72	99
7	356	37.71	3.63	452
8	464	28.70	3.36	608
13	1520	8.06	2.088	2441
14	2490	4.53	1.51	3122
18	7070	0.95	-0.052	6904
20	8480	0.63	-0.473	896
23	12020	0.15	-1.923	11355
25	12850	0.07	-2.63	12355
27	12480	0.10	-2.263	12972
30	13330	0.03	-3.383	13448
32	12970	0.06	-2.773	13598
35	13770	7.26	-7.23	13707

So the linear equation according to the Logistic Equation will be:

$$\alpha - rt = \log_e[(K-N)/N]$$

and the linear regression is:

$$\log_e((K-N)/N) = 5.54-0.31t$$

with an intercept α=5.54 and a slope of r=0.31. And the theoretic value according to Logistic Equation will be:

$$N=13780/(1+e^{5.54-0.31t}).$$

Figure 3 is the comparison between the real and theoretical value of *A. tamarense,* which coincide well.

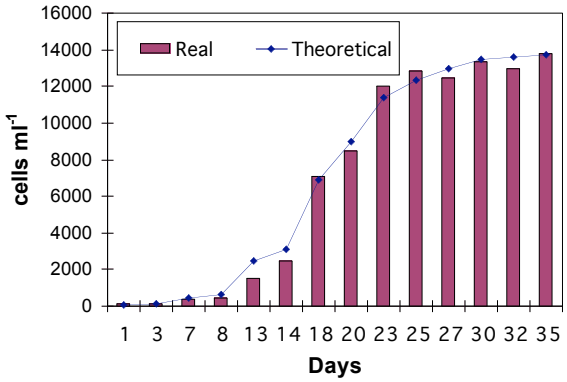


Figure 3. The comparison between real and theoretical *Alexandrium* populations.

The Growth efficiency (E) express the increase of cells increased in a geometric series in the exponentially increasing stage, this may be expressed as:

$$E = (\log_e N_0 - \log_e N_t)/T$$

or

$$E' = (\lg N_0 - \lg N_t)/T$$

where T is a length of growth time, N_0 is the cells in T moment, and N_t represents cells after T moment. The average double increasing time G is expressed as:

$$G = 0.301/E'.$$

The average doubling time in several groups was demonstrated in Figure 4. In the same condition, the average doubling time of *A. tamarense* population was 85 hours.

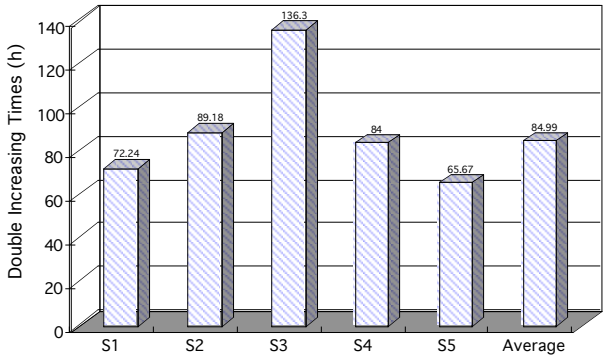


Figure 4. Average doubling times in different test groups. S1-5 are samples 1 through 5.

Figure 5 represented the specific growth rate (SGR, d^{-1}) of *A. tamarense*. The expression is as follows:

$$\mu = (lnX_t - lnX_0)/t$$

where μ is the specific growth rate (d^{-1}), X_0 represents the preliminary cell density, X_t is the cell density after t days, and t is time.

In the exponentially increasing stage, the specific growth rate is concurrent to the growth curve, especially from the third day to the 18th day. But it is antithetical between these two curves from the 18th day to 35th day. The curves became identical after 35 days.

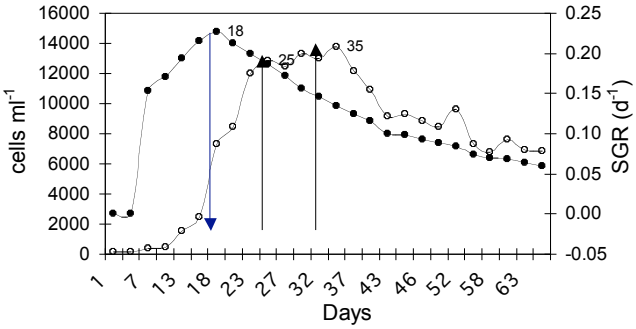


Figure 5. Comparison between growth patterns and the specific growth rates of *Alexandrium*. Open circles indicate the cell concentration; solid circles denote the specific growth rate.

DISCUSSION

*Alexandrium tamarense (*Lebour) Balech is distributed widely throughout the world and can be found in Dapeng Bay, Jiaozhou Bay and Xiamen in China. They often bloom in warm areas but can be sampled in summer and autumn in Huang Bo-hai Sea. The highest concentration observed was 2036 cells dm^{-3} and they accounted for 1-13% of the phytoplankton population in August 1993 in Jiaozhou Bay in China. The number of *A. tamarense* cells corresponded with temperature: higher algal concentrations were observed in water with temperatures between 14.3-25.4°C (July to November). In our experiment, the optimum conditions were f/2 and L1 media at 21-23°C, salinity of 28-31‰, and illumination of 1500-2500 lux on a 16:8 hr light:dark cycle [5, 7, 8].

The average doubling time for *A. tamarense* population was 85 hours, that means 3.5 days but this varies among isolates. There was a discrepancy between some strains, the longest doubling time was 136.3 hr and the shortest was 65.6 hours.

Alexandrium tamarense ages if fresh medium is not added. Most strains settled, formed amoebocytes or had protoplasm deviating. Some strains may rejuvenate when fresh medium is added. SHI Yanjun reported that the growth conditions had a significant effect on the uptake rate of phosphate by *A. tamarense* cells, and the cells grown in low-nitrate and low-phosphate cultures had a higher uptake rate, and lower ratio of N/P promoted the propagation of *A. tamarense* [12]. The optimum growth conditions suggested that we may forecast the blooming of this species through these circumstances, which may be very useful in harmful algae management and mitigation.

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THE TOXICITY STUDY ON *HETEROSIGMA AKASHIWO* USING AN ARTEMIA BIOASSAY

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ABSTRACT

The toxicity of a fish-killing HAB species, raphidophyte *Heterosigma akashiwo* (Hulburt) Taylor was studied using an *Artemia* bioassay. The mobility of *Artemia* was inhibited clearly when exposed to the alga. In the experiment, *Artemia* nauplii were exposed to different dilutions of the algal culture, cell-free medium, re-suspended cells, cell contents, and cell fragments. *Artemia*'s migration behavior in each treatment was recorded using a video camera at 0.1, 1.5, 6, 24 and 48 hr and swimming speed (mm s^{-1}) was calculated accordingly. The results indicated that the swimming speed of *Artemia* decreased with increased algal concentration and exposure time. EC50 values at 0.1, 1.5, 5 and 24 hr were 1.5×10^7, 7.5×10^6, 4.5×10^6 and 3×10^6 cells L^{-1}, respectively. Under higher algal concentrations of 1.5×10^7 cells L^{-1}, *Artemia* could not migrate after exposure to *H. akashiwo* for over 48 hr, but were observed to remain in a fixed location despite attempting to move. The majority of *Artemia* survived despite these adverse effects on migration. At lower concentration 3×10^6 cells L^{-1}, *Artemia* recovered to normal swimming after 48 hr. Study of the toxicity indicated that algal cells showed the highest toxicity at the middle exponential growth phase. No toxicity was detected from the alga at senescent phase. Re-suspended cells and cell fragments produced similar sublethal effect as algal culture, while the cell-free medium and cell contents had little effect. The results implied that substances on or attached to the algal cell surface were responsible for the toxicity. The algal fragments were extracted and the extract was examined with *Artemia* bioassay, Phenol-H_2SO_4 method. The visible and ultraviolet absorption spectra were obtained. The toxic substance(s) was suspected to be polysaccharide with ultraviolet-absorption.

INTRODUCTION

The raphidophyte *Heterosigma akashiwo* is commonly associated with fish kills, causing great commercial loss in many countries around the world [1,2]. Some studies have suggested that the mechanism by which of *H. akashiwo* kills fish is via gill and intestinal lesions than by mechanical obstruction or changing of gill permeability [3]. But its killing mechanism is still poorly understood [4]. Some zooplankton species such as Copepods *Calanus pacificus* and *Acartia omorii* are apparently not affected. Other species were found to have reduced feeding, or not to feed at all. Low fecundity or low survival was also reported when exposed to *H. akashiwo* [5]. However, it has been thought that this ichthyotoxic species of *Heterosigma* could not affect common laboratory animals so it has been difficult to study biological responses in the lab and many questions remained regarding the nature of the "toxins" [6]. During our toxin screening experiments on more than 40 strains of different harmful or toxic species using brine shrimp *Artemia salina* Leach, a commonly accepted experimental organism for zooplankton, it was found that the mobility of the brine shrimp was clearly inhibited by *H. akashiwo*. The observation raised many questions: How does the algae affect the shrimp? Where does the toxicity come from, algal cells or the culture media? Can this phenomenon provide thoughts regarding the fish-kill mechanism? In this paper, we report the results of the impact of *H. akashiwo* on *Artemia* mobility, toxicity dynamics during algal growth, the toxicity source as well as the chemical characteristics of crude extraction of the toxic substances.

MATERIAL AND METHODS

Algal Species and Culture Methods

Heterosigma akashiwo was isolated from Jiaozhou Bay, Qingdao, China. The diatom *Phaeodactylum tricornutum* was obtained from the Algal Culture Center in Institute of Oceanology, Chinese Academy of Sciences, Qingdao, P.R. China. Both algae were grown in f/2 enriched medium in 1 L glass flasks under 60 $\mu Em^{-2}s^{-1}$ white fluorescent light, 12:12 hr (L:D) cycle at 20°C. Cells were counted using a haemocytometer before each experiment.

Experiment on Algal Culture and Its Different Fractions

Dilutions (1, 2 and 5%) of *H. akashiwo* culture at 3.0×10^8 cells L^{-1} were used in the experiment. A seawater control and a control of *P. tricornutum* which was diluted to 1.5×10^7 cells L^{-1}, equivalent to the highest concentration of *H. akashiwo* in the experiment, were used. Each concentration was done in duplicate.

Cell-free medium and cells were obtained by centrifugation of algal culture dilution of 1.5×10^7 cells L^{-1} at 10,000 rpm for 10 minutes. Supernatant I was removed for use as cell-free medium and Pellet I was re-suspended with seawater to the original concentration. Half of the re-suspension was used in the experiment as re-suspended cells and another half was ultra-sonicated for 20 min in an ice bath, then centrifuged at 10,000 rpm for 10 minutes. Supernatant II was removed for use as cell contents and Pellet II was re-suspended with seawater to the original concentration and tested as cell fragments. Six ml 6-well plates containing 5 ml fresh algal culture, cell materials or seawater in each well were maintained at 20°C until *Artemia* were transferred in.

Artemia *Mobility Determination*

Artemia salina cysts were purchased from *Artemia* Center, Belgium, and kept at low temperature (4°C) during storage and transportation. *Artemia* were hatched three days before initiating the test at 20°C. Twenty instar II-III larvae were transferred into each well of the 6 ml 6-well plates. Each well was marked by two coordinate axes for determining *Artemia* position. Swimming behavior of the brine shrimps in each well was recorded by a video camera at 0.1, 1.5, 6, 24 and 48 hr for at least 10min, then displayed on a TV screen after experiments. The positions of a larva at a fixed duration can be easily obtained using determined pause and slow display functions. The period when *Artemia* was swimming horizontally was chosen so that the moving speed was calculated accordingly. Average speed and its standard deviation were calculated from at least ten individuals in each well.

Toxicity Dynamics During Algal Growth Cycle

During a whole growth cycle, algae were sampled for counting daily. Cultures at growth day 7, 9, 11, 12, 13, 16 and 18 were sampled and diluted to test their effects on *Artemia* mobility after 1.5 hr exposure. *Heterosigma aashiwo*'s 1.5 hr EC50 of these days were calculated, respectively. Using the reciprocal of the lowest value as 100%, relative toxicities were calculated and plotted with the growth day, together with the cell densities.

Crude toxic substances extracted from the algae

Crude toxic substances were extracted from algal cells according to the procedures in Figure 1.

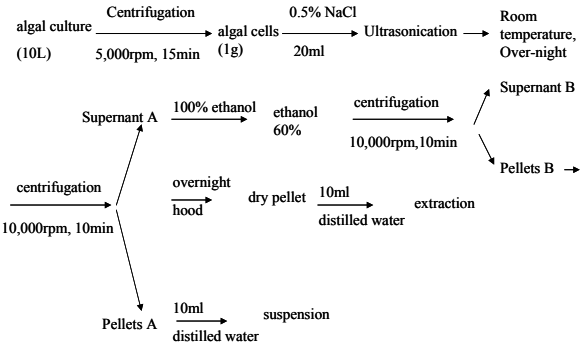

Figure 1. The procedures for crude toxic substances extraction from *H. akashiwo.*

The toxicity of the obtained extract and suspension were respectively tested using *Artemia* bioassay. The extract was tested with Phenol- H_2SO_4 for saccharide and its visible and ultraviolet absorption spectra were determined at wavelength from 200-800 nm with a Beckman UV 650 spectrophotometer with distilled water as a reference sample.

Data Analysis

Data were given as means and standard deviations. When comparing each treatment with the control, unpaired *t*-test was applied by *F*-test to compare variances. The relationship of *Artemia* swimming speed with algal concentration was determined by regression analyses, by which EC50 was calculated.

RESULTS

Inhibition of Artemia *mobility by* H. akashiwo *in 24 hr exposure*

Swimming speeds of *Artemia salina* at 0.1, 1.5, 6, 24 and 48 hr after exposure to different concentrations of *H. akashiwo* are shown in Figure 2.

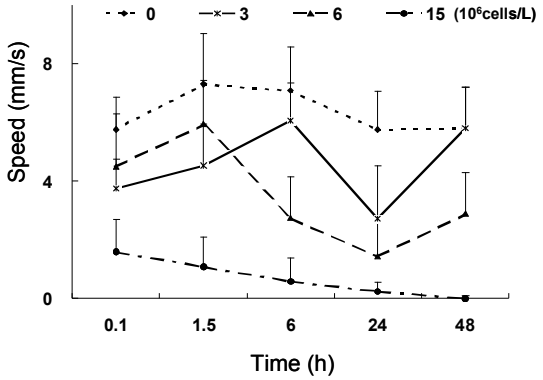

Figure 2. The effect of different concentrations of cultures on swimming speed of *A. salina* after exposure for 0.1, 1.5, 6, 24 and 48 hours. 1%: 3×10^6 cells L^{-1}; 2%: 6×10^6 cells L^{-1}; 5%: 1.5×10^7 cells L^{-1}.

In Figure 2, it is clear that the mobility of the brine shrimp was inhibited by *H. akashiwo* during the experiments. In higher concentrations of 1.5×10^7 cells L^{-1}, swimming speeds were very significantly lower than those of the control at 0.1, 1.5, 6 and 24 hr ($p<0.05$). In lower concentration of 3×10^6 cells L^{-1}, significant inhibition took place after longer exposure (as shown in Fig. 2), which was after 1.5 hr at 3×10^6- 6×10^6 cells L^{-1}, and after 24 hr at 3×10^6 cells L^{-1} ($p<0.05$). The swimming speeds decreased with increased concentrations and exposure time from 0.1 hr to 24 hr. The EC50 values of swimming speeds of *Artemia salina* at 0.1, 1.5, 6 and 24 hr were 1.5×10^7, 7.5×10^6, 4.5×10^6 and 3×10^6 cells L^{-1}, respectively, indicating the effect of *H. akashiwo* to *Artemia* movement was time and concentration dependent. *P. tricornutum* culture at 1.5×10^7 cells L^{-1} did not influence *Artemia* mobility ($p>0.05$), which indicated that significant inhibition of *Artemia* movement was not caused by viscosity of dense cells, but toxic substances from *H. akashiwo*.

The Effect of H. akashiwo *on* Artemia *After 48 hr Exposure*

Under higher algal concentrations of 1.5×10^7 cells L^{-1}, *Artemia* could not migrate any more after over 48 hr exposure (swimming speed at 0, shown in Fig.2). It was observed that these shrimps remained in a fixed location despite attempt to move, but the vibration frequency and amplitude of their appendage movement apparently reduced with increased concentration. The majority of *Artemia* survived despite these adverse effects on migration, but subsequently appeared not to grow. In lower concentration of $3\text{-}6\times10^6$ cells L^{-1}, *Artemia* recovered to normal swimming after 48 hours (see Fig. 2). At 48 hr, the swimming speed was not significantly different from that of control ($p>0.05$).

Toxicity Dynamics

Figure3 shows the growth of *H. akashiwo* in an algal growth cycle and relative toxicity at day 7, 9, 11, 12, 13, 16 and 18. The growth curve showed that algal density increased rapidly and dropped at day 13 dramatically in the whole growth period. The toxicity of the alga also changed during the whole growth period. The relative toxicity increased with growth time before day 11, then decreased, and no toxicity can be detected at senescent stage when the algal cell number began to decline. The results implied algal cells have more toxic substance or the toxic substances have more activity during exponential growth phase.

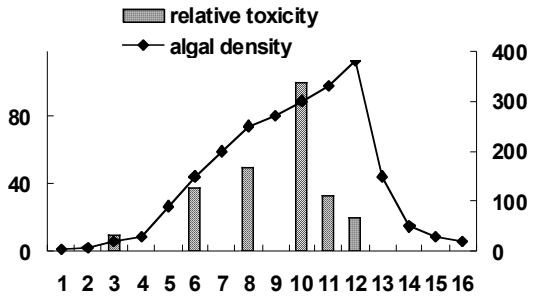

Figure 3. Growth of *H. akashiwo* during a growth cycle and 1.5 hr EC50 of cultures at different growth phases on *Artemia* mobility.

Toxicity Source

Different fractions of algal culture were studied to distinguish the toxicity source. Both re-suspended cells (P-I) and re-suspended cell fragments (P-II) had the inhibitory effect on *Artemia* mobility after 6 hr exposure, while cell-free medium (S-I) and cell contents (S-II) had little effect after 48 hr exposure (Fig. 4). The results implied that substances on or attached to algal cell surface were responsible for the toxicity.

Crude Extraction of Toxic Substances From the Alga

Crude extraction had an inhibitory effect on *Artemia* while the re-suspended leftover had little effect (Table 1). The crude extraction turned yellow when tested with Phenol-H_2SO_4 indicating monosaccharide or polysaccharide was present. The material exhibited little absorption in the visible spectrum, but the ultraviolet spectrum showed strong absorption with two peaks at 203 and 206 nm (Fig. 5). This result indicated there were little protein and DNA/RNA present which may have absorption at 260 and 280 nm, respectively. Therefore, we suspected that the water-soluble toxic substances were polysaccharide.

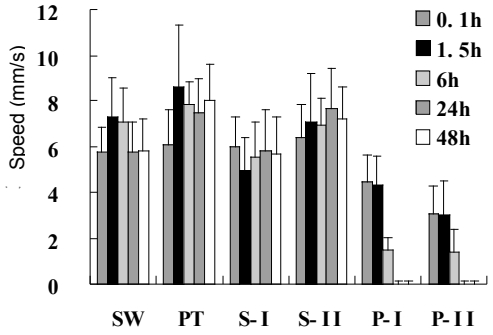

Figure 4. The effects of different factions of *H. akashiwo* cultures as well as a diatom *P. tricornutum* on swimming speed of *A. salina* after exposure for 0.1, 1.5, 6, 24 and 48 hours. SW: seawater; PT: *Phaeodactylum tricornutum*; S-I: cell-free medium; S-II: cell contents; P-I: re-suspended cells; P-II: re-suspended cell fragments.

Table 1. Crude extraction from *Heterosigma akashiwo* tested using *Artemia* bioassay and Phenol-H_2SO_4 method.

	Inhibition to *Artemia* movement	With Phenol - H_2SO_4
Extraction	+ (the swimming speed was 0 after 2h exposure)	turned yellow
Suspension	- (no effect at 0.1, 2, 6, 24 and 48h)	

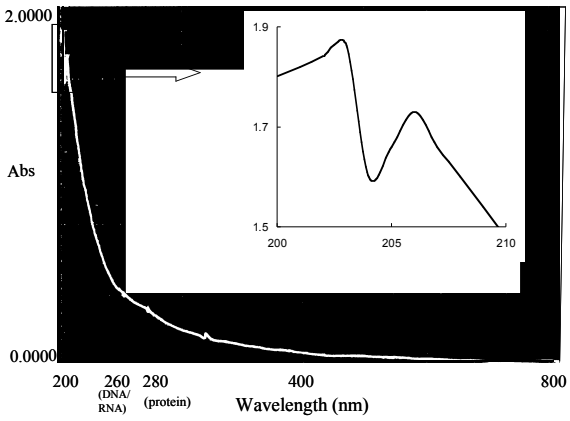

Figure 5. Visible and ultraviolet absorption spectra of crude extract from *H. akashiwo*.

DISCUSSION

Our results showed clearly that *H. akashiwo* cells could strongly inhibit *Artemia* (2-5D) mobility while no mortality was caused during 48 hr experiment. A similar adverse effect of *Heterosigma* on the same species of *Artemia* was also observed in Lush&Hallegraeff [7]. In their study, adult *Artemia* exposed to culture also showed uncoordinated and reduced swimming activity with long periods of total inactivity, which was consistent with our result. However, their results were different from ours in that mortality of *Artemia* at metanauplii stage (4d) was found when exposed to both *Heterosigma* cultures and cell-free culture medium in 24 hours. In our 24 hr experiment, over 90% *Artemia* survived and cell-free medium did not show any adverse effect. Besides centrifugation, cell-free medium was also made by filtration through 5 μm mesh then 0.45 μm GFC filter under gravity and tested for toxicity, according to the method which they used. The results also showed it had no obviously adverse effects on *Artemia*. We do not know the cause of these differences, however different strains of *H. akashiwo* and *Artemia* may vary in toxicity and sensitivity to toxic substance. *Heterosigma akashiwo* has been confused with the chrysophyte *Olisthodiscus luteus* Carter in many countries and most red tides ascribed to *O. luteus* actually involved *H. akashiwo* [8]. In previous studies of *H. akashiwo* reported as *O. luteus* or *H. akashiwo*, some species such as copepods *Calanus pacificus*, *Acatia omorii* were not apparently affected when exposed to this alga. Yet detrimental effects on some zooplankton species were found, such as no or low feeding, in some cases with low fecundity or low survival of copepods *Pseudodiaptomus marinus*, *A. hudsonica* and *A. tonsa*, rotifers *Synchaeta cecilia*, *Brachionus plicatilis* and ciliates *Tintinnopsis tubulosoides* and *Favella* sp. [5, 9]. For the above two tintinnid species, *T. tubulosoides* and *Favella* sp., growth rate inhibition was reduced in the presence of 10^5-10^6 *H. akashiwo* cells L^{-1} and lethal effect was observed at a concentration of 5×10^6 cells L^{-1} *H. akashiwo* in a 3 day experiment [10]. For the above two copepods, *A. hudsonica* and *A. tonsa*, mobility inhibition and no feeding occurred during 24 hr grazing period at a concentration of 7.3×10^6 cells L^{-1} *H. akashiwo* [11]. Similar filtration inhibition was also found in the rotifer *Brachionus plicatilis* Muller [12], five prosobranch larval species [13] and even mussels *M. edulis* [14], *O. edulis* and the clam *M. mercenaria* [15]. These results were consistent with our observations of adverse effect on *Artemia*. The observation of no growth of *Artemia* in our experiment may be also caused by filtration inhibition and may result in mortality in several days. Compared with the above tintinid and copepod species, the response of *Artemia* was relatively fast and sensitive. In our experiment, *Artemia* mobility was inhibited at 3.0×10^6 cells L^{-1} only after 24 hr exposure. This was probably due to the direct exposure of the gills of this branchiopod, which may be easily harmed by certain toxins.

The mechanism by which *H. akashiwo* harms *Artemia* remains unclear. As motility inhibition at higher concentration occurred immediately when *Artemia* was exposed to *H. akashiwo*, it is very likely that direct exposure or contact is the main cause of *Artemia* motility inhibition. The contact between *Artemia* and algal cells may further activate the release of the novel inhibitory substances. Ingestion may be an alternative or contributing factor as *Artemia* is a filter feeder. Another way may be by substances being released from algal cells, but the continued presence of *H. akashiwo* is required to maintain sufficient concentration or activity of the inhibitory factors over some time. At low concentration, after exposure for some time, concentration or activity of the inhibitory substance may be reduced, so *Artemia* could recover normal swimming behavior.

Our results indicated that polysaccharide substances related to cell surface of *H. akashiwo* showed the toxicity to the brine shrimps, which coincided with previous study that showed a glycocalyx on the cell surface of the alga [16]. Also polysaccharide secretions were considered to alter the rheological properties of seawater and result in fish death [17, 18]. The substances having effect on *Artemia* in our experiment were likely the same viscous compounds from algal cells. The nature of the toxic substances will be investigated in further studies.

As allelophathic substances from *Heterosigma* cells could suppress or enhance the growth of other phytoplankton species, *H. akashiwo* may play an important role in causing dramatic changes in cell numbers of other phytoplankton during harmful algal blooms [19]. On the other hand, the influence of grazing by marine organisms on *Heterosigma* dynamics may be indirect in reducing competition from diatom species which normally compete with the flagellate for resources, indirectly influencing the initiation and abundance patterns of this species in bloom formation.

The cell density of *H. akashiwo* dropped quickly after 13 days in our experiment, which was similar to the phenomenon of sudden disintegration of red tide of this species. This sudden disappearance of red tide was considered to possibly reflect the physiological changes related to cyst formation of this species [20], the presence of bacteria [21] and virus-like particles [22]. Whether the bacteria or virus could influence the

production of unknown toxic substance(s) remains unknown.

The *Artemia* test has been applied successfully in toxicity studies of *Alexandrium* and *Prorocentrum* species [7, 23-25]. Although *Artemia* generally does not live in marine environments, it is a member of the crustacean class, hence, related to shrimp and lobsters. The advantages of using the *Artemia* bioassay are the economy, small size, adaptability of the organism, and the ease with which it can be hatched and produce a homogenous population of *Artemia* larvae in the laboratory. Furthermore, the *Artemia* assay can reflect the direct interaction between the algae and the filtering organism and help in the study of toxins which may be related to the marine animal killing events. A new detection method using *Artemia* mobility inhibition as an index is under development. We hope the rapid, inexpensive and easy to use tool may aid in monitoring these kinds of harmful algae.

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ICHTHYOTOXICITY OF A HARMFUL DINOFLAGELLATE *COCHLODINIUM POLYKRIKOIDES*: ASPECT OF STRUCTURAL CHANGE OF FISH GILL AND SCREENING OF TOXIC COMPOUNDS

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ABSTRACT

In order to elucidate the ichthyotoxic mechanisms of *C. polykrikoides,* the existence of toxins in this algal species, and the physiological responses of fish exposed to this algal bloom were investigated. Based on our previous results [4-6], the loss of structural integrity of gill membranes was examined. The gills of red sea bream exposed to dense blooms (>3000 cells ml^{-1}) for 24 hr showed severe epithelial separation: a severe edema formation was found in the secondary lamellar epithelium and interlamella regions of primary lamellae. However, membrane swelling and/or lysis were found to be out of proportion to the level of algal cells and exposure time. The aqueous and lipid-soluble fractions of *Cochlodinium* extracts did not show toxicity against mice or fish. Ichthyotoxicity was also not observed in *C. polykrikoides*-free media. In addition, no effects of algal extracts on the growth of lymphoid cells as well as mammalian mitochondrial respiration were observed. Algal extracts did not show any peaks corresponding to PSP on fluorometric HPLC chromatograms. These results suggest that the mechanism of fish kills by live *C. polykrikoides* is distinct from ichthyotoxic compound-mediated toxic effects and this species should be considered a non-toxin producing dinoflagellate.

INTRODUCTION

Cochlodinum polykrikoides is one of the most frequently appearing harmful red tide dinoflagellates in Korea, and recent dense blooms caused serious damage to coastal fish farms [1]. The economic loss caused by the *C. polykrikoides* blooms was $8.4 million in 1993, $95.5 million in 1995, and about $6.5 million in 2001. Although the lethal effects of this dinoflagellate on fish have been investigated to some extent, there is yet not consensus among workers as to the mechanisms of fish kills by *Cochlodinium*. Nevertheless, it is generally accepted that suffocation is the direct cause of fish death. Namely, suffocation due to excessive covering of the fish gill surface by ruptured algal cells and/or mucus-like substances secreted by them [2, 3]. Furthermore, our previous results suggest that reactive oxygen species (ROS)-mediated oxidative damage is associated with ichthyotoxicity of *Cochlodinium* [4]. We reported that inactivation of ion transporting enzymes such as carbonic anhydrase and Na^+ /K^+-ATPase and abnormal mucus secretion in gill cells were one of the principal causes of the fish kills [5]. The aspects of hematological changes in fish exposed to these algal blooms have also been investigated [6].

Meanwhile, one group suggested that *Cochlodinium* species might secrete some ichthyotoxic substances such as neurotoxins, hemolysins and hemagglutinins [7, 8]. Moreover, they have reported that two unique paralytic shellfish poisoning (PSP) toxins were separated from the *Cochlodinium* type '78 Yatsushiro [9]. That is, they proposed that some toxic compounds released during the dense bloom might be responsible for the fish kill. However, reciprocal results were observed in extracts of Korean type *C. polykrikoides*. In one study, the water- and lipid-soluble fractions of *Cochlodnium* did not show ichthyotoxicity and we could not find any toxic compounds such as PSP [3]. But, recently, Cho and Kwak reported the possibility of the existence of toxins in *Cochlodinium*; a water-soluble fraction prepared from natural and cultured *Cochlodinium* showed toxicity against mice [10]. Thus, their insistence on the existence of ichthyotoxins might be viewed as being contradictory to the general consensus about suffocation. Particularly, the reciprocal observations give rise to confusion to the handling of dead fish, with anxiety arising about the impact of toxins on the health of people who eat these dead fish. Therefore, this point was scrutinized in the present study. In addition, we examined the structural changes in fish gills exposed to *Cochlodinium* blooms to elucidate the ichthyotoxic mechanism of this species.

MATERIALS AND METHODS

Plankton and Fish

The dinoflagellates, natural type *C. polykrikoides* and *Heterosigma akashiwo*, were collected in Namhae and Masan, Korea, in summer 2000, during blooms of these species. The *H. akashiwo*, which is known to be non-harmful red tide species in Korea though it is considered harmful in other regions, was used as the control sample. The collected samples were lyophilized and frozen at –20°C until use. Flounder, *Paralichthys olivaceus,* (ca. 160 g, 15 cm) and red sea bream, *Pagrus major,* (ca. 180 g, 15 cm) were purchased from an adjacent fish farm. They were transferred into tanks with a continuous water supply and subjected to 2 days of acclimation before use.

Preparation of Algal Extracts and Ichthyotoxicity Test

Crude extracts were prepared from *C. polykrikoides* and *H. akashiwo*, respectively, as described by Lee with a slight modification [3]. Briefly, the sample was extracted with 80% methanol three times. After evaporating the methanol phase, the residue was partitioned between diethyl ether and water. Salts in the

aqueous layer were removed by methanol precipitation and charcoal column, successively.

Toxicity tests were performed on various concentrations of extracts in 20 L of aerated seawater. Seawater containing algal extracts was poured into 50-L containers, and then test fish were introduced. Test fish were exposed to three different algal extracts of 300, 500 and 800 μg ml^{-1} for 24 hr, respectively. The toxicity of the algal cell-free medium (from which about 1 x 10^4 and 1-1.5 x 10^6 cells ml^{-1} of *C. polykrikoides* were removed), was also tested. Ichthyotoxicity of saponin was also investigated as a positive control. During the experiments, the water temperature, salinity and dissolved oxygen in the containers were 23-24°C, 32-33 psu and ca. 5 ppm, respectively.

Bioassays

Male mice (ddY strain) weighing 18-20 g were observed for 48 hr after intraperitoneal injection with an aqueous and lipid-soluble fraction containing 1% Tween 60. For hemolytic activity, mouse erythrocytes were washed in saline several times and used for the bioassay, 1% suspension of the red blood cells was incubated with different concentrations of extracts for 30 min at 35°C. After the well microplate was centrifuged at 3000 rpm for 5 min, the destruction of cells was observed [11]. Cytotoxicity of algal extracts was examined by the XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]- 2H-tetrazolium hydroxide] method as described by Scudiero *et al*. [12]. For the measurement of mitochondrial respiratory activity, the isolation of mitochondria from fresh beef heart was performed according to the procedures described by Rickwood *et al*. [13]. Respiration of mitochondria in a suspension medium was measured by monitoring oxygen uptake, using a polarograph in the presence of substrates as described previously [14]. For histological examinations, after the fish gills were excised out from test fish, the gill specimens were fixed in Bouins solution. Paraffin sections were made by the conventional method, and than stained with hematoxylin-eosin [15].

Analysis of PSP Toxins

Lypohilized samples were suspended in 1 ml of 0.5 N acetic acid in a plastic tube, sonicated for 2-3 min, and centrifuged at 3000 rpm for 5 minutes. The supernatant was then passed through a Sep-Pak C18 cartridge column (Waters) and an ultrafiltration membrane filter (10,000 MW cut-off, Ultrafree C3GC, Millipore). The filtrate was analyzed by a post column derivatization HPLC method for PSP toxins as described by Oshima [16]. When a peak corresponding to PSP toxin was detected, it was examined again by the HPLC system, in which the oxidizing reagent was replaced by distilled water.

RESULTS

Screening of Toxic Compounds in C. polykrikoides

The collected natural *C. polykrikoides* included a few *Ceratium furca* and *Chaetoceros* sp. (< 1%), respectively, which are well-known non-toxic algal species. *H. akashiwo* blooms were nearly mono-specific with only a very small number of *Prorocentrum* sp. present.

To investigate the toxicity of *Cochlodinium* and *Heterosigma*, a mouse bioassay test was carried out on the aqueous and lipid-soluble fractions prepared from each. Mice did not exhibit any abnormal symptoms at 10–20 mg ml^{-1} of the aqueous fractions of *C. polykrikoides*. On the other hand, mice showed abnormal activity such as convulsions and partial paralysis at about 5 min after intraperitoneal injection with higher doses of the aqueous fractions (30–50 mg ml^{-1}). But, these negative symptoms disappeared after about 10 hours. These symptoms were similar to those of mice treated with the *H. akashiwo* extracts of 40–50 mg ml^{-1}. Abnormal signs were not seen after intraperitoneal injections of lipid-soluble fractions of test samples as well as intestinal extracts of dead fish killed by live *C. polykrikoides*.

Table 1. Cytotoxic and hemolytic activity of *Cochlodinium* extracts.

Extracts	Conc. (mg ml^{-1})	Cyto-toxicity	Hemo-lysis	Inhibition of respiration
Water soluble fraction	1.0	– *(–)^x	– (–)	–
Lipid soluble fraction	1.0	– (–)	+ (+)	–
	0.5	– (–)	+ (+)	–
	0.25	– (–)	+ (–)	ND
	0.1	– (–)	+ (–)	ND
	0.05	– (–)	– (–)	ND

* – = negative; + = positive reaction; ND = not determined; ^{x}values in the parentheses are the effect of the extracts from *H. akashiwo.*

Table 1 shows cytotoxic and hemolytic activity of the crude algal extracts. In the case of cytotoxicity, morphological changes of lymphoid cells and the inhibition of XTT activity by the algal extracts were not observed. In contrast, when treated with lipid-soluble fractions, hemolytic activity was detected. The extent of hemolytic activity was considerably larger in the *Cochlodinium* fraction than that of *H. akashiwo*: the hemolysis that occurred in the extracts of *C. polykrikoides* required ≥0.1 mg ml^{-1} but for *H. akashiwo* ≥0.5 mg ml^{-1}. Inhibition of mammalian mitochondrial respiration by *Cochlodinium* extracts was also not found.

No mortality occurred when fish were exposed to *Cochlodinium* extracts at concentrations of 300–800 μg ml^{-1} (Table 2). In addition, *Cochlodinium*-free seawater also did not show ichthyotoxicity. These results suggest that, at the least, *Cochlodinium* does not secrete

ichthyotoxic substances outside of cells. In order to confirm the effect of hemolysins on fish, the mortality was measured in the seawater containing saponin, which is a well-known hemolysin. When test fish were exposed to saponin of concentration >75 μg ml^{-1}, they exerted every effort to escape and exhibited mortalities of 30–100% within 24 hr, whereas all species in the low concentration <50 μg ml^{-1} were unaffected.

Table 2. Mortalities of fish exposed to *Cochlodinium* extracts.

Treatments	Concentration (μg ml^{-1})	Mortality for 24 hr (%)
Aqueous fraction	300-500	0
Lipid soluble fraction	500-800	0
Saponin	10-50	0
	75	30
	100	80
	>120	100
Cochlodinium-free sea water	1-1.5 x 10^6 cells ml^{-1}	0

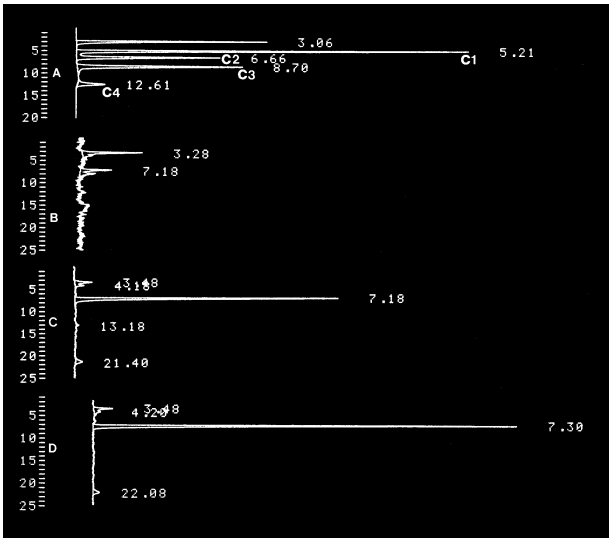

Figure 1. HPLC chromatograms of A) standards of N-sulfocarbamoyl-11-hydroxysulfate toxins, B) algal extracts of *C. polykrikoides,* and C) *H. akashiwo*. The peak of *H. akashiwo* in the absence of an oxidizing agent (D).

HPLC analysis of the extracts of *C. polykrikoides* and *H. akashiwo* did not show a peak corresponding to standard PSP toxins (Fig. 1). The test samples gave peaks indistinguishable to that of the standard of N-sulfocarbamoyl-11- hydroxysulfate toxins (C1-C4). However, when analyzed by the HPLC without an oxidizing reagent, the peaks of samples behaved differently from the standard toxins (Fig. 1D). In the case of saxitoxins and gonyautoxins, the appearance of unknown peaks in test samples was also present in the absence of an oxidizing reagent, periodic acid (data not shown). Thus, they were regarded as false peaks.

Structural Changes in Gills of Fish Exposed to C. polykrikoides

The gills of red sea bream exposed to dense blooms >3000 cells ml^{-1} for 24 hr (moribund and dead fish) showed severe epithelial separation: a severe edema formation was found in the secondary lamellar epithelium and interlamellar regions of primary lamellae

(Fig. 2). Moreover, membrane swelling and/or lysis were found to be out of all proportion to the level of algal cells and exposure time. Lipid peroxidaton of gill tissue was also found in *C. polykrikoides*-exposed fish as compared with the control fish: the values of TBARS averaged 18.2 ± 1.3, 40.5 ± 4.1 and 46.4 ± 5.6 nmol g^{-1} tissue in control, moribund and dead fish, respectively.

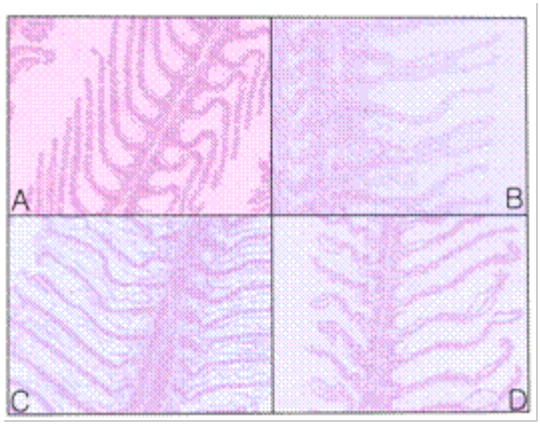

Figure 2. Histological changes of gill filament of the red sea bream exposed to A) *C. polykrikoides*, B) control, C) moribund fish, and D) dead fish; dead fish by environmental hypoxia.

DISCUSSION

As shown in Table 1 and 2, the toxic effects of *Cochlodinium* extracts on mouse and fish were not observed. However, abnormal symptoms such as convulsions and paralysis in mice treated with aqueous fractions of 30-50 mg ml^{-1} led us to speculate that, at least to a certain extent, some toxins of *C. polykrikoides* could be involved in physiological disorders in mice. However, this assumption is not reasonable because the *H. akashiwo*-treated mice had the same symptoms. Thus it would be reasonable to assume that the abnormal symptoms may be related with the shock of the presence of the injected alien substances. This result is consistent with that of Lee [3], but different from those of Cho and Kwak [10]. In the case of the latter, the aspects of the

imperfect desalting in the algal extracts and/or the purity of samples should probably be considered.

A few papers have reported that free fatty acids in phytoplanktons are associated with ichthyotoxicity [11, 17]. In addition, *C. polykrikoides* have unsaturatted fatty acids such as EPA (eicosapentaenoic acid, C20:5, n-3) and DHA (docosahexaenoic acid, C22:6, n-3) [3]. On the basis of these results, the possibility should not be entirely excluded that hemolysins such as highly unsaturated fatty acids present in *C. polykrikoides* may also be involved in the fish kills. However, the extent of fish kill by saponin appears to be related to the concentration; fish kills were not observed in the dose of $<50\ \mu g\ ml^{-1}$. Therefore, from the results of Table 1 and 2, we can guess that fish kills by *C. polykrikoides* are not directly linked to unsaturated fatty acids. Furthermore, this fact was also supported by the data of *H. akashiwo* extracts which also had hemolytic activity. Since suffocation is one of the causing factors leading to fish kills, when fishes are exposed to *C. polykrikoides* blooms, we could assume that respiration of fish is blocked by some respiratory inhibitors such as rotenone. However, the effect of algal extracts on mammalian respiration was not observed; this indicates that these particular inhibitors are not in existence in *C. polykrikoides*. Moreover, *Cochlodinium* cells do not contain the same inhibitors which inhibit the growth of mammalian cells.

In PSP analysis by HPLC, the test samples gave peaks indistinguishable to those of the standard PSP toxins (Fig. 2). However, when analyzed by the HPLC system without an oxidizing reagent, periodic acid, as described by Onodera *et al*. [18], these peaks behaved differently from the actual PSP toxins. Namely, the peaks of authentic toxins disappeared by replacing the periodate solution to distilled water, but the peaks of samples did not disappear under this condition. Therefore, we can guess that the peaks in test samples were regarded as false peaks.

Swelling or lysis of gill membrane by *C. polykrikoides* was very similar to those observed in the fishes exposed to *Chattonella* [15, 19]. Furthermore, interestingly, the histological alteration in the gills of killed fish caused by environmental hypoxia was different from those of *Cochlodinium*-exposed fish; lamellar epithelium was contracted. The histological difference between dead fish by harmful algal cells and hypoxia indicates that, to a certain extent, some substances containing chemical and/or physical stimuli are associated with fish kills by *Cochlodinium*. If it is so, what are the causative agents that are inducing structural disturbances in *Cochlodinium*-exposed fish? In this respect, we can guess that ROS generated from algal cells is one of the factors inducing membrane alteration. Since oxidative damages such as oxidation of lipid and protein in gill cells are induced by ROS [4], these damages may be associated with the edema and/or lysis of cell membrane.

Based on these findings, together with our previous results [4-6], the mechanism of fish kills by *C. polykrikoides* may be considered as the following: the gill of fish exposed to these blooms are damaged (e.g., the loss of functional and structural integrity of cell membranes) and these damages seem to be involved in a decrease of gas exchange in the gills. Ultimately, both physical clogging of gill by algal cell debris and abnormal mucus excretion as well as gill damage are involved in fish kills by *C. polykrikoides*. In conclusion, the results obtained in this work strongly indicate that the red tide dinoflagellate *C. polykrikoides* does not contain toxic substances that lead to fish kills. Consequently, *C. polykrikoides* should be considered a non-toxin producing marine plankton that contains a higher content of mucus-like substances, and the ichthyotoxicity of these blooms is distinct from toxic compound-mediated effects.

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APPLICATION FEASIBILITY OF UV BIO-OPTICAL PROPERTIES FOR RED TIDE MONITORING IN CASE 2 WATERS

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ABSTRACT

A survey of bio-optical characteristics was carried out during four cruises in the coastal waters near Hong Kong, including Peal River Estuary & Lamma Channel, Port Shelter, Tolo Harbor and Mirs Bay, from March to May 2001. Two red tides, the dominating species of which were *Chattonella ovata* and *Prorocentrum dentatum*, were measured in Port Shelter and Tolo Harbor, respectively. The samples were analyzed for chlorophyll (Chl) a, phytoplankton species composition and abundance, and absorption coefficients of different seawater components. Optical property profiles at the sampling stations were taken to obtain remote sensing reflectance (R_{rs}). Together with the laboratory analysis, the relationship among the absorption coefficients of particulates, phytoplankton, non-algal particles and soluble materials, remote sensing reflectance were obtained. It is found that there were spatial and temporal differences in the optical properties in coastal waters, and the bio-optical properties varied clearly during the algal blooms. In particular, both red tide causative species displayed high absorption characteristics in the UV region. Based on the results, the feasibility of using the bio-optical properties in the UV region to monitor red tide in Case 2 waters is discussed.

INTRODUCTION

Application of Remote Sensing to Monitor HABs and the Difficulty in Case 2 Waters

The attenuation of light, which travels from the atmosphere into seawater, is the co-result from the processes of absorption and scattering by water molecules and other suspended/soluble, biotic/abiotic substances in the water body. Marine phytoplankton plays an important role in the process of light transmission. Many biological and physiological factors of the algae, including the composition and distribution of pigments and other chromophoric components [1, 2], the pathway and efficiency of photosynthesis [3], the living status of cells and so on, influence the bio-optical characteristics of the phytoplankton. All these inherent properties may change in response to the ambient environmental factors, such as nutrients [4, 5], light [5] and suspended sediment [6]. Hence, the optical properties of phytoplankton, which interact with other ecosystem factors and influence the biogeochemical processes of the ocean, are the crucial components not only in application of the remote sensing but also in marine biological, physiological and ecological studies.

Present optical studies of marine phytoplankton mainly focus on the effects of photosynthically available radiation (PAR, 400-700 nm) and the application of remote sensing to monitor harmful algal blooms (HABs) [1, 7, 8]. These studies establish important principles for developing algorithms and models for the remote sensing of ocean color [9, 10]. For example, Mitchell and Kahru [11] reported that the algorithms using cubic regressions of remote sensing reflectance (R_{rs}) ratios provided the best estimation of Chl a (0.05-22.3 mg m^{-3}) in oceanic waters. Case 1 water is considered as the ordinary open ocean, wherein scattering and absorption are dominated by phytoplankton, pigments, and Gelbstoff concentrations, and where global blue-green color ratio algorithms for chlorophyll a concentration and K (490 nm) work well. Most of the deep ocean belongs to this case. Water masses that do not satisfy these criteria will be grouped to Case 2. Within Case 2, by this definition, water masses with a wide diversity of bio-optical characteristics will be found. Prominent subcategories include: coccolithophorid blooms, coastal areas, phytoplankton blooms and inorganic particles dominating waters [12]. The data-assimilative modeling as well as the advanced instrumentation offers the bases for developing an algal monitoring and forecasting system. However, in optically complex Case 2 waters, in which HABs occur much more frequently than in Case 1 waters, the absorption, scattering or fluorescence values of phytoplankton are strongly affected by signals from suspended materials, CDOM and other light-sensitive components [13]. To establish a monitoring and forecasting system for HABs in coastal waters, more bio-optical studies are needed to isolate the phytoplankton, especially red tide species, information from the bulk optical signals measured by remote sensing.

MAAs Contribution to UV Attenuation in Aquatic POM & DOM Pools and to the Algal Bloom

With regard to the depletion of atmospheric ozone (O_3) associated with enhancement of ultraviolet (UV) radiation, more researchers recognize the pronounced effects of UV on biological processes and the defense mechanisms of living organisms. Four UV defense mechanisms of marine phytoplankton have been identified: avoidance, screening, quenching and repair [14, 15]. Screening is directly related to the optical characteristics of phytoplankton because it is caused by UV-absorbing compounds such as mycosporine-like amino acids (MAAs). MAAs, water soluble compounds with a cyclohexenone chromophore exhibiting absorption maxima ranging from 310 nm to 360 nm [16],

are found in the different phytoplankton divisions such as cyanobacteria, diatoms, dinoflagellates, cryptomonads, prymnesiophytes, and raphidophytes [17, 18]. The compounds are only synthesized by the primary producers with or without the UV induction, but can be transferred to other organisms through the food chains, where they perform the same UV-screening function [15]. Furthermore, MAAs also play a significant role on the UV attenuation of DOM in seawater, if exuded from phytoplankton [19, 20]. MAA degradation not only decreases the UV radiation in water, but it may produce inorganic carbon and nutrients into the water column that may subsequently stimulate bacteria [21]. Both of these processes will benefit the algal growth

The occurrence of MAAs in certain phytoplankton species may be one of the strategies that allow algal blooms to form in surface waters. Firstly, species with MAAs in cells may manage to dominate under the UV exposure [7, 18]. Secondly, MAAs in seawater may also protect the algae from UV radiation as a screen. Thirdly, MAAs in seawater may be degraded to inorganic compounds, which will stimulate the growth of phytoplankton. MAAs may contribute to the UV attenuation before and during the algal bloom in the above interactive manners, especially in areas with ozone depletion. These specific optical characteristics of red tide causative species may offer a key to the application of monitoring HABs with remote sensing in coastal waters.

To understand photobiological processes in waters, it is essential to quantify the contributions of the individual constituents to the total absorption coefficients of the spectrum from ultraviolet to visible region. Methods have been developed, including the quantitative filter technique (QFT) for determining the absorption coefficient of particulates and soluble absorption measurement for colored dissolved organic mater (CDOM) [22]. The spectra got by the methods are important evidence to know how the red tide species use light and if they resist UV radiation by producing screening materials, such as MAAs.

In this study we aim to know the bio-optical characteristics of coastal waters in Hong Kong (HK) through a series of field surveys. Benefits of the study include understanding bio-optical characteristics of phytoplankton, the relationship between reflectance and Chl a, and the effects by other environmental factors in coastal water. Based on the phytoplankton absorbing characteristics in the UV region, we may enhance our ability to detect HABs by adding an accessory channel in the UV to remote sensors.

METHODS

Stations and Dates of the Cruises

Four areas of HK waters (Pearl River Estuary & Channel, Port shelter, Tolo Harbor and Mirs Bay) were surveyed in the spring of 2001. Details about the position of the stations are shown in Figure 1. The cruise dates of Pearl River Estuary & Channel were 2 & 16 March and 27 April, of Port shelter were 1 & 15 March and 26 April, of Tolo Harbor was 10 May and of Mirs Bay was 11 May.

Parameters

In-situ measurements from the surface to the layer near the bottom included downwelling irradiance (E_d) and upwelling radiance (L_u) using a PRR-800 (profiling reflectance radiance system, Biosperical Instruments Inc), as well as temperature, salinity, dissolved oxygen (DO), pH, depth, turbidity and in-situ Chl fluorescence using a YSI model 6600 (Yellow Springs Instrument Co Inc). The sensors of PRR-800 measure E_d and L_u at 18 wavelengths, including the SeaWiFs standard wavelengths (380, 412, 443, 465, 490, 510, 532, 555, 565, 589, 625, 665, 683, 694 nm and PAR) and the extra ones (340, 395, 710 and 765 nm).

Discrete water samples were collected by boat to be analyzed in the laboratory. Samples from two depths (surface and middle layers) were collected at every station and filtered for different indices. Sampling and measuring methods as well as data processing follow the standard ones [22-24].

Chlorophyll (Chl) a concentration was determined by filtering seawater of certain volume onto GF/F filters, freezing them in the dark, extracting them with acetone, and measuring with high performance liquid chromatography (HPLC) (WATERS) [25].

Seawater (250 ml) was preserved with Lugol's solution and condensed to 10 ml and condensed to determine phytoplankton species composition and abundance (ZEIZZ, RC160 microscope).

Seawater of certain volume was filtered on 47 mm glass-fiber filter (WHATMAN, GF/F). Optical density at wavelength (λ) of the particles ($OD_{fp}(\lambda)$) were measured on a double beam UV-visible spectrophotometer (VARIAN, Cary-100) and referenced to a blank filter soaked with 0.2 μm filtered seawater, between 300-800 nm with 1 nm increment. Filters were replaced on filtration system, extracted for the pigments with 100% methanol, and rinsed with 0.2 μm filtered seawater. Optical density at wavelength (λ) of the de-pigment particles ($OD_{fd}(\lambda)$) were measured with the spectrophotometer again. Both absorption coefficients of particles $a_p(\lambda)$ and absorption coefficients of de-pigment particles $a_d(\lambda)$ were derived with the $OD_{fp}(\lambda)$ and $OD_{fd}(\lambda)$ respectively, using the path length amplification factor given by Mitchell [26]. The absorption coefficient of phytoplankton, $a_{ph}(\lambda)$, was calculated as the difference $a_p(\lambda)$- $a_d(\lambda)$.

Seawater samplers were filtered through 25 mm polycarbonate filters (NUCLPORE, 0.2 μm), which were pre-soaked in 10% HCl for 15 minutes and rinsed thoroughly with ultra pure Milli-Q water. The filtrate samples were stored in combusted bottles in dark and cold until being measured. Ultra pure Milli-Q water was also filtered as a blank. The optical density at wavelength λ ($OD_g(\lambda)$) were measured in 10 cm quartz cuvettes on a double beam UV-visible spectrophotometer (VARIAN, Cary-100) and referenced to ultra pure Milli-Q water, over the 250-800 nm range with 1 nm increment. The

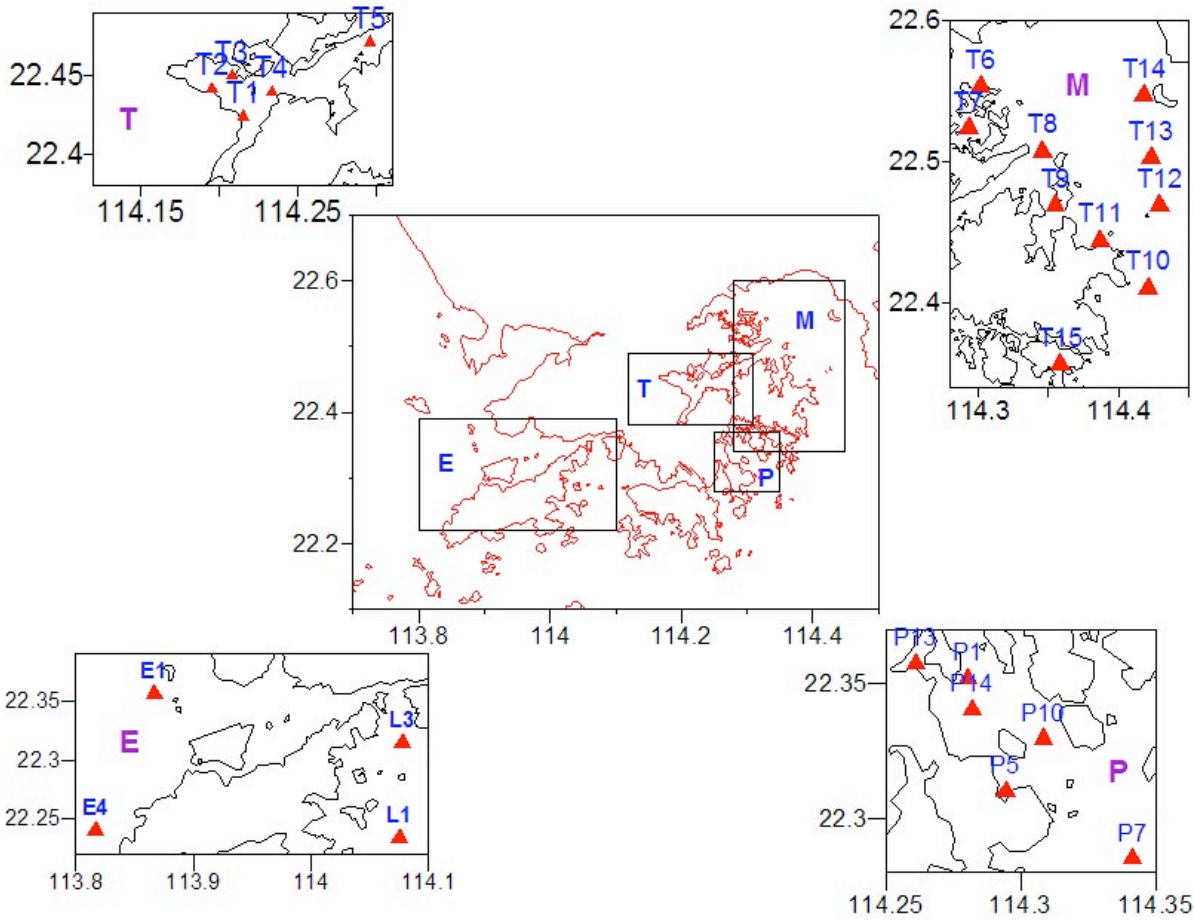

Figure 1. Sampling stations in Hong Kong waters.

absorption coefficients were obtained by:

$$a_g(\lambda) = 2.303\ OD_g(\lambda)/L$$

where L was the cuvette path length.

RESULT AND DISCUSSION

Phytoplankton Abundance and Chl a Concentration in the Four HK Coastal Regions

The results of phytoplankton abundance and Chl a concentration show there are spatial and temporal differences in the four coastal waters (Fig. 2A-D). Port shelter and Tolo Harbor generally had higher phytoplankton abundance and Chl a concentrations than the other two regions. During the red tide bloom in Port Shelter the density of *Chattonella ovata* reached approximately 1.0 X 10^9 cells m^{-3}, much higher than the other stations during the same cruise (Fig. 2B). The *Prorocentrum dentatum* bloom in Tolo Harbor caused Chl a concentrations to reach 28.5 mg m^{-3}, while the cell abundance was about 7.1x10^8 cells m^{-3} (Fig. 2C). On the other hand, at most of the stations phytoplankton abundance during the April cruises was higher than during the March cruises in Port Shelter and Estuary (Fig. 2A and B).

Table 1 summarizes the key species during the different cruises in the four regions. All these species were the red tide causative ones and were very common in HK waters. *Scrippsiella trochoidea* dominated both in Estuary and in Port shelter in the March cruises, while *Heterosigma akashiwo* dominated during the April cruises. The key dinoflagellate species were different in Tolo Harbor and in Mirs Bay. *Prorocentrum dentatum* and *Ceratium furca* had relatively high densities in Tolo Harbor, whereas *Gymnodinium catenatum* was dominant in Mirs bay.

Absorption Characteristics in the Four Coastal Regions and of Algal Blooms

The spectra of a_p, a_d, a_{ph} and a_g from the surface waters at each station were sorted by different waters, and an average value with standard deviation of these absorption coefficients values at specific wavelengths was also evaluated in order to distinguish the spatial difference of the four waters (Fig. 3-6).

The statistical results for a_p at 440 nm indicated that Estuary and Port Shelter had similar particulate absorption coefficients, and was further confirmed by their TSM concentration (Fig. 3E and F). The different spectral features demonstrated that the light-absorbing materials of these two places were different. There are significant peaks in the algal-blooming stations

corresponding to pigment absorption (Fig. 3B and C). Mirs bay exhibited the lowest value of a_p in spectra (Fig. 3D), which was correlative with the lowest TSM concentration.

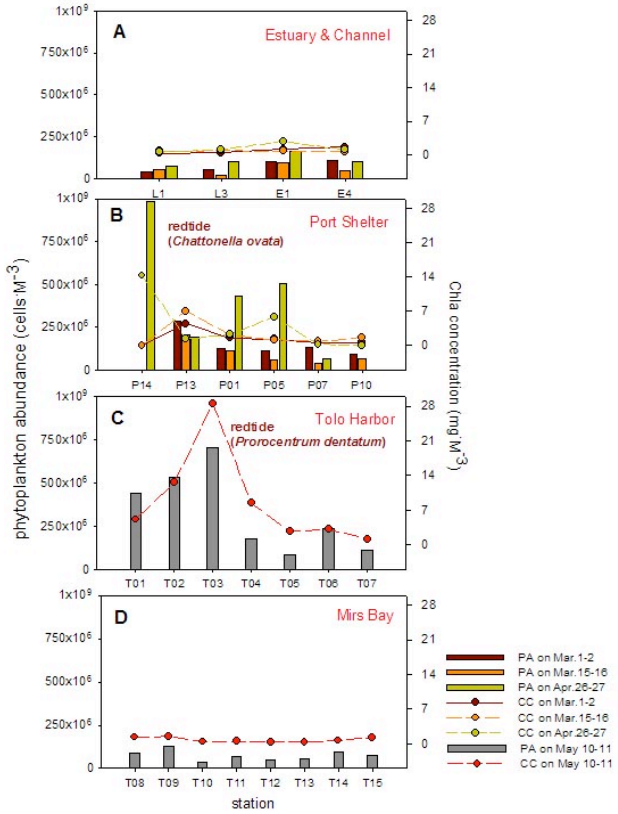

Figure 2. Phytoplankton abundance (PA) and Chl a concentration (CC) in different cruises and waters.

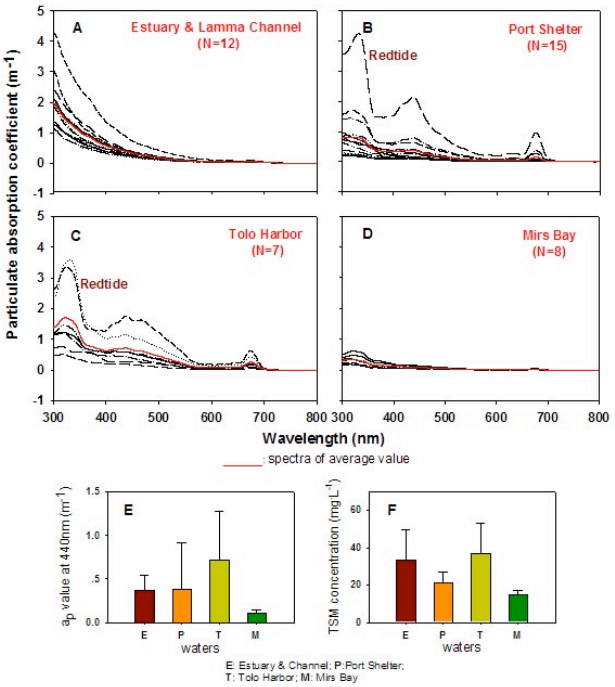

Figure 3. Comparison of a_p spectra in four different waters.

Figure 4 suggests that non-algal particulates were responsible for the high a_p in Estuary. After the phytoplankton pigments were extracted with methanol, the remaining material on the filter represented particulate organic and inorganic substances (e.g., suspended sediment as well as non-pigmented cellular material). Estuary had highest absorption by the non-algal particles, distinctly different from the other waters (Fig. 4 A and E). There were much fewer algal boom cases in turbid Pearl River Estuary than in other areas of HK waters. One of the reasons may be that plenty of inorganic suspended sediments shaded the light through the water body, which affected the algal growth directly. The a_d value at 400 nm in Mirs bay was the lowest (Fig. 4F). Figure 3 (D and F) indicates the lowest $a_p$values as well as the TSM value in Mirs Bay compared to the other regions, all showing that Mirs Bay is the clearest water with the lowest level of suspended particles.

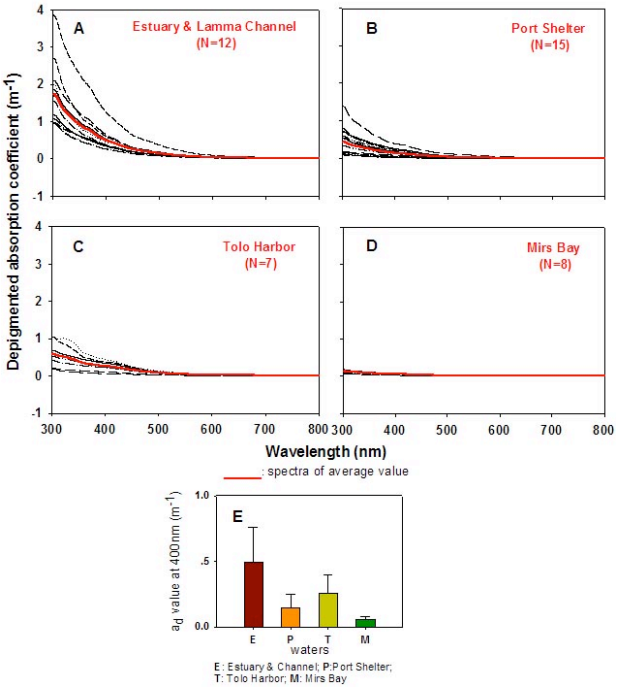

Figure 4. Comparison of a_d spectra in four different waters.

The spectra of a_{ph} show markedly that two red tide causative species, *Chattonella ovata* and *Prorocentrum dentatum*, had high absorption characteristics in the UV region from 310-340 nm, which is also the absorption range of MAAs (Fig. 5B and C). Further studies are needed to determine if these species have these UV-absorbing compounds, which affect absorption characteristics in the UV. Kahru and Mitchell [27] studied the spectral reflectance and absorption of a massive *Lingulodinium (Gonyaulax) polyedra* red tide in 1995 off southern California and found high UV absorption properties by the phytoplankton compared to the "background" situation. Accordingly Mirs Bay and Estuary had lower pigment concentrations and lead to the very low absorption coefficients (Fig. 5A and D). Figure

5 (E-G) shows the good agreement between the values of a_{ph} at 330 nm, a_{ph} at 440 nm and the Chl a concentration.

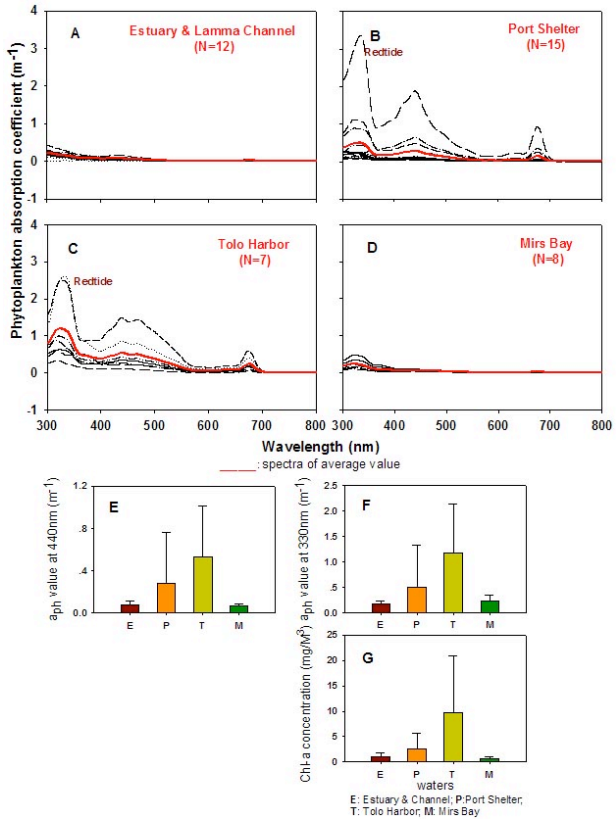

Figure 5. Comparison of a_{ph} spectra in four different waters.

The a_g coefficient is closely correlated to the CDOM concentration. The average a_g value at 400 nm indicated Tolo Harbor had highest average value of a_g, which means there were high concentrations of CDOM there (Fig. 6E). It is possible that the high abundance of CDOM could be due to the presence and activity of the phytoplankton, which has the potential for excreting CDOM into to seawater. Mirs bay has the lowest value of a_g (Fig. 6D and E).

Relationship Between Profile Reflectance and Chl a Concentration

It is difficult to find a correlation between L_{wn} or R_{rs} and Chl a in Case 2 waters compared to Case 1 waters. The optically complex components may result in low water transparency and complicated radiation signals. On the other hand, the optical signals of UV wavelengths may give us some ideas on how to monitor algal blooms with remote sensing. The first small graph in Figure 7 indicated the L_{wn} at 340 nm decreased rapidly when Chl a concentration increased, especially during the algal bloom. Other wavelengths did not have similar characteristics. The relationship between Chl a concentration and R_{rs} ratios (340:412 nm or 380:412 nm) was compared with the similar study by Mitchell [11]. He reported the decreasing trend of the R_{rs} ratio (380:412 nm) during red tide blooms. A similar conclusion can be found in our study. Figure 7 (A and B) shows R_{rs}(340 nm)/R_{rs}(412 nm) decreased greater than R_{rs}(380 nm)/R_{rs}(412 nm), the trend line of which is very flat. It suggested that UV radiation attenuation at 340 nm was higher than at 380 nm. The difference between our studies may be due to the particularity of phytoplankton species and/or other environmental factors.

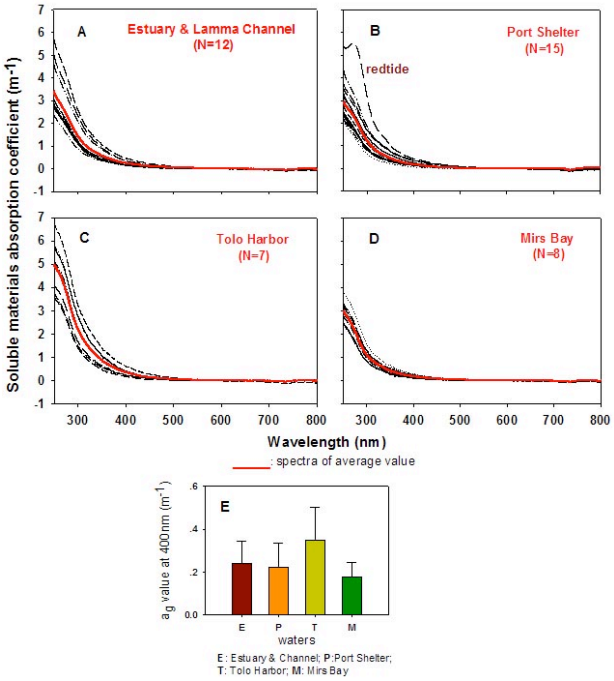

Figure 6. Comparison of a_g spectra in four different waters.

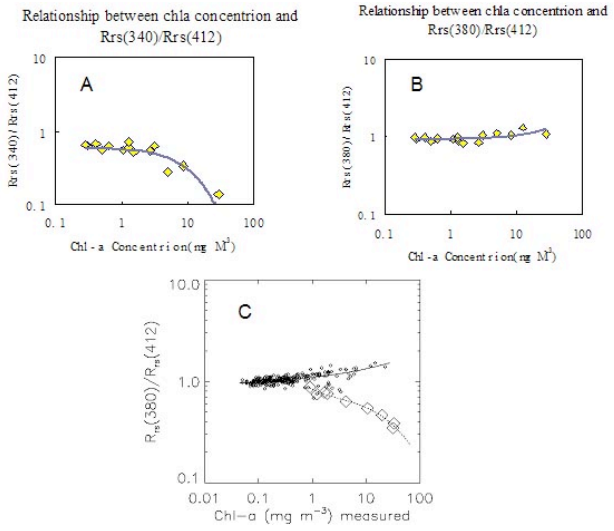

Figure 7. R_{rs} ratio versus Chl a considering UV wavelength (May cruise).

Kahru and Mitchell [27] presented the possibility of red tide remote detection by extending the study of absorption and reflectance into the UV region. Considering the absorption characteristics of phytoplankton, especially certain red tide causative species, and the UV light attenuation characteristics in

Case 2 waters, our study also offers the evidence of temporal and spatial changes in UV absorption characteristics in HK waters, especially during the period of algal blooms. The study results indicate the application feasibility of UV bio-optical properties for HAB monitoring in coastal waters.

CONCLUSION

There are spatial and temporal differences in the bio-optical characteristics of the four coastal waters in HK.

Phytoplankton abundance and the species composition varied in different waters. There was lower phytoplankton density in Estuary & Channel and Mirs bay. Abundance of phytoplankton was higher in April than in March. Inorganic suspended sediment was the major component contributing to the absorption characteristics in Estuary & Channel. The composition absorbing the light in Tolo Harbor was most complex, especially with respect to the high concentration of UV-absorbing substances.

Two algal blooms had high absorption properties in the UV region, especially from 310 to 340 nm. There is application feasibility for using the bio-optical properties in the UV region to monitor red tide in Case 2 waters.

Table 1. Key species of phytoplankton in Estuary & Channel (E), Port Shelter (P), Tolo Harbor (T), and Mirs Bay (M).

	Cruise and Waters	Mar.1-2		Mar.15-16		Apr.26-27		May 10-11	
	Key species	E	P	E	P	E	P	T	M
Diatoms	*Nitzschia longissima*		●		●				
	Nitzschia sp.	●		●		●			
	Pseudonitzschia delicatissima	●						●	●
	Pseudonitzschia pungens	●	●						●
	Skeletonema costatum	●		●				●	●
Dinoflagellates	*Ceratium furca*							●	
	Gymnodinium catenatum								●
	Prorocentrum.dentatum							●	
	Prorocentrum. triestinum				●	●	●		
	Scrippsiella trochoidea	●	●	●	●	●			
Others	*Chattonella ovata*						●		
	Heterosigma akashiwo					●	●		

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CHARACTERIZATION AND DETECTION OF HARMFUL BLOOMS OF *CHATTONELLA* (RAPHIDOPHYTES) IN THE EASTERN NORTH SEA

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ABSTRACT

Several massive blooms caused by potentially harmful raphidophytes, *Chattonella sp*. cells, occurred from the German Bight to north Skagen between late March and early May and have caused fish kills since 1998. The observations from microscopy and pigment profiles suggest that *Chattonella* sp. in the German Bight is different from the expected *Chattonella verruculosa*. Shape, size of cells and morphology are distinctively different. The cells of the observed *Chattonella* species from the North Sea are about 19–28 μm. The main difference from *Chattonella verruculosa* in the pigment profile is the occurrence of unidentified carotenoids in the profile of the North Sea species. The extent and intensity of the blooms in the sea surface could be observed by a series of remote sensing pictures in the near real-time situation. Though satellite images alone will not identify the specific presence of *Chattonella*, they can be used to track the blooms once an identification has been made. The reoccurrence of *Chattonella* blooms may indicate the response of algae to some kind of environmental change in the North Sea.

INTRODUCTION

The genera *Chattonella* and *Heterosigma* are phytoflagellates of the class Raphidophyceae to which several toxic species belong [1-3]. Toxic *Chattonella* species are well known from Japanese waters, where they have been observed since 1969 [4-6]. From European waters, *Chattonella* was described for the first time in the coastal waters off the Netherlands in 1991 [7]. Fish kills brought attention to the occurrence of the toxic species *Chattonella* in the Skagerrak area in 1998. Since then, high cell densities of this genus have been found in the northeastern waters of the North Sea almost every spring [8-9]. During the first days of May 1998, more than 400 tons of salmon from mariculture were killed at 9 salmon farms along the southwestern coast of Norway. The toxic phytoflagellate *Chattonella cf. verruculosa* Y. Hara et Chihara, which appeared in this area in relatively high cell numbers (0.5–6.8 million cells L^{-1}) was held responsible for the fish kills. In 1999 no toxic algal blooms were observed in the region of interest and relatively low cell numbers of raphidophytes (≤1000 cells L^{-1}) were recorded by the Swedish and German monitoring programs. In 2000, an algal bloom of raphidophytes was observed on satellite images and was verified from the German and the Swedish monitoring program, but no detrimental effects were reported. During the last days of March 2001, an extensive bloom of *Chattonella cf. verruculosa* and *Heterosigma cf. akashiwo* was responsible for the loss of 640 tons of cultivated salmon in the Old Hellesund (south Norway) [10]. Since cloud-free weather conditions persisted, the advection and/or progression of the bloom could be tracked on satellite images. This paper aims to provide some information obtained from observations by microscopy, pigment measurements and satellite remote sensing on the tiny *Chattonella* that newly appeared in the North Sea.

MATERIALS AND METHODS

Characterization of Chattonella *sp.*

Chattonella sp. was found in samples that were collected by the routine sampling by helicopter for the Early Algal Detection System of Schleswig-Holstein. The cell was isolated by serial dilution method [11] from a station that is located in the northwest of the sampling area (coordinates: 55°00.80 N, 08°07.00 E; date of sampling: 2 May 2000). The cells were maintained in f/2 as well as in Erdschreiber medium of salinity 30 psu. The light cycle was 12:12 hours. The strain of *Chattonella verruculosa* was cultured under the same condition as *Chattonella* species.

The high performance liquid chromatography (HPLC) analysis was carried out for cultured *Chattonella*. Extractions of pigments followed the method of Derenbach [12]. Analysis of pigments was performed with a reverse phase (rp) HPLC technique after the method from Barlow et al. [13].

Remote Detection

SeaWiFS (Sea-viewing Wide Field-of-view Sensor) data (1998) were obtained from the Dundee Receiving Station. Data processing was done by the Plymouth marine laboratory, applying the SeaWiFS Data Analysis System (SeaDAS) provided by NASA. High-resolution data (HRPT) recorded from the SeaWiFS receiving station from 1999–2001 were obtained from Goddard Space Flight Center (GSFC), Distributed Active Archive Center (DAAC). For handling of this Level 1A data as well as for visualization and analysis of the products, the software provided by NASA-SeaDAS v.4.0 (SeaWiFS Data Analysis Software) was used. The SeaWiFS Chl a data were calculated using the maximum band ratio (OC4) algorithm as described in O'Reilly et al. [14]. For visualization of the pigment maps, the SeaDAS implemented color table and geographical projections were used.

MOS-data were obtained from DLR-Institute for Space Sensor Technology Berlin. Chlorophyll data were calculated using algorithms developed at DLR based on the information from all 13 spectral bands of MOS using principal component analysis [15].

NOAA-AVHRR images of the eastern North Sea were used to conclude from sea surface temperature anomalies on the advection of water bodies, especially with respect to the Juetland current.

To ground truth satellite imagery, discrete water samples were also collected. Helicopter sampling of the surface water was done along the west coast of Schleswig-Holstein, Germany (Landesamt für Natur und Umwelt) to trace the toxic bloom. In 1998 Norwegian and Danish scientists investigated the distribution of *Chattonella. cf. verruculosa* on several transects in the Skagerrak and along the west coast of Jütland. At several stations Chl a measurements were made and hydrographic parameters were measured.

From 1999 to 2001 information of phytoplankton species distribution was collected from the Danish, Norwegian and Swedish monitoring programs (Internet pages) and from the German monitoring program along the German North Sea coast. The received data used included phytoplankton species distribution and Chl a concentrations.

RESULTS

Observation in the Light Microscope and Pigment Measurement



Figure 1. Living cells of *Chatonella* species.

Chattonella sp. (Fig. 1)

Status: living cells
Size: variable
Length: 12-23 µm
Shape: ellipsoid to obovoid, slightly flattened, with anterior depression, flagella emerge from the bottom of the depression, a lot of osmophilic particles present in peripheral cytoplasm
Chloroplasts: numerous, small, roundish
Flagella: two sub-equal, one is pulling the cell forward, pointed, slightly curved, in the swimming direction, the other one trailing, close to the cell body, sometimes hidden

These cells have mucocysts and display phototactic behavior. Live cells are very sensitive; they can be dead in a few minutes under light microscopy. They stop swimming and loose their flagella just before dying. Then the shape of the body changes immediately. Lugols-fixed cells usually loose their original cell-shape or break. However, chloroplasts are visible.

The pigment profiles of *Chattonella* sp. and *Chattonella verruculosa* show that they contain large amounts of 19'-butanoyloxyfucoxanthin. In addition to 19'-butanoyloxyfucoxanthin, the *Chattonella sp.* in the North Sea contains three unidentified carotenoids that *Chattonella verruculosa* does not have (Fig. 2).

The observations from microscopy and pigment profiles from HPLC suggest that *Chattonella sp*. in the German Bight is different from *Chattonella verruculosa*.

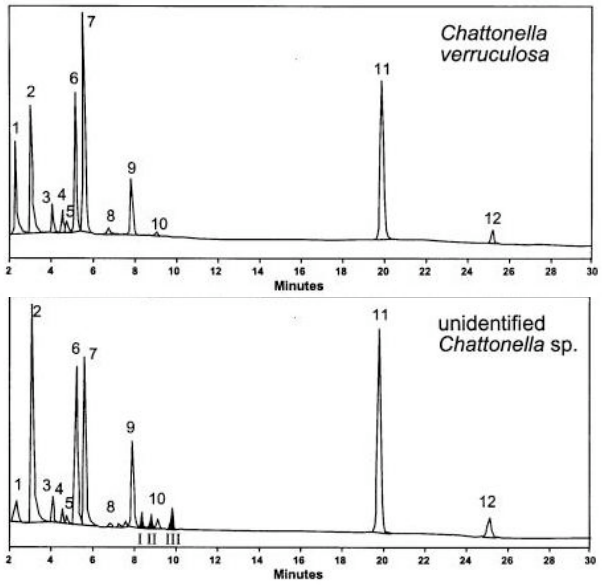

Figure 2. HPLC chromatograms of pigments from two Chatonella species: 1) Chl c3, 2) Chl c2, 3-5) derivatives of 19'-butanoyloxyfucoxanthin, 6) 19'-butanoyloxyfucoxanthin, 7) fucoxanthin, 8) violaxanthin, 9) diadinoxanthin, 10) unidentified carotenoid, 11) Chl a, and 12) beta-carotene.

Detection of the Bloom

In the visible spectrum, satellite images show high Chl concentrations in 1998, 2000 and 2001 (Figs. 3 and 4). In 1999 high Chl values were also observed in the same region on 27 and 29 April and to a lesser extend on 17 May (Fig. 4a). However, no ground truth data exist to confirm the raphidophyte phytoplankton species in 1999.

High numbers of *Chattonella* cells (up to10 million cells L^{-1}) were first observed in surface water samples north of Skagen on 19-23 April 1998 (Backe-Hansen 1999, personal communication). On 2 May the bloom of *Chattonella* cf. *verruculosa* was observed along the coast of southern Norway and the west coast of Jütland with cell numbers of 2-7 million cells L^{-1}. On 12 May lower cell numbers (1.6 million cells L^{-1}) were counted at a

station north west of Sylt Island, which was about the most southerly edge of the *Chattonella* bloom in that year. The peak of the bloom in the waters west of Jütland occurred on 15 and 16 May with cell numbers exceeding 24 million cells L^{-1} and Chl values >20 µg L^{-1} [8].

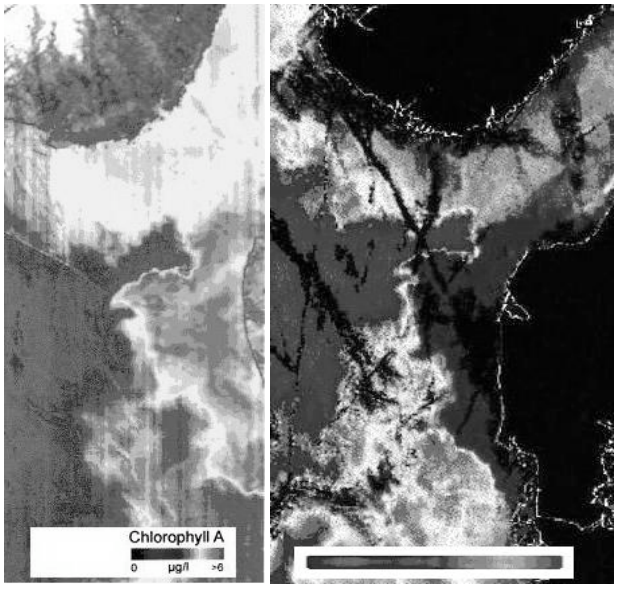

Figure 3. Extension of *Chattonella* bloom west of Jütland on 14 May (MOS) and 15 May (SeaWiFS) 1998. Chlorophyll data were obtained by applying different algorithms.

In April and May 1999, low numbers of Raphidophytes were recorded from the coastal waters of Germany, Sweden, Denmark and Norway [10]. However, satellite images of SeaWiFs exhibited distinct patterns of high Chl west of Jütland on cloud free scenes on 27 and 29 April as well as on 17 May. In the year 2000, high numbers of Raphidophytes were observed along the northwest coast of Jütland at the end of April and on 2 May. On 11 May, 1.7 million cells L^{-1} were counted off Flødevigen, south Norway (Algeinfo, 2 and 11 May 2000). Cell counting from helicopter sampling along the German North Sea coast showed 10 million cells L^{-1} on 2 May, northwest of the island of Sylt. The surface water samples contained as much as 80 µg L^{-1} of Chl a. From the Swedish and Norwegian coast no toxic effects of the algal bloom were reported.

In 2001, the bloom started at the end of March, considerably earlier than in the previous years. Data from the Norwegian and Swedish monitoring program showed increasing numbers of *Chattonella* as well as of *Heterosigma* in their samples from the Kattegatt and Skagerrak with time [10].

On 23 March, the loss of 650 tons of salmon was reported from Old Hellesund (South Norway) due to very high concentrations of toxic *Chattonella sp.* and/or *Heterosigma* species. On 29 March, 10 million cells L^{-1} were counted in samples from the Oslofjord area in the Skagerrak. Within the first days of April the cell numbers decreased rapidly. Only a few cells L^{-1} were detected in the samples in the German North Sea on 7 May.

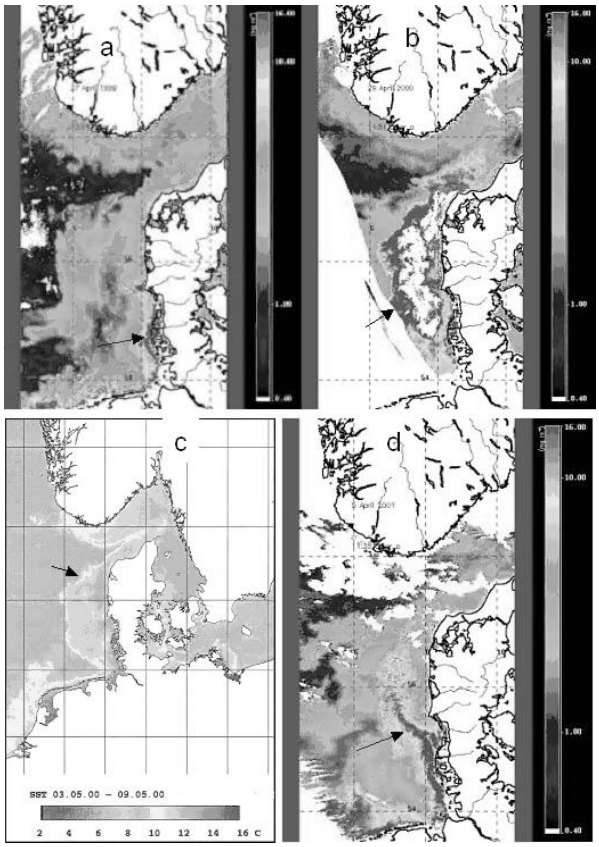

Figure 4. a) SeaWiFS image of 1999 showing higher Chl values in the German Bight; b) SeaWiFS images of 2001 showing higher Chl values in the German Bight when the *Chattonella* bloom was identified from ground truth data; c) summarized image of AVHRR – surface temperature image 3-9 May 2000, showing higher temperature in the west of Jütland and advective pattern in the area of the Jütland current (produced by BSH, Germany); and d) SeaWiFS image of 2001 showing higher Chl values in the German Bight.

We assume that the initial area of the bloom is located in the eastern North Sea, west of Jütland (Denmark) with its center at 56° N 8° E. Water masses in this area, highly affected by river water, are transported by the anti-cyclonic current of the North Sea. These waters are especially influenced by the River Elbe [16]. The Jütland current, accounting for water transport from west of Jütland towards the northeast, is the most important factor for advection of toxic algal blooms in the transitional area North Sea/Baltic Sea. When the water masses from the North Sea reach the entrance to the Kattegatt east of Skagen, the extensions of the Baltic current cause north to northwesterly advection of surface water masses towards the Oslofjord (Norway). Finally, water masses are advected with the Norwegian coastal current along the south coast of Norway. Depending on the prevailing wind conditions and the amount of in and outflow to/from the Baltic, the Jütland current as well as

the Baltic current system can be interrupted or change its directions. Hence, North Sea surface water can be advected into the Kattegatt.

DISCUSSION

Concerning the taxonomy of the toxic Raphidophyceae species some uncertainties still exist. The present working name that is used in the algal reports of the Scandinavian countries, is *Chattonella* c*f/aff. verruculosa*. However we recently found that there are a number of different characteristics, morphological aspects, as well as the pigment profiles that indicate one of the two *Chattonella* species from the North Sea is different from the species *Chattonella verruculosa* that is described from Japanese waters. We have found 3 additional accessory pigments, which were not present in the cultures from Japan.

The cell shape and size of the Japanese species is reported to be variable from ovoid to round with 12-45µm in length [2]. However, we found one species that looks like *Chattonella* cf. *verruculosa* with a round-like cell shape and a size of 10-15 µm. The other, dominant form/species of *Chattonella* was considerably larger (20–30 µm) compared to the *Chattonella cf. verruculosa* type in the North Sea, and it had some distinctive features. The cell is much more elongated with one truncated end at the swimming direction. The opposite end of the cell is more or less ovoid.

We have isolated and cultivated *Chattonella sp.* from the North Sea and have observed a much faster swimming velocity and a considerably higher cell division rate than the *Chattonella verruculosa* from Japan under comparable culture conditions. Further culture experiments showed, that the species avoids high light intensities (>250 µmoles m^{-2} s^{-1}). From this, we may conclude that on sunny days the highest *Chattonella* concentration may not be at the sea surface. Vertical distribution measurements in Danish waters have shown highest concentrations of *Chattonella sp*. at a depth of 5 meters.

Since *Chattonella* sp. seems associated with *Heterosigma akashiwo*, from which toxins have been well investigated, it is presently not clear, whether *Heterosigma* or *Chattonella* causes the death of the cultivated salmon along the Norwegian south coast. The killing mechanism of raphidophytes to fish is not quite clear yet. It maybe related with both physical clogging of fish gills and toxins. The toxicity of raphidophytes, though not yet fully investigated, seems to originate from the production of neurotoxins, as reported for *C. antiqua* and *C. marina* [17].

Tracking algal blooms using satellite remote sensing techniques require cloud-free weather situations. Before and during the blooming period of the raphidophytes in the North Sea, the weather was relatively calm and sunny. Possibly, this calm weather might stimulate the toxic raphidophyte bloom that may be similar to dinoflagellate blooms [18-19].

The temperature in the west of Jütland during these blooms is usually slightly higher (2-3°C) as in the adjacent areas. The satellite images of 1998, 2000 and 2001 show higher Chl concentrations in the west of Jütland and in the area where the Jütland current occurs. There is also evidence from the satellite images that higher Chl concentrations occur in the extensions of the Baltic current along the Swedish and Norwegian Skagerrak coast and also in the Norwegian coastal current. It cannot be clearly distinguished, weather the requirements for these harmful algal blooms (nutrients, temperature) are advected in the current system or the bloom itself. In order to make more specific statements about this, further *in-situ* data are needed.

The reoccurrence of the raphidophyte species within a certain time of the year during the periods of March, April and early May leads to the conclusion that this species has adapted to special environmental conditions, which predominate in the eastern North Sea after the early spring bloom in silica-depleted waters when phosphate and nitrogen compounds are still available in the surface water. Satellite remote sensing of water color will be an important and economically relevant tool for identifying areas of high Chl concentrations where *Chattonella* may be present and tracking this reappearing harmful algal bloom.

It is reported that the flux of nitrogen to the rivers of the North Sea has increased 10-fold since pre-industrial, pre-agricultural levels while **t**he history of *Chattonella* in the North Sea is not long. The appearance of these toxic raphidophytes has become questionable whether they belong to exotic or indigenous species in the Europen waters [20]. Because the cells are so small, fragile and difficult to preserve, they might be overlooked if cell density is low. On the other hand, *Chattonella* species was never found in such high cell concentrations and formed large blooms in this area before 1998. It seems that they have established their ecological niche and potentiality of recurrence to form spring blooms in the Eastern North Sea. It assumes that changes in nutrient inputs and composition may stimulate the development of some harmful species and change the algal composition [21]. Does this phenomenon indicate the response of algae to some kind of environmental change in the North Sea?

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EXTRACTING RED TIDE INFORMATION FROM HYPERSPECTRAL REMOTE SENSING DATA THROUGH SPECTRAL ANALYSIS

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ABSTRACT

As a new synthetical probing technology for more than 10 years, hyperspectral remote sensing has been successfully used in such fields as geological investigation and vegetation researching. However, in the area of red tide monitoring and forecast, its research and application lag far behind. Based on four groups of hyperspectral remote sensing data obtained from red tide and non-red tide areas (each area has two groups, respectively) in Bohai Sea on 1 & 15 July 2001. Using several red tide and non-red tide spectral curves this paper discusses three approaches of extracting red tide information: 1) spectral sharpness analysis (comparison of the sharpness of spectral curves), 2) spectral parameter analysis (determination and comparison of the spectral absorption characteristics parameters of the curves after being smoothed including the position, width, and symmetry), 3) spectral derivative analysis (use of the spectral derivative technique - get the 1st-order and 2nd order derivative spectral curves for preliminary analysis). The three approaches can extract red tide information effectively.

INTRODUCTION

As a combination of spectral and imaging technology, hyperspectral remote sensing is a young synthetic probing technology. When creating a 3-dimensional image for an object, it can have several, even hundreds, of pieces of information of each image point within a band range, presenting the spectral characteristics of the given object directly in its spectral image, making the determination of materials on the earth surface directly from space possible. Because of its high identification rate and its integration of multi-band images and spectra, hyperspectral remote sensing techniques are well developed and widely used in earth research. Up to now, it has been successfully used in such fields as geological investigation and vegetation research. However, in the area of red tide monitoring and forecasting, its application lags behind. How to take advantage of the hyperspectral remote sensing technique to improve red tide monitoring and forecast is an important branch of hyperspectral remoting sensing research.

Based on four groups of hyperspectral remote sensing data obtained from Bohai Sea red tide and non-red tide areas (each area has two groups, respectively) on 1 & 15 July 2001. This paper uses several red tide and non-red tide area spectral curves and discusses three approaches of extracting red tide information: 1) spectral sharpness analysis (comparison of the sharpness of spectral curves, 2) spectral parameter analysis (determination and comparison of the spectral absorption characteristics parameters of the curves after being smoothed including the positions, width, and symmetries), and 3) spectral derivative analysis (use of the spectral derivative technique - get 1st-order and 2nd-order derivative spectral curves for making preliminary analyses). We hope this paper can facilitate further research and application of hyperspectral remote sensing techniques in red tide monitoring and forecast.

DATA SOURCES, SELECTION, AND PREPROCESSING

Data Sources and Selection

On 1, 15 and 16 July 2001 (the 8th time zone), large patches of red tide waters were observed near Yingkou anchorage ground in Liaodong Bay. They were timely monitored and sensed from an airpcraft on 1 and 15 July by North China Sea Marine Forecasting Center of State Oceanic Administration using a PHI and hyperspectral remote sensing data of both red tide water and non-red tide water. Selecting among these data, this paper uses four groups of data (two groups each from red tide and non-red tide waters) from which spectral curves are extracted.

It can be seen from the original spectral curves of the image points that there exists false information at some bands, which should be defused before analysis. In order to avoid peculiarity of each image point and to eliminate noise and keep as many spectral absorption characteristics within each group of data as possible, we selected thirty typical image points and averaged the spectral data of the thirty points after error eliminating. Finally, within one group which was obtained from red tide water and from which we extracted a spectral curve, we obtained another red tide water spectral curve which represented a red tide area with different plankton density by the same way afore said.

Normalization of the Curves

Because the curves we obtained come from different data, and the data are obtained at different times and places with different sunlight conditions, their radiation values differ significantly. In order to compare these curves effectively, we normalized them using the normalization function as below,

$$R_s(\lambda) = \frac{\rho(\lambda) - \rho_{\min}}{\rho_{\max} - \rho_{\min}}$$

Applying this function to the five curves we get the curves demonstrated in Figures 1 through 5. Figures 1 and 2 are the normalized curves for non-red tide water, and Figures 3-5 are the normalized curves for red tide water. Figures 4 and 5 are extracted from one group of data, but Figure 4 represents a red tide area with higher plankton density compared to Figure 5.

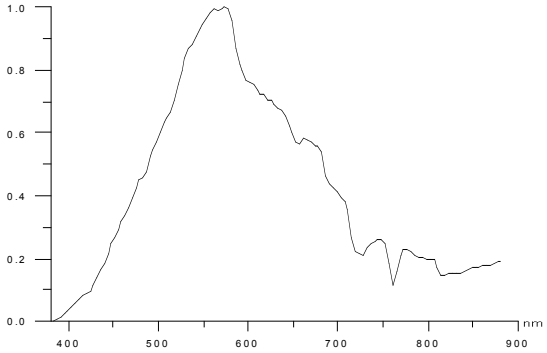

Figure 1. Non-red tide spectral curve-1.

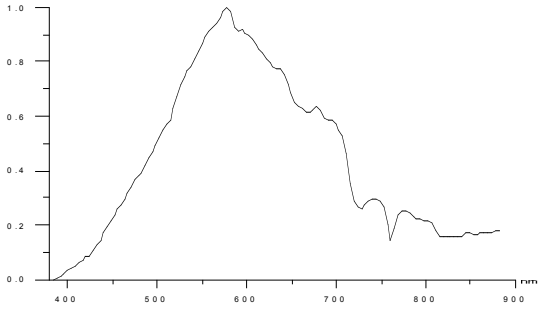

Figure 2. Non-redtide spectral curve-2.

SPECTRAL ANALYSIS

Preliminary Comparison of the Curves

It can be seen from the figures that, compared with non-red tide water curves, the red tide water spectral curves have their peaks' band position shifted. Table 1 contains the peak positions of the curves. The non-red tide spectral curves have their peaks at 573.8 nm and 577.8 nm, within yellow and green regions. But red tide water spectral curves shifted their peaks within band range 575–640 nm to band positions 617.7 nm, 598 nm and 577.8 nm, within orange region and red regions (the orange region being 585-620 nm; the red region 620-760 nm). It is remarkable that, although R3 has the same peak as S2, which is 577.8 nm (Fig. 5), the peak values remain high. The color difference between red tide water and non-red tide water is clearly observed in the curves.

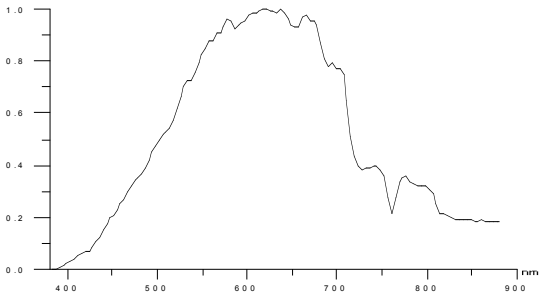

Figure 3. Red tide water spectral curve-1.

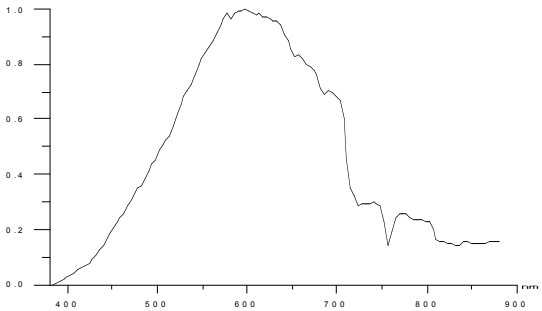

Figure 4. Red tide water spectral curve-2.

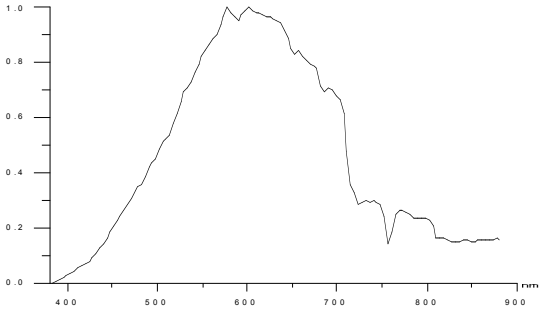

Figure 5. Red tide water spectral curve-3.

Table 1. Peak positions of the spectral curves.

	S1	S2	R1	R2	R3
nm	573.8	577.8	617.7	598	577.8

S symbols refer to non-red tide water spectral curves; R indicates red tide water curves.

Abstraction of Red Tide Information

Spectral Sharpness Analysis

We can see from the figures that red tide curves have quite different shapes than the non-red tide water curves. We used the spectral sharpness factor, originally used in wave analysis, to demonstrate the difference. To calculate the sharpness factor, we used the formula below:

$$SS = \lambda_P \rho(\lambda_P) / m_0$$

where m_0 symbols spectral zero order matrix and

$$m_0 = \int_0^{\infty} \rho(\lambda) d\lambda .$$

Using the normalized data, we determined the spectral sharpness factors of red tide water curves and non-red tide water curves and the results are shown in Table 2.

Table 2. Spectral sharpness comparison.

	S1	S2	R1	R2	R3
SS	2.71	2.64	2.46	2.60	2.52

S symbols refer to non-red tide water spectral curves; R indicates red tide water spectral curves.

We can see from the above analysis that the red tide water spectral curves have larger sharpness factors than those of non-red tide water spectral curves. By calculating spectral sharpness factors, red tides can be distinguished from non-red tide water.

Spectral Parameters Analysis

Spectral absorption characteristics parameters well reflect a probed object both by character and amount. A lot of information about red tides can be obtained from the absorption characteristics parameters of red tide water spectral curves. Turning to the normalized red tide spectral curves (R2, R3) with different plankton densities, we calculated the band positions of the absorption bands, the widths, and symmetries (Table 3).

Table 3 shows that widths and symmetries of the two curves differ only by a little (e.g., -1 nm and 0.06), but positions changed obviously by an amount of 20.2 nm. That is, with a higher plankton density, red tide spectral curves have their peaks shifted toward the red region, but their widths remain basically the same. Spectral absorption characteristics parameters show the difference between spectral curves with different plankton densities. We can take advantage of this to extract plankton density information from red tides.

Table 3. Spectral absorption characteristics parameters comparison.

	P (nm)	W (nm)	S
R2	598	206	0.43
R3	577.8	205	0.37
R2-R3	20.2	1	0.06

R2 symbols the spectral curve with higher plankton density; R3 indicates the spectral curve with lower plankton density.

Spectral Derivative Analysis

The spectral derivative technique is one of the most important techniques used to analyze hyperspectral remote sensing data. It can directly ascertain the positions of spectral bend points and the positions of radiation maximum and minimum values. This technique can be applied to the normalized red tide spectral curves with different plankton densities to get information about plankton density of red tides.

Using the following formulas, we get approximately the 1st-order and 2nd-order spectral derivative curves:

$$\rho'(\lambda_i) = [\rho(\lambda_{i+1}) - \rho(\lambda_{i-1})] / 2\Delta\lambda$$

and

$$\rho''(\lambda_i) = [\rho(\lambda_{i+1}) - 2\rho(\lambda_i) + \rho(\lambda_{i-1})] / \Delta\lambda^2$$

where λ_i symbols band width of each band, $\rho'(\lambda_i)$ and $\rho''(\lambda_i)$ symbols the 1st-order and 2nd-order derivative curves of λ_i ,and $\Delta\lambda$ is the distance between $\rho(\lambda_{i-1})$ and $\rho(\lambda_i)$.

Figures 6 and 7 present the 1st-order and 2nd-order spectral derivative curves of red tide water with different plankton density. We can see obvious vibrations at bend points in the two figures. Within the red tide plankton sensitive spectral region, the derivative curves with higher plankton density have a rather smaller vibration breadth compared with the curves with lower plankton density. But out of the region, two curves in each figure have a similar vibration breadth. We can use 1st-order and 2nd-order derivative curves in order to get information about the plankton density of red tides.

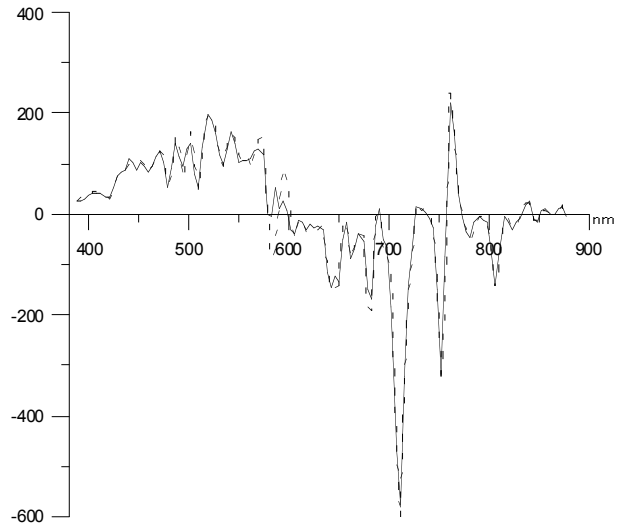

Figure 6. First-order spectral derivative curves (solid line indicates red tide water 2 and broken line indicates red tide water 3.

CONCLUSIONS AND DISCUSSIONS

Through the above analysis, we can say:

a) Compared with non-red tide water spectral curves, red tide spectral curves have the positions of the peak points shifted.

b) Spectral curves can show the color difference between red tide water and non-red tide waters.

c) Red tide spectral curves have a higher spectral sharpness value than that of non-red tide water. By calculating spectral sharpness factors, we can distinguish red tide areas from non-red tide areas.

d) Spectral absorption characteristics parameters discern the difference between red tide spectral curves with different plankton densities. Using this approach, we can extract information about the plankton density of a red tide.

e) 1st-order and 2nd-order derivative curves undergo obvious vibration at their bend points. Within the red tide plankton sensitive spectral region, the derivative curves with higher plankton density have a rather smaller vibration breadth compared with the curves with lower plankton density.

f) The three approaches do well in extracting red tide information.

Our analysis is based on the hyperspectral remote sensing data of two voyages. However, we need more data to test whether our conclusions are applicable to more red tide phenomena. At the same time, how to take full advantage of red tide information hidden in spectral curves to facilitate red tide monitoring and forecast needs further efforts.

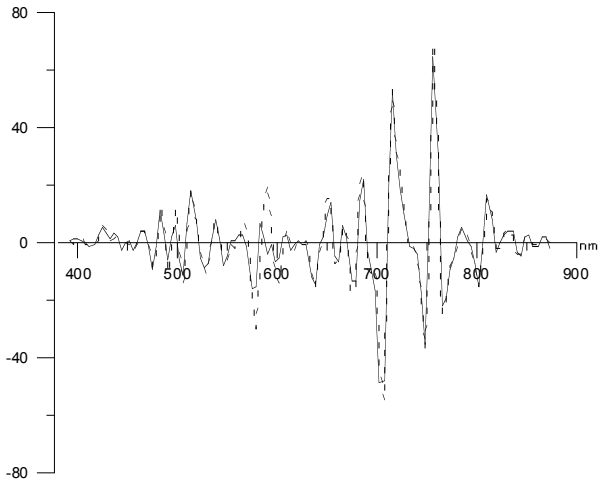

Figure 7. Second-order spectral derivative curves (solid line indicates red tide water 2 and broken line indicates red tide water 3).

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Asia-Pacific Economic Coooperation (Singapore): APEC Publication #204-MR-04.2

APPLICATION OF RADIANCE-REFLECTANCE TRANSFORMATIONS TO HARMFUL ALGAL BLOOM WATER

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ABSTRACT

In order to retrieve spectral features of harmful algal bloom water, it is necessary to correct for the variations in the measured radiance due to the solar output, atmosphere and sensor noise, in an attempt to retain variations in reflectance by the harmful algal bloom. In this paper, two methods of radiance-reflectance transformation, which are the empirical linear approach and transformation based on the data, are discussed and applied to the PHI (Pushbroom Hyperspectral Imager, a remote sensor on aircraft) hyperspectral data of the North Sea of China in 2001. As a result, the spectral features and spectral differences among normal seawater and different algae forming water are obtained, and specific characteristics may assist in the identification of these organisms.

INTRODUCTION

In recent years, the harmful algal bloom calamity has been becoming one of the most serious calamities of the marine environment. With high resolution and continuous spectra, hyperspectral data can be used to retrieve marine signals, including information about the various constituents present in the water. Actually, it can be an effective means for surveying harmful algal blooms. From July to August in 2001, during which period harmful algal blooms occurred frequently, PHI hyperspectral data were obtained from the North Sea of China by the remote sensing platform.

As is well known, different constituents have different spectral reflection features [1]. Therefore, once radiance measurements are converted to reflectance, differences in the spectral shape can be used to distinguish harmful algal blooms from "normal" seawater (the seawater which is not polluted by algae, oil and sewage, etc.). It is then possible to retrieve the optical characteristics of the components present in the seawater, which in certain cases may provide information about the phytoplankton causing the bloom. In this paper, the empirical linear approach and transformation based on the data are discussed and applied to the PHI hyperspectral data.

METHODOLOGY

From 7-19 August 2001, in Bayuquan of the North Sea of China, two kinds of red tide forming algae were cultured in two large-scale enclosed 3-m diameter equipments, which were placed in the seawater. In order to reduce the interference of the light reflecting off the equipment, the enclosed frames and floating balls were painted black, and the light-reflecting accessories were removed. We added nutrient salt and *Skeletonema costatum* dominated seawater into the enclosed water of one enclosed sack (sack A) and *Skeletonema costatum* was cultured. We added nutrient salt and local seawater into the other enclosed sack (sack B) and *Leptocylindrus danicus* was cultured.

At the same time, two sets of hyperspectral data of the two sacks and normal seawater were obtained respectively by ASD in the sea surface and by PHI on aircraft. ASD (Analytic Spectral Device) delivers data spaced about 3 nm apart in the spectral region from 350 to 1050 nm. Of the ASD data, the reflectance of the sample has a linear relationship with its radiance value [2, 3]:

$$D_\omega / D_b = R_\omega / R_b$$

where R_ω is the reflectance of the sample, D_ω is the radiance value of the sample, D*b* is the radiance value of the baffle-board, and R_b is the reflectance value of the baffle-board.

$$R_\omega = R_b * D_\omega / D_b$$

The reflectance of the sample is:

$$R_r = R_\omega \cdot A$$

where A is the reflectance coefficient of the baffle-board.

The remote sensing platform by the PHI provides 3-D hyperspectral data [1]: one dimension is the scanning direction, one dimension is the flying and the other is the continuous spectrum. The data are in 124 contiguous spectral channels spaced about 5 nm apart in the spectral region from 400 to 850 nm.

In order to obtain reflectance spectra, transformation must be done to convert the radiance measurements to reflectance. Two of the methods proposed for the transformation are the empirical linear approach and transformation based on the data [4, 5].

Empirical Linear Approach

This approach is useful when atmospheric parameters are not available. It transforms the radiance spectra measured by a remote sensing platform into reflectance. It requires that the reflectance spectra of field samples from a few sites in the scene be measured strictly and synchronously on the ground. It also requires that the field samples be physically flat, the reflectance

be different, and the scanning angle be small. Otherwise, errors may be introduced.

It is assumed that the radiance value of a sample has a linear relationship with its reflectance value [5]:

$$DN = k \bullet R + b$$

where DN is the radiance value of the sample, R is the reflectance of the sample, and k and b are the coefficients of the linear equation. For each spectrum band i:

$$k = \frac{N\sum(DN_iR_i) - \sum R_i \sum DN_i}{N\sum R_i^2 - (\sum R_i)^2}$$

$$b = \frac{\sum(DN_iR_i) - K\sum R_i^2}{\sum R_i}$$

Transformation Based on the Data of the Scene

These kinds of methods are based entirely on the actual data collected. They do not require any external measurements of the atmospheric conditions [5, 6].

With the IARR (Internal Average Relative Reflectance) approach, the radiance values in each band are divided by the geometric mean taken across the spectrum of sample.

For the FFR (Flat Field Correction), the image must contain an area that is known to be spectrally and physically flat. The radiance values in each band of the entire image are divided by the average of the radiance value over this flat area. Thus, it is expected that this accounts for the effects of the atmosphere and solar contributions [5].

The LRC (Log Residual Correction) adjustment may suppress atmospheric effects and the topology effects. The log residual is computed by the following formula:

$$Y_{ik} = \frac{X_{ik}X..}{X_i.\ X.k}$$

where Y_{ik} is the log residual value of sample i and spectral band k, X_{ik} is the raw radiance data measured by the sensor, $X_i.$ is the geometric mean taken across the spectrum of sample i, $X.k$ is the geometric mean of spectral band i taken across all samples, and X.. is the geometric mean taken across all samples and all spectral bands. In order to avoid overflow problems, logarithms are used to compute the geometric means which gives rise to the name of the method:

$$\log(Y_{ik}) = \log(X_{ik}) - \log(X_i.) + \log(X..) - \log(X.k).$$

EXPERIMENTAL RESULTS

Empirical Linear Approach

Figure 1 shows the different spectral features of the samples measured by ASD in the sea surface, including normal seawater, enclosed experimental sacks A and B. Sacks A and B represent two different kinds of red tide biological species - *Skeletonema costatum* and *Leptocylindrus danicus*, respectively, which were cultured by artificial technique.

In Figure 1, it can be seen that the spectral features between red tide water and normal seawater are different and the differences between the two species are obvious. The three kinds of water all have reflecting peaks near 580 nm, the reflectance value of normal seawater is bigger than that of the water of sack A and B. The reflectance value of the water of sack A is bigger than that of sack B. From 690 to 710 nm, the two red tide waters have the second reflecting peaks while normal seawater does not, and the reflectance value of the water of sack A is bigger than that of sack B. Additionally, the water of sack A and B have absorption characteristics in their reflectance spectra near 670 nm.

Figure 2 shows the radiance spectral curves as measured of the same samples by the remote sensing platform.

With the formula described in the empirical linear approach, the coefficients k and b can be solved to provide the best fit in the least-squares sense of the radiance values to the corresponding reflectance values (Fig. 3).

Figure 3 shows the absorption feature of the two algal species forming the red tides near 670 nm and the reflection feature from 690 to 710 nm. They (the red tide spectra) are more similar as shown in Figure 2. Unfortunately, the effect of the transformation is not satisfying from 400 to 600 nm, and the reason for the distortion can be explained by Figure 4. This shows the relationship between in site reflectance and the reflectance measured with the remote sensing platform and transformed using the empirical linear approach. It appears that the two are not correlated between 400-600 nm; however, there is close agreement from 600 to 800 nm.

Methods Based on the Actual Data

The results generated from these kinds of methods are not the reflectance of actually meaning. But they also show some absorption and reflection features of red tide.

Figure 5 shows the log residual spectra derived from the same radiance spectra shown in Figure 2. Though the reflecting peak from 690 to 710 nm is very obvious, the first reflecting peak nears 580 nm and the first feature indicative of absorption at 670 nm disappeared.

Figure 6 shows the internal average correction spectra and Figure 7 shows the flat-field correction spectra. Of the three transformation methods applied to the remote sensing data, the flat-field correction method may be more suitable for analyzing red tide than the other two methods. By using the flat-field correction method, normal seawater can be selected as the average so its curve can nearly reach a straight line while the curves of red tide water can be very different from it.

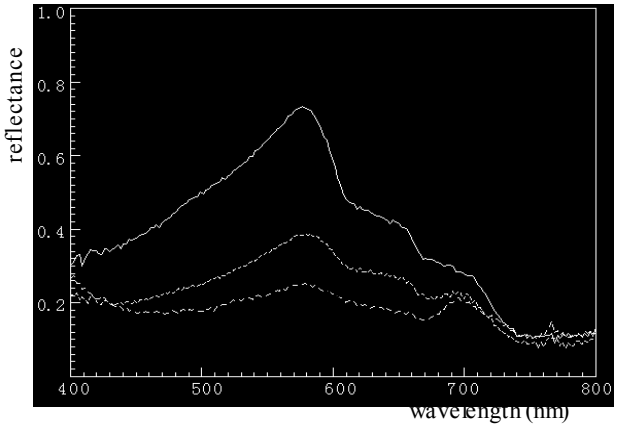

Fig. 1. The reflectance measured in the sea. surface

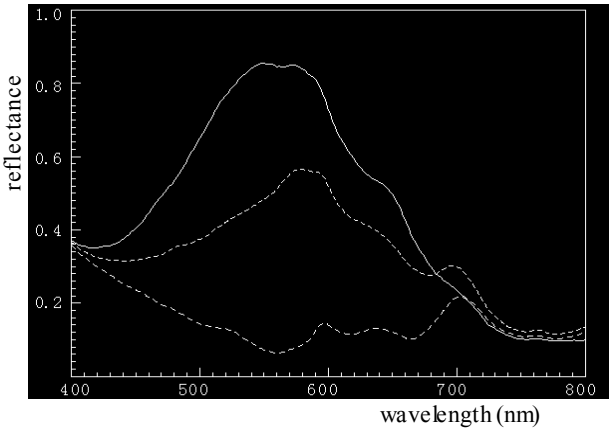

Fig. 3. The reflectance caculated by the Empirical Linear Approach.

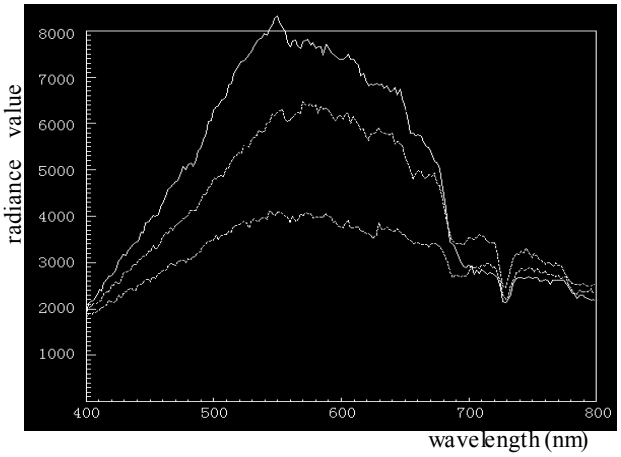

Fig. 2. The radiance of the raw data measured by the remote sensing platform.

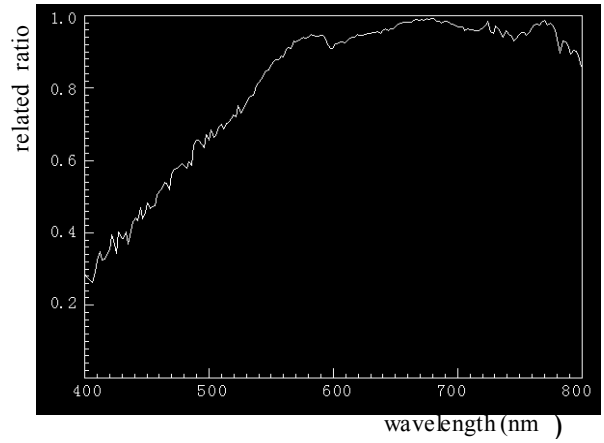

Fig. 4. The relationship between the data measured in the sea surface and the data measured by PHI.

Analyzing the Harmful Algal Bloom Characteristic Spectra

From the data shown above, the red tide reflectance spectra are different from that of normal seawater. As shown in Figures 1, 3, 5 and 6, the following conclusions can be made:

a) Red tide seawater and normal seawater have different spectral features.
b) The spectral features between different red tide biological species are different.
c) Red tide water has two reflecting peaks, one is near 580 nm and the other from 690 to 710 nm. Its absorption feature is near 670 nm.
d) The characteristic spectra of red tide water is the reflecting peak which is the local maximum from 690 to 710 nm can be used to distinguish algal forming water and normal seawater. Following are two examples for distinguishing.

On 8 July 2001, the seawater near Yingkou harbor turned yellow and on 12 August 2001, the seawater near Bayuquan turned brown. Figure 8 shows the reflectance cspectral features of the two kinds of algal water compared with that of normal seawater by applying the Empirical Linear Approach to PHI data. In Figure 8, no characteristic spectra of the yellow water can be found from 690 to 710 nm, so the discoloration was not caused by the harmful algae. Actually it was found that this was caused by floating sand. But the brown water has a reflecting peak value which is much smaller than that of normal seawater near 570 nm and a second reflecting peak form 670 to 710 nm which can be confirmed that the discoloration was caused by algae. Indeed, there was an algal bloom that took place in that area reported in the sea surface.

CONCLUSIONS

With high resolution and spectrally continuous data, the hyperspectral reflectance measurements are very convenient for harmful algal bloom analysis. This work represents our initial attempts to use remote sensing reflectance to analyze harmful algal blooms and current research is underway to improve our ability to detect, monitor, and forecast harmful algal blooms.

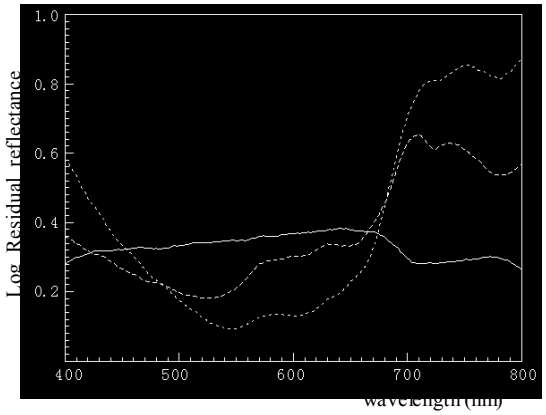

Fig. 5. Log Residual reflectance Spectra.

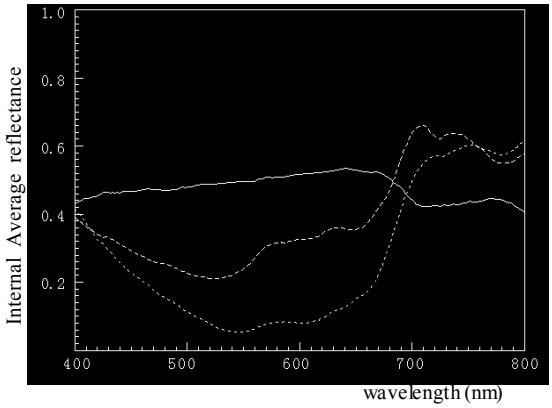

Fig. 6. Internal Average relative reflectance Spectra.

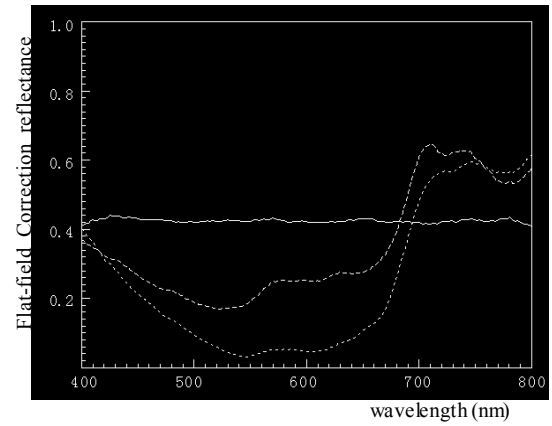

Fig. 7. Flat-field Correction Spectra.

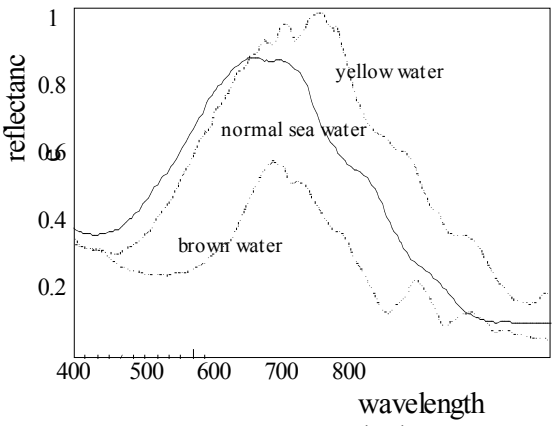

Fig.8. The reflectance of yellow water, brown water and normal sea water.

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