Monitoring and Management Strategies for Harmful Algal Blooms in Coastal Waters

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This report to be cited as:

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LIST OF ACRONYMS

ADEC   Alaska Department of Environmental Conservation
AFCD   Agriculture and Fisheries Conservation Department (Hong Kong)
AOAC   Association of Official Analytical Chemists
APEC   Asia Pacific Economic Cooperation Program
APHA   American Public Health Association
ASP   amnesic shellfish poisoning
ATS   Aquaculture Technology Section
AVIRIS   Airborne Visible/Infrared Imaging Spectrometer
AZA   azaspiracid
AZP   azaspiracid shellfish poisoning
CDOM   colored dissolved organic matter
CEC   Commission of European Communities
CFIA   Canadian Food Inspection Agency
CFP   ciguatera fish poisoning
COIS   Coastal Ocean Imaging Spectrometer
CSSP   Canadian Shellfish Sanitation Program
CTD   conductivity, temperature, depth
CTX   ciguatoxin
CV   coefficient of variation
CWC   Chemical Weapons Convention
CZCS   Coastal Zone Color Scanner
DA   domoic acid
DEC   Department of Environmental Conservation (Alaska, US)
DFO   Department of Fisheries and Oceans (Canada)
DMR   Department of Marine Resources (Maine, US)
DO   dissolved oxygen
DOC   dissolved organic carbon
DON   dissolved organic nitrogen
DOP   dissolved organic phosphorus
DSP   diarrhetic shellfish poisoning
DTX   dinophysistoxin
ELISA   enzyme-linked immunosorbent assay
EC   Environment Canada
ECOHAB   Ecology and Oceanography of Harmful Algal Blooms (US)
EEC   European Economic Community
EPA   Environmental Protection Agency (US)
EU   European Union
FAQ   frequently asked questions
FCZ   fish culture zone
FDA   Food and Drug Administration (US)
FISH   fluorescent *in situ* hybridization
GEOHAB   Global Ecology and Oceanography of Harmful Algal Blooms
GIS   geographic information system
GOOS   Coastal Global Ocean Observing System
GTX   gonyautoxin
HAB   harmful algal bloom
HACCP   Hazard Analysis Critical Point Control Program (US)
HFS   hydrodynamic filtration system
HMA   heteroduplex mobility assay

HPLC	high performance liquid chromatography
IANZ	International Accreditation New Zealand
IFREMER	Institut Français pour la Recherche et l'Exploitation de la Mer
IMO	International Maritime Organization
IOC	International Oceanographic Commission
ISSC	Interstate Shellfish Sanitation Conference (US)
IUPAC	International Union for Pure and Applied Chemistry
LBA	light-beam attenuation
LC	liquid chromatography
LDPE	low-density polyethylene
MAb	monoclonal antibody
MAF	Ministry of Agriculture and Forestry (New Zealand)
MBMB	Marine Biotoxin Management Board (New Zealand)
MoH	Ministry of Health (New Zealand)
MOU	Memorandum of Understanding
MS	mass spectrometry
MU	mouse units
NEO	neosaxitoxin
NERI	National Environmental Research Institute (Denmark)
NMFS	National Marine Fisheries Service (US)
NRC	National Research Council (Canada)
NSP	neurotoxic shellfish poisoning
NSSP	National Shellfish Sanitation Program (US)
OA	okadaic acid
OBS	optical backscatter
ORHAB	Olympic Region Harmful Algal Blooms
PAb	polyclonal antibody
PAC	polyhydroxy aluminum chloride
PbTx	brevetoxin
PCR	polymerase chain reaction
PIC	Panchromatic Imaging Camera
PSP	paralytic shellfish poisoning
PUFA	polyunsaturated fatty acid
PTX	pectenotoxin
QMPI	Quality Management Program for Importers (Canada)
REOS	Remote Electro-Optical Sensor
REPHY	French Phytoplankton Monitoring Network
RIA	radioimmunoassay
RL	regulatory level
RT	red tide
RT/HAB	red tide/HAB
SAX	strong anion exchange
SCOR	The Scientific Committee on Oceanic Research
SDE	sediment oxygen demand
SeaWIFS	Sea-viewing Wide Field-of-view Sensor
SPIA	solid phase immunobed assay
SQAPDC	Shellfish Quality Assurance Program Delivery Center (New Zealand)
SST	sea surface temperature
STX	saxitoxin
TOC	total organic carbon
TSP	toxic shellfish poisoning
UK	United Kingdom

US	United States
WHO	World Health Organization
WWW	World Wide Web
YTX	yessotoxin

ACKNOWLEDGMENTS

PREFACE

In early 1998, a toxic red tide devastated the fish farming industry in Hong Kong. In response to the attention given to this event by the general public and the fisheries industry, the government commissioned a study of the existing red tide monitoring management program in Hong Kong, which included recommendations on ways to update and change it. One element of that study involved a compilation of existing monitoring, management, and mitigation technologies used on red tides and harmful algal blooms throughout the world. The resulting report: Technical Report No. 2: Red Tide Monitoring and Management Strategies (Anderson et al., 1999) was only available to officials within the Hong Kong government. The authors felt that the information that had been compiled would be of interest and use to many others faced with harmful algal bloom problems globally. Accordingly, upon the authors' request, the Hong Kong Agriculture and Fisheries Conservation Department kindly granted permission to publish an edited version of this report. Specific references to Hong Kong were removed at the AFCD's request to make the report more useful globally. The editing and printing of the report were facilitated by the *Project on Management of Red Tides and Harmful Algal Blooms* of the Asia Pacific Economic Cooperation Program (APEC) Marine Resource Conservation Working Group. The distribution and mailing of the edited report outside of the APEC region was supported by the Harmful Algal Bloom Program of the Intergovernmental Oceanographic Commission (IOC) of UNESCO.

Several issues should be noted with respect to the information compiled in this report. These relate mostly to rapid changes in technology and policy as well as the occurrence of new toxic events since the report was initially written in early 1999. First, prices for the various instruments described here are estimates made in 1999 and many new products or pricings are not listed. It was simply not possible to update many of the figures and product descriptions in this edited report given the limited resources available for the editing and publication process. Second, web site addresses are provided throughout the text, though the addresses or sites are expected to change or to become inactive through time. Readers are encouraged to use an appropriate search engine to find equivalent sites if the addresses given here are no longer valid. Third, species names have not been changed to reflect a very recent publication (Daugbjerg et al., 2000) that proposed sweeping changes to the genera *Gymnodinium* and *Gyrodinium*. Finally, we emphasize that although this is an extensive compilation of monitoring and management methods, it is far from complete or even up-to-date. Specific national monitoring programs were selected as case studies, but many countries with excellent programs are not included. We apologize to individuals, companies, programs or countries who are not mentioned or whose recent studies, results, or policy changes are not included. We hope all realize that our objective is to provide a broad but necessarily incomplete overview of the many different technologies, methods and approaches that were being used in early 1999 to monitor and manage HABs in coastal waters worldwide.

1 INTRODUCTION

The world's oceans teem with countless single-celled algae called phytoplankton. Among the thousands of living species are a few dozen that produce potent neurotoxins or that cause harm in other ways. Impacts from "blooms" or "red tides" of these tiny organisms are many and diverse, ranging from the death or illness of humans, whales, manatees, or other marine animals to discoloration of the water and fouling of beaches with foam and dead fish. Ecosystem effects also occur, as toxins are transferred through the food chain, affecting larval as well as adult forms of many marine organisms.

In some areas the term "red tide" is used to describe all phenomena in which the water is discolored by high algal biomass. This term is potentially misleading, however, because it includes many blooms which discolor the water but cause no harm, and ignores blooms of highly toxic cells, which cause problems at very low (and essentially invisible), cell densities. As a result, many bloom events which are harmless end up having negative impacts because skittish consumers avoid purchasing or eating seafood which is perfectly safe, or tourists and residents avoid using beaches because of a mistaken concern over swimming safety. Because of this confusion, the term harmful algal bloom (HAB) is now used by scientists and government officials in most countries to describe the subset of these phenomena which are toxic or cause harm. **In this report, the term "harmful algal bloom" or HAB will be used in its most general or inclusive sense – it will refer to blooms of toxic and non-toxic algae which discolor the water, as well as to blooms which are not sufficiently dense to change water color but which are dangerous because of the algal toxins they contain or the physical damage they cause to other biota.** We do recommend that an effort be made to alter the usage of "red tide" in those countries that still use this term. It should be possible to decrease negative impacts by ensuring that negative public reactions are confined to only those events that are toxic or potentially harmful.

HABs are truly global phenomena, and evidence is mounting that the nature and extent of the problem has been expanding over the last several decades (Anderson 1989; Smayda 1989). Formerly only a few regions were affected in scattered locations, but now virtually every coastal country is threatened, in many cases over large geographic areas and by more than one harmful or toxic species. It is still a matter of debate as to the causes behind this expansion, with possible explanations ranging from natural mechanisms of species dispersal to a host of human-related activities such as nutrient enrichment, climatic shifts, or transport of algal species via ship ballast water. Whatever the reasons, we are now subject to a bewildering array of toxic or harmful species and impacts, and are faced with disturbing trends of increasing incidence throughout the world.

Given this significant problem and an increasing reliance on the coastal zone for habitation, food, recreation, commerce, and even waste disposal, how can we achieve the balance between these needs and the effect they may have on nearshore waters, and in particular, on coastal ecosystems? What actions are necessary to manage resources affected by HABs, and what research is needed to provide the scientific basis for policy decisions? Perhaps most importantly, can anything be done to reverse the trend in bloom incidence – will improvements in coastal water quality lead to fewer or smaller blooms of toxic species or can strategies be employed to directly intervene in the bloom process, to destroy the bloom organisms? These are important questions, made all the more compelling by the expansion of the problem through time.

1.1 HAB Impacts

HAB phenomena take a variety of forms and have multiple impacts. One major category of impact occurs when toxic phytoplankton are filtered from the water as food by shellfish such as clams, mussels, oysters, or scallops, which pump large volumes of water and hence can rapidly accumulate the algal toxins to

levels which can be lethal to humans or other consumers (reviewed in Shumway 1990). These poisoning syndromes have been given the names paralytic, diarrhetic, neurotoxic, amnesic and azaspiracid shellfish poisoning (PSP, DSP, NSP, ASP and AZP respectively; Table 1.1). Carnivorous gastropods (snails, whelks), either predatory species (e.g. *Nassarius succinctus* and *Babylonia areolata*) which consume live bivalve prey, or scavengers which consume dead bivalve prey, can also act as important vectors of PSP toxins (Shumway et al. 1995). For example, the large snail, *N. succinctus*, is a popular food item in some Asian countries, and has been frequently implicated as a vector of PSP in mainland China (Chen and Gu 1993, Qiu 1990 in Lin et al. 1993). These two main vectors for the food web transfer of PSP toxins to humans are illustrated in Fig. 1.1. A sixth human illness, ciguatera fish poisoning (CFP) is caused by biotoxins produced by dinoflagellates attached to surfaces in many coral reef communities (reviewed in Anderson and Lobel 1987). Ciguatera toxins are transferred through the food chain from herbivorous reef fishes to larger carnivorous, commercially valuable finfish. In a similar manner, the viscera of other important fish such as herring or sardines can contain PSP toxins, endangering human health following consumption of whole fish. Whales, dolphins, seabirds, and other animals can be victims as well, receiving toxins through the food chain via contaminated zooplankton or fish (e.g., Geraci et al. 1989).

Another type of HAB impact occurs when marine fauna are killed by algal species that produce exogenous toxins associated with the cell surface, release toxins and other compounds into the water, or that kill without toxins by physically damaging gills, by creating low oxygen conditions as bloom biomass decays or by causing light attenuation as thus affecting submerged aquatic vegetation. Some algae (including but not restricted to those that produce chemically well-characterized toxins known to affect humans), can adversely affect growth and survival of larvae or adults of commercially important shellfish populations. For example, red tides of the dinoflagellate *Heterocapsa circularisquama* in Japan are not a public health concern and do not appear to affect finfish, but have caused mass mortalities of valuable cultured pearl oysters (*Pinctada fucata*) as well as edible bivalves including Pacific oysters (*Crassostrea gigas*), clams (*Tapes philippinarum*) and mussels (*Mytilus galloprovincialis*) (Matsuyama et al. 1996). Similarly, brown tides of the picoplanktonic alga *Aureococcus anophagefferens* (Pelagophycea) have caused mass mortalities (not linked to hypoxia) of mussels, and devastated bay scallop fisheries in the mid-Atlantic USA, but are not known to affect finfish or humans (Bricelj and Lonsdale 1997). Finally, some algal toxins that are of human health concern also have direct negative effects on shellfish populations. For example, PSP toxins produced by *Alexandrium* spp. also were shown in laboratory studies to cause burrowing and feeding incapacitation, and even mortalities of softshell clams, *Mya arenaria* (MacQuarrie and Bricelj 2000). Farmed fish mortalities from HABs have increased considerably in recent years, and are now a major concern to fish farmers and their insurance companies. The list of finfish, shellfish and wildlife affected by algal toxins is long and diverse (Anderson 1995) and accentuates the magnitude and complexity of the red tide phenomena. In some ways, however, this list does not adequately document the scale of red tide effects, as adverse impacts can occur throughout coastal ecosystems in subtle ways that are difficult to detect. In virtually all trophic compartments of the marine food web, there can be impacts from toxic or harmful blooms.

Finally, economic impacts can also result from the so-called "halo effect", or avoidance of safe, uncontaminated seafood because of mistaken public perceptions that the red tide has affected all fish and shellfish and that toxins that kill these organisms are retained within their tissues. Management strategies must address this public overreaction and devise strategies (e.g. via public education) to reduce these impacts.

TABLE 1.1 Human illnesses associated with harmful algal blooms. (Modified from Morris 1999.)

Syndrome	Causative organisms	Toxins produced	Route of acquisition	Clinical manifestations
Ciguatera fish poisoning (CFP)	*Gambierdiscus toxicus* (benthic) and others	Ciguatoxins	Toxin transfer up the marine food chain; illness generally results from eating large, carnivorous reef fish	Acute gastroenteritis, paresthesias and other neurological symptoms
Paralytic shellfish poisoning (PSP)	*Alexandrium* spp, *Gymnodinium catenatum, Pyrodinium bahamense* var. *compressum* and others	Saxitoxin family	Eating shellfish harvested from affected areas	Acute paresthesias and other neurological manifestations; may progress rapidly to respiratory distress, muscular paralysis and death
Neurotoxic shellfish poisoning (NSP)	*Gymnodinium breve, G. brevisulcatum* and others	Brevetoxins	Eating shellfish harvested from affected areas; toxins may be aerosolized by wave action	Gastrointestinal and neurological symptoms; respiratory and eye irritation with aerosols
Diarrhetic shellfish poisoning (DSP)	*Dinophysis* spp.	Okadaic acid and dinophysistoxins (DTXs)	Eating shellfish harvested from affected areas	Acute gastroenteritis
Azaspiracid shellfish poisoning (AZP)	*Protoperidinium crassipes*	Azaspiracids	Eating shellfish harvested from affected areas	Neurotoxic effects with severe damage to the intestine, spleen, and liver tissues in animal tests
Amnesic shellfish poisoning (ASP)	*Pseudo-nitzchia* spp.	Domoic acid and isomers	Eating shellfish (or, possibly, fish) harvested from affected areas	Gastroenteritis, neurological manifestations, leading in severe cases to amnesia (permanent short-term memory loss), coma, and death
Possible estuary-associated syndrome	*Pfiesteria piscicida* and other *Pfiesteria* spp.	Unidentified	Exposure to water or aerosols containing toxins	Deficiencies in learning and memory; acute respiratory and eye irritation, acute confusional syndrome
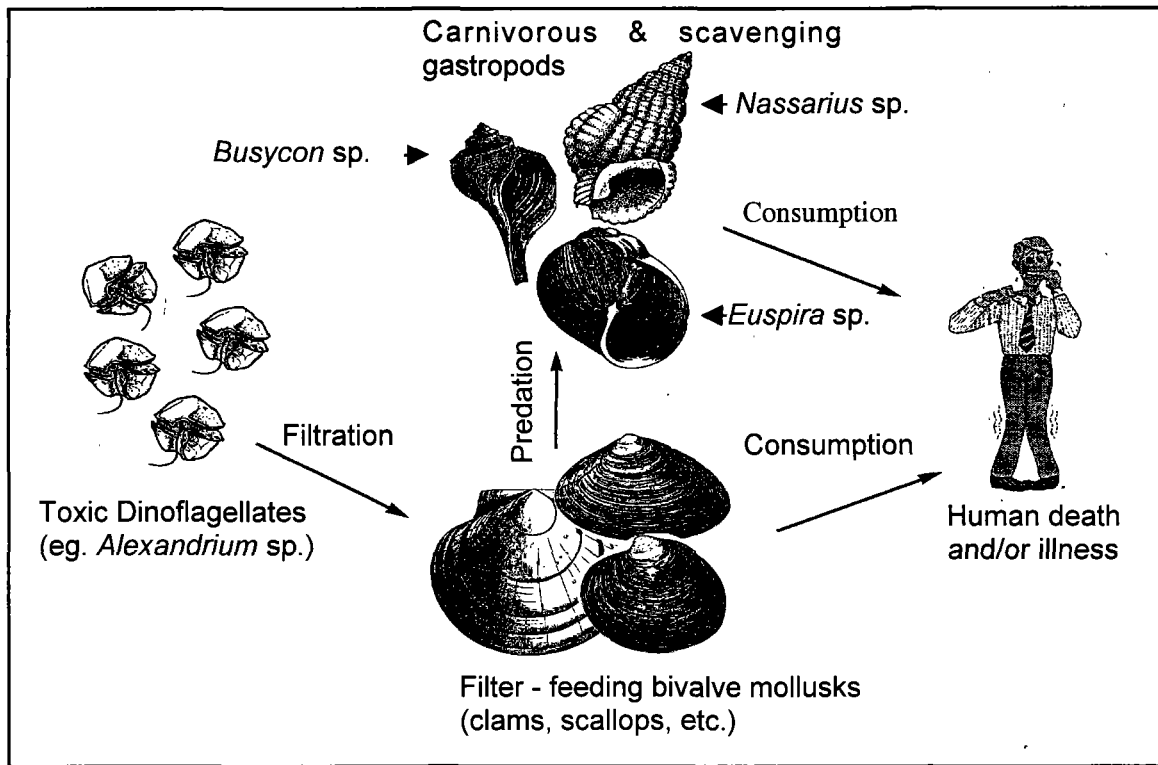
FIGURE 1.1. Generalized pathways of human intoxication with molluscan shellfish toxins via filter-feeding bivalves and carnivorous and scavenging gastropods. (Modified from Shumway et al. 1995.)

2 BASIC COMPONENTS OF HAB MANAGEMENT SYSTEMS

2.1 General issues

The goal of all HAB research and monitoring efforts is to protect public health, fisheries resources, ecosystem structure and function, and coastal aesthetics. This requires an understanding of the many factors that regulate the dynamics of HABs and the manner in which they cause harm, but by itself, that knowledge does not provide protection. Management and mitigation strategies of many different types are needed. An effective management system for HABs therefore must have a variety of elements. At the core of those programs are the monitoring programs needed to detect cells or toxins sufficiently early to take management actions. Those management actions should be clearly defined for each of the many different types of HAB impacts (e.g., shellfish toxicity, fish mortalities).

The following section provides background on basic or generic components of HAB monitoring programs. More detail will be provided in Section 4 in the form of case studies highlighting specific programs in a number of different countries.

The design elements of HAB monitoring programs must reflect the goals of those programs, the facilities and resources available, and the specific demands of the end-users of the data, as well as the rules and regulations imposed by the responsible national or regional authorities. Monitoring programs must be adapted to local conditions and circumstances, and wherever possible, should be interfaced with other monitoring efforts, such as those for general water quality, taking into account the physical and biological regime, available technology, expertise and competence of the staff to carry out the monitoring and management procedure, as well as local administrative tradition (Andersen 1996).

2.2 Basic elements

The basic or generic elements of a HAB monitoring program (Figure 2.1) are:
- Environmental observations including plankton observations, fish kills and anomalous animal behavior
- Sampling of plankton, shellfish or fish
- Analysis of the samples (identification of harmful algae, quantification of harmful algae, measuring toxicity in shellfish or fish)
- Evaluation of results
- Dissemination of information and implementation of regulatory action
- Action plans /Mitigation measures

The structure of a monitoring program can be complex depending upon the number of institutions involved in the procedures at each level in the network. Some involve a single agency that collects the samples and analyzes them for toxins. Others split the responsibilities, sometimes with private industry or user groups. For example, in Denmark the sampling of algae and mussels is carried out by the fishermen, but the analysis of those samples is conducted by private consultancy companies. Those companies report to the Ministry of Fisheries, which is ultimately responsible for management decisions.

The structure of the HAB monitoring program must be kept as simple as possible to facilitate fast and uncomplicated flow of information. It must be clear to everyone involved who is responsible for the different parts of the program. The operational structure should be well documented in the form of a report distributed to all users, containing information on which institutions are involved, the responsible persons in the different institutions (addresses, phone and fax numbers, e-mail addresses etc.) and a clear

description of the tasks for which each institution/person is responsible. It is also useful to have flow charts or action plans outlining the steps to be taken in different circumstances, such as a human poisoning or fish mortality episode, the detection of high levels of a known toxin, or the identification of a new toxin. In preparing these plans, it is useful to assume that the individual faced with management decision has little or no prior experience with HABs or their toxins. This helps to insure that a suitable level of detail is provided in the written documentation. Some examples of action plans developed by individual countries and regions are presented in Section 4.
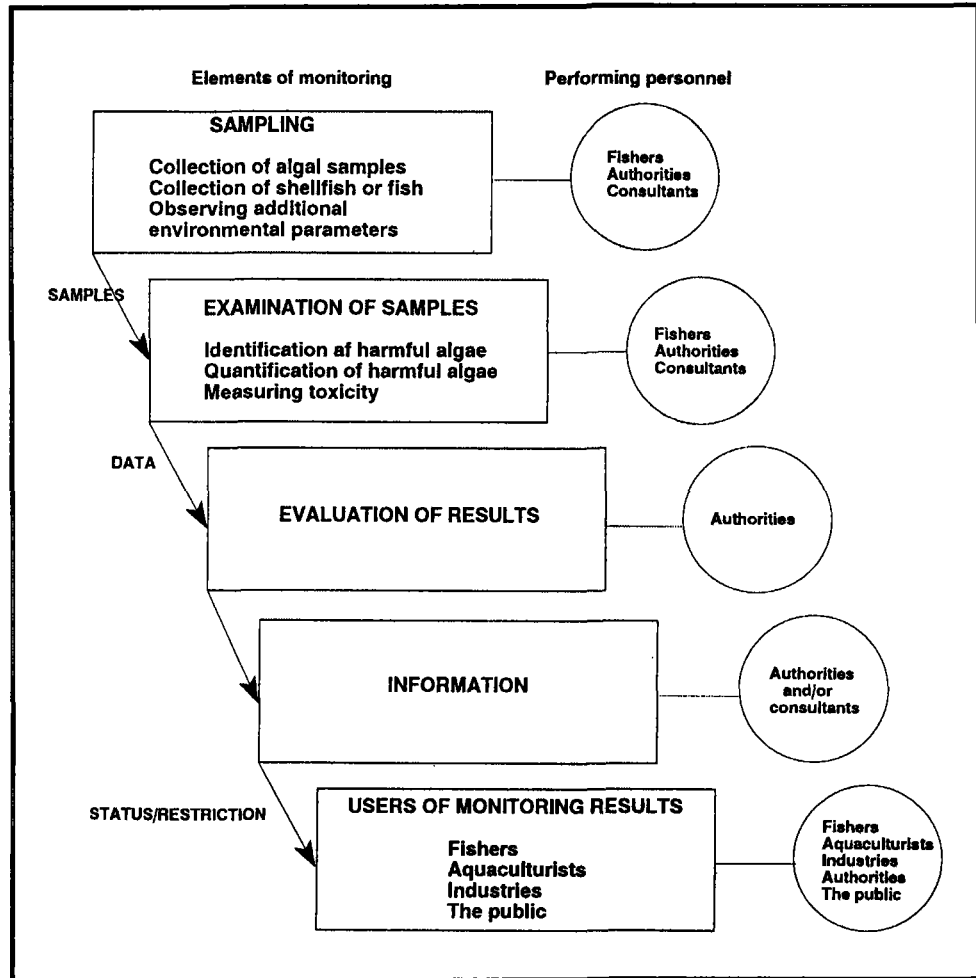
FIGURE 2.1. Theoretical monitoring network for HABs. (Source: Andersen 1996.)

Monitoring marine environmental conditions in relation to red tides/HABs can be carried out at different levels of detail, that is with different levels of temporal and geographical as well as vertical and horizontal resolution, depending upon which kind of HAB is to be monitored. Furthermore, depending upon the goal of the monitoring program, it can include a range of environmental parameters (Table 2.1). This list is meant only as a general guideline. Specific programs may find that only a subset of these parameters is appropriate for the goal of the program. Others may add many other parameters to the list.

TABLE 2.1. Examples of environmental parameters that could be included in a HAB monitoring program.

Physical	Chemical	Biological

Temperature
 	
Wind speed and direction
Light attenuation/turbidity
	Salinity
	Oxygen
	Chlorophyll
	Nutrients
	  - Nitrogen
	  - Phosphorous
	  - Silicate
		Phytoplankton
		- Toxic species
		- Other species
		Zooplankton

Data acquisition. When monitoring data are collected in the field, it is important that the staff responsible for sampling have detailed guidance on what and how to sample. The information should be available in an official manual that defines:

- which kinds of samples should be collected and analyzed
- which forms are to be used
- the methods used for sampling, the analyses to be performed, and units used
- the institution/individual responsible for collecting the samples
- the institution/individual responsible for working up the samples
- how the data are to be archived and analyzed.

Preprinted forms should be filled in with the monitoring data as well as additional information on the sampling, such as location/position, station name/number, and identification code for the staff responsible for sampling. An example of a well-designed manual is that of the National Marine Biotoxin Program of New Zealand, discussed further in Section 4.3.5. The manual is set up so that pages are easily removed and replaced with updated versions without compromising the flow or utility of the program. Material in the manual includes details of the administration of the national plan, methodological details, harvesting closure and re-opening procedures, methods for investigating toxic shellfish poisoning cases, product control, as well as a range of appendices with definitions, forms, and other details. Once the results of the different analyses are available, it is important to create well-defined routes for communication. In addition to providing information during actual outbreaks, monitoring data can be used to provide forecasts which define risk-zones in time and space, such as areas with a high incidence of toxic outbreaks, or conversely, areas where HABs are rare. Site selection of aquaculture facilities often requires careful analysis of long term monitoring data to identify sites which have a low risk of HAB damage. It is often the case that the personal experience of individuals who have been directly involved in the analysis of HAB data over many years allows those individuals to make forecasts or predictions of trends in HAB incidence or transport. These individuals are extremely valuable to monitoring programs, and every effort should be made to keep them involved in program activities. Though non-quantitative, this type of "experience-based" analysis is often quite accurate for predictive purposes and for guiding management decisions.

Distribution of information to users.

It must be clearly defined which institution/person is responsible for compilation/synthesis of the monitoring results, and how the results of the analyses are to be presented to the public and users of the program. A single spokesperson or communications node is desirable to avoid conflicting reports from

multiple government agencies or organizations. Results can be distributed instantly to the users of the monitoring system by telephone, telephone answering machine, fax, e-mail and Internet. The use of the Internet to distribute HAB monitoring data is common in many countries/regions, though in some cases, restricted access web sites or listservers available only to government officials are used to control the release of sensitive information. Data on toxicity of shellfish, for example, is often highly sensitive with major economic implications, and it may, therefore, be prudent to keep such results private, making sure, however, that appropriate and acceptable management actions are taken. The following WWW pages provide examples of how monitoring information is presented in this manner:

- The Baltic Sea Algaline WWW-page: **http://www2.fimr.fi/project/algaline/**

   This page contains information on the quantitative occurrence of phytoplankton in general, including HAB species in the Baltic Sea.

- The Baltic WWW-page: **http://www.ab.lst.se/infobalt/infooff.htm**

   This page focuses on HABs in the coastal areas of the Baltic Sea.

- The Irish HAB WWW-page: **http://www.marine.ie/frc/toxins/**

   This page focuses on HABs as they affect Irish shellfisheries and contains general information on HABs as well as practical instructions (e.g. how to sample phytoplankton for HAB analysis). More detailed information on the current situation on algal toxins and toxic algae in relation to the Irish shellfisheries is available for persons with an appropriate password, which is granted on request.

- The Norwegian HAB WWW-page: **http://algeinfo.imr.no/**

   This page focuses on the occurrence of toxic algae and algal toxins in shellfish in Norwegian coastal areas. The users of the information are the recreational shellfishermen, commercial mussel culturists, and the Norwegian mariculture industry.

- The Korean HAB WWW-page: **http://www.nfrda.re.kr**

This page focuses on distribution of HAB information to aquaculturists, fishermen and the municipal administrative authorities.

3   MONITORING AND MANAGEMENT METHODS

3.1   Methods of Toxin Analysis

3.1.1   General Considerations

The section below focuses primarily on methods currently used in routine toxin monitoring. However, some of the most promising emerging technologies, which may offer alternative methods in the near future, are also described. Chapters on algal biotoxin assays in the IOC Manual on Harmful Marine Algae (Cembella et al 1995; Wright and Quilliam 1995 in Hallegraeff et al. 1995) and a recent report (Cembella 1998) provide the main sources for this summary. The IOC manual is presently out of print, but is available on the web at: **http://unescodoc.unesco.org/images/0012/00122021eo.pdf**. A second edition of the manual is in preparation at this time, and should be available in 2002.

Monitoring of algal toxins involves assays and/or analytical or instrumental methodologies. Assay methods yield a single quantitative value representative of the overall toxicity or toxin content of the sample. They include *in vivo* bioassays using live animals (e.g. the Association of Official Analytical Chemists, AOAC, mouse bioassay) or *in vitro* assays, including:

- Cytotoxicity assays (these can eliminate the need for live animals by using immortal cell lines);
- Receptor assays (in which binding affinity of a toxin is related to its potency);
- Immunological or structural assays;

Analytical methods are often used by regulatory agencies for confirmatory analysis of toxin components, and in some cases as certified methods in routine monitoring, e.g. high-performance liquid chromatography (HPLC) is the approved method for analysis of domoic acid worldwide. These methods usually cannot be used for rapid screening, require costly equipment, and are carried out in centralized laboratories with highly trained personnel. Most countries, with the exception of two European countries, still rely on the use of mouse bioassays. These are especially useful to assess the toxicity of unknown toxins. For example, the first indications of the toxicity associated with ASP, and subsequently attributed to domoic acid, were obtained from the AOAC bioassay used for PSP toxin analysis.

Mouse bioassays measure the biological response of the whole animal, thus allowing correlation with human toxicity effects. They do not require expensive equipment or extensive sample cleanup procedures. Their main disadvantages are that they involve use of live animals (a practice which has become increasingly unpopular with animal rights groups), require experienced personnel and careful standardization of assay conditions to obtain reproducible results, cannot be automated, show lower sensitivity than other methods, and provide no information on specific toxin composition. This is a problem especially for samples that contain multiple toxin derivatives (e.g. PSP toxins) or where co-occurrence of different toxins can cause synergistic or antagonistic effects. False positive or negative reactions may also occur due to interference by compounds co-extracted during sample preparation. Bioassays are often less sensitive and precise than analytical methods, and tend to be more reliable for toxins with acute toxicity, i.e., which yield a low LD50 and short death times.

While false negatives are not tolerable because of their potentially serious consequences to human health, a high incidence of false positives can cause undue economic hardship, due to lost product and/or the need for costly confirmatory toxin analysis.

The Asia Pacific Economic Cooperation (APEC) Task Team on Algal Biotoxin Regulations has provided (Report from the May13-14 1998 meeting) recommendations on toxicity testing as follows:

- For fresh product, the entire animal is to be tested (except the shell), but if only parts of the animal are offered for sale, these are the parts that should be tested.

- For frozen product, the entire product should be tested (except the shell) but including the liquid drainings.

- For canned or other sealed, processed product, the entire contents of the can or package should be tested, excluding the shell if whole animals are included.

- A problem was identified for dried product, in that it is not clear whether the product should be tested before drying, or rehydrated before testing. Problems with salt on dried product were also identified. These have not yet been resolved within this Task Team.

APEC's Task Team on Analytical Methods and Standards for Marine Algal Toxins has established performance criteria for various algal toxins.

For PSP, DSP and ASP toxins, it was established that:

- A proper sampling scheme must be in place to ensure that a representative sample is analyzed,

- The upper limit of uncertainty for the toxin assay method employed must not exceed the specified action limit.

For DSP (okadaic acid series) toxins:

- The extraction and cleanup procedure should recover the entire OA series quantitatively.

Efforts are underway to specify action limits within APEC economies, but this will take some time. It was also determined that for other toxins, such as NSP toxins, pectenotoxins, yessotoxins and ciguatoxins, there is at present inadequate information on their link to human oral potency. Therefore, the Task Team was unable to define action limits, or set performance criteria for these groups of toxins.

Representative sampling is an important consideration given that individual bivalves of the same species are known to vary greatly in their toxin content even when sampled from the same general location (reviewed by Bricelj and Shumway 1998). Some of this variation can be attributed to differences in body size, since small individuals accumulate higher toxin levels per unit body mass than large ones. However, 8-fold differences in PSP toxicity, on average, have been found among individual surfclams sampled from the same station on Georges Bank, USA (White et al. 1993), and 10-fold differences occurred among mussels sampled within a 1.2 km distance in the Bay of Fundy (Prakash et al. 1971).

The APEC Task Team on Analytical Methods and Standards is presently undertaking a full evaluation of methods of detection for various algal toxins, and is compiling a list of available sources of standards and reference materials, with an aim towards the establishment of standard methods for APEC's member economies. Current prescriptive analytical methods (as implemented in the EU) are considered a barrier to effective international trade of seafood products by APEC. This organization has therefore recommended the adoption of 'performance based' methods for detection of algal toxins, and generally recognized the need for "equivalence" among international food safety programs (i.e. provision of equivalent levels of seafood safety and public health protection despite programmatic differences which reflect the needs of specific economies), in order to reduce trade barriers. APEC's Task Team on Algal Biotoxin Regulations is focused on establishment of standard regulations (action limits) for various algal toxins, development of a set of guidelines for the design of toxin monitoring programs, and compilation of existing materials for

public education on HABs and their impacts. It has recommended the creation of a network of APEC government authorities that would be responsible for ensuring that seafood commodities for export or import are safe with respect to marine algal toxins. The nomination of several APEC Reference Laboratories for Algal Toxin Analysis and Standards has also been recommended (Report from the APEC meeting, Haikou, PRC, April 18-19, 2000). These laboratories, distributed worldwide, would provide advice on analytical methods and standards, promote the development of standard reference materials and assist in the development of a distribution network for standards and reference materials.

Once a method is developed, it has to undergo inter-laboratory validation to determine its performance (accuracy, precision and other performance parameters). Method validation programs, such as the ones administered by the AOAC (Official Methods Program) and the International Union for Pure and Applied Chemistry (IUPAC) are expensive and time-consuming to conduct, but are critical for acceptance of new methods.

Liquid chromatography-mass spectrometry (LC-MS) has been advocated by Quilliam (1998) as a universal analytical method of toxin analysis suited for a centralized laboratory. Its main advantages are detection of multiple toxins (DTX1, OA and DSP ester derivatives, ASP, NSP toxins as well as ciguatoxins, spirolides, PTXs and YTX), high specificity, high sensitivity (detection in the ng g^{-1} range), speed, automation, elimination of false positives, and limited sample preparation compared to other methods which require complex derivatization and cleanup protocols. Its main drawbacks for use in routine monitoring are the high capital equipment cost involved, which demands a continuous, high throughput of samples, the need for considerable technical expertise, and especially the need for calibration standards, which are not available for all biotoxins. There is also a need for validation and international acceptance of this method for routine monitoring, although LC-MS is undergoing validation in New Zealand (see sec. 4.3.5). Additionally, reliance on a single instrument is not recommended for monitoring purposes. Development of a universal extraction protocol (extraction solvent and cleanup method) that allows good recovery and thus detection of all toxins present in a geographic region in a single analysis is also required and is presently under investigation. Tandem mass spectrometry (LC-MS/MS) is required for definitive identification of toxins in complex mixtures.

3.1.2 Paralytic Shellfish Poisoning (PSP) Toxins

PSP toxins are water-soluble neurotoxins which cause reversible and highly specific blockage of ion transport by the sodium channel and thus of the action potential in excitable membranes. Human fatalities resulting from consumption of contaminated shellfish are caused by respiratory paralysis. There is no known antidote for PSP toxins and treatment is limited to artificial respiration in life-threatening situations. PSP toxins include a number of structurally related derivatives which vary widely in their potency: carbamate toxins (including saxitoxin, STX, neosaxitoxin, NSP, and gonyautoxins, GTXs), the most potent, decarbamoyl toxins of intermediate potency, and N-sulfocarbamoyl toxins (B and C toxins), the least potent.

The internationally recognized method used for analysis of PSP toxins is the **standard AOAC mouse bioassay** (AOAC 1990, AOAC International 1995). This is the only live animal bioassay method that has been fully validated in a collaborative study. The detection level of this method is about 40 µg STXeq per 100g wet weight of tissue, i.e., only half the accepted action limit or regulatory limit for safe human consumption (= 80 µg STXeq per 100g), providing little margin for technical error. Precision of the assay is ± 20%. It involves boiling of 100g of tissue with 100 ml of 0.1 N HCl for 5 min, adjustment of volume back to 200 ml, of the pH ideally to 3, and intraperitoneal (i.p.) injection of the acidified extract into mice. Results are calibrated against a STX standard and expressed in mouse units (MU) which are converted to toxicity units [STX equivalents (STXeq)] using a conversion factor which varies with the sensitivity of the mouse strain used (1MU = 0.18 to 0.23 µg STXeq). Maximum reliability of results is obtained by

standardization of methods: mouse weight, age, sex, strain, pH of extract (typically adjusted to about 3.0), salt concentration, etc. Toxicity values are derived from standard toxicity tables relating dose to death time. The bioassay is only quantitative when mouse death occurs between 5 and 7 min. Therefore several dilutions may be required to obtain an extract concentration within this range.

There can be substantial variation in the results of the mouse bioassay depending on pH conditions during extraction. It has been recently recommended that a narrower pH range is required to improve the reproducibility of the assay. The AOAC method recommends pH adjustment to 3, but a range of 2 to 4 is deemed acceptable. These conditions result in substantial conversion (40 to 60% depending on the tissue's buffering capacity) of low potency and more labile N-sulfocarbamoyl toxins (C_{1-4} and $B_{1,2}$) into their respective high-potency carbamate analogues. Between a pH of 3 to 4 all PSP toxins are relatively stable, but they become unstable under more alkaline conditions. Complete conversion of N-sulfocarbamoyl toxins to carbamate toxins would lead to determination of the maximum potential toxicity, which many believe would overestimate human health risk. The AOAC extraction protocol for the mouse bioassay results in partial conversion and therefore yields an intermediate value between actual and potential toxicity. The argument in favor of this approach is that acidic conditions in the human digestive tract could also result in partial conversion of the individual toxins, and therefore the AOAC extraction protocol provides a more realistic estimate of human health hazard. A lower pH (=2) of the extract injected into mice can introduce artifacts caused by acidosis. Addition of NaOH is sometimes necessary to adjust the pH prior to injection, but if this is not done carefully (dropwise and with stirring) localized pH changes may cause toxin degradation. An additional concern is that high levels of Zn cause apparent neurotoxic symptoms in mice, which may result in false positives. However, this artifact can be detected by experienced personnel because Zn causes death more than 15 min after injection (McCulloch et al. 1988). Yet another concern is that salt effects can occur at low dilution. Sodium ions present in the extract can reduce toxicity to mice by as much as 20 to 50%, such that extract dilution can yield higher values than a full strength extract. Because of this so-called "salt effect", the mouse bioassay is not very accurate at low toxicity levels.

Calibration standard for saxitoxin is presently available from the US FDA, Washington D.C., and certified STX, NEO and gonyautoxin standards are marketed (at cost recovery) by the Institute of Marine Biosciences (IMB), National Research Council (NRC), Halifax, Canada, as part of its Certified Reference Materials Program (CRMP) (**www.nrc.ca/crmp_e.html**). The former is not certified. Available standards were compared in terms of their chemical form and purity by Quilliam et al. (1998). The activity of the FDA and NRC standards is equal to 2100 and 1990 MU μmol^{-1} respectively. CRMP/NRC supplies PSP-1B, a kit containing individual ampoules of STX, NEO, $GTX_{1,4}$ and $GTX_{2,3}$ in 0.1M acetic acid, suitable for analysis by HPLC-FD, as well as an STX calibration standard in HCl, intended specifically for bioassay methods. Calibration standards for N-sulfocarbamoyl (B and C) toxins are not commercially available but limited amounts of purified toxins can be obtained from research institutions. A recent issue of concern has been the 1997 classification of STX as a chemical weapon. As a result, permits are required from both the importing and exporting countries through the Organization for the Prohibition of Chemical Weapons for shipping of STX from one country to another. This has caused substantial delays in shipping. It is also not possible to ship STX to countries that are not signatories to the Chemical Weapons Convention (CWC). This classification could also affect the export of shellfish containing trace amounts of STX (below the quarantine level). These problems can be circumvented, however, as the CWC classification applies specifically to the free base of STX and does not include other STX forms. International shipment of STX has thus resumed, but it is important to specify the proper chemical name of the compound if CWC constraints are to be avoided.

The cost of mice and supplies alone for the AOAC mouse bioassay is estimated at ~10US$ per sample (Table 3.1), excluding shipment cost for mice and based on 3 mice per sample. However, when the assay gives a death time less than ~5 min, it is necessary to repeat it on a diluted extract to get a death time in the 5-7 min range where quantitation is most accurate, in which case more mice (4-6) may be required per

TABLE 3.1. A comparison of whole animal and *in vitro* assay techniques, and the high performance liquid chromatography – fluorescence detection (HPLC-FD) method, for PSP toxin determination in shellfish samples. Cost in $ Can. 1 Can.$ ~ 0.65 US$. (Source: Cembella 1998.) (See text for additional, updated information.)

	Standard AOAC Mouse Bioassay	Fly Bioassay	Radioimmuno Assay (RIA)	Cytotoxicity Assay (MIST kit)	Radio-receptor Assay	HPLC-FD Analysis
Cost per assay/ analysis (supplies only)	$15 (3 mouse assay)	$1 (10 fly assay)	$20 (3 tubes)	$50	$2	$8
Time per assay (start to finish; prepared extract)	5-7 min	20 min	40 min	9 h (1 h preparation + 8 h incubation)	4 h*/8 h (*with µBeta counter)	2 h (analysis of 3 toxin groups)
Maximum # Assays per 8 h day (1 technician)	60	20	100	200 (manual operation)	50	15
Major expense	animals	labor	antibody; scintillation counter ($30K)	MIST Kit; microplate reader ($15K)	µBeta counter ($75K) or scintillation counter ($30K)	columns and HPLC-FD system ($75K)
Advantages (general)	standard method; widely used; historical data base	organism inexpensive and readily available; few animal rights concerns	assay is rapid and can be automated	except for incubation period assay is rapid and can be automated	assay can be automated	analysis can be automated
	measures total toxicity	measures total toxicity	low labor cost per assay	low labor cost per assay	low labor cost per assay	determines total spectrum of toxin present
	no expensive equipment required	no expensive equipment or facility needed	only small sample size required	measures total toxicity without requiring whole animal	measures total toxicity without requiring whole animal	widely used; well calibrated method
	can be performed in simple facility	only small sample size required	high sensitivity and precision	only small sample size required	only small sample size required	only small sample size required
				sensitivity and precision superior to mouse assay	sensitivity and precision superior to mouse assay	sensitivity and precision superior to mouse assay

TABLE 3.1. (Continued).

	Standard AOAC Mouse Bioassay	Fly Bioassay	Radioimmuno Assay (RIA)	Cytotoxicity Assay (MIST kit)	Radio-receptor Assay	HPLC-FD Analysis
Disdvantages (general)	animal care certification required; pressure against use of mammalian bioassays	not accepted as an official method; not widely accepted	not accepted as an official method; not widely accepted	not yet accepted as an officialmethod	not accepted as an official method; not widely accepted	multiple injections required for total toxin profile
	manipulation requires high manual dexterity; injection procedure distasteful to some	some find micro-manipulation clumsy	only sensitive to some PSP toxins, i.e. can underestimate toxicity	prolonged incubation period	need license for radioisotopes; can be dangerous to manipulate and dispose of	method is compliated and requires high level of expertise
	may be difficult to procure or maintain standard mice; mice need care	relatively low precision and sensitivity; poor dynamic range	need license for radioisotopes; can be dangerous to manipulate and dispose of	relatively high cost per assay	need standard curve for each run	samples can only be run in series (one at a time)
	relatively low precision and sensitivity; poor dynamic range; detection limit close to the regulatory limit		need standard curve for each run		scintillation or µBeta counter costly	needs more refined extract than bioassays
			shelf life questionable			does not measure toxicity – must be calculated indirectly
			scintillation counter costly			high labor cost per analysis HPLC instrument costly and must be dedicated to this specific analysis for extended period

sample. The total cost of the bioassay, including supplies (US$14) and labor ($42.83/hr x 1.35 hr = US$58) was estimated at ~$72 per sample (by Jellett Biotek Ltd.), and $52 per sample by Mariño et al. (1998). Typically, an experienced technician can carry out 10 to 30 mouse bioassays per day (van Egmond et al. 1994).

High-performance liquid chromatography with fluorescence detection HPLC-FD (Oshima et al. 1989; Oshima 1995; Lawrence et al. 1991) is the most common analytical method currently used for PSP analysis in support of mouse bioassay data, but is not used in routine shellfish toxin monitoring programs because of the high instrumentation costs and technical expertise required. The method of Lawrence et al. (1991) uses pre-column derivatization with peroxide or periodate as oxidation agent, whereas Oshima's method (1989, 1995) uses post-column periodate oxidation. These later methods use C18 HPLC columns, which have better separation properties than the polymer-based columns used in Sullivan and Wekell's earlier (1987) method. Net toxicity (expressed in µg STXeq) is calculated from the molar specific potencies (MU µmol^{-1}) of individual PSP toxins (Oshima 1995). However, there is some uncertainty around these conversion factors and a need for their validation. Sample extraction is carried out following the AOAC protocol to allow more direct comparison with the AOAC mouse bioassay, or under mild acidic conditions (e.g. 0.03N acetic acid, without boiling) in research studies that require preserving the integrity of the individual toxins (especially the N-sulfocarbamoyl toxins). It is important to note that within the European Community, if a chemical method of toxin analysis is used in association with a biological one, and when results are challenged, the reference or accepted method is the bioassay (Directive 91/492/EEC in Van Egmond et al. 1994).

3.1.2.1   Emerging technologies.

A few *in vitro* assays show promise for use by regulatory agencies, but have not yet been certified. These methods are much more sensitive than the mouse bioassay, and relative to analytical methods have the advantage that they can typically use relatively impure extracts such as those produced by the AOAC extraction protocol. Functional assays such as the receptor-based and cell-based assays described below, allow regulations to be based on toxicological evidence, because they are based on the biological activity of the toxin. False positives can occur with these tests, however, due to interference by other ion channel active compounds that may or may not be toxic. In contrast, structural assays, such as immunoassays, may or may not reflect biological activity. An effective immunoassay test for PSP toxins should express cross-reactivity proportional to the abundance and specific toxicity of the various individual toxins present in shellfish tissues, as shellfish from different locations, and different species within a location, are known to vary greatly in their toxin profiles (Bricelj and Shumway 1998). Costs, advantages and disadvantages of various methods used for PSP analysis are shown in Table 3.1.

An enzyme-linked immunoabsorbent assay (ELISA) is currently marketed as a test kit for detection of STX (RIDASCREEN® R-Biopharm, Darmstad, Germany). This method has been subjected to collaborative testing by the European Commission's Measurement and Testing Program (Van Egmond et al. 1994), but it has not been certified by either the US FDA or the AOAC. A major disadvantage of this technique is its limited cross-reactivity with other PSP toxins.

The Maritime *In Vitro* Shellfish Test, MIST Alert™ immunoassay has been recently developed by Jellett Biotek Ltd. (Dartmouth, NS, Canada). This rapid test kit, based on lateral-flow immunochromatography, can provide qualitative results (positive/negative) in about 20 min (Laycock et al. in press). It is designed as a pre-screening method to eliminate negative samples (below the regulatory limit), and reduce the requirement for mouse bioassays. It is being tested by regulatory agencies in the USA (Maine, Alaska and California), Canada (British Columbia) and Scotland (Jellett 2000). The simplicity of this kit (based on the elimination of one of the two colored lines on the test strip by

competition with free toxin in the sample) is comparable to that of the universally used home pregnancy test kit. Shellfish AOAC extracts are diluted in a buffer solution and applied to a test strip. Therefore, no laboratory equipment is required, except for a homogenizer to prepare shellfish extracts. All PSP toxins tested to date (including the sulfocarbamoyl toxins $C_{1,2}$ and B_1) were detectable within or close to the regulatory level. The main advantages of the qualitative test include its speed and simplicity of use, allowing rapid elimination of positive samples by regulatory agencies, shellfish processing plants or the aquaculture industry. Positive samples can be subjected to further quantitative analysis using other methods. Its main disadvantage is the differential affinity of the antibody mixture to individual PSP toxins. The test shows highest sensitivity for STX, dcSTX and $GTX_{2,3}$, which are detectable at concentrations of ~200 nM, intermediate sensitivity for $C_{1,2}$ and B_1, and lowest sensitivity for NEO and $GTX_{1,4}$ (detected at ~ 400 and 600 nM) (Laycock et al., in press). Therefore, the detection limit may vary somewhat [between ~100 and 800nM (= 7 - 60 µg STXeq/100g)] depending on the toxin profile of the shellfish tested, and may require calibration for optimal response in a specific geographic region, especially if shellfish are relatively rich in NEO and $GTX_{1,4}$.

Trials conducted to date by regulatory agencies are very promising. Results of tests conducted in Scotland show 100% agreement between the MIST Alert[TM] and the AOAC mouse bioassay for shellfish (mussel and scallop gonad samples) with toxin levels >80 µg STXeq 100g[-1]) and 79 to 82% agreement for samples containing <40 µg STXeq 100g[-1] (Gallacher et al., unpublished results presented at the 2001 Canadian Workshop on Harmful Marine Algae, Nanaimo, BC). Thus, ambiguities may occur near the detection limit of the strip assay, in cases where the intensity of the test line is less than that of a negative control, but not when toxin levels exceed the regulatory level. Agreement between results obtained with this technology and the AOAC mouse bioassay during trials conducted in Alaska and Maine was about 90-95% (Jellett 2000). The MIST Alert[TM] immunoassay is also being developed for testing of PSP-producing phytoplankton and for detection of ASP toxins in shellfish. The cost per test kit is estimated at US$20 (J. Jellett, Jellett Biotek Ltd., pers. comm.).

STX radio-receptor binding assay: this assay (Doucette et al. 1994, reviewed by Cembella et al. 1995) is being developed at the Charleston Laboratory, US National Marine Fisheries Service (NMFS), Charleston, South Carolina. Although it is not yet available commercially, it has been validated by the US NMFS for use with various shellfish species and phytoplankton extracts, substantially automated by use of microtitre plates and automated plate readers, and yields results which compare well with the AOAC mouse bioassay. Inter-laboratory comparative trials are underway. The method is highly sensitive, with a detection limit of about 4 ng STXeq ml^{-1}. The main disadvantages of the method are the requirement of a scintillation counter and the need to use radioisotopes, which restricts its use to centralized, high-technology facilities.

This is a competitive displacement assay in which radiolabelled (^{3}H-STX) and unlabelled STX and/or its derivatives compete for a given number of receptor sites in a preparation of rat brain synaptosomes. The percent reduction in radiolabelled STX binding is directly proportional to the amount of unlabelled toxin present in a certified reference standard or an unknown sample. Because the affinity of a toxin for its receptor is directly proportional to its potency, this method yields a response representative of the integrated potencies of all PSP toxins (or other Na^+ channel blockers) present and can thus be correlated with human response. Although the method still requires fresh mammalian tissue, the amount required is minimal. This need may be eliminated in future by the use of isolated ion channels or cloned receptors.

The Maritime *In Vitro* Shellfish Test, MIST[TM] Cell Bioassay: the cytotoxicity or neuroblastoma cell assay (Jellett et al. 1992, 1995, 1998) was developed by Jellett Biotek Ltd. (Dartmouth, Nova Scotia, Canada) as a shippable kit with ~ 3 weeks of shelf life. The colorimetric method is highly specific for Na^+ channel blockers (including PSP toxins) and is suitable for the AOAC extraction procedure. The basic principle involved is that two biologically active drugs added in combination to an established

neuroblastoma cell line culture increase the influx of Na$^+$ causing swelling and cell death. The presence of a Na$^+$ channel blocker prevents this effect and the cells remain viable. Vital staining is used to determine cell viability, and the intensity of the color reaction is proportional to the amount of Na$^+$ channel blocker (toxin) present. An alternative technique based on the same principle was developed by Gallacher and Birkbeck (1992).

The MIST™ kit was developed in three configurations depending on the degree of accuracy required, the number of samples to be assayed, and the detection hardware available. The Mini-MIST™ kit indicates only presence/absence of toxins and the results (color change) can be evaluated visually. This can be used for pre-screening to determine which samples require further analysis and thus reduce the requirement for mouse bioassays. Cell incubations require a 10 hr period, but up to about 200 samples day^{-1} can be analyzed with this method (J. Jellett, pers. comm.). The semi-quantitative version yields a range of toxicity, i.e., it can indicate whether the sample exceeds the regulatory limit. The fully quantitative version has a sensitivity level of 2μg STXeq 100g^{-1} (i.e. about 20x more sensitive than the standard mouse bioassay) and is performed most effectively with a scanning spectrophotometer for colorimetric detection in microtitre plates. The MIST™ kits can be performed in the field or on a ship, and have been used to determine toxicity in both shellfish and phytoplankton samples (Jellett et al. 1998). Live mouse neuroblastoma cell cultures need to be maintained at 20-25°C and activated at a higher temperature for 48 hrs prior to their use. Therefore, one of the limitations of the MIST kit is that it has a limited shelf life (ca. 2-3 weeks) and therefore must be purchased on the basis of demand. Problems with the viability of cell cultures during shipping have also been encountered, as existing shipping containers can only ensure a 15 hr transit time (J. Jellett, pers. comm.).

The MIST cell assay for PSP detection has undergone a collaborative, inter-laboratory calibration study following AOAC certification protocol, which was completed in 1999. This study involved 10 countries, including Portugal, Spain, France, Italy, and Scotland. Mussel homogenates with known profiles of PSP toxins were tested using the kit to determine reproducibility of results, and were also compared with results of the mouse bioassay. However, delays caused by issuing of permits, and the need to ship material to distant locations resulted in the need to use frozen samples, which partly compromised the results of this study. A subsequent study, using fresh tissue samples of a number of different shellfish species, was undertaken in collaboration with the Alaska Department of Environmental Conservation (ADEC). The cell-based assay awaits AOAC certification and is not commercially available at the present time. A modified neuroblastoma cell-based assay has been developed for the detection of all toxins active on voltage-sensitive sodium channels, either with blocking (PSP toxins) or enhancing activity (brevetoxins associated with NSP and CTXs, involved in ciguatera fish poisoning) (Manger et al. 1993, 1994, reviewed by Manger 1995) (see sec 3.1.5).

3.1.3   Amnesic Shellfish Poisoning (ASP) Toxin

ASP toxins are comprised of domoic acid (DA), the major toxin present in contaminated shellfish or plankton, and its isomers. Domoic acid is a water-soluble excitatory aminoacid, which binds strongly to glutamate receptors of the kainate sub-type, present throughout the nervous system. It causes continuous depolarization of neurons leading to cell rupture. Its symptoms in humans include gastrointestinal effects such as nausea, vomiting, gastric distress, gastric bleeding and diarrhea, followed by neurological symptoms including dizziness, confusion, weakness, lethargy, somnolence, coma, seizures, and permanent short-term memory loss (Wright and Quilliam 1995). Neonates, elderly individuals and individuals with compromised renal function are considered high-risk groups for death or memory impairment by ASP.

High-performance liquid chromatography (HPLC). The HPLC method for analysis of domoic acid, using UV/diode-array detection (LC-UV/DAD) (Quilliam et al. 1989; Lawrence et al. 1989, 1991) is the

only other fully validated method for testing of marine algal toxins in seafood (in addition to the PSP mouse bioassay). The HPLC-UV method is the preferred method for determination of DA in shellfish tissues, and is used by regulatory agencies for domoic acid detection in both fish and shellfish tissues. It allows separation of domoic acid from its isomers, which although found in small quantities in shellfish, may not be as toxic as DA. Confirmatory analysis of DA can be achieved by HPLC-MS.

The AOAC aqueous extraction protocol with only slight modifications (Lawrence et al. 1989, 1991, AOAC 1991) was initially adopted in Canada for regulatory purposes because it allowed the extract to be used for both PSP and DA analysis. However, there are several problems with this procedure (outlined by Wright and Quilliam 1995), especially the inability to store the extract due to decomposition of DA in acidic solutions. Rapid extraction and cleanup procedures have recently been developed for fish and shellfish tissues based on extraction with aqueous methanol and cleanup by strong anion exchange (SAX) solid phase extraction (Quilliam et al. 1995). This method allows > 90% recovery of DA even at trace amounts and eliminates the problem of false positives due to interference of compounds present in shellfish and fish tissues, such as tryptophan. The detection limit of the HPLC/UV method is dependent on the method of extraction and cleanup. If crude extracts (either acidic or aqueous methanol) are analyzed without cleanup, the practical limit for quantitation is $1\mu g\ g^{-1}$ (ppm). With the cleanup procedure described by Quilliam et al. (1995) the detection limit drops to 20-30 ng g^{-1} (ppb). The LC-UV/DAD method using SAX-cleaned aqueous methanol extracts was validated for use of routine monitoring of DA in Scottish shellfish, including scallop (*Pecten maximus*) whole tissues and gonad tissue (Hess et al., in press).

Pocklington et al. (1990) developed a sensitive HPLC method for DA in seawater and phytoplankton, with fluorometric detection. The detection limit is 15 pg ml^{-1}, allowing analysis of *Pseudo-nitzschia* samples with cell densities as low as 1000 cells ml^{-1}, assuming production of DA at 1-20 pg $cell^{-1}$. This method has been extended to shellfish tissues.

The AOAC mouse bioassay (intraperitoneal injection) was used for detection of DA during the early stages of the ASP crisis in Atlantic Canada, prior to development of a simple HPLC analytical method. It required observation of mice beyond the 15 minutes established for PSP toxins (up to 18 hrs, with continuous observation during the first 4 hrs; National Health and Welfare, Health Protection Branch, Ottawa, Canada, 1988). Symptoms associated with DA are a unique scratching of the shoulders by the hind leg, uncoordinated limb movements, and loss of equilibrium, followed by convulsions. The sensitivity of the mouse bioassay (detection limit ca. 40 $\mu g\ g^{-1}$) is inadequate for the action level (= 20μg g^{-1}) set by Canada following the crisis, and later adopted by other countries (Wright and Quilliam 1995). The method still has some value because of its characteristic symptomology, which is apparent at the detection level and becomes very obvious at DA concentrations > 100$\mu g\ g^{-1}$.

Pure DA is commercially available from several sources. Domoic acid calibration standard (DACS-1C), and certified mussel tissue reference material containing DA (MUS-1B) are available from NRC's Certified Reference Materials Program (IMB, Halifax, Canada). Domoic acid is also available from the US FDA, but is not certified.

Emerging technologies. A competitive neuroreceptor binding assay has been developed for detection of domoic acid (Van Dolah et al. 1994). This is a very sensitive, highly specific, functional detection method. It has been used to assay shellfish and algal extracts, as well as marine mammal serum, feces and urine (Scholin et al. 2000). It uses frog brain synaptosomes and is based upon binding competition of DA with radiolabelled [^{3}H]-kainic acid (a structural analogue of DA) for the glutamate receptor. Further improvement of this assay involved use of cloned rat glutamate receptors, which avoid the use of animals and provide greater consistency in resujlts, and inclusion of a pretreatment to eliminate interference by glutamate in shellfish tissues (Van Dolah et al. 1997). The main drawback of this method is that it requires

the use of radioisotopes. Assay kits are not yet commercially available. An assay based on the fluorometric detection of Ca^{2+} in rat cortical primary culture has been described for ASP (and PSP) but was only tested with toxic phytoplankton (Beani et al. 2000). Several ELISA immunoassays for DA have been developed (e.g. Smith and Kitts 1995, used for detection in shellfish extracts, and most recently by Garthwaite et al. (1998) and Branaa et al. (1999), which show potential as screening methods but require validation. The latter were suitable for analysis of DA-spiked, shellfish extracts and showed high specificity (no cross-reactivity with kainic acid) and high sensitivity. The Branaa et al. (1999) study was able to quantify DA at levels of 2-180 $\mu g\ g^{-1}$, corresponding to 0.02-1.8 $\mu g\ g^{-1}$ of mussel tissue.

An LC-MS method has also been developed for the detection of DA in a variety of shellfish, providing a detection limit of 0.4 $\mu g\ g^{-1}$ shellfish extracts (Hess et al., in press). This study provided the first confirmation of DA in shellfish harvested in UK waters (samples collected in 1998).

3.1.4 Diarrhetic Shellfish Poisoning (DSP) Toxins

Polyether DSP toxins in shellfish include the okadaic acid (OA) group (OA and dinophysistoxins DTX1, DTX2, DTX4 and acyl-derivatives DTX3), pectenotoxins (PTX) and yessotoxins (YTX). Pectenotoxins are produced by *Dinophysis*, and YTX by *Protoceratium reticulatum* (Satake et al. 1997). Only the OA group has diarrhetic effects: OA and DTX1 are the main toxins responsible for DSP outbreaks, although DTX2 has also been implicated in DSP episodes. Pectenotoxins are reported to be hepatotoxic and yessotoxins are lethal to mice when injected intraperitoneally, causing histopathological changes in heart, liver and pancreas (Terao et al., 1990), but their oral toxicity to humans remains unknown. Human poisonings due to PTXs and YTXs have not been reported to date. There are no official action limits in most countries for YTX or PTX, but the EU is considering establishing limits in the near future. OA and DTX1 are potent inhibitors of protein phosphatases and also have tumor-promoting activity. Mammalian (rat and mouse) bioassays are generally used in monitoring programs to determine DSP toxins. However, there is a lack of international methods standardization, and there are inconsistencies in extraction efficiencies of these lipophilic toxins (see review by Fremy et al. 1999, which includes a comparison of benefits and limitations of various methods). Numerous methods are used, but none has been officially validated. Extracts for the bioassays are usually prepared from viscera rather than whole soft tissues, because DSP toxins, which have relatively low acute toxicity, are largely localized in the digestive gland; therefore higher concentrates can be obtained from this tissue, resulting in reduced matrix interference. The response of mammalian assays (below) can vary depending on the extraction protocol used, the animal species used and the method of administration.

Oral dosage rat bioassay: this bioassay (Kat 1983) is used for monitoring purposes in some European countries. It is realistic in that it simulates oral toxification in humans, and does not require extraction of the sample, but measures only the diarrhetic effects of DSP toxins (i.e., only detects the OA group), and is only semi-quantitative. Pre-starved, female Wistar rats (*Rattus norvegicus*) (100-120 g) are fed shellfish viscera, and the consistency of the feces produced the following day is scored qualitatively.

Intraperitoneal (i.p.) mouse bioassay: this functional assay (Yasumoto et al. 1995) is the most common method used in monitoring programs worldwide, and provides a measure of overall toxicity. It is used quantitatively: 1 MU is defined as the minimum amount of toxin required to kill 2 to 3 mice in 24 hrs following i.p. injection (= ca. 3.2μg DTX1 = 4μg OA). The detection limit of the mouse bioassay is ~ 0.8 μg OAeq g^{-1} digestive gland (Fremy et al. 1999). Disadvantages of this method are that there is no international agreement on the time of observation required, which can range from 2 to 3 deaths in < 5 hrs to < 24 hrs, and a number of different toxin extraction methods are used which can cause a large variability in results. Most extraction procedures for DSP toxins co-extract other lipophilic toxins (e.g. brevetoxins and some icthyotoxins), and fatty acids, which yield false positives in the bioassay.

Interference may also be caused by PSP toxins found at undetectable levels with the AOAC mouse bioassay, which are concentrated by the evaporation protocol.

Four variations of the mouse bioassay are detailed by Cembella et al. (1995). The original method uses acetone extraction of viscera, which is then evaporated and resuspended in surfactant prior to injection. A modified extraction protocol (Yasumoto et al. 1984) is now used as the official method for monitoring in Japan and some European countries, which uses diethyl-ether as solvent and eliminates PSP interferences. The main drawback is that this method is not suitable for extraction of YTX, which is found in the Japanese scallop, *Patinopecten yessoensis*. An alternative extraction procedure (Lee et al. 1987) is used for DSP toxin monitoring in Norway, which may result in loss of low-polarity DSP toxins (i.e. DTX3), and yet another extraction method using hexane wash is used in Portugal and France, which removes interference by fatty acids but not by salt and PSP toxins. A standard okadaic acid calibration solution (OACS-1) and mussel tissue reference material containing OA and DSP1 (MUS-2) is distributed by NRC's Certified Reference Materials Program (IMB, Halifax, Canada).

Analytical methods: Some countries, such as Sweden and Germany, use HPLC methods with fluorometric detection to analyze for DSP toxins. However, the conventional HPLC-FD method (Lee et al. 1987; limit of detection = 0.4 μg g^{-1} digestive) is unable to detect ester derivatives of OA which do not have a free carboxylic acid end group, such as okadaic acid diol-ester. Improvements to Lee's original method, which involved methanol extraction and 9-anthryl-di-azo-methane (ADAM) derivatization, are reviewed by Fremy et al. (1999). Using other methods, such as liquid chromatography with ion spray mass spectrometry (LS-MS) (Quilliam 1995; limit of detection = 1 ng g^{-1} digestive), Bauder (1997) showed that OA diol-ester comprised a major portion of the total DSP toxin load in tissues of scallops exposed to *Prorocentrum lima*. His study showed that although OA diol-ester is not a phosphatase inhibitor, it can be hydrolyzed to active OA in the bivalve digestive tract, via release of esterase enzymes from ruptured *P. lima* cells, and therefore represents a "cryptic" toxin that is not measured by HPLC. It was suggested that an alternative solution for regulatory programs, which do not have access to a mass spectrometer, would be to hydrolyze all OA-esters in shellfish extracts to OA prior to HPLC-FD.

Emerging technologies. The DSP-Check competitive enzyme-linked immunoabsorbent (ELISA) test kit (UBE Industries, Tokyo, Japan) is a commercially available immunoassay-based kit used for rapid (2-4 hrs) semi-quantitative screening of OA and DTX1. The detection level is rated at 20 ng OA eq g^{-1}. However, it shows low cross-reactivity with DTX1 and may therefore underestimate the total toxin present as determined by mouse bioassay, and inconsistencies including false positives, have been reported with this method.

Several phosphatase inhibition assays, based on the known inhibitory effect of OA and DTX1 on serine-threonine protein phosphatases, have been developed (Kinoshita et al. 1995; Zhang et al. 1994). The latter has been used successfully with shellfish and natural and cultured DSP-producing algae, but it requires the use of the isotope ^{32}P and therefore must be conducted in a regulated laboratory. A more promising assay is the **protein phosphatase 2A inhibition assay (PP2A-IA)** (Tubaro et al. 1996), which is very sensitive for the detection of OA and its derivatives in shellfish tissues. The detection limit is 10 ng OA g^{-1} digestive gland, two orders of magnitude lower than that of the mouse bioassay. This colorimetric method is rapid to perform (~5 hrs) and does not require sophisticated laboratory equipment (a spectrophotometer and microplate reader are needed). Since the assay does not discriminate among OA derivatives, results are expressed in OA equivalents (eq). Temporal patterns of DSP mussel contamination in the Adriatic Sea, Italy, using this method showed excellent agreement with those determined by the mouse bioassay (Della Loggia et al. 1998). The PP2A enzyme is expensive to obtain commercially, but can be extracted from mussel tissues (Mondeguer et al. 1998). Recent modifications of the colorimetric assay involve use of fluorimetric (e.g. Vieytes et al. 1997) or bioluminescence/chemoluminescence detection. The assay using fluorogenic substrates is more accurate and sensitive than the colorimetric assay, which is prone to giving

false positives at low levels of OA approaching the regulatory level (20 μg 100 g⁻¹ soft tissues) (Mountfort et al. 1999). This study provides a detailed comparison of PP2A-IA, ELISA, LC-MS and the mouse bioassay using naturally contaminated shellfish samples. Their high sensitivity may make fluorimetric methods particularly suitable for early detection of DSP in phytoplankton.

3.1.5 Neurotoxic Shellfish Poisoning (NSP) Toxins

Brevetoxins (PbTx) are lipid-soluble, polyether toxins implicated in NSP. Although they are considered primarily ichtyotoxins, they are known to also adversely affect bivalve molluscs. They bind to voltage-sensitive sodium channels, leading to channel activation.

The accepted method for determination of NSP toxins in the US is the American Public Health Association (APHA 1985) mouse bioassay procedure based on diethyl-ether extraction of shellfish tissue (Cembella et al. 1995). In New Zealand, where NSP toxins were first detected in 1993, an acetone extraction followed by partitioning into dichloromethane was used (Hannah et al. 1995). This method has a higher extraction efficiency and allows more rapid separation of extraction phases. However, the regulatory authorities reverted to the APHA method after discovery of a new bioactive (gymnodimine). The APHA method is used because gymnodimine, a compound that is not known to pose a health hazard to humans, is not extracted by the APHA method. Shellfish have been recently shown to produce metabolites of NSP toxins exhibiting receptor-binding activity and thus toxicity, which are likely to affect the accuracy of analytical methods for these toxins (Poli et al. 2000).

Emerging technologies. HPLC methods for these toxins are not in widespread use due to the need for extensive sample pre-treatment. A **neuroblastoma cell assay** for sodium-channel activating toxins (brevetoxins and ciguatoxins, see Section 3.1.7) has been developed by Manger et al. (1993, 1994) and granted a US patent (Manger et al. 1999). Cytotoxicity (reduced cell viability) is assessed colorimetrically in a microplate reader, based on measurement of mitochondrial dehydrogenase activity (reduction of a tetrazolium compound, MTT, to a blue formazan product in metabolically active cells). Measurement of cytotoxic effects by PbTxs and CTXs requires that cells be sensitized with a treatment combining veratridine (a site-2 specific Na⁺ channel activator) and ouabain (a specific inhibitor of Na⁺/K⁺ ATPase. The assay takes ~4-6 hrs, and is highly tolerant of extract impurities, thus requiring minimal sample preparation.

3.1.6 Other algal toxins

Fast-acting toxins are a group of novel, structurally related toxins containing a bioactive cyclic imine group. When isolated from shellfish, these induce rapid death and characteristic neurotoxic symptoms in mice. They include spirolides (A-D), pinnatoxins and gymnodimine. Although their human health significance has not yet been determined, and they are not included in routine biotoxin monitoring programs, their toxicity, detectable by the mouse bioassay, makes them a serious cause of concern as a potential food poisoning agent. *Alexandrium ostenfeldii*, a dinoflagellate species which occurs worldwide, has been confirmed as the primary source of spirolides in SE Nova Scotia, Canada, and Denmark (Cembella et al. 2000). Analysis of spirolides has been conducted by liquid chromatography combined with ion-spray mass spectrometry (LC-MS) (Hu et al., 1996). Mouse symptoms have been induced by intraperitoneal injection of lipophilic extracts of bivalve viscera (scallops and mussels).

Azaspiracids. Azaspiracid poisoning is caused by the AZP toxins azaspiracid, AZ-1, and its methyl and dimethyl analogues AZ-2 and AZ-3 respectively, which have been identified in mussels from several European countries, including Ireland, England and Norway (James et al. 2000). They are presently

monitored only in Ireland, as a result of an episode in which consumption of contaminated Irish mussels caused human intoxications in several European countries: the Netherlands (1995), and France and Italy (1998). A risk assessment analysis is in progress at this time, and until it is complete, the Food Safety Authority of Ireland is using a level of 0.1 µg g^{-1} of whole flesh as an interim action limit (T. McMahon, pers. comm.). There is as yet no official EU Level for AZP toxins although a provisional level of 0.16 µg g^{-1} is under consideration. In animal tests, AZPs are neurotoxic and also produce damage to intestinal, spleen and liver tissues (Ito et al. 2000). *Protoperidinium crassipes* has been identified as the algal source of AZP (T. Yasumoto, unpub. data).

Much of the difficulty with identifying AZP incidents has been due to the problems of detecting azaspiracid (AZA). The unique structure of this phycotoxin required the development of new analytical methods. The first reported method utilized liquid chromatography coupled with mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS/MS) using an ionspray interface (Draisci et al 2000). The LC-MS/MS method has an estimated lower limit of detection of 20 pg g^{-1} but requires very expensive equipment. The Yasumoto DSP mouse bioassay (Yasumoto et al, 1978) can be used for detection of AZA. However, it is reported to only be capable of detecting high levels of AZA and may not be suitable for detecting the toxin in monitoring programs due to the low extraction efficiency (10%-40%) of AZA present in mussels (Draisci et al 2000). These authors also quote a lower level of detection for the mouse bioassay of about 2.8 mg g^{-1} of AZA. Other workers have referred to a level of 2-4 mg g^{-1} of AZA and 24 hrs of observation as the lower level of detection of the mouse bioassay (Ofuji et al. 2000). An alternative detection method is under development at the National Diagnostic Center, Galway, Ireland. The method is a cytotoxicity assay that is reported to distinguish between the okadaic acid family of toxins (DSP) and azaspiracid. A tentative lower detection limit for this assay is approximately 0.45mg g^{-1} (Flanagan et al. 2000).

Pfiesteria. One toxin syndrome that remains problematic for monitoring programs is that caused by *Pfiesteria piscicida* and related organisms (Burkholder and Glasgow 1997). At present, there is no standard procedure for measuring *Pfiesteria* toxins in water or in fish or shellfish tissues. Fairey et al. (1999) developed a C-fos assay that uses a reporter gene to reveal the presence of putative *Pfiesteria* toxin(s). Thus far, a lack of sufficient toxic material has precluded the chemical characterization of *Pfiesteria* toxin(s) or the development of analytical methods for their measurement. Likewise, no toxin standards are available.

3.1.7 Ciguatera Fish Poisoning (CFP) Toxins

Ciguatera or CFP is a syndrome caused by the consumption of tropical and subtropical fish contaminated primarily by ciguatoxins (CTX), a group of low molecular weight polyether, lipid-soluble, heat stable Na^{+} channel activator toxins. Two structurally distinct CTX families occur in the Pacific Ocean and Caribbean Sea (P-CTX and C-CTX respectively). P-CTX1 is the most potent and is the principal toxin in large (> 1 kg in weight) carnivorous fish, which cause most cases of CFP, on the basis of both lethality and toxin quantity. In the Pacific Ocean, P-CTX1 contributes ~ 90% of total lethality in these fish and poses a health risk at levels > 0.1 ppb. The risk level of C-CTX1 was established at > 0.25 ppb (Lewis et al. 1999). However, CFP may also be caused by other toxins, such as maitotoxins, which occur primarily in the viscera of herbivorous fish feeding on *Gambierdiscus toxicus* cells, although the role for other toxins remains unproven. Suspension-feeding bivalve molluscs, which feed predominantly on planktonic algae, typically do not act as vectors of CFP, although gastropods, which graze on benthic algae, have occasionally been implicated. CFP is characterized by multiple short- and long-term symptoms, including gastrointestinal, cardiovascular and neurological disorders. It has caused human fatalities in the Indian Ocean, although it is rarely fatal in the Pacific Ocean and the Caribbean Sea. Ciguatera toxins are odorless and tasteless. Boiling, cooking, and salting do not remove ciguatoxins from fish. The head, gonad and

viscera of fish contain higher toxin levels (up to 100x greater) than the flesh. Repeated exposure to ciguatoxins in the diet does not confer immunity in humans, and indeed, results in sensitization.

A number of assays have been used to detect ciguatoxins in fish extracts. These include a range of *in vivo* assays (e.g., mouse, brine shrimp, diptera larvae, cat, chicken, mosquito), a number of *in vitro* assays utilizing antibodies or isolated tissues, and chemical assays involving derivatization and HPLC separation with fluorescence detection. Biosensor assays are also under development. It is beyond the scope of this study to elaborate on these analytical methods (see review by Lewis 1995). Nevertheless, some discussion is worthwhile on the mouse bioassay, as well as on a recently developed, promising immunoassay and cell-based assay.

The mouse bioassay is at present the most widely used method for detection of ciguatoxins in fish. Fish samples must be immediately frozen at –20°C for storage. Toxins from tissues of carnivorous fish (typically > 50 g) are usually extracted in acetone, partitioned into a series of liquid-liquid phases (e.g. methanol, hexane, chloroform), and semi-purified by thin layer or column chromatography. The toxin fraction is eluted from a Fluorosil column in acetone:methanol, dried, emulsified, and injected intraperitoneally in mice, which are observed for at least 24 hrs for symptom development (see Lewis 1995 for a detailed description of the extraction protocol and mouse bioassay).

Lewis and Sellin (1993) tested the mouse bioassay for ciguatoxin in ether extracts of fish and found that the assay was only able to detect ciguatoxin at > 0.5 ppb in fish flesh. Based on clinical poisoning data, an appropriate method should be capable of detecting ciguatoxin at levels as low as 0.05 ppb. The study found that $63\pm14\%$ of spiked ciguatoxin was recovered using a standard extraction procedure, so the extraction method was deemed suitable for testing purposes. The authors concluded that the "cost of such an assay, as well as its insufficient sensitivity and ethical considerations all preclude the use of the mouse assay for routine seafood monitoring programs" (Lewis 1994).

The main disadvantages of the mouse bioassay for detection of ciguatera toxins are that extraction protocols are poorly standardized for different toxin components, the assay is time-consuming (due to the requirement for complex extraction procedures and prolonged observation of mice), and has low specificity for individual toxins. Identification of toxins requires confirmation by analytical methods, e.g. LC/MS, HPLC-FD/MS or gradient reversed-phase HPLC coupled with tandem electrospray mass spectrometry (HPLC-MS/MS) (Lewis et al. 1998). HPLCMS is not sufficiently sensitive to detect clinically relevant CTX levels in crude fish extracts. However, HPLC-MS/MS is highly sensitive, as it is able to detect both Pacific and Caribbean ciguatoxins at sub-ppb levels in crude extracts of fish flesh (Lewis et al. 1999). Detection limits using this method are 0.04 ppb P-CTX1 and 0.1 ppb C-CTX1 and good correlation was found with results obtained with the mouse bioassay. Confirmatory analysis is especially important in cases of false positives, which may have legal ramifications.

Additional research is needed to develop rapid, simplified and high efficiency extraction methods for ciguatera toxins. The dinoflagellate *Gambierdiscus toxicus*, the causative agent of CFP, produces gambiertoxins and maitotoxins. These toxins are also present in herbivorous fish. Maitotoxins are water-soluble and induce effects in mice that are sometimes confused with those of CTXs, although they are distinguishable from these on careful observation. Extraction procedures can be modified to improve the separation of CTXs and maitotoxins. Thus initial extraction from algal cells uses methanol, a less polar solvent than acetone. Lower potency ciguatoxins (CTX2 and CTX3) cause longer times to death (up to 4 days) than CTX1 over a wider range of doses. Unlike CTX1, they induce hind-limb paralysis symptoms not unlike those of gambiertoxins. Toxicity (in MU) is determined from the relationship: $\log (MU) = 2.3$ $(1 + T^{-1})$, where $MU = LD_{50}$ dose for a 20 g mouse, and T is the time of death in hrs (Lewis and Sellin 1993). This relationship should be determined for the mouse strain available in a given region, and ideally should be established for each species of fish to be assayed.

Emerging technologies. A number of *in vitro* antibody-based assays have been developed for the detection of ciguatoxin and related polyethers. These are simple to conduct, allow screening of a large number of samples, and allow toxin detection directly using fish tissues. **The solid-phase membrane immunobed assay (MIA)** developed by Hokama et al. (1998a and b) is available commercially as the "Cigua-Check™" test kit (Oceanit Test Systems, Inc., Honolulu, Hawaii USA) and represents a modification of an earlier stick enzyme immunoassay (S-EIA). Cigua-Check™ is presently undergoing testing for certification by the AOAC International. The AOAC certification involves testing by a number of laboratories and is generally recognized as the "gold-standard" for any analytical method seeking worldwide approval. If the company obtains certification by the AOAC, it will approach the World Health Organization for their endorsement of the product.

The Cigua-Check™ kit is available directly from Oceanit Test Systems for US$20. Each kit can test five fish in less than an hour without any special equipment. The kit is reported to detect ciguatoxin at levels down to 0.1 ppb, which is comparable to the threshold for human symptomology of 0.05-0.1 ppb. The immunoassay consists of a hydrophobic membrane laminated on a solid, plastic stick, which is immersed with a rice grain-sized piece of fish tissue (ca. 5 mg obtained with a punch biopsy tool or razor blade), in methanol. The membrane is then dried, placed into an immunobed suspension containing polystyrene particles coated with monoclonal antibody to ciguatoxin. The color intensity of the membrane is related to the concentration of toxin bound to the membrane, yielding negative, borderline or positive results. The assay includes negative controls, and fish samples with both high and low concentrations of toxins as positive controls. The relative concentration of CTX in the crude extract can be assessed using various concentrations of pure CTX.

The method can be used in the field, and has been used extensively to survey areas in Hawaii where CFP is endemic, and for analysis of fish implicated in clinically documented cases of CFP by the State of Hawaii Department of Health. Of 176 fish implicated in clinically diagnosed ciguatera cases in Hawaii, 171 (97.2%) gave borderline or positive responses with the Cigua-Check™ system, and five (2.8%) gave negative responses. The possible false-negative responses may have been due to the implicated fish containing toxins other than ciguatoxin but which may cause ciguatera-like symptoms (palytoxin, maitotoxin). Overall, the sensitivity of Cigua-Check™ for the ciguatera-implicated fish was 92.3%. The rated specificity and sensitivity values of Cigua-Check™ are well within acceptable ranges for a biological test system.

The main problems associated with the application of this kit are: 1) the difficulty in obtaining pure CTX1 for calibration; 2) the false positives obtained due to cross-reactivity of the CTX1 antibodies with other polyether compounds, including less potent ciguatoxins, palytoxin, maitotoxins, and brevetoxins and okadaic acid (causative agent of NSP and DSP) that are structurally related to CTX; and 3) the structural differences between ciguatoxins from other regions of the world, potentially leading to false negatives. The antibody used in the kit was developed for detection of Pacific ciguatoxins, and it has been determined that there are differences in toxin composition between ciguatoxic fish in the Pacific, Indian Ocean and Caribbean region. Nevertheless, if validated, this kit could represent a major development in the fight against CFP.

Ciguatoxins are sodium channel activators rather than blockers, as is the case with PSP toxins. *In vitro* cell-based assays, which measure the effects of CTX-induced sodium channel opening (cytotoxicity on mouse neuroblastoma cells) or the inhibition of $[H^3]$-brevetoxin binding (brevetoxin competitive displacement assay using rat brain synaptosomes), are more sensitive than *in vivo* methods. In particular, the *in vitro* **neuroblastoma cell-based assay** developed by Manger et al. (1993, 1994) offers another promising alternative to the mouse bioassay. This method takes advantage of the high-binding affinity of CTXs to voltage sensitive sodium channels. Its main advantages are that it is 10^4 times more sensitive than

the mouse bioassay, provides a direct measure of human potency (unlike the immunoassay described earlier), and can be automated. This type of assay, however, cannot be conducted *in situ,* since it requires specialized laboratory equipment and is less cost effective than the Cigua-Check[TM]. It is presently undergoing validation by the US FDA.

HPLC-MS/MS (see above) shows promise as an analytical method to replace the mouse bioassay. At present, it requires expensive instrumentation but the costs of MS/MS are expected to decline.

3.1.8 Toxin in Finfish and Consumption by Humans

Toxins that harm or kill fish, or transfer toxins further into the food web may be classified into several main categories as shown in Table 3.2. The categories are by no means exclusive and represent a collection of toxin effects on fish in general, and on specific organs or cell types. The state of knowledge is not well advanced regarding fish toxins, especially their physiological effects on fish, and the fate of toxins in tissues. Examples of harmful species, the involved toxin or chemical and the primary cellular or organ targets in fish are noted in Table 3.3.

A key point in this regard is that fish death due to many HAB fish toxins is so rapid that accumulation in non-visceral tissues (e.g., the portions often consumed by humans) is unlikely. In reviewing the literature we came to this conclusion on our own. We later found that Brusele (1995) had reached a similar conclusion. This is important because it means much of the thrust of human health protection in the HAB context is focused on shellfish intoxication, not on concerns that mariculture fish will harm or kill humans. There are probably exceptions, however, such as low levels of NSP toxin in fish killed by *Gymnodinium breve* (D. Baden, pers. comm). Authorities in Korea, Japan, Norway, New Zealand, Chile, British Columbia and Washington State in the US were contacted regarding rules and practices for disposition of dead fish killed by icthyotoxins. In most cases we were told that human consumption is not an issue due to the poor quality and large quantity of fish from a catastrophic fish kill. In practice, countries with large-scale mariculture industries such as Japan, Korea and Norway have prohibitions in this regard. In some countries, such as Norway and Canada, dead fish are sometimes used as food in animal husbandry such as mink farms (fur production) where humans do not eat the end product.

There are several possible characterization schemes for fish toxins, such as toxins that are located within HAB cells (endotoxins) and those associated with the cell's exterior or surrounding waters (exotoxins) or combinations. For example, PSP toxins are typically found within cells, whereas brevetoxins can be released into the water by cell breakage during blooms. Recent work off the west coast of Florida shows that most of these toxins are actually associated with small particles, not truly dissolved in the water column and increase relative to intracellular toxins as a bloom progressed (Landsberg et al. 2000).

Toxins are also characterized by their solubility, e.g., fat-soluble or water-soluble. For the purposes of this review, we divide the toxins into two overall categories: algal toxins known to accumulate in fish and those that do not (or have not been detected in tissues).

Toxins Not Known to Accumulate in Fish Tissues: A variety of disparate HAB species and their toxins apparently do not accumulate in non-visceral fish tissues. Most of these share one important characteristic: they injure or destroy gill tissue very quickly. In this manner, fish are unable to respire and may quickly die of blood hypoxia. In some of these cases, there is conflicting information regarding whether or not damage to the gills occurs in all cases (e.g., *Heterosigma akashiwo*) but in other cases all reports generally concur (e.g., *Gyrodinium aureolum*). The toxic dinoflagellate *G. aureolum* may produce mucilage that can accumulate on the gills. The accumulation may add to the reduction of oxygen transport into the gills that may be already impacted by tissue damage to the epithelium.

TABLE 3.2. Categorization of phytoplankton toxins according to their cellular targets and identified toxin. (Source: Brusele 1995.)

I	=	Icthyotoxins	= general term for HAB toxins that kill fish
N	=	Neurotoxins	= interference with nervous system
Hep	=	Hepatotoxins	= damage or accumulation in the liver
Hem	=	Hemolysins	= destroys red blood cells
C	=	Cytotoxins	= centered mainly on biological membranes

TABLE 3.3. Examples of specific HAB species, associated toxin or chemical, category of toxins, and organ or tissue in fish primarily targeted by that toxin. Key to toxin category given in Table 3.2.

HAB Species	Associated Toxin or Chemical	Toxin Category	Organ or Tissue Targets
Gyrodinium digitatum	?	I, ?	Gills, possibly other tissues
Gymnodinium breve	Brevetoxin	I, N, Hem	Nervous System
Gyrodinium aureolum	Not described ?	I, C	Gill, Gut
Gymnodinium mikimotoi	Not described ?	I, C	Gill
Chattonella marina	PUFA	I, N	Heart
Chattonella antiqua	PUFA	I, N, C	Gill
Heterosigma akashiwo	Superoxide radicals or hydrogen peroxide; brevetoxin-like compound	I, N, C	Gills, Gut (?)
Noctiluca scintillans	Ammonia	I	Gills
Pfiesteria piscicida	Cytotoxic substances?	I, C?	Skin Ulcers, Scale Loss, Secondary Fungus and Bacterial Infections, Inflammation
Prymnesium parvum	prymnesium	I, Hep	Gills

PUFA = polyunsaturated fatty acid. Sources: Okaichi 1991, Brusele 1995; brevetoxin effects from D. Baden, pers. comm.; Ramsdell et al. 2000, Vogelbein et al. 2000.

Toxins Known to Accumulate in Fish Tissues: Several fish toxins discussed elsewhere in this report accumulate in wild fish tissues, via food web sources. The same toxins can affect mariculture fish, via contaminated feed, although this is not known to occur in large, commercial feed preparation. It is more likely to occur in small-scale aquaculture that could utilize raw or unprocessed feeds gathered by individual mariculturists. Ciguatera toxins are well known for their ability to accumulate in fish tissues (e.g., Anderson and Lobel 1987), but there is also good evidence that PSP toxins can do the same. Typically, fish are sensitive to PSP toxins and will die before they accumulate doses that represent a health risk to humans (White 1981). Whales and other marine mammals have died from consuming PSP toxin-contaminated fish (e.g., Geraci et al. 1989), but the quantity of fish consumed was enormous. Filter feeding fish such as sardines and herring can accumulate these toxins (or more appropriately can carry large amounts of undigested algae in their stomachs), and this has resulted in human poisonings in populations who eat whole fish. Another group of toxins (brevetoxins produced by *Gymnodinium* spp.) may contaminate fish flesh as well, but so far the evidence is limited and only showed low levels not thought to represent a threat to humans (D. Baden, pers. comm).

Unfortunately, the group of toxins that kill the most mariculture fish (those from *Heterosigma*, *Chattonella*, and the *Gymnodinium/Gyrodinium* complex) remain poorly or only partially characterized chemically and poorly studied with respect to accumulation in fish. Although we believe these toxins do not accumulate in fish to any significant extent, as discussed above, this is an area where information is clearly lacking, and thus where considerable research effort is needed.

3.2   Action or Regulatory Limits for Toxins and Cells

3.2.1   Shellfish

At present there is a general consensus in the international community on the action or regulatory limits for algal toxins in shellfish, though there are some differences as well. Regulatory (action) limits and details of the analytical methods and assays used for these toxins are given in Section 3.1, but these limits are summarized below as well.

ASP toxins are monitored in many countries/regions with commercial shellfisheries. HPLC analysis is used alone or in combination with the mouse bioassay (Table 3.4). The action limit in Canada is 2 mg100g^{-1} g (= 2 ppm) of bivalve meat. In the USA, the action limit in crab meat is 3 mg 100g^{-1}. In the EU, testing for DA has been required since 1997, when an action limit of 20 µg g^{-1} was established for shellfish (whole animal or edible part) (Council Dire4ctive 97/6/EC of 20 October 1997).

TABLE 3.4. ASP toxin detection methods and action limits. (Source: Andersen 1996).

Country	ASP Action limit	Method of analysis
Canada	2 mg 100 g^{-1}	HPLC
Denmark	2 mg 100 g^{-1}	HPLC
France	2 mg 100 g^{-1}	HPLC
Netherlands	2 mg 100 g^{-1}	HPLC
New Zealand	2 mg 100 g^{-1}	HPLC
Spain (Galicia)	2 mg 100 g^{-1}	Mouse bioassay and HPLC
USA	2 mg 100 g^{-1} (3 mg 100 g^{-1} crab meat)	HLPC

DSP toxins are monitored using bioassay in many countries, in some cases supplemented by chemical methods, most frequently HPLC. The mouse bioassay is used in most countries/regions, whereas the rat bioassay is used in a few countries (Netherlands and UK-Northern Ireland) (Table 3.5). For all European Union (EU) countries, Council Directive No L 268, of 15 July 1991 states that "the customary biological testing methods must not give positive results to the presence of Diarrhetic Shellfish Poison (DSP) in the edible parts of molluscs (the whole body or any part edible separately)." It is likely that a new EU directive will approve analysis using HPLC with fluorimetric detection providing that it can detect all analogues.

TABLE 3.5. DSP toxin detection methods and action limits. (Source: Andersen 1996).

Country	DSP	
	Action limit	**Method of analysis**
Canada	20 µg 100 g^{-1}	Mouse bioassay, HPLC, ELISA
Denmark	Presence (2 of 3 mice die within 24 h)	Mouse bioassay, HPLC
France	Presence (2 of 3 mice die within 5 h)	Mouse bioassay
Italy	5 hour mouse test	Mouse bioassay
Ireland	Positive bioassay	Mouse bioassay & LC-MS
Japan	5MU/100 g (=20 µg 100 g^{-1})	Mouse bioassay
Korea	5MU/100 g (=20 µg 100 g^{-1})	Mouse bioassay
Netherlands	0.2-0.4 µg g^{-1} digestive gland	Rat bioassay
New Zealand	20 µg 100 g^{-1}	Mouse bioassay & LC-MS
Norway	5-7MU100g^{-1} (= 20-30 µg 100 g^{-1})	Mouse bioassay
Portugal	Presence (20 µg 100g^{-1})	Mouse bioassay
Spain	Presence	Mouse bioassay
Sweden	16 µg 100 g^{-1}	HPLC*
Uruguay	Mortality in 24 h.	Mouse bioassay
UK (Northern Ireland)	20 µg 100 g^{-1}	Rat bioassay

*Ann-Sofi Rehnstam-Holm, Goteborg University (pers. comm.)

PSP toxins are monitored in most countries using mouse bioassay (AOAC 1995), except for the Netherlands where HPLC analysis is used alone (Table 3.6). In Denmark, Japan and UK-Scotland the bioassay is supplemented by HPLC analysis. The action limit of 80 µg 100g^{-1}, equivalent to ~ 400MU100g^{-1} if the conversion factor is 0.2 µg STXeq MU^{-1}, is used in most countries for PSP toxins. This concentration is the official action limit in all EU countries. In the Philippines and Norway the critical action limit is only 40 µg 100g^{-1} (200MU100g^{-1}). In UK-Northern Ireland the action limit is 32 µg 100g^{-1}. This is simply the detection limit of PSP toxins measured by mouse bioassay (Table 3.6).

For Canada, products having PSP-toxin concentrations up to 160 µg 100g^{-1} may be canned. For southern Spain, some canned shellfish products can contain PSP toxins up to 300 µg 100g^{-1}.

NSP toxins: the action limits presently used in the US include detectable toxin levels in shellfish tissues, as measured by the APHA method, and *Gymnodinium breve* concentrations exceeding 5000 cells L^{-1} (ISSC, 1997). Harvesting areas are re-opened when mouse bioassay results indicate that shellfish from closed areas contain < 20MU 100g^{-1}. New Zealand also uses an action limit in shellfish tissues of 20MU 100g^{-1} using ether extraction and a 5-mouse bioassay.

TABLE 3.6. PSP toxin detection methods and action limits. N/A = not available. (Source: Andersen 1996.) 1MU 100g^{-1} ~ = 0.18 μgSTX-equivalents 100g^{-1} (Premazzi and Volterra 1993).

Country	PSP	
	Action limit	Method of analysis
Australia	80 μg 100g^{-1}	Mouse bioassay
Austria	80 μg 100g^{-1}	Mouse bioassay
Canada	80 μg 100g^{-1}	Mouse bioassay
Denmark	80 μg 100g^{-1}	Mouse bioassay, HPLC
Finland	80 μg 100g^{-1}	N/A
France	80 μg 100g^{-1}	Mouse bioassay
Germany	80 μg 100g^{-1}	N/A
Greece	80 μg 100g^{-1}	N/A
Guatemala	400MU100g^{-1} (~μg 100g^{-1})	Mouse bioassay
Hong Kong	400MU100g^{-1} (~μg 100g^{-1})	Mouse bioassay
Italy	80 μg 100g^{-1}	Mouse bioassay
Ireland	Positive bioassay	Mouse bioassay
Japan	400MU100g^{-1} (~μg 100g^{-1})	Mouse bioassay
Korea	400MU100g^{-1} (~ 30 μg 100g^{-1})	Mouse bioassay
Netherlands	80 μg 100g^{-1}	HPLC
New Zealand	80 μg 100g^{-1}	Mouse bioassay
Norway	200MU100g^{-1} (~ 15 μg 100g^{-1})	Mouse bioassay
Panama	400MU100g^{-1} (~ 30 μg 100g^{-1})	Mouse bioassay
Philippines	40 μg 100g^{-1}	Mouse bioassay
Portugal	80 μg 100g^{-1}	Mouse bioassay
Spain	80 μg 100g^{-1}	Mouse bioassay
Singapore	80 μg 100g^{-1}	Mouse bioassay
Sweden	80 μg 100g^{-1}	Mouse bioassay
Uruguay	80 μg 100g^{-1}	Mouse bioassay
USA	80 μg 100g^{-1}	Mouse bioassay
UK (Scotland)	80 μg 100g^{-1}	Mouse bioassay, HPLC
UK (England and Wales)	80 μg 100g^{-1}	Mouse bioassay
UK (Northern Ireland)	80 μg 100g^{-1}	Mouse bioassay
Venezuela	200-400 MU 100g^{-1} (~ 15-30 μg 100g^{-1})	Mouse bioassay

AZP toxins are presently only monitored in Ireland. The Food Safety Authority of Ireland is using a level of 0.1 μg g^{-1} of whole flesh as an interim action limit (T. McMahon, pers. comm.). There is as yet no official EU action limit for AZP toxins, but discussions are ongoing and action is likely. If adopted, LC-MS can be used as long as it can detect the analogues AZA1, AZA2 and AZA3. Likewise, functional asays and ELISA tests may be used if they can be shown to detect all analogues.

Cell concentrations of HAB species used as action limits. At present there is no general consensus on action limits for algal cell concentrations that should be used for the management of potentially toxic shellfish (Table 3.7). In many cases, cell concentrations are used as guidance to initiate flesh testing in shellfish, but in some cases, such as with NSP in Florida, cell concentrations of *Gymnodinium breve* are used as the decision criterion for harvesting closures. A questionnaire circulated in 1995 showed that cell concentration limits existed for 27 HAB species or species-groups, belonging to the genera *Alexandrium*, *Aureococcus*, *Dinophysis*, *Gonyaulax*, *Gymnodinium*, *Nodularia*, *Prorocentrum*, *Pseudo-nitzschia*,

Ptychodiscus (= *Gymnodinium*) and *Pyrodinium* (Andersen 1996). The results of the survey (summarized in Table 3.7) indicate that action limits based on cell concentrations can be highly variable between countries, and eventual restrictions implemented are not clear in many cases.

TABLE 3.7. Cell concentrations of HAB species that result in implementation of restrictions on shellfisheries. (Source: Andersen 1996)

Species/country-region	Cell concentration (cells L^{-1})	Implemented actions
Alexandrium catenella Australia, Spain-Valencia	$> 4 \times 10^4$ 2×10^7-5×10^7	measure toxins?
Alexandrium fundyense Canada	Presence	measure toxins
Alexandrium minutum Spain-Balearic Islands	10^3	intensified monitoring/closed
Alexandrium ostenfeldii Denmark UK-Northern Ireland UK-Scotland	500 presence presence	intens. monitor., closures, restrictions?? restrictions??
Alexandrium tamarense Denmark	500	intensified monitoring, closures
Alexandrium sp. Denmark	500	intensified monitoring, closures
Alexandrium spp. Netherlands Norway Spain-Balearic Islands	10^3-10^4 presence in net-hauls 10^3	restrictions-alert/closures? restrictions/closed restrictions/closed??
Dinophysis acuminata Denmark Portugal Spain-Balearic Islands Spain-Valencia Region	500 200 10^3 2×10^7-5×10^7	intensified monitoring/closures restricted restrictions/closures?? measure toxins??
Dinophysis acuta Denmark Portugal	500 200	intensified monitoring, closures restricted
Dinophysis norvegica Denmark	10^3	intensified monitoring, closures
Dinophysis rotundata Denmark	10^3	intensified monitoring, closures
Dinophysis sacculus Spain-Valencia	2×10^7-5×10^7	measure toxins
Dinophysis spp.* Italy Netherlands Norway UK-Northern Ireland UK-Scotland Spain-Andalusia	10^3 100 10^3 >100 >100 ?	restrictions?? restrictions=alert closure restrictions?? restrictions?? restrictions??

TABLE 3.7. (Continued)

Species/country-region	Cell concentration (cells L^{-1})	Implemented actions
Total *Dinophysis* spp. Denmark Italy Norway	1.2 x 10^3 10^3 and DSP in mussels 500-1.2 x 10^3	Intensified monitoring, closures restrictions/closure - depending on species
Gymnodinium catenatum Spain-Andalucia UK-Northern Ireland	>500 presence	restrictions? restrictions?
***Gymnodinium* sp.** Venezuela	no concentration limit	restricted
Nodularia spumigena Denmark	1 x 10^5-2 x 10^5 coloniesL^{-1}	intensified monitoring
Prorocentrum lima Denmark UK-Northern Ireland UK-Scotland	500 presence presence	intensified monitoring/closures restrictions? restrictions?
***Pseudo-nitzschia seriata*-group** Denmark	2 x 10^5	intensified monitoring/ closures
***Pseudo-nitzschia delicatissima*-group** Denmark	2 x 10^5	intensified monitoring/ closed
Pseudo-nitzschia multiseries Canada	5 x 10^4	monitor shellfish
Pseudo-nitzschia pseudodelicatissima Canada	1 x 10^5	measure toxins
Pseudo-nitzschia pungens UK-Northern Ireland	>10^3	restrictions??
***Pseudo-nitzschia* spp.** Netherlands	10^4-10^5	restrictions-alert/closures
Gymnodinium breve USA-Florida	>5 x 10^3	remain closed if toxins previously detected
Pyrodinium bahamense* var. *compressum Philippines	200	restrictions?

In several countries, the presence of the PSP-toxin producing *Alexandrium fundyense, Alexandrium minutum, Alexandrium ostenfeldii, Alexandrium tamarense* and an unidentified *Alexandrium*-species in concentrations from detectable to 10^3 cells L^{-1} requires analysis for toxins in shellfish or closing of areas for harvesting in several countries. Concentrations of *Alexandrium catenella* in Australia and part of Spain may reach >10^4 cells L^{-1} before closures are initiated (Table 3.7). In Norway a semi-quantitative measure based on net samples leads to analysis of toxins in shellfish if *Alexandrium* is present.

For the different species within the DSP-toxin producing genus *Dinophysis,* concentrations from < 10^2 - 10^3 cells L^{-1} impose restrictions in most countries/regions, with the exception of the Valencia region in Spain, where the concentration of *Dinophysis sacculus* and *Dinophysis acuminata* may reach much higher concentrations (> 10^7 cells L^{-1}) before action is taken (Table 3.7). For *Gymnodinium catenatum,* regulations are initiated at concentrations from presence to 2 x 10^3 cells L^{-1}. Concentration limits for the

filamentous cyanobacterium (blue-green alga) *Nodularia spumigena* only exist in Denmark (10^5 colonies/L). For *Prorocentrum*, action limits only exist for *Prorocentrum lima*, and they are within the range from detectable to 500 cells L^{-1}. For the ASP toxin-producing diatom genus *Pseudo-nitzschia*, concentrations from 10^3 to 2×10^5 trigger actions such as intensified monitoring (Table 3.7). In most cases intensified monitoring involves HPLC analysis of shellfish extracts. For *Gymnodinium breve* shellfish harvesting restrictions are imposed in Florida at concentrations > 5×10^3 cells L^{-1}. For *Pyrodinium bahamense* var. *compressum*, in the Philippines, closures of shellfish harvests occur at a concentration of 200 cells L^{-1}.

There are clearly large differences in concentration levels among different species from the same genus that trigger actions. This is the result of variations in toxicity between those species. The difference in cell concentration limits within a single species presumably reflects variability in the toxicity of strains or populations of the species, or simply the manner in which the regulations were determined by each country.

3.2.2 Finfish

There are no regulatory limits for toxins in fish tissue, for reasons explained elsewhere in this document. Recently, Rensel and Whyte (2001) assembled data from various sources to illustrate concentrations of fish killing HABs that should elicit alert or mitigation responses from fish growers. The table is reproduced here as Table 3.8.

TABLE 3.8. Harmful phytoplankton species known or suspected of causing fish losses in mariculture, recommended action concentrations and a few pertinent references. Most cited cell concentrations are gross approximations, and will vary considerably from case to case. (Source: Rensel and Whyte 2001).

HAB Category and Species	Action Level to Intensify Management	Action Level to Initiate Mitigation	References
DIATOMS and MIXTURES			
Chaetoceros concavicornis, C. convolutus and other. Subgenus Phaeoceros, possibly *C. danicus*	> 2-5 cells ml^{-1}	> 5 cells ml^{-1}	Bell 1961; Bruno et al. 1989; Rensel 1992, 1993; Taylor 1993, Albright et al. 1993; Taylor and Horner 1994; Yang and Albright 1994b
Leptocylindrus minimus	1,000 to 10,000 cells ml^{-1}	> 10,000 cells ml^{-1}	Clément 1994; Clément and Lembeye 1993
Other Harmful Diatoms Including some *Chaetoceros* subgenus Hyalochaete and in some cases: *Corethron criophilum, Skeletonema costatum, Thalassiosira spp.*	> 50,000 to 100,000 cells ml^{-1}, depending on sensitivity of fish and life stage	> 100,000 cells ml^{-1}, especially if juvenile fish reared	Kim 1998b; Speare et al. 1989; Kent and Poppe 1998
Other Diatoms and Dinoflagellates mixtures	> 40,000 to 80,000 cells ml^{-1} if > 50% dinoflagellates	> 80,000 cells ml^{-1} if > 50% dinoflagellates	Kim 1998b

TABLE 3.8. (Continued)

HAB Category and Species	Action Level to Intensify Management	Action Level to Initiate Mitigation	References
DINOFLAGELLATES			
Alexandrium tamarense	Unknown; acute mortality to farmed fish not well documented and may not occur; mortality and potential food web toxin accumulation for wild fish		Mortenson 1985; Erickson 1988 (for *A. catenella*)
Ceratium fusus	Unknown; gill irritation, poorly understood, affects oyster larvae and shrimp		Rensel and Prentice 1980; Cardwell et al. 1979
Cochlodinium spp. *C. polykrikoides*	300 to 1,000 cells ml^{-1}, Aeration induces lethality	1,000 cells ml^{-1}	Kim 1998a; Yuki and Yoshimatsu 1989; Whyte et al. 2000
Gymnodinium breve	5 -10 cells ml^{-1}, can co-occur with *G. mikimotoi*	>10 – 25 cells ml^{-1}	Ray and Wilson 1957 Steidinger et al. 1998a
Gymnodinium digitatum	unknown, order of magnitude estimates possible		Anderson et al. 1999
Gymnodinium mikimotoi	1,000 to 3,000 cells ml^{-1}	> 3,000 cells ml^{-1}	Kim 1998b
Gyrodinium aureolum	500 to 2,000 cells ml^{-1}	> 2,000 cells ml^{-1}	Kim 1998b; Takayama and Adachi 1984; Okaichi 1989; Tangen, 1977
Noctiluca scintillans	Unlikely to be a problem, un-ionized ammonia dependent on pH and temperature		Okaichi and Nishio 1976
PRYMNESIOPHYTE FLAGELLATES			
Chrysochromulina polylepis, *C. leadbeateri*	unknown; causes gill damage and osmoregulatory problems, threshold of damage not defined		Estep and MacIntyre 1989; Aune et al. 1992; Skreslet et al. 1993
Phaeocystis pouchetii	unknown; irritant substances and the alga's mucus can clog gills		Lancelot et al. 1987; Gaines and Taylor 1986
Prymnesium parvum, *P. patelliferum*	unknown; toxins cause tissue, blood-cell and neurological damage		Shilo 1982; Guo et al. 1996; Larsen and Bryant 1998
RAPHIDOPHYTE FLAGELLATES			
Chattonella antiqua (possibly *C. marina*)	1 - 50 cells ml^{-1}	Conflicting data. 50 to 500,000 cells ml^{-1}, may be dependent on fish species, size, etc.	Okaichi et al. 1989; Onoue et al. 1990; Tanaka et al. 1994; Kim 1998b, Hallegraeff et al. 1998.
Heterosigma akashiwo	> about 50 cells ml^{-1}, less if very calm and warm weather	variable to non-toxic, some cases, in others >750-1,000 cells ml^{-1}	Black et al. 1991; Taylor and Haigh 1993; Chang et al. 1990, 1993.
SILICOFLAGELLATES			
Dictyocha speculum (= *Distephanus speculum*)	unknown; siliceous skeleton may irritate gills, also possible toxin action.		Thomsen and Moestrup 1985; Larsen and Moestrup 1989; Erard-Le Denn and Ryckaert 1990

3.3 Phytoplankton Cell Detection

3.3.1 Sampling of planktonic algae

One important area of monitoring involves that of HAB algae directly – counts of individual species that are used to guide either management decisions or additional monitoring activities. A detailed compilation of methodologies used for HAB monitoring is provided in the IOC *Manual on Harmful Marine Microplankton* (Hallegraeff et al. 1997). This manual is presently out of print, but is available on the web at: **http://unescodoc.unesco.org/images/0012/001220021eo.pdf**. A second edition of the manual is in preparation at this time, and should be available in 2002. Another resource is a brief review of routine light/epifluorescence microscopy-based methods used for quantitative analysis of phytoplankton samples, presented in Andersen (1996).

Within all EU countries, or for countries wishing to export shellfish product to the EU, Council Directive No L 268 of 15 July 1991 provides the following general guidance with respect to monitoring of HAB cells:

"The control system must include:

1. Periodic monitoring of live bivalve molluscs in relaying and production areas in order to:
    (a) [not relevant]
    (b) [not relevant]
    (c) check the possible presence of toxin-producing plankton in production and relaying waters and biotoxins in live bivalve molluscs."

2. Sampling plans, as provided for in point 1, must in particular take account of:
    (a) possible variations in production at relaying areas in the presence of plankton containing marine biotoxins. The sampling must be carried out as follows:

> (i) monitoring: periodic sampling organized to detect changes in the composition of the plankton containing toxins and the geographical distribution thereof. Information leading to a suspicion of accumulation of toxins in mollusc flesh must be followed by intensive sampling.

> (ii) intensive sampling: monitoring plankton in the growing and fishing waters by increasing the number of sampling points and the number of samples, [supplemented with] toxicity testing using the molluscs from the affected area which are most susceptible to contamination."

Sampling of harmful algae should take place as close as possible to the resources to be protected. Furthermore, additional sampling should be conducted at "key" stations representing the different water masses in the investigation area - see Franks (1995) for further information on strategies of station location. In periods of higher risk of HABs, sampling should be intensified in time and space, that is, carried out at least weekly and at more stations than during normal monitoring. During HAB development, sampling should be further intensified, e.g., to sampling every few days. It is important to recognize that the relatively high growth rates of phytoplankton can result in very rapid increases in the concentrations of blooming species. For that reason it is very important to monitor HAB species at low concentrations and to utilize methods that can detect cells at less than bloom densities. Consideration of time of day and possibly tidal period should also be made in constructing a sampling plan, for example, land breezes on hot afternoon days can cause mixing of cells in the surface waters that may result in lower cell counts than at

other times of the day. Similarly, temporal effects of vertical migration of some species can mask peak cell concentrations.

Qualitative samples are best obtained using a plankton net (mesh size ~20 μm). The samples are collected by vertical tows to cover the depth range of interest. In shallow waters (depth < 20 m) the net should be towed several times from the bottom to the surface of the water or until the water in the sample collector becomes unclear or colored by the concentrated algae.

Quantitative samples are collected using a water bottle (e.g., Niskin Bottle, Nansen Bottle) or a sampling hose capable of collecting water at different depths over the depth range of interest. Depth intervals between samplings should be 2-5 m, depending on local conditions. If resources are limited, samples from the different depths are sometimes pooled to yield a single count representative of the whole water column or mixed layer. If there is a pycnocline at a certain depth, or if the measurements of fluorescence or light attenuation indicate a subsurface bloom of phytoplankton, a quantitative sample should be collected from that subsurface layer for analysis of the species composition and the actual concentration and /or biomass of HAB species.

An alternative to sampling using water bottles utilizes a segmented hose as described by Lindahl (1986). This is a low cost method that provides integrated vertical profiles of cell distributions, but is more suited for quiescent waters than in tidally active areas unless conducted from a drifting boat.

It is very important that all equipment used for sampling be properly cleaned before or after use. This should be done to remove corrosive salt residue and to avoid contamination of the samples from phytoplankton from previous samplings. Furthermore, it is recommended that all equipment used for sampling be rinsed with water from the sampling location before the actual samples are collected.

3.3.2   Sampling of benthic microalgae

Several methods have been used to quantify benthic dinoflagellates that can cause ciguatera (Bagnis et al. 1980; Quod et al. 1995; McCafferey et al. 1992). According to Quod et al. (1995), the following procedure can be used:

1. Collection of macroalgae (20g).
2. The macroalgae are placed in sealed plastic bags and vigorously shaken in seawater.
3. The seawater is sieved (mesh size 150μm) to separate the microalgae from the macroalgae and other large particulate material.
4. The dinoflagellates in the < 150μm fraction are counted using a compound microscope and an appropriate counting chamber. Calcafluor and epifluorescence microscopy can also be used, according to Andersen and Kristensen (1995).

3.3.3   Fixation/preservation of algal samples

Immediately after collection, phytoplankton samples must be preserved for subsequent analysis. (Live samples can be very useful as a supplement to the fixed samples for taxonomic investigations, especially for fragile HAB genera such as *Chattonella, Heterosigma, Gymnodinium,* and *Pfiesteria*). In other cases, swimming action and flagella arrangement that are diagnostic of certain species can be viewed with live samples. Phytoplankton samples (and especially the fragile forms listed above) can be preserved using either neutral or acidic Lugol's, which produce preparations suitable for light microscopy, and which are non-toxic to humans. If the brownish staining of the phytoplankton caused by Lugol's poses a problem in

the taxonomic investigations, the color can be removed by adding a few drops of a sodium thiosulfate stock solution prepared at 3 g $Na_2S_2O_3$ to 100 ml of water; Pomeroy 1984). If the algal samples are to be stored for longer periods (> 6 months), more Lugol's will have to be added to keep the samples properly preserved. Glutaraldehyde (2-5% solution) is also fairly effective for some species, especially when fixation is carried out at 4°C. Once again, care should be taken to avoid exposure to glutaraldehyde fumes.

Formaldehyde is also a useful fixative, especially for armored cells such as dinoflagellates, but it should be used with care. Not only is it dangerous to humans as a chemical, but it can destroy fragile phytoplankton species. A fume hood and well-ventilated working area are recommended. A 2-5% solution of formalin (buffered to saturation with sodium bicarbonate or borate) is commonly used for plankton samples.

3.3.4   Labeling and storage

All samples must be properly labeled with information on station number/name and position, date, content of the sample, which preservative was used (Lugol's, formaldehyde, etc.) and the identification code of the staff responsible for sampling. Phytoplankton samples are best stored in glass bottles. For long term storage (>1/2 year), glass bottles with sealing lids are preferred because formaldehyde will not evaporate from the sample. In the case where the samples are preserved using Lugol's, glass bottles are also preferred because oxygen will not enter the sample and oxidize the iodine. On the other hand, if samples are to be analyzed immediately after sampling, plastic bottles are preferred because they are easier and safer to handle than glass. A cool, dark environment is generally best for storage of preserved samples in bottles.

3.3.5   Volunteer plankton monitoring programs

In response to the onset of domoic acid shellfish poisoning events along the U.S. west coast in the early 1990's, the US FDA provided funds for monitoring of harmful phytoplankton species by a volunteer network of interested individuals. The goal of the program was to learn more about the distribution and occurrence of the species, to help in management of the problem and possibly lead to early warning mechanisms, all at a reasonable cost. Another goal was to get the public involved in HAB monitoring so that the topic would not be so foreign and worrisome to many. From that initial US West Coast program, other volunteer programs for HABs have been established, such as those in Maine and Massachusetts.

In the California program, a series of workshops was organized to educate and standardize procedures for sampling and analysis of phytoplankton samples. Training was conducted in all three Pacific coastal states and was attended by fish farmers, shellfish farmers/fishers, agency staff and private citizens who would help in sample collection. A collection, co-ordination, and analysis scheme was prepared and sampling was initiated. The samples were collected by the volunteers, who would examine them briefly and then send the preserved material to a skilled phytoplankton expert for enumeration. Monthly newsletters are then issued summarizing the distribution and density of HAB species, especially *Alexandrium catenella* and *Pseudonitszchia* spp. The California Department of Health conducts this program, and has a part-time phytoplankton taxonomist dedicated to the analyses. Volunteer samplers continue to make a valuable contribution to the effort by collecting and shipping the samples from remote locations. All of the above is done in addition to the normal biomonitoring of shellfish toxins that is funded and conducted by each state.

3.3.6 New Cell Detection Methods

A common problem in monitoring programs focused on HAB species occurs when the species of interest is only a minor component of the planktonic assemblage. Many potentially useful measurements are not feasible because of the co-occurrence of numerous organisms and detritus. Another constraint arises from difficulties in identifying and distinguishing between morphologically similar species or strains. This is a problem not only for those with limited taxonomic training, but also for skilled taxonomists, since considerable time and effort are required to identify a species when its distinguishing characteristics are difficult to discern under the light microscope. Such fine levels of discrimination are often not feasible in monitoring programs or studies that generate large numbers of samples for cell enumeration.

This situation is encountered frequently in studies of HABs. For example, the diatom *Pseudo-nitzschia pungens* occurs in two varieties, one toxic and the other non-toxic, but these cannot be distinguished from each other using the light microscope (Smith et al. 1990). Likewise, toxic and non-toxic varieties of the dinoflagellates *Alexandrium tamarense* and *Gymnodinium catenatum* occur (Yentsch et al. 1978; Oshima et al. 1993), as do morphologically similar *A. tamarense* and *A. catenella* (Cembella et al. 1987).

As a result of these problems and constraints, the scientific community has been working towards the development of species- or strain-specific "probes" that can be used to label only the cells of interest so they can then be detected visually, electronically, or chemically. Progress has been rapid and probes of several different types are now available for many of the harmful algae, along with techniques for their application in the rapid and accurate identification, enumeration, and isolation of individual species. Reviews of these methods are available in Anderson (1995), Scholin and Anderson (1998), and in a new chapter by Scholin et al., in the second edition of the *Manual for Harmful Marine Microalgae* (Hallegraeff et al., in press.) The latter was used extensively in updating the following sections.

3.3.6.1 Antibodies

Basic Principles. Shapiro et al. (1989) provide a useful review of the application of immunological techniques to marine phytoplankton identification. The approach involves the use of antibodies that bind specifically to proteins in the cell walls of the algal species of interest. Antibodies are produced by inoculating cells of target species into animals, which then produce antibodies in response to the presence of the intact foreign organism or compounds derived from it. The target molecule against which the antibody is directed (termed an antigen) is typically, but not necessarily, a cell wall protein. Fortunately, it is not necessary to purify specific proteins in order to produce antibodies.

Most immunological assay methods for cell identification use indirect immunofluorescence for visualization or detection of the label (e.g., Anderson et al. 1989). Cells are first exposed to the primary antibody (i.e., the one produced by the inoculated, host animal) and then to a secondary antibody which will bind to all antibodies produced by that particular host animal. The secondary antibody is typically conjugated to a reporter molecule such as fluorescein isothiocyanate (FITC). Visual detection of the labeling is possible using an epifluorescence microscope. Alternatively, samples can be processed using a flow cytometer or other instrument that can detect and quantify fluorescence. Assays can also be conducted on filters using fluorescent or colorimetric detection. Under the microscope, the fluorescent label is often visible as a colored "halo" or ring outlining the periphery of the cell.

Table 3.9 lists the harmful algal species for which high-specificity polyclonal and monoclonal antibodies (PAbs and MAbs) have been developed. To the surprise of many, the specificity obtained thus far with PAbs has been remarkable, despite the potential for cross-reactions inherent in such preparations. For example, a PAb developed for the brown tide chrysophyte *Aureococcus anophagefferens* is species-

specific, showing no cross reactions with 46 phytoplankton cultures representing 5 algal classes, including 20 species from the Chrysophycophyta (Anderson et al. 1989). An even higher level of specificity was demonstrated for the diatom *Pseudo-nitzschia pungens*, where PAbs were able to distinguish toxic from non-toxic varieties of the same species (Bates et al. 1993b). These strains were later separated into different species (Hasle 1994).

Similar specificities have been obtained using MAbs. An MAb produced by Nagasaki et al. (1991) labeled nine strains of *G. nagasakiense* from Japan, but not *G. breve*, *G. catenatum*, or a western European strain of *Gyrodinium aureolum*. In contrast, sixteen MAb antisera produced against *G. aureolum* from western Europe cross-reacted with *G. nagasakiense* and *G. mikimotoi* (Vrieling et al. 1994). These results emphasize the need to establish multiple monoclonal cell lines, as MAbs are highly specific for individual epitopes that may not be unique to the target species. A related concern is that the number of epitopes labeled by an antiserum and the number of those epitopes on a cell directly affects the intensity of immunofluorescence and therefore the detectability of the signal.

TABLE 3.9. Antibody probes developed for HAB species. (Modified from Anderson 1995.)

Species	Type*	Specificity	Reference
Alexandrium catenella *Alexandrium tamarense*	MAb	strain	Sako et al. (1993); Adachi et al.(1993)
Aureococcus anophageferens	PAb MAb	species	Anderson et al. (1989) D. Caron (unpub.)
Chatonella marina *Chatonella antiqua*	MAb	strain or species	Nagasaki et al. (1989)
Cryptoperidiniopsis sp.	PAb	species	D. M. Anderson (unpub.)
Gymnodinium nagasakiense	MAb	species	Nagasaki et al. (1991)
Gyrodinium aureolum	MAb	species or complex	Vrieling et al. (1993; 1994)
Pseudo-nitzschia pungens *Pseudo-nitzschia multiseries*	PAb	strain	Bates et al. (1993b)

*MAb = Monoclonal antibody; PAb = Polyclonal antibody

3.3.6.2 Nucelotide probes

Basic Principles. In recent years, use of nucleic acid probe technology to detect microorganisms has expanded considerably. This technology is used extensively in the detection of pathogenic bacteria and other microbes, and is now being applied to HAB species. The procedure involves the detection of target nucleic acid sequences by binding (hybridizing) those sequences to a short strand of DNA containing a homologous complementary sequence. Extraordinary sensitivity and specificity are possible with well-designed probes. Many DNA or RNA sequences can be targeted in the organism of interest, including fragments of genes, spacer regions between genes, repeated (non-transcribed) sequences, and transcribed genes.

The first step in probe development is the identification of a unique series of RNA or DNA bases that are only found in that organism. Typically, target genes have sequence domains that are highly conserved among all organisms, and that are thus not useful in discrimination, as well as other domains that are variable to different degrees. It is the latter which are the target areas for probe development. If resolution is sought at the genus, species, or even sub-species levels, the most rapidly evolving, highly variable,

domains are targeted, such as those in the intergenic transcribed spacer regions of ribosomal RNA (rRNA). Short, contiguous segments (approximately 20 nucleotides) of sequence are identified that serve as targets for probes. "Oligonucleotide" probes are synthesized and used in a variety of formats to detect the cells of interest.

Oligonucleotide (DNA) probes for identifying HAB species applied in the whole cell format are typically directed against sequences of the small subunit (18S or SSU), large subunit (28S or LSU) and the intergenic transcribed spacers (ITS1, ITS2) of the rRNA cistron (e.g., Adachi et al. 1996; Anderson et al. 1999; Cangelosi et al. 1997; Miller and Scholin 1996, 1998; Rublee et al. 1999). Much of this work, especially as it relates to field surveys, has focused on species of *Alexandrium, Pseudo-nitzschia, Pfiesteria*, and *Pfiesteria*-like organisms, but *Heterosigma akashiwo, Chattonella* and *Fibrocapsa* (J. Tyrell, pers. comm.; Edvardsen and Medlin, unpub. data), and *Dinophysis* spp. (Edvardsen and Medlin, unpub. data; Rhenstam-Holm and Anderson, unpub. data), have also been sequenced and targeted for probe design.

One way to use these probes is through fluorescent *in situ* hybridization (FISH) using intact cells that are either immobilized on a microscope slide or suspended in solution. In this "whole cell" format, the probe enters the cell and binds to target sequences, excess probe is washed out, and the complex is detected with fluorescence or radioactivity. Similar to antibodies, oligonucleotide probes can be labeled with a variety of fluorescent dyes.

Many assays for bacteria and other prokaryotes immobilize extracted DNA on a solid surface such as a nitrocellulose or nylon membrane to which the probe is added and allowed to hybridize and establish a double-stranded molecule (Macario and Macario 1990). Excess unbound probe is washed off, and the hybrid (target + probe) sequence is detected using radioactivity, fluorescence, chemiluminescence, or colorimetric methods. Modifications of this procedure exist, such as the sandwich hybridization assay in which two probes are used - one to capture the target DNA and bind it to a solid surface, and the other to permit detection.

For HAB species, the sandwich hybridization technique involves collection of a sample onto a filter, after which a lysis solution and heat are used to break cells and liberate nucleic acids. The resulting cell lysate is then dispensed to a pre-packaged 96-well test plate and processed automatically in a relatively simple benchtop system (Scholin et al. 1997, 1999). One hybridization reaction captures the target nucleic acid sequences (DNA or RNA) from the crude lysate using an oligonucleotide tethered to a solid support, and a second reaction binds a signal probe to a different sequence on the target nucleic acid. Visualization of the probe "sandwich" can be enzymatic, yielding colorimetric or chemiluminescent products that provide a measure of the abundance of target species in the original sample (e.g., Scholin et al. 1999, 2000). Sandwich hybridization offers a potentially faster mode of sample processing than whole cell assays, especially when large numbers of samples must be processed rapidly. Detection of multiple species in a single sample simultaneously is also possible. The assay can be performed in the laboratory as well as aboard ships.

Detection methods for HAB species have also been developed that employ the polymerase chain reaction (PCR). As with other probe techniques, one needs an appropriate nucleic acid target sequence that has the desired specificity at the species, genus or other taxonomic level. Here again, rRNA genes (rDNA) have been most common for HAB assays since they include highly conserved regions common to most organisms. Once a potential signature sequence is identified, a pair of oligonucleotide primers (forward and reverse) is designed to bind to unique sequences within or bordering that target. The PCR reaction is run in a thermalcycler that regulates temperature during the reaction process. Examples of specific reaction conditions and components of the reaction mixture can be found in Bolch et al. (1999) and Penna and Magnani (1999, 2000) for *Alexandrium* sp., and in Rublee, et al. (1999) and Oldach et al. (2000) for

Pfiesteria spp. Reaction products are generally visualized by agarose gel electrophoresis followed by staining with ethidium bromide, syber green, or some other nucleic acid stain.

Recently, Bowers et al. (2000) described real-time PCR assays for *Pfiesteria* sp. In this assay, an oligonucleotide probe with both a fluorophore and a quencher molecule (Taqman™) are used in addition to oligonucleotide primers, and an instrument capable of excitation and detection of fluorescent signals. Fluorescence is related to the number of amplicons (= free fluorescent molecules in solution) and thus to the number of target molecules in the initial reaction mixture.

Yet another PCR-based method is the heteroduplex mobility assay (HMA) (Uribe et al. 1999; Oldach et al. (2000). These are particularly valuable for identifying unknown cultures or for determining the purity or clonality of a culture. Their use in evaluation of field samples can be problematic however, since a field sample containing multiple species in the taxon of interest will generate multiple bands that can rapidly become impossible to analyze.

3.3.6.3 Lectins

Lectins are non-enzymatic proteins (commonly glycoproteins) that bind non-covalently to specific sugar residues at cell surfaces. Potential binding sites associated with microalgae include cell surface glycoproteins, polysaccharides, and chitin (Sengbusch and Müller 1983; Waite et al. 1995; Hori et al. 1996). Fluorescently labeled lectins with a range of different binding specificities have been used to differentiate between algal species and even between clones of the same species. For example, they have been used to discriminate between Spanish strains of the *toxic G. catenatum* and morphologically similar, but non-toxic, *Gymnodinium* sp. (*G. impudicum*; Costas and Rodas 1994). Likewise, in Korea, lectins are used as a discriminatory tool for the fish-killing alga *C. polykrikoides* (Cho et al. 1998). Rhodes et al. (1995) used lectins to differentiate between morphologically similar *Gymnodinium* species, and demonstrated that *Gymnodinium mikimotoi* (Waimangu, New Zealand) differed from a Japanese strain of *G. mikimotoi*. The New Zealand *G. mikimotoi* also differed from a Korean isolate on the basis of lectin binding. The use of lectins for the differentiation of toxic and non-toxic *Pseudo-nitzschia* species has also been explored and is promising for a given species in a particular geographic region. Using a suite of lectins, Rhodes et al. (1998) were able to discriminate between six of seven *Pseudo-nitzschia* species.

Results of lectin binding studies clearly demonstrate that binding patterns can differ for "strains" of the same (morphologically defined) species. Despite some obvious limitations, lectin probes show promise in laboratory and field studies of HAB species.

3.3.6.4 Application of molecular probes to natural populations

Much of the effort in immunological detection of HAB species has been focused on development and characterization of antibodies for individual species, so applications of this probe technology to field populations are limited to date. Experience with a PAb for the brown tide organism *Aureococcus anophagefferens* and an MAb for the fish-killing alga *Gymnodinium mikimotoi* suggest that immunofluorescence has a major role to play in HAB monitoring and research programs. The brown tide antibody has been used in cell enumeration and in grazing studies (Caron et al. 1989), and was used to map the geographic distribution of this species over a large region (Anderson et al. 1993). The latter study detected *A. anophagefferens* at extremely low concentrations (10-20 cells ml^{-1}), demonstrating that the species was present in many areas with no known history of harmful brown tides. This degree of resolution is of note since *A. anophagefferens* is so small and non-descript that microscopic identification and enumeration are highly uncertain at low cell concentrations. The antibody is now used for routine

monitoring of this harmful species. A MAb to the same organism is now available as well and is being used for whole cell assays and in a semi-automated ELISA plate format (D. Caron, pers. comm.).

Vrieling et al. (1994, 1995a) developed and tested a series of MAbs to *G. mikimotoi* using cultured and natural samples. A direct labeling technique is now used to identify this species at densities of about 1000 cells L^{-1} in a tube-assay format combined with flow cytometry (Vrieling et al. 1996) and 100 cells L^{-1} in a filter assay followed by epifluorescence microscopy (Peperzak et al. 1998). Although the two methods differ in sensitivity, *G. mikimotoi* can readily be detected at concentrations below those of concern. These assays have been used for routine detection of *G. mikimotoi* in Dutch coastal waters since 1996.

With respect to nucleic acid probes, sandwich hybridization assays have been devised for *Alexandrium tamarense/catenella/fundyense* (North American, Western European and temperate Asian Strains), *Pseudo-nitzschia australis, P. multiseries, P. pseudodelicatissima, P. pungens* (reference strains from central California, USA), *Heterosigma akashiwo, Fibrocapsa japonica, Chatonella antiqua/subsalsa*, and a Cryptoperdiniopsoid species (from Florida, USA) not yet formerly described. At the time of this writing, prototype sandwich hybridization kits are available from the Saigene Corporation (Seattle, WA, US), but the kits are still undergoing an active phase of development, testing and refinement. It is reasonable to expect these products will be available readily by 2002. The sandwich hybridization assay, as well as whole cell assays using rRNA probes have been used in field trials in several areas of the world, including both the east and west coasts of the U.S. where *Alexandrium* species cause PSP (Scholin and Anderson, unpub data), and in several countries where *Pseudo-nitzschia* species cause ASP (Scholin, unpub data).

The most extensive field applications of PCR-based molecular probe technologies to HAB species are probably in the monitoring for *Pfiesteria piscicida* and other *Pfiesteria*-like species in the southeastern U.S. Heteroduplex mobility assays, as well as real-time PCR have been used for several years in numerous state monitoring programs. Whole cell rRNA probing has also been used by Rublee et al. (1999) in field studies of *Pfiesteria* species. These molecular techniques have proven invaluable in detecting and enumerating *Pfiesteria*-like species, which are otherwise difficult to distinguish from each other and from co-occurring gymnodinioid species.

3.3.6.5 Use of molecular probes in new areas

The most likely application of molecular probes would be in the detection of toxic and harmful species, in order to address both aquaculture and public health concerns. The dinoflagellate species complex belonging to the genera *Alexandrium, Pseudo-nitzschia, Heterosigma, Chattonella, Gyrodinium* and *Gymnodinium* are among the most common agents of HABs worldwide, and all have been targets for probe design to some extent. No single type of probe or assay strategy presently stands out as preferable over the others. In fact, some HAB species are best detected using a variety of probes. The choice of probe for a given species in a given region should be a function of the technical background and available laboratory equipment in a given area.

It is critical that probes be "tuned" to suit the geographic region of interest, regardless of the type of probe or application format. Many species exhibit genetic diversity that is not always apparent morphologically. Thus probes that work well to identify a particular organism in one region may not work well on the same species in another. For example, there is no single *Alexandrium tamarense*-specific probe because that species exists as a series of molecularly distinct strains recognizable through application of lectin, antibody and rRNA-targeted probes.

With the exception of lectins and the prototype sandwich hybridization assays, none of the probes discussed above are available commercially. The lack of readily available probe "kits," complete with

control samples, limits the extent to which these tools are applied outside of those laboratories directly involved with probe development and testing. This situation is likely to change with increased demand and use of the probes for routine monitoring.

3.3.7 Fish Indicators

This section discusses how fish reared in mariculture facilities may be utilized as indicators of harmful microalgae in the water and a possible incipient fish kill. This topic is not well studied and documented, but some general observations are possible based on commonalties observed in mariculture worldwide. Fish farms represent ongoing bioassays of the quality of the water in the vicinity of the mariculture site, depending on the sensitivity of the cultured fish species. The often repeated comparison is that fish farms can be "canaries in the mine shaft", referring to the historical use of small caged birds in industrial mining to detect dangerous air quality conditions.

It is a common practice for fish farmers to observe their fish during feeding, either directly in small scale mariculture, or by submerged camera for large, corporate farms. Fish farmers will also pay attention to fish activity and orientation while working on rafts and support equipment. Feed is often the major cost component of fish farms anywhere, and farmers have tried to gauge levels of fish appetite satiation by observing the fish during feeding. Compared to fish faeces, waste feed is rich in nutrients and carbon and sinks much faster, so it is logical to try to reduce its loss to minimize benthic or water column impacts and to maintain profitability of the venture.

In large, corporate fish farms there is a well-established trend toward the use of automated feed transport, delivery and utilization management systems. In part this is due to the very large size of these farms, but also it is now well established that it is impossible for human observers to exactly gauge when feeding should cease for these large systems. Many large mariculture farms use relatively inexpensive underwater cameras to monitor feeding behavior, which also allows operators a useful view of fish swimming and feeding behavior. Other farms use "feedback" systems consisting of small airlift pumps connected to large funnels in the bottom center of the cage to recycle excess feed to the surface. This is not for the purpose of reuse, but to monitor when to cease feeding.

Fish may reduce or cease feeding and change their average position in the water column when stressed by environmental or fish health problems. Perhaps the most common occurrence of this has been due to bacterial disease infections that slowly spread as bacterial septicaemia in individual fish and the population if fish are not previously vaccinated. Cessation of feeding certainly is noticeable to the fish farmer, but in some cases it would be too late to take mitigation actions. In corporate fish farms, fish are routinely vaccinated for all common diseases, which has replaced the use of antibiotics in feed to a large extent.

In comparison to bacterial disease infection, however, fish affected by HABs may die very quickly or they may be chronically affected, depending on HAB cell concentrations and toxin dose, as well as the physiological condition of the fish. For environmental hypoxia (low dissolved oxygen), teleost fish usually display a generalized pattern of response that varies among species and individual fish but may have some common facets. It begins with unusual swimming behavior in most cases, sometimes gulping behavior near the surface, diagonal orientation in the water column (rather than horizontal), followed by periods of abrupt flight response or jerky swimming, progressing to loss of balance or orientation (homeostasis), and finally cessation of ventilation through the gills. The pattern of response may end at any time with no apparent ill effects once levels of dissolved oxygen return to minimal levels needed to support basal metabolism, but there are probably residual stress effects.

For noxious blooms, i.e., those that affect the gills of the fish mechanically or through overstimulation of mucus production, the behavioral response pattern is similar to that of fish affected by environmental hypoxia depending on dose. If mucus production by the gills occurs over chronic (long) exposure periods, however, it is possible to notice strands of mucus trailing from the gills, or at a minimum, when observing freshly killed specimens in the field. This is well established for some temperate water fish, such as salmonids, but we know of no documented examples for subtropical fish.

For toxic blooms, there may be a gradient of fish response depending on the dose, but this is an area of little knowledge. Experience in mariculture shows that patches of cells may move through some of a fish farm's cages, causing waves of fish death, but not all pens at a farm site may be affected simultaneously. Patchy cell distribution is common in phytoplankton distribution. A key point is that most fish mortalities due to toxic microalgae occur quite rapidly, due to destruction of gill structures or neurotoxic responses. The fish in these instances may display episodic fits of twitching and jerking, or may simply be inactive. Other types of phytotoxins, known as hepatotoxins, may affect the liver of the fish, and in these cases it appears that some impacts may be from chronic exposure to the causative alga. Hepatoxins may not be exclusive from toxins that cause gill damage, but in any case, we know of no literature documenting behavioral changes indicative of different types.

Another gray area is documentation of fish behavior after exposure of fish to low levels of toxic microalgae. Rensel (1996) reviewed literature indicating the threshold levels of certain harmful microalgae that cause death of fish. There are but a few examples, but below these thresholds it is reasonable to expect sublethal or chronic effects that should be manifested in behavior changes. This could be a research area, but we are not aware of any ongoing efforts.

Certain species of fish may be used as sensitive early warning indicators for other mariculture fish populations subjected to environmental hypoxia or HAB stresses. These fish may be stocked by fish farms and watched more closely as indicators of environmental hypoxia or anoxia. Included in this category are *Chysophrys major* (red seabream) and *Rabdosarga sarba* (gold-line seabream), (Wu and Lee 1989; Wu 1990).

How different genera or species of fish respond to toxic marine microalgae is not known, and thus this is an area of speculation. Again, from experience we know that there are differences, but they have not been documented. For example, species of trout (now in the genus *Oncorhynchus* and of the specie *mykiss*) are often the first fish to succumb to *Heterosigma* blooms in Pacific Northwest mariculture pens. Atlantic salmon (*Salmo salar*) seem more resistant to low exposure levels, and cultured Pacific salmon (e.g., *O. kisutch*) are typically intermediate. The cause of the differences are unknown, but may be related to differences in gill structure, permeability, sensitivity to epithelial damage or basal dissolved oxygen minimums.

3.4 Early Warning, Detection and Prediction of Blooms

Early warning and prediction of algal blooms requires observations to characterize algal distributions in relation to environmental factors (e.g., advection, mixing, light, nutrients), and models that relate algal population dynamics to the observed properties of the environment. Observations range from visual detection of discolored water and analysis of water samples to autonomous measurements from moorings to remote sensing. Models can range from empirical predictions (e.g., blooms will occur after major runoff events) to detailed numerical forecasts based on simulations of algal growth and behavior in hydrodynamic models. Predictive models can be developed and validated only if appropriate observations are available. Thus, physical-chemical-biological observation systems are essential to early warning and prediction of algal blooms.

Because algal blooms are episodic and patchy, observations of algal distributions in relation to physical and chemical properties should be both continuous and synoptic. Although this ideal is unachievable, a new generation of oceanographic instruments can provide continuous measurements of many physical, chemical and biological properties from autonomous moorings, in vertical profile and along ship-tracks. Also, remote sensing from aircraft and satellites can provide synoptic views of coastal processes when conditions allow. If analyzed carefully, data from well-designed observation systems could contribute effectively to early warning and prediction of algal blooms. However, costs for instruments are high, some of the measurements are difficult to interpret or to correct for interference, and autonomous systems (e.g., moorings) are subject to fouling, disturbance, vandalism or theft. Therefore, an evaluation of different strategies for early warning and prediction of algal blooms involves careful consideration of costs versus effectiveness and risk. Several observation technologies are described in the following section, including the simplest instruments and complex systems that are still under development. Generally, as the systems become more complex, our evaluations become much less quantitative.

3.4.1   Observing algal distributions in relation to environmental variability

Hands-on collection of water is an essential component of all programs to detect and monitor algal blooms in coastal waters. Samples are generally taken for enumeration of phytoplankton and determination of several environmental parameters, such as temperature, salinity, turbidity, dissolved oxygen, and nutrient concentrations. This sampling is conducted from boats, or from wharves or piers. In many countries, regular monitoring is conducted at fixed stations, with samples taken at a few depths in the water column. These sampling programs can miss episodic events such as initiation and termination of blooms, and subsurface features in the distributions of phytoplankton. Supplementary technologies for detection and monitoring of algal distributions in relation to environmental factors can help to improve the temporal and spatial coverage of sampling programs. They are discussed below.

3.4.1.1   Secchi disk

The Secchi disk, introduced to oceanography in the 19th century, is an extremely effective tool for observing variations in water clarity. It is a 30-cm white (or white and black) disk, which is lowered into the water until it is no longer visible (the Secchi depth). The Secchi depth is correlated with the penetration of solar irradiance into the water column, and it is influenced by phytoplankton, dissolved organic matter and suspended sediment. Measurements of Secchi depth in isolation are not very helpful for describing algal dynamics in relation to environmental forcing, but long-term records from a network of stations can reveal important trends, such as the long-term decline of water clarity in the Seto Inland Sea, associated with eutrophication, that was stopped by the imposition of environmental controls in 1973 (Yanagi and Okaichi 1997). A dramatic increase in algal blooms occurred during the final stages of the decline in water clarity (Figure 3.1).

A lesson from the Secchi disk is that a simple but quantitative measurement, made often enough, can be extremely useful in describing environmental variability associated with algal blooms. The technology is inexpensive, and appropriate for frequent, routine measurements by a network of observers, such as fish farmers. It is also easy to incorporate into any monitoring program for algal blooms. The measurements are of little use, however, unless patterns are analyzed in the context of environmental forcing and the dynamics of algal blooms.
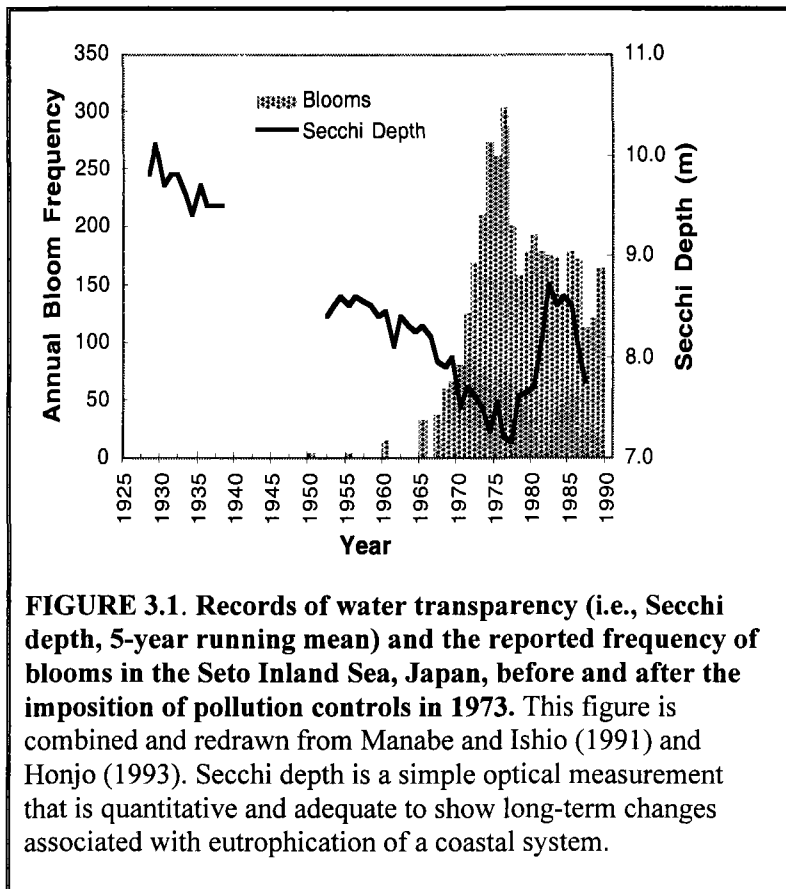
FIGURE 3.1. Records of water transparency (i.e., Secchi depth, 5-year running mean) and the reported frequency of blooms in the Seto Inland Sea, Japan, before and after the imposition of pollution controls in 1973. This figure is combined and redrawn from Manabe and Ishio (1991) and Honjo (1993). Secchi depth is a simple optical measurement that is quantitative and adequate to show long-term changes associated with eutrophication of a coastal system.

3.4.1.2 Chlorophyll *a*

Because all of the photosynthetic phytoplankton contain chlorophyll *a* (the prochlorophytes contain the closely related divinyl chlorophyll *a*), the measurement of chlorophyll is routine in research and monitoring. Samples are filtered, extracted in solvent, and the extract is analyzed for pigment. Spectrophotometric and fluorometric methods for the determination of chlorophyll are both relatively simple and affordable, but fluorometric methods are significantly more sensitive than spectrophotometric methods (i.e., much more sample must be filtered for a spectrophotometric determination). Even when a correction is made for degradation products (e.g., phaeopigments) or accessory pigments, both methods are subject to interference from pigments other than chlorophyll *a* (Lorenzen and Jeffrey 1980). A recent improvement in the fluorometric method minimizes this interference with a modest loss in sensitivity (Welschmeyer 1994). However, if long term records for a region have been acquired using established methods, it may not be prudent to switch to a different technique, even if it is somewhat better, because continuity in the record would be broken. It should be recognized that chlorophyll *a* is an imprecise indicator of phytoplankton biomass (Cullen 1982), so that even accurate determinations of chlorophyll *a* bear uncertain relationships to the abundance and species composition of phytoplankton.

Chlorophyll *a*, along with most other pigments of phytoplankton and their degradation products, can be determined accurately using high performance liquid chromatography (HPLC). Because certain pigments are characteristic of particular phytoplankton taxa, including some groups that are predominantly harmful (Johnsen et al. 1994), HPLC analysis can be an effective tool in characterizing variability in dominant species groups - it is therefore a good complement and a partial replacement for microscopic analysis, especially when a large number of samples is taken and resources for enumerating samples are strained. Costs for HPLC analysis are significantly higher than for fluorometric or spectrophotometric methods.

3.4.1.3 Fluorescence of chlorophyll *in vivo*

The chlorophyll *a* in live phytoplankton fluoresces red when photosynthetic pigments absorb light, so fluorometers can be used to assess distributions of phytoplankton *in situ* (Lorenzen 1966). Natural samples can be pumped continuously through an on-deck fluorometer (e.g., Turner Designs 10-AU Fluorometer) to assess distributions in vertical profile or during transects. Several types of compact, submersible, *in situ* fluorometers are commercially available from manufacturers including Chelsea Instruments, Dr Haardt, SeaPoint, Turner Designs, and WET Labs. These are generally less expensive

than bench-top fluorometers (several have prices ranging from about $3,000 to $5,000 US), and they are more convenient and effective than flow-through fluorometers for use in the field. They are designed for deployment on small instrument packages for monitoring or profiling salinity, temperature and other properties. Submersible fluorometers are readily adapted for flow-through systems, such as on ferries (e.g., McKenzie et al. 1998) or for moorings with samples pumped sequentially from one or several depths (Lee and Lee 1995). The WET Labs *ECO* sensors have an integrated anti-fouling shutter as an option; the Turner Designs SCUFA can be fitted with a copper screen that retards fouling. When used for vertical profiling, submersible fluorometers can provide critical information on the distributions of phytoplankton relative to density structure and light in the water column. They are sensitive enough to detect the lowest concentrations of phytoplankton in coastal waters. Response times (which are not necessarily the same as sampling rate) should be sufficiently fast to resolve thin features (about 10-30 cm) in vertical profiles during routine deployments.

Calibration of *in vivo* fluorescence. Fluorescence is an indicator of chlorophyll, and an imprecise one at that (Cullen 1982). The ratio of *in vivo* fluorescence to chlorophyll *a* varies widely (see Kiefer 1973) as a function of irradiance and irradiance history, nutritional state, accessory pigmentation and taxonomic grouping. Also, the patterns of variability with these factors differ, depending on the manner of measurement (excitation irradiance and duration), which is different for each instrument (Neale et al. 1989). Consequently, *in situ* fluorometry is much better suited for characterizing patterns in distributions of chlorophyll, such as subsurface layers (Derenbach et al. 1979) or patchiness along transects, than for quantifying accurately either chlorophyll *a* or the biomass of phytoplankton. Nonetheless, fluorometry can provide high-resolution records of chlorophyll if the measurements are carefully calibrated with discrete samples from the same waters, paying special attention to the influence of irradiance on fluorescence yield (Cullen and Lewis 1995). This may not be possible when fluorometers are used in autonomous systems. For example, there will be a depression in near-surface fluorescence when it is sunny (a physiological response to bright light) that could be interpreted as avoidance of the surface by phytoplankton. Besides making efforts to understand such natural variations in fluorescence responses of phytoplankton, it is essential to characterize shifts in instrument sensitivity and instrument blank on a regular basis. Distilled water and a fluorescent standard such as rhodamine can be used for this purpose in most coastal waters (McKenzie et al. 1998), but more rigorous procedures are preferable, especially for oceanic waters, where filtered sea water is the appropriate blank.

3.4.1.4   Spectral fluorescence excitation and emission *in situ*

Spectral characteristics of algal fluorescence reveal taxonomically important differences in algal pigmentation (Yentsch and Phinney 1985). That is, by measuring the red fluorescence emitted by algal chlorophyll *a* when excited by a spectrum of blue to green wavelengths (i.e., an excitation spectrum), one can discern influences of different accessory pigments, some of which are characteristic of particular taxa. In turn, the emission spectrum of algal fluorescence, when stimulated by blue light for example, can reveal taxonomically significant differences in the pigmentation and organization of photosynthetic systems (e.g., some cyanobacteria and cryptophytes fluoresce orange). Until recently, spectral fluorescence of phytoplankton has been measured for research purposes using laboratory instruments (Nelson et al. 1993; Sosik and Mitchell 1995) or highly specialized systems (Cowles et al. 1993). Now, a robust, commercially available instrument (WET Labs SAFIRE; about $30,000 US) can be used to measure spectral fluorescence. The SAFIRE is designed for *in situ* characterization of water fluorescence from the UV throughout the visible spectrum. The instrument employs a flashlamp source and a rotating filter wheel that provides excitation light at six wavelengths. Sixteen emission detectors built into the flow tube provide a 6 excitation, 16 emission data matrix. The resulting 96 channels are sampled at 5 Hz, providing information on colored dissolved organic matter as well as on algal pigmentation and aspects of physiology (Nelson et al. 1993; Sosik and Mitchell 1995). However, interpretations of spectral

fluorescence as measured by SAFIRE are still very much under development, so it is not yet a tool for operational monitoring.

The fast repetition rate fluorometer (FRRF; Chelsea FASTracka, about $70,000 US) directly assesses photosynthetic physiology of the phytoplankton assemblage (Falkowski and Kolber 1995). It is an extremely useful tool for research that could, in principle, be used for monitoring. Interpretations of the measurements require some refinement and validation, and instrument performance has yet to be fully evaluated under a broad range of conditions. Also, the cost of the instrument and requirements for careful maintenance are issues. The use of chemical anti-fouling agents during moored applications might affect phytoplankton physiology, compromising system performance.

3.4.1.5 Spectral attenuation and absorption

Absorption spectra. Phytoplankton in suspension absorb and scatter light. Attenuation is the sum of absorption and scatter: it can be quantified by measuring the transmission of a beam of light through a path of water to a target. It is well recognized that differences in pigmentation between algal groups can be detected in measurements of their absorption spectra (Johnsen et al. 1994). Absorption spectra of phytoplankton can be measured spectrophotometrically if modifications are made so that photons scattered out of the light path (scattering by phytoplankton is principally in the forward direction) are detected with the same efficiency as those that are neither scattered nor absorbed (Shibata 1958). This can be accomplished by collecting particulate matter on a glass-fiber filter, which serves the dual purpose of concentrating the sample and acting as a diffuser for ensuring the detection of forward-scattered light (Yentsch 1962). The measurement requires hands-on manipulation of samples and results must be corrected for the tortuous path of light through the sample (Mitchell and Kiefer 1988; Cleveland and Weidemann 1993) and the contribution of detritus to particulate absorption (Kishino et al. 1985). Thus, filter-pad measurements of phytoplankton absorption are used primarily for research, not monitoring. It is conceivable, however, that estimates of absorption could be made autonomously by the measurement of reflectance on filters (Balch and Kirkpatrick 1992) prepared in an autonomous unit. (See Kirkpatrick et al. 2000 for discussion of an alternate approach.)

Spectral absorption and scatter *in situ*. Systems have recently been developed for continuous *in situ* measurement of the absorption coefficient of natural waters (see Cullen et al. 1997; Schofield et al. 1999). The commercially available WET Labs ac-9 dual-path absorption meter (about $20,000 US) uses a quartz cylinder to reflect scattered photons toward the detector (Zaneveld et al. 1990). Attenuation (scattering plus absorption) is measured in a second chamber, so spectral scattering can be calculated by difference. The scattering coefficient is used in the calculation of a correction factor for photons scattered away from the detector of the absorption meter (Zaneveld et al. 1994). Using selectable filters, the ac-9 concurrently determines the spectral transmittance and spectral absorption of water over nine wavelengths. The ac-9 is available with 25 and 10 cm pathlength configurations, appropriate for coastal waters. More highly resolved spectra (3.3 nm spectral resolution throughout the visible range) can be obtained with the WET Labs HISTAR high-spectral-resolution absorption and attenuation meter (about $32,000 US).

Great care must be taken to acquire accurate data with the WETLabs absorption and attenuation meters, especially in oceanic waters, where the determination of blanks has a large influence on results. Also, fouling, bubbles and temperature shifts can cause problems. Nonetheless, instruments like the ac-9 have been shown to be effective for detecting vertical (Roesler and Zaneveld 1994) and temporal (Cullen et al. 1997) variability of phytoplankton in coastal or shelf waters. Fouling of the reflecting tube during long deployments can be retarded by using copper tubing on the intake port and pumping water through the instrument only periodically (T.D. Dickey, pers. comm.). Absorption meters with sensors for backscatter and fluorescence are also sold by HOBI Labs (**www.hobilabs.com**).

To date, the measurements of spectral absorption have been used more to resolve the red peak of chlorophyll absorption (a reasonably robust measure of chlorophyll a; see Figure 3.2) than to distinguish groups of phytoplankton according to their absorption characteristics (Johnsen et al. 1994; Millie et al. 1996; Schofield et al. 1999).
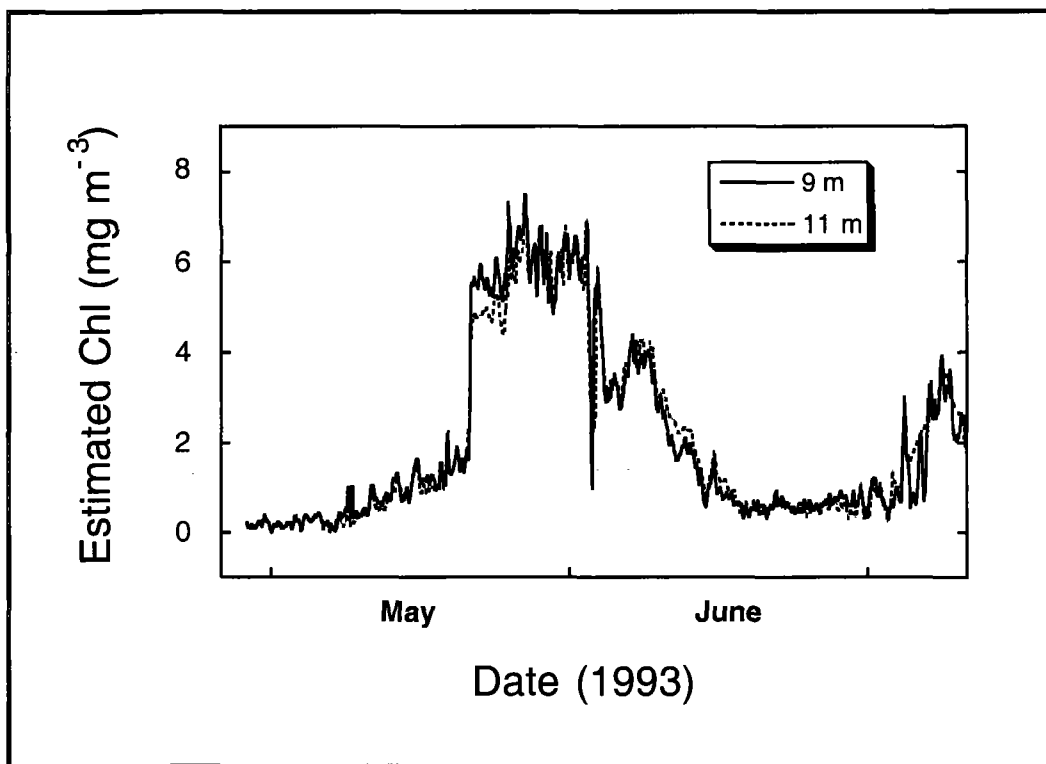
FIGURE 3.2. **Estimates of chlorophyll concentration based on measurements obtained with moored spectral absorption meters in the southeast Bering Sea, 1993.** A three-waveband absorbance meter (a-3: 650 nm, 676 nm and 710 nm; WET Labs, Inc.) was deployed at 9 m and a six-waveband meter (WET Labs ac-6: relevant wavelengths, 650 nm, 676 nm and 694 nm) was deployed at 11 m. Absorption associated with the red peak for chlorophyll a was estimated by subtracting from measured absorption at 676 nm a baseline described by a simple exponential curve connecting measurements at the lower and higher wavelengths. The concentration of chlorophyll was calculated from absorption using a coefficient from the literature (Bricaud et al. 1995). Later during the same deployment, the instruments became fouled and performance degraded. Records like this would now be obtained with an ac-9 instrument. (Source: Cullen et al. 1997.)

Limitations. It should be recognized that the technology for measuring absorption *in situ* is still emerging. Despite the sound theoretical foundations of the measurement systems, accuracy and precision is not assured; for example, although several methods show the same general patterns in absorption, measured ratios between wavelengths, as well as absorption coefficients, differ between instruments (Pegau et al. 1995). Thus, it may be some time before detailed analysis of *in situ* absorption spectra (Johnsen et al. 1994; Millie et al. 1996) will be practical. However, new techniques with better resolution of phytoplankton absorption provide some promise for detecting some species of phytoplankton from their spectral signatures (Kirkpatrick et al. 2000).

Spectral attenuation *in situ*. Measurements of spectral light-beam attenuation (LBA) have been made in a variety of environments (Voss 1992; Volent and Johnsen 1993), and Optisens (OCEANOR), a three-waveband LBA meter (blue/480 nm, green/550 nm, red/650 nm), has been incorporated into the SEAWATCH system (Johnsen et al. 1997; Tangen 1997). Chelsea Instruments can provide similar measurements. It is suggested that the ratio of attenuation in these different wavebands may indicate which component of the water - phytoplankton, colored dissolved organic matter, or inorganic particulate material - is dominating (see Tangen 1997). Descriptions of Optisens generally include references to research showing how the absorption characteristics of phytoplankton can be used to distinguish taxa, including some harmful species (Johnsen et al. 1994), supporting the implication that measurements of spectral attenuation are potentially useful for distinguishing phytoplankton *in situ*.

It is important to recognize, however, that spectral attenuation includes both scattering and absorption, and that in surface waters, the scattering coefficient of phytoplankton is generally much greater than the absorption coefficient (Morel 1990). Consequently, measures of LBA are dominated by scattering, which for phytoplankton has weaker spectral features in the visible range than does absorption (Morel 1990; Stramski and Reynolds 1993; Roesler and Zaneveld 1994). Thus, although it is feasible to measure spectral LBA to detect phytoplankton (Volent and Johnsen 1993), on first principles the approach is less sensitive than absorption-based techniques (Stramski and Reynolds 1993). The same conclusions apply to measurements of spectral backscatter using commercially available instruments (e.g., HOBI Labs): they are very important for optical oceanography (Voss and Smart 1994), but are not especially effective on their own for discerning the contribution of phytoplankton, except, perhaps, in fluorescence mode. New applications may be developed because several research groups are pursuing the discrimination of algal groups from the measurement of a suite of optical properties, and progress is likely.

Attenuation at one wavelength, measured *in situ*. Profiling transmissometers that measure attenuation at 660 nm (e.g., from WET Labs, Chelsea Instruments, and HOBI Labs; from about $4,000 to $7,000 US) are now used routinely in oceanographic research. Data have proved to be extremely useful in several contexts (Pak et al. 1988; Siegel et al. 1989; Stramska and Dickey 1992); an important feature of attenuation at 660 nm is that it is a measure of particle concentration, not strongly affected by algal pigments (Cullen and Lewis 1995). Thus the comparison of beam attenuation vs. chlorophyll fluorescence is potentially useful for distinguishing some microbial assemblages (weakly pigmented vs. strongly pigmented plankton; cf. Mitchell and Holm-Hansen 1991) and for characterizing the relative contributions to subsurface layers of suspended sediment vs phytoplankton. Profiling instruments that measure attenuation in more than one wavelength are becoming more common.

Turbidity. Long used in programs to monitor water quality, turbidity sensors (e.g., instruments that measure sidescatter or backscatter in the infrared) quantify water clarity by measuring an optical signal related to particle load. Instruments for measuring optical backscatter (OBS) *in situ* range in price from about US $1,000 to $4,000 and can be calibrated in turbidity units tied to standards. Measurements can be related empirically to water clarity (diffuse attenuation coefficient) or to particle load, but turbidity is not a direct measurement of either property. Turbidity sensors are not suited for detecting variability of phytoplankton *per se*, but like transmissometers, they can be used in conjunction with fluorometers to detect subsurface layers of particles and to characterize to some extent the relative contribution of microalgae to the particle load. Oceanographers tend to work with beam attenuation, because the attenuation coefficient (c: units, m^{-1}) is an absolute measure (an inherent optical property) that can be compared directly between instruments.
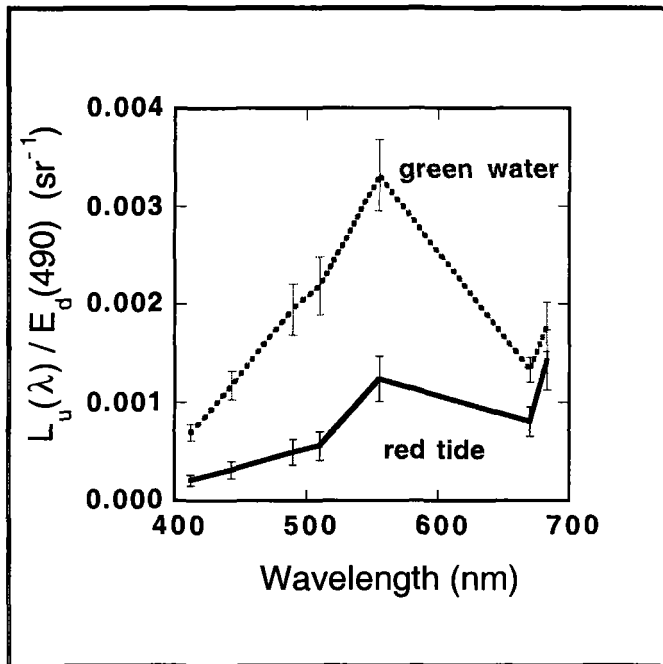
FIGURE 3.3. Detection of a dinoflagellate bloom with a radiometer buoy measuring ocean color, Aug. 18, 1993, during patchy discoloration of surface waters by high concentrations of the non-toxic dinoflagellate *Gonyaulax digitale*. The red water spectrum is the average for 1345 - 1350 h, and the green water spectrum is for 1354 - 1359 h. Error bars are s.d. for measures recorded 1 s^{-1}. The signal near 673 nm is sun-induced fluorescence of chlorophyll, which can be helpful in distinguishing red tide from muddy red water. (Source: Cullen et al. 1997.)

3.4.1.6   Ocean color

The absorption and scattering of light by algae, other micro-organisms, particles, dissolved substances and water modify both the underwater and upwelling (emergent) light fields. The influences of algae, which are generally distinct from those of other components (Morel 1990), can be detected and quantified by measuring reflected and fluoresced light using near-surface and above-water radiometers and satellite sensors. Ocean color is generally measured as upwelling spectral radiance, $L_u(\lambda)$ (W m^{-2} nm^{-1} sr^{-1}) and normalized to downwelling solar irradiance ($E_d(\lambda)$; W m^{-2} nm^{-1}) to calculate radiance reflectance (Rr; sr^{-1}).

Where algal blooms occur at sufficient biomass, they may be detected by passive optical instruments (radiometers), including ocean-color sensors on moorings, aircraft, or satellites (Figure 3.3). Passive optical sensors cannot detect toxic algae that occur as minor components of the phytoplankton, but estimates of total pigment and information such as spectral attenuation from these sensors can provide important data for biological-chemical-physical models of algal dynamics (Schofield et al. 1999; Glenn et al. 2000). Well-recognized limitations of satellite remote sensing, including interference by clouds, relatively coarse spatial resolution (for coastal processes), and discrete observation periods can be overcome by deployment of *in situ* ocean-color radiometers on moorings or drifters (Abbott and Letelier 1996) and by using radiometers on aircraft for surveys during events or process studies (Pettersson et al. 1993; Harding et al. 1995; Davis et al. 1997). One great strength of ocean-color measurements is that they are radiometric quantities that retain their validity for long-term and wide-ranging comparisons over time or between sites (e.g., for resolving influences of eutrophication or climate variability). Interpretations of the measurements may change for the better, but the data should never become obsolete.

Ocean color is often related to near-surface chlorophyll concentration through empirically derived algorithms. These have been particularly successful in open ocean (Case I) waters where bio-optical variability results principally from algal biomass. Coastal waters (Case II), where algal blooms occur, present problems since the algorithms have to discriminate the absorption and scattering of algae from the absorption and scattering of the terrigenous inputs of colored dissolved organic matter and sediment. These problems are being addressed vigorously by the ocean-color remote sensing community, and progress has been good (Sathyendranath 2000). Local algorithms can be developed by sampling for chlorophyll and particulate absorption while measuring ocean color with a radiometer buoy or profiler. Although absorption contributes strongly to ocean color (reviewed by Cullen et al. 1997) and groups of phytoplankton can be distinguished on the basis of highly resolved absorption spectra (Johnsen et al.

1994; Millie et al. 1996), research to date indicates that species composition of phytoplankton cannot be determined from measurements of ocean color alone (e.g., Garver et al. 1994; but see Schofield et al. 1999).

Instruments for measuring ocean color from boats or ships. Ocean color and downwelling irradiance are measured *in situ* with profiling radiometers (about $25,000 to $75,000 US, depending on numbers of wavebands and extra sensors; e.g., from Biospherical Inc. or Satlantic, Inc.) or hand-deployed radiometer buoys (about US $15,000 for simple systems from either supplier, to about $35,000 for a hyperspectral version from Satlantic). Radiometers can also be used on the deck of a ship or boat for some applications (Carder and Steward 1985; Lazin 1998): hand-held, high-resolution units are available for about US $20,000 from Analytical Spectral Devices, and deck-mountable, multiple-waveband units (including downwelling irradiance) are available from Biospherical and Satlantic for prices in a similar range. These boat-deployed tools are extremely useful for the development of local algorithms to interpret remote sensing from aircraft or satellites, and they can be used for estimating variability in surface pigments during transects. Ocean color sensors on moorings and aircraft can be very useful in the early warning and prediction of algal blooms. Those applications will be discussed below.
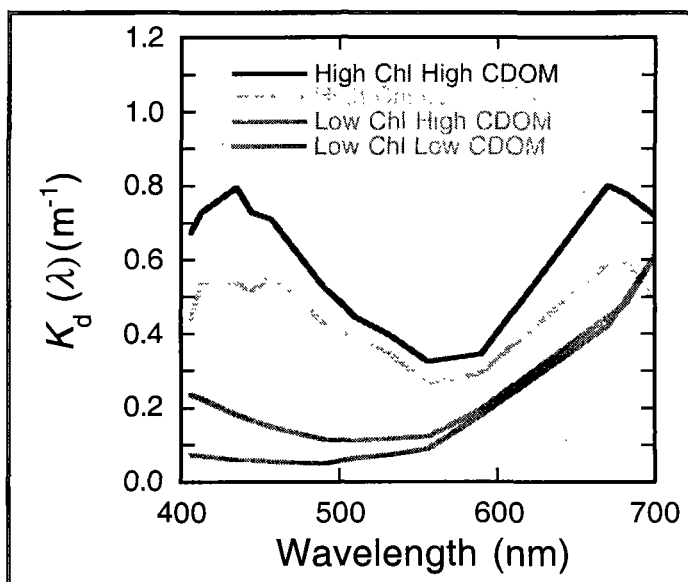
FIGURE 3.4. Measurements of spectral diffuse attenuation coefficients $[K_d(\lambda)$, m$^{-1}]$ in coastal waters off Oregon (US). Vertical profiles of $E_d(\lambda)$ were obtained with a multichannel profiling radiometer (Satlantic, Inc.), and $K_d(\lambda)$ was computed as the slope of $\ln(E_d(\lambda))$ vs depth for the upper 5 - 10 m of the water column. The contributions of chlorophyll (Chl) and CDOM (gelbstoff) to absorption can be seen in these spectra: Chl concentrations for the highest-absorbance to lowest-absorbance spectra were 24.5, 20.8, 0.57, and 0.25 mg m^{-3}, respectively. (Source: Cullen et al. 1997.)

Instruments for measuring attenuation of solar irradiance in the water. While ocean color is largely determined by both absorption and scattering by phytoplankton and other constituents of the water, spectral characteristics of the vertical attenuation of solar irradiance are mostly affected by absorption. Thus, measurements of the diffuse attenuation of solar irradiance $[K_d(\lambda)$; m$^{-1}]$, generally made with a profiling radiometer or a chain of sensors, can reveal the influences of phytoplankton as well as other absorbing constituents, such as colored dissolved organic matter, or gelbstoff (Figure 3.4). Another important characteristic of diffuse attenuation is that the measurement integrates all influences in the depth range monitored. That is, a thin layer of highly concentrated particles will be detected through its attenuation of light, even if it is not directly resolved by the radiometer. Measurements of attenuation at one wavelength should be effective at detecting subsurface layers of absorbing material. Estimates of attenuation at multiple wavelengths will help to resolve contributions of phytoplankton from other constituents of the water.

3.4.1.7 Flow cytometry

Flow cytometers are extremely powerful tools for characterizing algal assemblages with respect to taxonomic composition and cellular

properties, such as chlorophyll content (Li et al. 1993). A suspension of particles is directed into a very narrow stream, such that one particle at a time encounters a focused beam of light. Detectors measure properties such as forward light scatter and side-scatter (to characterize cell size) as well as the fluorescence of chlorophyll a, phycoerythrin, or a fluorescent stain (e.g., Pan and Cembella 1998). Some systems are capable of sorting cells on the basis of user-defined characteristics. Flow cytometry has proven to be effective in distinguishing and quantifying different groups on the basis of scattering properties (i.e., size) and fluorescence of photosynthetic pigments (Olson et al. 1989; Li et al. 1993). With the use of stains, flow cytometry can be effective in identifying taxa and quantifying important cell constituents (see Vrieling and Anderson 1996). Flow cytometers are expensive, and not suitable for use on small boats. However, many types of analyses can be done on preserved samples (Vaulot et al. 1989), so routine monitoring or surveys could be practical. Also, new instruments are under development, including the novel Cytobuoy system (**www.cytobuoy.com/**), which have strong potential for use on moorings for autonomous detection and monitoring of algal blooms. Preliminary results from the Cytobuoy system are promising, but it appears that the system is still in the experimental stages. A flow-system, which collects images of larger plankton, including some harmful species, is now being marketed (**www.fluidimaging.com**).

3.4.2 Characterizing Environmental Variability Relevant to Algal Blooms

Most programs to monitor coastal waters for algal blooms include measurements of temperature, salinity, dissolved oxygen and nutrients at the surface and usually at one or more depths. Many programs include continuous profiles of conductivity (salinity) and temperature vs depth (CTD). These sampling programs can miss episodic events such as initiation and termination of blooms, and subsurface features in the distributions of phytoplankton. For example, transport of subsurface populations was found to be extremely important in the dynamics of dinoflagellate populations off France (Gentien et al. 1998) and near the Irish coast (McMahon et al. 1998). Detection of subsurface populations and incorporation of the information into measurement-guided circulation models was critical to resolving important factors that influence the occurrence of these blooms. The information could not have been obtained effectively with CTD profiles and fixed-depth sampling alone. In the preceding sections, we described supplementary technologies for detection and monitoring of algal distributions in relation to environmental factors. Here we describe approaches for characterizing environmental variability concurrently with observations of properties related to algal distributions.

3.4.2.1 Profiling systems

Compact, high-quality CTD systems are widely used for coastal monitoring and research. These systems are designed for use with supplementary sensors that can effectively describe the distributions of microalgae relative to important properties of the water column. For example, the Harmful Algae Monitoring Program of Galicia, Spain (Mariño et al. 1998) has developed an effective system for describing distributions of algae relative to environmental factors. Their profiling system measures temperature, salinity, oxygen, pH, fluorescence, and transmittance (attenuation). Similar systems can be obtained from several suppliers (Table 3.10), with prices ranging from about US $25,000 and up for compact systems that provide good temporal (and thus good spatial) response, real-time read-out (important so that subsurface features can be targeted for sampling and enumeration of phytoplankton), and software for presentation and analysis. Manufacturers such as YSI and Hydrolab market profilers that are widely used and substantially cheaper. Some trade-offs in instrument performance (such as depth tolerance and sensor precision) are made. Nonetheless, they may be a good choice for many applications.

A detailed comparison of different products is difficult, because evaluations depend on who will be using the system, under what conditions the profiler will be deployed (e.g., from a well equipped monitoring

vessel or from a small boat), what configuration is chosen, and the level of internal technical support that is available to the user.

A fluorometer should be included in profiling packages, so subsurface distributions of phytoplankton can be characterized and discrete sampling can be targeted to subsurface layers, when they are encountered. With appropriate modifications for mounting and moving water past sensors, the instrument systems from Table 3.10 can be deployed on moorings, but different factors influence the evaluation of relative merits. These will be discussed in the section on moorings.

TABLE 3.10. Compact profiling systems. Prices start at about $25,000 US. Not all systems have exactly the same configuration and some include spare parts or set-up and testing.

Model	Manufacturer	Features	Comments
SBE-19 SEA-CAT Profiler	Sea-Bird Electronics	C, T, Depth, DO, Fl, LBA, OBS	Pump required; 2 Hz sampling
Micro-CTD 3"	Falmouth Scientific, Inc.	C, T, Depth, DO, Fl, LBA, OBS	No pump needed; 6 Hz
AQUApack CTD	Chelsea Instruments	C, T, Depth, Fl, LBA, nephelometer	No pump; includes integration and test
OS200 CTD	Ocean Sensors	C, T, Depth, DO, Fl, LBA, OBS	No pump needed; good sensor response
EMP 2000	Applied Microsystems	C, T, Depth, DO, Fl, LBA	No pump needed

Notes: Laptop computer and cable (about 50 m; cost of about $500 - $800 US) would be required for each. Direct comparisons on the basis of price would be misleading, because there are many differences in sensor response, system integration, and simplicity of use.

3.4.2.2 Underway sampling on ferries

Ferries or other ships offer excellent opportunities for regular sampling of environmental parameters relevant to monitoring and detection of algal blooms. Water can be sampled through the hull, fed through a de-bubbler, and directed through a series of sensors. A Japanese system (Harashima et al. 1997) measures conductivity, temperature, pH and fluorescence, and an automated filtration system collects samples for determination of nitrate, nitrite, ammonium, phosphate, dissolved silica, chlorophyll and phaeopigments. A simpler system, designed for use on fishing vessels, measures conductivity, temperature and fluorescence (McKenzie et al. 1998). The costs for such a simple system would be on the same order as a profiling instrument package: more money would be spent on water handling and calibration systems, but fewer sensors would be operated, because dissolved oxygen cannot be measured reliably in such systems, and the measurement of beam attenuation requires special precautions because of bubbles. Ferry-based sampling is being established elsewhere in the world, and some systems employ underway measurement of ocean color with an on-deck radiometer.

Although it seems reasonable to expect that dramatic surface blooms will be detected through underway sampling, day or night, other benefits of underway sampling systems become evident once long time-series are analyzed (Harashima et al. 1997). Regional patterns of blooms can be determined and related to eutrophication, relationships between blooms and hydrographic features such as fronts can be resolved, and long-term shifts in patterns can be related to nutrient loading. The analysis presented by Harashima et

al. (1997) relies heavily on measurements of nutrients, which certainly require a large investment of resources. The measurements were not continuous, but they were numerous and regular.

Autonomous measurement of nutrients. The measurement of nutrient concentrations in discrete samples of seawater is not considered an issue in early warning and prediction of algal blooms, as the two principal options, analysis by hand and using an automated nutrient analyzer, are well known. Since the capability for autonomous measurement of dissolved nutrients in seawater is potentially useful for describing and understanding the dynamics of algal blooms, the technology deserves consideration here.

The concentrations of dissolved nutrients are commonly measured during monitoring programs, and when blooms are detected and sampled intensively. It is problematic, however, to correlate nutrient concentrations with algal biomass: the systems are dynamic, so that even if the initiation of blooms is correlated with higher nutrient concentrations, a negative correlation would exist between algal biomass and nutrients during the development phase of the bloom. Monthly or bimonthly sampling could not resolve these dynamics, but continuous measurement of nutrient concentrations, in conjunction with measures of algal biomass, might. As discussed in the previous section, semi-continuous sampling (including filtration) during underway transects (Harashima et al. 1997) can be effective. Alternatively, nitrate and phosphate can be continuously measured with the commercially available systems from, for example, Chelsea Instruments, WS Oceans or WET Labs. This technology can be used on moorings, though the WET Labs system is designed for profiling applications. In the context of early warning and prediction of algal blooms, the expense of continuous nutrient analysis (roughly US $25,000 per nutrient module) would have to be justified carefully, either by comparing expense to that for discrete samples (Japanese model), or by showing that there were good reasons to believe that patchy or episodic changes of nutrients might be involved with the initiation or termination of blooms (justification for measuring nutrients from a mooring). Even if the information were not necessary for early warning of blooms, continuous measurements of nutrients would be better than data from routine monitoring programs for development and validation of simulation models.

3.4.2.3   Bio-optical moorings

Instrument systems on moorings can describe biological dynamics in the context of physical and chemical forcing, making pertinent observations on appropriate scales (Dickey 1991). Development of moored monitoring systems has begun (Johnsen et al. 1997; Tangen 1997; Glenn et al. 2000), and several ocean observation projects have been established, including the MBARI Ocean Observing System (MOOS), the Bermuda Testbed Mooring (both components of the O-SCOPE program; see the web page of Dr. Tommy Dickey, UCSB), the Coastal Ocean Observation Lab / LEO-15 project (Rutgers University), the Chesapeake Bay Observing System, and the Marine Optical Buoy (MOBY) time series off Hawaii (all have web pages). All are still under active development. Operational monitoring has been conducted by OCEANOR (the SEAWATCH program, www.oceanor.com; Hansen 1995; Johnsen et al. 1997; Tangen 1997). Less comprehensive bio-optical moorings have been deployed by Biospherical, Inc (REOS; biospherical.com/products/reos.html) and Satlantic, Inc. (TACCS; www.satlantic.com). Many of the instruments on these moorings have been discussed in preceding sections. They will be evaluated below in the context of early warning and detection of algal blooms using mooring technology.

Passive optical sensors. In principle, passive optical sensors (radiometers) are extremely well suited for early warning and detection, as well as long-term monitoring, of algal blooms. The presence of algae at the surface is detected continuously (during daylight) in ocean color and sun-induced fluorescence, and subsurface accumulations can be discerned in measurements of diffuse attenuation, measured with a string of sensors. Passive sensors have low power consumption (an important consideration for moorings) and, unlike fluorometers, which have to deal with physiological interactions between the light field and

photosynthetic systems, they can be rigorously calibrated. In practice, problems are encountered with biofouling, some consequences of shading, and confounding optical influences of colored dissolved organic matter and suspended sediment. Still, there are very good reasons for believing that passive optical measurements from moorings will become a cornerstone of detection and monitoring of biological variability in aquatic systems, including algal blooms (Dickey 1991; White et al. 1991; Abbott and Letelier 1996; Cullen et al. 1997).

One operational sensor system is the Remote Electro-Optical Sensor (REOS) from Biospherical, Inc. (White et al. 1991; Morrow et al. 1999). Systems have been installed in several water reservoirs operated by the Los Angeles Department of Water and Power. The systems were designed to provide reservoir managers with the daily data needed to recognize and react to incipient algal blooms before water quality is degraded. The first prototype was deployed in 1989, with some success. The third generation systems measure spectral reflectance and spectral diffuse attenuation (upwelling and downwelling sensors, seven wavebands each, at 2 and 5m), ancillary measurements (YSI 600 for oxidation-reduction potential, pH and temperature), and surface irradiance. Algorithms were developed to estimate chlorophyll concentration accurately from passive optical measurements. Real-time data from the system are sent through armored cable (hydrowire), but other communications could be employed. The system supports early intervention of nuisance algal blooms and complements monitoring. Continuous daytime data from the system also provide useful information on episodic processes such as algal blooms, thermal destratification, and rain runoff events. A REOS system costs about $100,000 US, exclusive of the cost of the mooring. They have been effective in reservoirs - deployment in busy, rough coastal waters has not been tested. It is nonetheless noteworthy that this bio-optical system has worked well to describe biological variability, and that optical sensors from Biospherical have been deployed on many coastal and oceanic moorings. A thermistor string would provide more information on physical structure of the water column.

Passive optical systems have been developed by Satlantic, Inc. and deployed in coastal waters using simple weight-and-float moorings. The Tethered Attenuation Coefficient Chain Sensor (TACCS) has proven effective in characterizing biological and optical variability in coastal waters of Nova Scotia over deployments of several months. It measures ocean color (upwelling radiance in seven wavebands) at the surface, and downwelling irradiance above the surface (one or three wavebands) and at four depths (one waveband), with communication by cell phone or other options. Variation in ocean color revealed day-to-day variability of algal biomass, and patterns in the diffuse attenuation coefficient showed not only seasonal patterns in the vertical distributions of phytoplankton, but also the vertical migration of layers of phytoplankton and the sinking of a surface bloom (Figure 3.5). Cost for a system would be about US$25,000-35,000, depending on options. Incorporation in a rugged, proven mooring system might raise the cost to about US$125,000 US. The next generation of TACCS moorings can accommodate hyperspectral radiance and irradiance at the surface and four wavebands of downwelling irradiance at four depths. Satlantic reports that they have developed a mechanism to clean fouling from the windows of their radiance sensors. This could be an important development, because fouling would require fairly frequent checking of the sensors during coastal deployments.

Commercially available radiometric sensors can be incorporated into moorings in a variety of configurations. However, care must be taken to avoid problems with shading and changes in orientation. For many applications, these problems can be countered. Depending on requirements, optical systems can be configured to minimize cost (perhaps US $10,000 for rudiments of ocean color) or to maximize information ($100,000 for REOS-type suite of sensors).
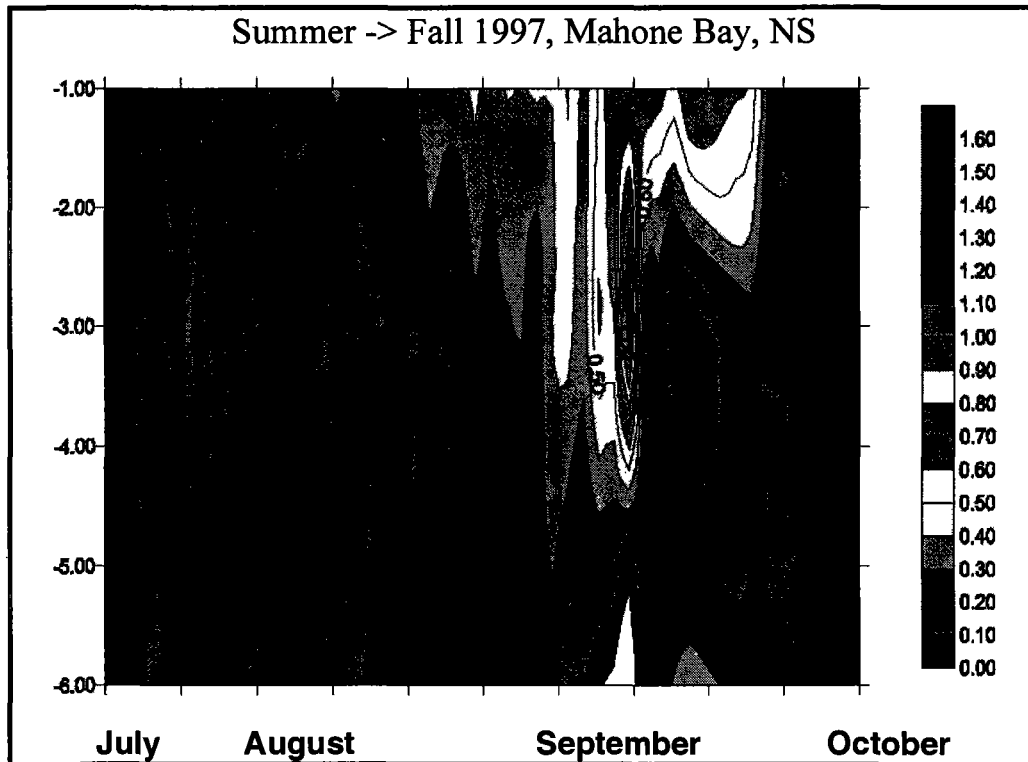
FIGURE 3.5. Diffuse attenuation coefficient at 490 nm (K_d(490); m^{-1}) in the upper 6 m, summer to fall 1997, Mahone Bay, Nova Scotia, Canada measured with a Tethered Attenuation Coefficient Chain Sensor. The sensor chain detected the consequences of summer stratification and the occurrence of a fall bloom. (Source: Biospherical Instruments, Inc., San Diego, CA.)

Active optical sensors. Fluorometers and instruments to measure absorption and attenuation were discussed in Section 3.4.1.5. If these instruments are to be deployed on moorings, biofouling and power consumption must be considered. If moorings are to be tended frequently (perhaps once per week), then fouling may not be much of a problem, provided instruments can be easily cleaned. Toxic coatings have been used to retard fouling, but these have only limited success. More recently, copper screening close to *in situ* sensors and copper tubing on flow-through sensors has effectively retarded fouling. It thus appears that moored active optical instruments should be used in the flow-through mode. This could be arranged for most of the instruments, with the added advantage of permitting more than one depth to be sampled sequentially through the same sensor package. Power consumption is still a problem: the SAFIRE spectral fluorescence meter may provide some of the most informative data on phytoplankton *in situ*, but it is also more power-hungry than many of the other instruments. The fluorescence of phytoplankton is depressed in bright light near the surface (Marra 1992; Cullen and Lewis 1995; Falkowski and Kolber 1995), so records of fluorescence at the surface during the day must be interpreted with caution.

CTD profiles. In coastal environments where salinity contributes significantly to density, a mooring should record temperature and conductivity. Much more could be described and understood if salinity and temperature could be measured in vertical profile. One approach is a string of CTD sensors (e.g., OCEANOR SeaProfiler, offered as one possible component of the Seawatch system). Strings could also be built from commercially available CTD units, but integration and communication would have to be developed. These problems have already been addressed in the Seaprofiler, and through the use of inductive modem technology, for example from SeaBird Electronics.

Currents. Currents at one point can be measured using several approaches (e.g., Marsh-McBirney electromagnetic current meter as used by Lee and Lee 1995). In many environments, depth-profiles of currents are a better complement to measurements of salinity and temperature, providing crucial data for the development and validation of models. An acoustic doppler current profiler is appropriate for this task. Downward looking and upward-looking acoustic doppler profilers are available. For example, SonTek (San Diego, CA; www.sontek.com/) can recommend a bottom-mounted, upward-looking unit for autonomous deployment for roughly $25,000 US. A 500 kHz unit can profile currents with maximum resolution of 1-m in depth and 0.1 cm/s in velocity. Choices for current-measuring devices would be strongly influenced by the needs of the user, for example the nature of the environment where the mooring is deployed (is advection important to algal dynamics at the site?), resolution of the model(s) to which the data might be applied, and the vertical resolution of the biological measurements that are made from the mooring.

3.4.2.4   Moored profiler

Brooke Ocean Technology, Ltd. offers a novel solution to the problem of characterizing vertical profiles of properties from moorings. Their SeaHorse Wave-Powered Moored Profiler (**www.brooke-ocean.com/s_horse1.html**) resides at depth and, on programmed cues, rises to the surface while recording data from a suite of sensors. At the surface, it can communicate its data. Then, a ratchet system engages, and the package climbs down the wire by harnessing wave action. A SeaHorse mooring was operated off Nova Scotia for five weeks in late 1997, profiling conductivity, temperature and turbidity over about 15 m of water (report by Jim Hamilton, Fisheries and Oceans Canada). The system performed well, as it has during several other deployments, for example with CTD and fluorescence (**www.brooke-ocean.com/graphs-sh-01.html**). Radiometric sensors (7 wavebands downwelling irradiance) could be incorporated into a SeaHorse package with CTD for about US $75,000 total. A fluorometer could also be included. A comprehensive system would include a surface buoy with downwelling irradiance at the surface and optional ocean color, CTD and ancillary measurements. In principle, such a system would be very effective and probably less susceptible to some of the hazards encountered by moorings. However, an integrated optics-SeaHorse package has not been tested to date.

SeaHorse technology may be particularly well suited for areas where tampering with buoys by third parties is a real concern. The SeaHorse instrument package resides near the bottom most of the time; only a float appears at the surface. If the mooring is attached to a very heavy weight (e.g., several railroad wheels), it could not easily be taken by small, fast boats. If a frustrated vandal cut the float, the instrument package would remain on the bottom, where it could be retrieved. Some modifications would have to be made to prevent the module from deploying when the line goes slack, and an alarm system with GPS should be installed to notify the user if the instrument is moved. These modifications would have to be developed.

Apprise Technologies, Inc. (www.apprisetech.com), has developed profiling systems based on buoyancy modulation. Their Remote Underwater Sampling Station (RUSS) can be outfitted with third-party sensors: it is generally connected to a buoy or platform with solar panels. The price range seems to be similar to SeaHorse.

3.4.3   SEAWATCH™ system

OCEANOR has developed SEAWATCH™, the only complete operational marine monitoring and information system available on the open market (Sørås et al. 1998). SEAWATCH is an integrated monitoring system, including: buoys equipped with instruments to assess a range of processes; other data sources such as satellites, coastal stations and research vessels; networks of observers, communication

links; information processing (including analysis and forecasting), and information distribution (Johnsen et al. 1997; Tangen 1997). Applications include acquisition of surface meteorological observations and coastal zone management, oil spill contingency and oil spill forecasting, environmental monitoring and documentation (including radioactivity), as well as monitoring and forecasting algal blooms.

As of 1998, SEAWATCH systems had been installed in Europe, Thailand, Vietnam, and Indonesia, with deployments planned for Spain, Latvia and India (Sørås et al. 1998). Each system is tailor-made, representing a wide range of choices, depending on objectives. For example: SEAWATCH Europe is an on-line monitoring surveillance system for the North Sea, and a regional component of the Global Ocean Observing System (Stel and Mannix 1996); SEAWATCH Indonesia is an environmental monitoring, forecasting and information system; while SEAWATCH Vietnam is tailored to improve typhoon forecasting and monitoring capabilities (Sørås et al. 1998).

It is clear that SEAWATCH systems have a wide range of potential benefits that should be considered when contemplating the cost of such systems (Stel and Mannix 1996). Estimating costs of a new system is complicated, because each system is tailor-made, and different countries have different needs in terms of the complexity of their coastal environments and the amount of existing infrastructure available to support a system. It is difficult, and in fact unfair, to compare the costs of SEAWATCH to the other technologies for early warning and prediction of algal blooms, because only SEAWATCH is a fully integrated system, and in all likelihood SEAWATCH systems would serve a much broader range of users than an observation system specially designed to monitor and predict algal blooms. Still, it is necessary to have a rough idea of costs in order to evaluate SEAWATCH technology in the context of early warning and prediction of algal blooms.

3.4.3.1   Estimated costs and requirements for support

Buoys. Because each system is custom-built, it would be essential to consult with OCEANOR to obtain cost estimates. In a benefit-cost analysis of SEAWATCH, Stel and Mannix (1996) consider a 'planning unit' consisting of 10 buoys, with an annual cost of between US$2 and $3 million. The costs mentioned only relate to the buoys. The distribution of data is another expense that largely depends on the availability of software and hardware at the customer's offices. The estimated costs for buoys would not include third party damage or other accidents. In busy waters, it is important to consider the potential consequences of such losses, and who bears the financial risk. Education of the local fishing community about the benefits of the system can reduce incidents of third party damage to buoys.

According to Mr. P. Sørås of OCEANOR, there is no minimum commitment related to number of years or number of buoys. Also, OCEANOR can include third party sensors, etc. on their buoys/systems. This flexibility is important, as the technologies for ocean observation from buoys is improving rapidly, and some third party sensors might be more effective than OCEANOR's for particular applications. Mr. Sørås indicates that OCEANOR is quite happy to work with their potential customers and to conduct their own investigations to design and price the systems that best serves the needs of the user.

3.4.3.2   Monitoring algal blooms with SEAWATCH™

Capabilities of the system. The capabilities of SEAWATCH for monitoring algal blooms is well described by Johnsen et al.(1997) and Tangen (1997). Blooms have been observed, and their progression along the coastline has been well described. The Optisens LBA sensor has detected variations in attenuation that reflect algal dynamics, but identification of the species has been accomplished through conventional enumeration of samples collected manually (Stel and Mannix 1996). A network of observers in the coastal region is thus necessary for operational monitoring and forecasting of potentially harmful

algae (Tangen 1997). Successes of SEAWATCH can be attributed to the efforts spent designing the structure, management and integration of the phytoplankton monitoring, which includes both high-tech (buoys) and low-tech (network of fish-farmers who act as samplers and observers) components. It is not clear from available information the degree to which the buoy system enhanced the monitoring capability of the network of observers. A published benefit-cost analysis (Stel and Mannix 1996) did not break down the relative benefits and costs of hi-tech vs. low-tech components of the SEAWATCH system.

3.4.3.3 Forecasting algal blooms with SEAWATCH™

OCEANOR employs the integration of data from several sources for producing forecasts of phytoplankton dynamics. During regular forecasting meetings, an analysis or assessment of present conditions is use as a basis for formulating predictions. The process is comparable to meteorological forecasting (Tangen 1997). However, as Tangen states, "phytoplankton forecasting is more primitive or non-mature than meteorological forecasting in the sense that operational, prognostic models are not yet available, and it is not realistic to expect that models in the near future will give indications on the species specific level for safe forecasting of toxic phytoplankton." This cautious assessment is consistent with a generally held view in the oceanographic/harmful algal bloom community that, although new technologies and approaches show great promise for the development of predictive simulation models for algal blooms using real-time data, the capability has not yet been developed. That is, "models are not presently capable of predicting the occurrence, distribution, toxicity, and environmental response of HABs" (GEOHAB 1998). Despite this somewhat gloomy assessment, planning groups for both GEOHAB (Global Ecology and Oceanography of Harmful Algal Blooms) and the Coastal Global Ocean Observing System (C-GOOS) have strongly endorsed further research and technological development toward coastal observation systems linked with forecasting models.

3.4.3.4 A general assessment of SEAWATCH for monitoring and predicting algal blooms

OCEANOR's approach to marine monitoring and prediction is consistent with strong trends in oceanography, and in many ways, SEAWATCH has led the way. As the only complete operational marine monitoring and information system available on the open market, SEAWATCH deserves very careful consideration by any user group committed to substantial improvements in marine monitoring. The capabilities of SEAWATCH are consistent with some key requirements of GOOS, the Global Ocean Observing System (Hansen 1995). If similar technologies are applied broadly throughout the world, capabilities for environmental assessment will improve, to the benefit of all. It seems appropriate, however, for the systems to include the most appropriate sensors, regardless of supplier.

The great strengths of OCEANOR and SEAWATCH, then, are their capabilities to integrate observation systems, and their real-world operational experience. Also, the data from SEAWATCH systems are valuable to a broad range of users, independent of the benefits for monitoring and prediction of algal blooms. This must be kept in mind when costs are considered. Consider an initial investment of several million dollars US, plus about US $500,000 per year for upkeep, roughly enough for an integrated system of 10 buoys. This investment could probably support a networked system of 30 vertically profiling moorings with CTD, fluorescence, and measurements of spectral diffuse attenuation and ocean color. Or, it would be enough for perhaps 20 fixed moorings with spectral absorption meters and CTD, and 20 underway systems on ferries, including nutrient analyses (these are very rough guesses). Taking another direction, it would be possible to support inexpensive monitoring systems for measuring temperature, salinity, fluorescence, oxygen, at several depths, plus irradiance and winds on fish farms (after Lee and Lee 1995) in 25 mariculture regions, plus several telemetered buoys in more open water, leaving money for personnel committed to working with the data in the context of early warning and prediction of algal

blooms. Each of these alternatives lacks the critical elements that OCEANOR can offer: integration of the system and proven success at implementation.

To summarize, SEAWATCH is a special product, reflecting OCEANOR's well developed capabilities for designing and operating ocean observation systems. Given the rapid developments in ocean observation technology over the past several years, it seems that, with respect to the harmful algal bloom problem, the best value could be obtained by designing new systems that exploit improved capabilities of third-party sensors.

3.4.4   Observations from Aircraft

Many blooms are visible at the sea surface, and sometimes the boundaries of blooms are clearly evident to an observer. It is thus reasonable to use aerial surveys to monitor and detect algal blooms, a practice that has been conducted for decades, for example off California in the 1960's. Blooms can be observed visually, through aerial photography or video, or by using a radiometer, either fixed-point or imaging. Phytoplankton can also be detected with laser-induced fluorescence (Hoge and Swift 1983), but this approach does not offer significant benefit-cost advantages for algal bloom detection.

3.4.4.1   Visual detection of blooms

Visual surveys of algal blooms can be very effective, if conducted by trained observers, such as is being done in the Puget Sound region (WA State, US) by fish farmers routinely and more intensively on emergency basis. Aerial surveys in conjunction with regular monitoring could be very useful in the early warning and prediction of blooms. For surveys near coastlines, the observer could sketch the distributions of blooms on a chart, which could be digitized and used for purposes of short-term forecasts (according to oil-spill models or more sophisticated hydraulic models). They could also be archived for the development of empirical models of the occurrence and movements of blooms. Visual observations can also be backed up by video or digital photography. Costs for this type of aerial monitoring would be low, especially if the observer could join surveys that were underway for other purposes. New systems integrate photographic images with GIS. A contingency plan would have to be in place for special surveys when harmful blooms are detected.

There are several limitations to visual detection of blooms. First, the identification and delineation of blooms is subjective. Also, the human eye can detect only fairly large differences in concentrations of phytoplankton in the water. Sometimes it can be difficult to distinguish reddish-brown blooms from muddy water, and subsurface blooms cannot be detected.

3.4.4.2   Quantitative observations of ocean color from aircraft

As discussed in Section 3.4.1.6, ocean color is a useful, though imperfect indicator of the distributions of phytoplankton in coastal waters. Generally, blooms of phytoplankton are detectable even in the presence of colored dissolved organic matter and suspended sediment (Carder and Steward 1985); sun-induced fluorescence is helpful in this regard (Gower and Borstad 1981), even though the relationships between fluorescence and chlorophyll are poorly resolved for phytoplankton near the surface (Cullen and Lewis 1995). Quantitative measures of ocean color are much more sensitive than the human eye to small changes in chlorophyll, and quantitative measurements can be entered directly into databases, so aerial surveys with quantitative radiometry are in principle far superior to visual surveys for characterizing the variability of surface phytoplankton in coastal waters.

Technical considerations. It is not a simple matter to obtain estimates of ocean color from an airborne radiometer, because upwelled light from the ocean is attenuated through the atmosphere, and light from other sources reaches the sensor and contaminates the signal (Figure 3.6). Consequently, steps must be taken to minimize and correct for contributions from: atmospheric scattering (sunlight that is scattered into the path of the sensor), sun-glint (sunlight that is reflected off the surface), and sky-glint (diffuse light from the sky that is reflected off the sea surface). It is also necessary to characterize solar irradiance (the reflectance of which constitutes ocean color), and the attenuation of irradiance through the atmosphere.
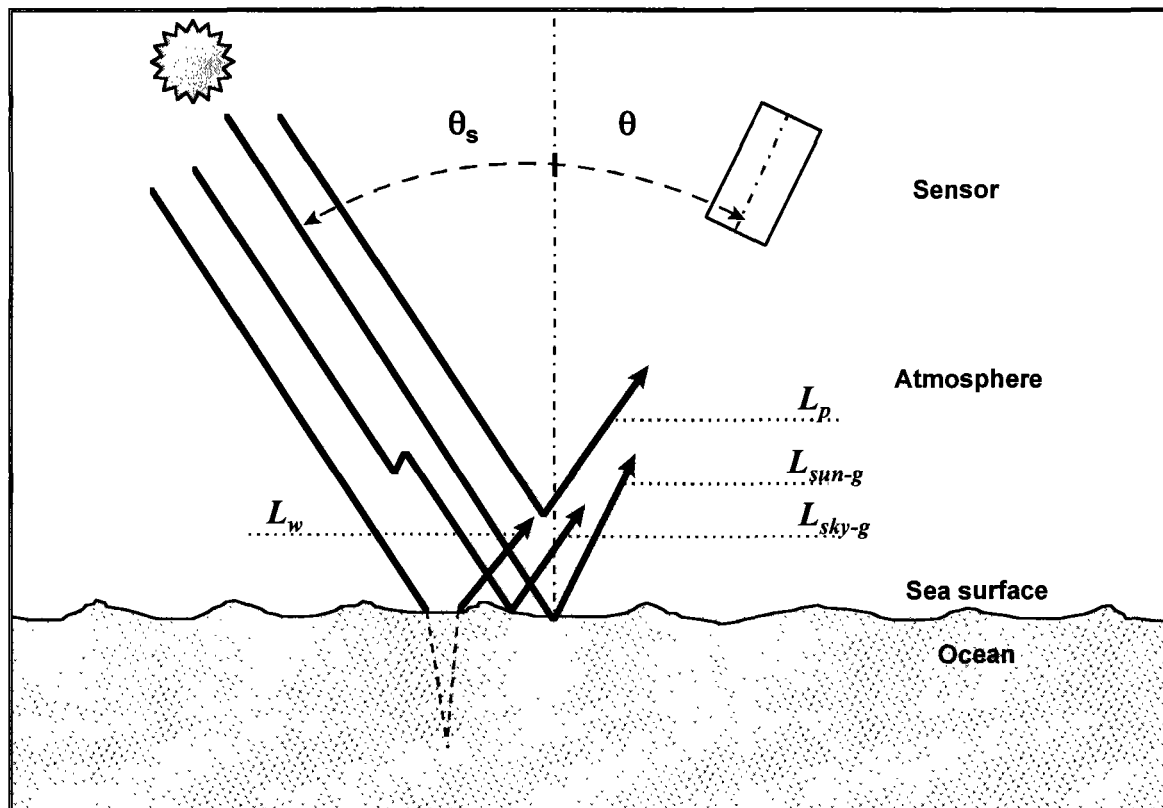
FIGURE 3.6. The different origins of light received by a remote sensor pointed to the ocean surface. L_p is path radiance (due to atmospheric scattering), L_{sun-g} is sun-glint radiance, L_{sky-g} is sky-glint radiance, and L_w is water-leaving radiance. To quantify ocean color (L_w in this figure), it is necessary to minimize or correct for the other contributions. (Source: Lazin 1998.)

Procedures have been developed to characterize atmospheric attenuation and to correct for atmospheric scatter. Corrections are relatively small for low-flying aircraft, and they can be checked for particular situations. Problems from sun-glint can be minimized by pointing the sensor away from the direction of the sun; bright outliers in the data are discarded for being contaminated by glint. This procedure works well when color is measured with a simple radiometer (i.e., a spectral sensor directed at one point, for example the SAS-II SeaWiFS Airborne Simulator from Satlantic — roughly US$26,000, including GPS). An imaging radiometer (discussed below) looks in more directions, so problems with glint can be greater. There are several methods for dealing with sky-glint (see Lazin 1998 and references therein). Perhaps the best approach to getting quantitative data would be to fly an imaging radiometer with a SAS-II radiometer pointed in the appropriate direction to log more easily corrected and calibrated data for one line along the

track. Corrections can be made under clear skies or uniform clouds, but partial clouds seriously compromise any correction scheme.

The problem of partial clouds can be visualized by looking at greenish waters under a sky with patchy clouds. A pattern of light green patches contrasts with darker, bluer water. An ocean-color sensor would interpret these green patches as having more chlorophyll (Gordon et al. 1988), and the pattern might be regarded as a patchy bloom. However, the real cause is that the indirect sunlight in the cloud-shaded water surrounding these patches is bluer, diffuse skylight, so the patches exposed to direct sun appear greener by comparison (Cullen et al. 1994). Consequently, it is recommended that quantitative remote sensing of ocean color be conducted only under clear skies or uniform cloud (Mueller and Austin 1995). Algal blooms do not respect such rules.

An additional problem with remote sensing of ocean color in coastal waters is the influence of submerged vegetation and the bottom, which can be significant in shallow waters. It can be concluded that any program of quantitative ocean-color remote sensing in coastal waters from aircraft would have to incorporate stringent quality control, and it would have to expect difficult-to-interpret data under unfavorable conditions. Still, it is very likely that imaging radiometry would be very useful for detecting algal blooms (see Figure 3.6).

3.4.4.3   Imaging spectroradiometer

There are a number of imaging spectroradiometers, appropriate for detecting algal blooms, that are in use or under development (e.g., Gower and Borstad 1990; Millie et al. 1992; Pettersson et al. 1993; Harding et al. 1995). We discuss here a commercially available turnkey system that has been widely used.

The Compact Airborne Spectrographic Imager (casi), from ITRES Research Ltd. (www.itres.com/) can be used from most aircraft platforms. It is one of the few commercially available imaging spectrographs in use worldwide. According to ITRES, multiple systems have been sold throughout Canada, Europe, Japan, and the United States. In addition, systems have been leased and utilized in the South Pacific, Southeast Asia, Africa, the West Indies, Central and South America. The casi technology has developed through time, and, judging by performance demonstrated in publications, the imager can be considered an effective tool for characterizing optical variability in coastal waters.

The instrument can be mounted on small fixed-wing aircraft, with some minor modifications, like a hole in which to mount the sensor head. Flying at 600m, casi can image a swath about 300 m wide, with a resolution of 60 cm. At 1200 m, it would be about 500 m wide with 1-m resolution. Data can be integrated into a GIS system. There are several other features to make the system appropriate for operational use. As mentioned above, imaging under patchy clouds is problematic. Under cloudy skies, the signal (upwelling radiance) is sometimes too small to measure precisely.

The cost for a casi system is in the range of about US$500,000. About 4 people would be required for support: a trained survey pilot, an operator/technician, a data analyst, and a scientist who understood the system and who could analyze the data critically and effectively. This represents a major commitment of money and resources, and would likely require recruitment of personnel with appropriate skills. It is likely that, in the near future, other systems will come on the market for lower prices, but a monitoring program would still require a large commitment of resources.

3.4.4.4 Satellite remote sensing

Remote sensing has long been considered a tool with great potential for monitoring the distribution of red tide organisms over larger spatial and shorter time scales than is possible with ship-based sampling. It has not yet fully lived up to this promise, however. Although multi-spectral scanners (e.g. Coastal Zone Color Scanner; CZCS, and Sea-viewing Wide Field-of-view Sensor, SeaWiFS) can be used to detect chlorophyll and other pigments from algae, these efforts have been constrained by the inability of the sensors to discriminate phytoplankton populations at the species level (Garver et al. 1994). In established, nearly mono-specific red tides, ocean color can nevertheless be useful, as was shown for several blooms of *Gymnodinium breve* (Steidinger and Haddad 1981; Tester and Steidinger 1997).

Another approach that is not dependent on identifiable pigments requires that specific water masses be linked to red tide blooms, and those water masses are then tracked with an appropriate remote sensing technique. Remotely-sensed sea surface temperatures (SST) have been used to follow the movement of fronts, water masses, or other physical features where toxic species accumulate. A coastal current that dominates the dynamics of *Alexandrium tamarense* in the southwestern Gulf of Maine is easily identified by its temperature signature (Keafer and Anderson 1993). Likewise, the long-distance advection of *Gymnodinium breve* from Florida into the nearshore waters of North Carolina via the Gulf Stream was documented with this SST approach (Tester et al. 1991). These successes in tracking blooms within water masses (see also Gentien et al. 1998) suggest that, if blooms are detected and water masses defined, movements of the blooms can be forecast.

Even though ocean color is unlikely to provide adequate information to determine species composition (but see Schofield et al. 1999), remote sensing can be very useful in studies of HABs. For example, remote sensing can provide the oceanographic context for areas where HABs occur. Satellite sensors can provide data to describe patterns of wind, rainfall (between 40°N to 40°S), sea surface temperature, sea surface height (thus geostrophic currents), salinity and ocean color, although of coarser resolution than desirable to describe key aspects of algal bloom dynamics (cf. Uno and Yokota 1989). These data would provide insights into models of algal development, in some cases providing good information on the transport of blooms. New satellite sensors will have better spatial and spectral resolution for ocean color, and thus may be even more useful.

3.4.4.5 Remote sensing and forecasts of bloom dynamics

This integration of real-time and near-real-time environmental observations into atmospherically-forced numerical simulations of ocean processes is an example of data assimilation modeling (the procedure used in operational meteorology). The essential feature of data assimilation models is that information is used not only to initialize a forecast model, but newer observations are used to correct the model, so simulations of present conditions and predictions of future changes are as accurate as possible (see Schofield et al. 1999). Data-assimilation modeling of coastal processes is being actively pursued by research groups in many countries. The incorporation of biological processes into these models is only in its infancy, so operational forecasting of algal blooms does not yet utilize operational, prognostic models of species-specific bloom dynamics (see Section 3.4.3.2).

3.4.4.6 Remote sensing and research on algal blooms

The present situation is still one of potential rather than actual application of remote sensing as a forecasting or prediction tool for HABs. Cloud cover and the need for high-resolution imagery may obviate the use of remote sensing for operational forecasting. However, there are strong reasons for using remote sensing as a research tool to develop empirical and conceptual models of bloom development and

transport. Studies are needed that obtain satellite images of ocean color and SST concurrent with field measurements on bloom distribution or toxicity under a variety of meteorological conditions. With sufficient background information of this type, development of conceptual models will be possible, allowing observations from a variety of sources, including remote sensing images (if available) to be used for actual forecasts of impending outbreaks along specific sections of the coast. Progress in this area should be rapid in the immediate future, due to the launch of several satellites designed to collect ocean color data, including satellites (**www.ioccg.org/sensors/500m.html#2**).

The US Department of the Navy is developing a satellite-based hyperspectral ocean color imager with a planned launch date in the year 2001. The satellite, Navy EarthMap Observer (NEMO), will have a 2.5 day site reaccess time, and a 3-5 year mission lifetime. The hyperspectral sensor, Coastal Ocean Imaging Spectrometer (COIS), will have 10 nm spectral resolution in the visible to near-infrared and also short-wave to infrared, with excellent signal-to-noise ratio. A typical scene will be 30 km wide by 200 km in length, with 30 m by 30 m pixel resolution, yielding nine image strips per orbit (number of strips limited by on-board data storage capacity). A co-aligned 0.45-0.68 micron Panchromatic Imaging Camera (PIC) with 5 m resolution will also be on board. The aircraft sensor that most resembles COIS in signal-to-noise ratio, footprint, and spectral resolution is the Airborne Visible / Infrared Imaging Spectrometer (AVIRIS). COIS and PIC are designed to provide hyperspectral data on spatial scales comparable to optical features typical of coastal regions. This tool will be used in targeted research by the US Office of Naval Research. Commercial applications (mostly land-based) will also be developed. The technology, if shown to be robust, should be very effective in the remote sensing of algal blooms in coastal waters.

A great deal of research on remote sensing in coastal waters is being conducted in many countries. Advances are likely to be rapid. Research is especially cost-effective, because operational use of satellite data can be very expensive.

3.4.5 Modeling

As discussed above, operational forecasting of algal bloom dynamics seems not to be possible at this time, even though retrospective analyses have been quite successful at describing important factors that influence the distributions and persistence of algal blooms. The lesson to date is that as better and better observations of algal distributions in relation to environmental factors are accumulated, better and better models follow. Eventually, predictive capabilities will develop and improve. Availability of observations to test and refine these predictions (i.e., data from a network of sensors and information from monitoring programs) is essential for progress toward the goal of prediction with measurable accuracy.

Important decisions relevant to mitigation of harmful blooms rely on empirical or conceptual models relating algal population dynamics to environmental forcing, such as climate variability (e.g., El Niño) or human influences such as nutrient loading. In some regions a great deal of work has been done addressing the influences of nutrient loading on the occurrence and nature of algal blooms. Evaluation of the conclusions and implicit predictions of these studies can only be improved if observation systems are upgraded so that more data can be acquired with improved temporal and spatial resolution.

Simulation models can be effective in revealing which factors dominate in the control of algal bloom dynamics. Water quality models incorporate information on many processes that influence the distributions of nutrients, oxygen, phytoplankton and light. Rarely can the models be parameterized using data from the environment to be modeled, or the target species to be considered. Consequently, although species groups can be treated simultaneously, the conditions that lead to the dominance of a particular species are difficult to resolve with such detailed, but still generalized, models. Nevertheless, multi-

parameter simulation models can be an important tool to explore the controls (e.g., nutrient loading, light, tidal flushing) on the biomass and growth rates of phytoplankton in particular environments.

Large amounts of information on the physiology and behavior (or sinking characteristics) of individual species is key to understanding its dominance in particular environments - the better the information, the better the description. Knowledge of the interactions of algal behavior and physiology with hydrographic forcing (e.g., Yamamoto and Okai 2000) has allowed the description of important features of the dynamics of *Prorocentrum mariae labouriae* in the Chesapeake Bay (Tyler and Seliger 1981), *Gymnodinium catenatum* off the coast of Spain (Figueiras et al. 1998), and *Heterosigma akashiwo* (Taylor 1993; Rensel 1995) and *Chatonella antigua* in the Seto Inland Sea of Japan. A recent study by Amano et al. (1998) epitomizes what can be done with the results of years of study on an organism. They simulate nutrient uptake (nitrate, ammonium, phosphate) of *C. antiqua*, as affected by light, temperature and vertical migration in a model that includes zooplankton grazing, advection and dispersion. The results of their modeling are quantitatively consistent with hypotheses that had been developed about the environmental conditions that promote the growth of *C. antiqua* in the Seto Inland Sea. This work exemplifies the best that can be done at this time. The model relies on species-specific information that required many years of targeted research. Application of this approach to different species would likely require similar background work. Another complication is that information on a species isolated from one region might not apply to the same species from elsewhere. For example, the vertical migration patterns and nocturnal nutrient uptake of *Alexandrium tamarense* from the Gulf of St. Lawrence, Canada (MacIntyre et al. 1997) are not shared by a strain of *A. tamarense* from Casco Bay, Maine (Poulton 2000).

One can conclude that continued research is extremely important to understanding and modeling algal dynamics, and that environmentally relevant work on individual species (local strains) should be emphasized, if it is feasible. However, it seems that the best predictions, for some time to come, will be made by people who have worked on the problem for a very long time, and have accumulated a great deal of information on which to base assessments and forecasts.

4 HAB MONITORING PROGRAMS

4.1 Fish Mariculture Monitoring

Fish mariculture has become a large industry worldwide, and as it has grown, so has the need to protect that industry from the negative impacts of red tides and HABs. In year 2000, global farmed-salmon production, mostly of Atlantic salmon, increased 18 percent from the prior year to exceed 1.1 million metric tons, far outweighing the 0.75 million metric tons produced by wild-salmon harvests (Seattle Times, Sept. 2, 2001). Many countries have instituted monitoring programs and action plans to provide early warning to their fishermen and to guide mitigation strategies. Selected programs are highlighted in the sections that follow.

4.1.1 Norway

The history of HABs in Norwegian waters goes back to the start of this century. The first reported human DSP intoxication was observed in 1870; the first reported fish mortality from phytoplankton in fish farms was observed in 1976. This section focuses on the Norwegian monitoring of HABs in relation to fish farming and is based upon information from Andersen (1996), Johnsen et al. (1997) as well as additional information obtained from E. Dahl (pers. comm.) and K. Tangen (pers. comm.).

Norway produces almost 50% of the total world production of farmed salmon. In 1971, production was approximately 600 tonnes. Production has increased significantly since that time, reaching 215,000 tonnes of Atlantic salmon in 1994 and 460,000 tonnes in 2000. Atlantic salmon is the dominant species. Approximately 95% of Norway's farmed fish production is exported. Norway has a long and protected coastline with coastal water temperatures favorable to growing salmon due to the influence of the Gulf Stream. Almost 99% of the production occurs in marine environments. The main farming system employs open fish cages (SAR 1997).

The Norwegian phytoplankton monitoring program in relation to the fish farming industry was initiated by the Norwegian Association of Fishfarmers in 1987. The justification for the program was extensive mortality of salmon in fish farms situated along Norwegian coastal waters and fjords that resulted in great economic losses to the fish farmers. The initial mortalities were caused by several blooms of the fish-killing, toxic dinoflagellate *Gyrodinium aureolum* in 1976. In more recent years, several other species caused massive fish kills in Norway including *Chrysochromulina* spp. and *Prymnesium* spp., as well as different diatoms (Table 4.1). According to the largest fish farm insurance company in Norway, there are major losses of fish in Norway approximately once every 10 years (e.g., 1989 and 1998) but the losses are small compared to those in British Columbia (T. Thorsen, Vesta Insurance, pers. comm.). The largest single year economic losses to all Norwegian farms and insurance companies occurred in 1988 and totalled about US$ 6 million.

Current Monitoring Program. The present Norwegian monitoring program covers most of the coastal waters and fjords of Norway (Figure 4.1). Information on the occurrence of HABs is gathered from different sources including "the fish farm observation network" covering 40 sites along the Norwegian coast, including fish farmers, aquaculture research stations, and lighthouses as well as mussel farmers. Furthermore, data are collected by the State Food Control Authority as well as from offshore moorings known as SEAWATCH-buoys (see Section 3.4.3.2). The buoys currently measure a range of physical and biological parameters (wind speed and direction, air pressure, air temperature, wave height and period, current speed and direction, light attenuation, oxygen saturation, water temperature, salinity and radio-

activity). The different sensors are located at 3 m depth, except the 11 temperature sensors that are situated on a cable extending from the surface to 50 m.

TABLE 4.1. Harmful algae of concern to Norwegian fish and shellfish industries.

Dinoflagellates	Diatoms	Other algae
Alexandrium excavatum	*Chaetoceros borealis*	*Chrysochromulina leadbeateri*
Alexandrium minutum	*Chaetoceros concavicornis*	*Chrysochromulina polylepis*
Alexandrium ostenfeldii	*Chaetoceros convolutus*	*Chrysochromulina spp.*
Alexandrium pseudogonyaulax	*Chaetoceros spp.*	*Phaeocystis pouchetii*
Amphidinium carterae	*Leptocylindrus minimus*	*Prymnesium parvum*
Dinophysis acuminata	*Pseudo-nitzschia pseudodelicatissima*	*Prymnesium patelliferum*
Dinophysis acuta		*Dictyocha speculum*
Dinophysis norvegica	*Pseudo-nitzschia multiseries*	*Dictyocha fibula*
Dinophysis rotundata	*Pseudo-nitzschia delicatissima*	*Heterosigma akashiwo*
Dinophysis spp.	*Rhizosolenia spp.*	*Nodularia spumigena*
Gymnodinium galatheanum	*Skeletonema costatum*	
Gyrodinium aureolum		
Prorocentrum lima		
Prorocentrum minimum		
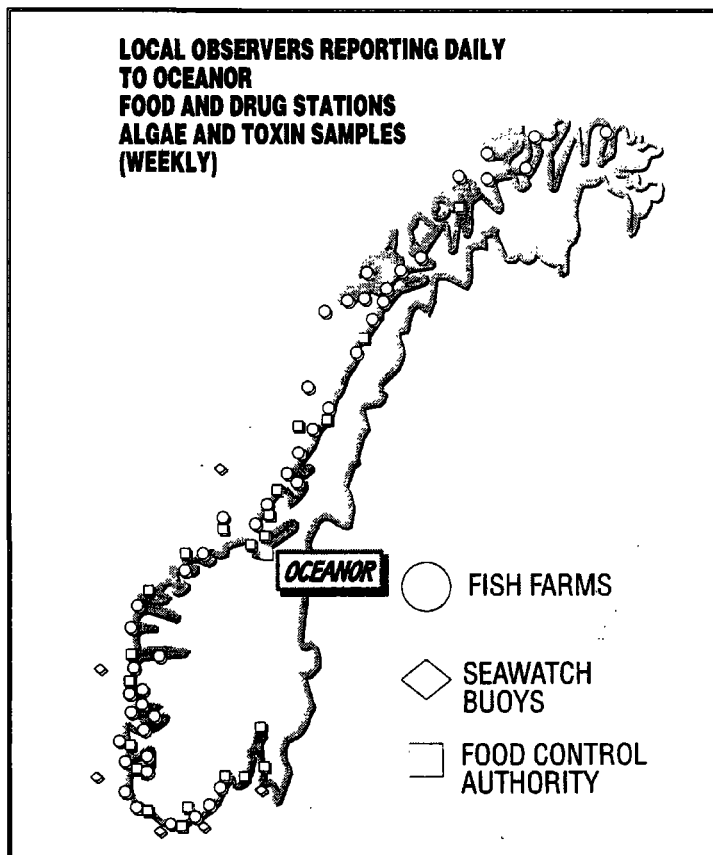
FIGURE 4.1. Sources of information in the Norwegian HAB monitoring program. Symbols denote locations of fish farms, SEAWATCH buoys, and stations monitored by OCEANOR. (Source: Andersen 1996.)

All data are collected and compiled by the private consultancy company OCEANOR, which is responsible for advising fish farmers if an HAB should occur. The major portion of the monitoring program cost is paid by the aquaculture industry. Additional information is obtained from the monitoring of phytoplankton in the context of shellfish harvesting, conducted by the State Food Control Authority.

The national monitoring program is currently under revision based upon experience in Norway and internationally. Over the years, revisions have involved moving sampling stations and/or increasing the number of stations to improve the coverage of the monitoring as well as updating and adding new species to the list of HAB species in Norwegian waters. The latest addition is *Chatonella* spp., which is new to Norwegian waters and which was involved in fish kills in the spring of 1998 (Einar Dahl, pers. comm).

Monitoring program purpose and objectives. The purpose of the monitoring program is to minimize losses to fish farms due to fishkills caused by HABs. The objectives are to collect monitoring information on the occurrence and distribution of HAB species in Norwegian waters concurrent with hydrographic conditions. These data are used for risk analysis and evaluation in the case of an HAB event. Based upon the risk analysis, proper mitigation measures to minimize losses are recommended and often implemented.

Sampling and analysis. A total of 80 stations are currently sampled in Norwegian coastal waters, covering the Norwegian coastline from the Swedish border in the south to the Russian border in the north. Samples for monitoring of harmful algae are collected weekly by a number of fish farmers, mussel farmers and the State Food Control Authority. If algal concentrations are high, based upon Secchi disk transparency measurements and water color, fish farmers will collect additional samples. Guidelines for sampling as well as Secchi depth measurement are provided to the fish farmers.

Fish farmers play a very important role in the Norwegian monitoring program. As mentioned earlier, the farmers routinely measure and report Secchi disk depth, color of the water and water temperature and collect phytoplankton samples for analysis for any occurrence of HAB species. Furthermore, the fish farmers are urged to contact OCEANOR if the fish behave abnormally or if mortalities occur (Table 4.2). The farmers are also advised to routinely consult with veterinarians, because problems can be caused by bacterial or viral infections.

Phytoplankton samples are collected by the observer at the farm site using a water sampler, usually at 0.5 m and 3 or 4 m depths. From each depth the sample is split in two: one unpreserved sample is used for live analysis and the other is preserved with formaldehyde. Standard samples are collected in 25 ml sterile plastic containers protected by transport containers and then sent by mail to OCEANOR for analysis. Since prompt analysis and response are necessary, the sampling is timed with the local postal routines to ensure that the transportation is as fast as possible. The samples, which are sent by ordinary mail, arrive at OCEANOR usually the day after the sampling or after two days from the more remote locations. Express mail is received from any part of the coast within 6-24 hours. In addition to the quantitative water samples, qualitative net samples are also collected as vertical hauls (0-15 m depth) using plankton net (mesh size : 20 μm). These samples are preserved using formaldehyde.

Another set of samples is received from the local food hygiene control authorities from 23 locations as part of the monitoring for toxic algae and algal toxins in mussels.

Quantitative water samples are analyzed using Palmer-Maloney counting chambers (volume 0.1 ml), or by using a Pasteur-pipette, two-drop sample on a standard glass slide, which equals 0.1 ml. This procedure is considered to be satisfactory for fish farm monitoring, since problems are observed only when concentrations of potentially harmful algae are fairly high (e.g. above 0.5×10^6 cells L^{-1}). A few diatoms, which may be harmful at low concentrations (*Chaetoceros convolutus/concavicornis*), are monitored

primarily in the qualitative net samples. If these *Chaetoceros* species are detected, the colonies are quantified using the quantitative water samples, either after settling in 2 ml chambers or concentration on membrane filters of 20-25 ml.

TABLE 4.2. Norwegian guidelines to fish farmers.

Contact OCEANOR:

- If the Secchi depth decreases to less than 4 m, or a rapid decrease is observed

- If the water is discolored

- If high concentrations of medusae are observed

- If abnormal fish behavior or death, which cannot be explained, is observed (in your own culture or neighboring cultures).

- If acute pollution is threatening your culture or neighboring cultures (oil, etc.)

Alert phones- OCEANOR
08.00-16.00   73 52 xx xx
16.00-08.00   Cellular phone: xx xx xx xx
Beeper: xx xx xx xx

Mitigation measures. It is considered very important that the fish farmers themselves take immediate action if a fish-killing bloom is reported in an area, or if they observe the fish beginning to behave abnormally. The action that must be taken to reduce losses involves different mitigation measures, which are carefully planned and tested in advance. The following mitigation and management measures are used in Norway. Of course, each farmer has different opinions on which is most effective, but all are aware of the choices:

- Prepare a prevention and management plan in advance, detailing possible actions to be taken, including consideration of all of the techniques below and early warning steps to be taken.
- Stop feeding the fish.
- Prepare for and conducting a pre-emptive harvest.
- Use a current generating propeller, which can dilute or spread a local bloom. However, this can increase exposure to harmful medusae, because the medusae are cut or broken into many small pieces.
- Move the culture pens into waters with less risk of contact with HABs.
- Move part of, or the entire fish farm away from the blooms, as occurred in 1988. This is less useful as the cages become larger.
- Install perimeter nets ("plankton curtain"), although their effectiveness is debated.
- Use aeration in combination with the above.

Fish farmers have arrangements with insurance companies that they may (free of charge) consult with OCEANOR experts for evaluation of HAB situations and advice on what to do to minimize losses. The

monitoring results are evaluated at OCEANOR using information from many sources including computer models, to simulate currents and to forecast the spread of blooms (see Section 3.4.3.3).

Dissemination of information. Data are communicated by fax, phone and the Internet. The updated information from the monitoring system is evaluated by a marine biologist and an oceanographer at OCEANOR during a routine surveillance meeting at the beginning of each day (Figure 4.2). If a HAB situation is under development or is already present, necessary action is taken to warn fish farmers and insurance companies about the situation for action to be taken to minimize losses. Furthermore, consultants are in a standby position to initiate emergency action at fish farms for a more accurate evaluation of the actual situation.
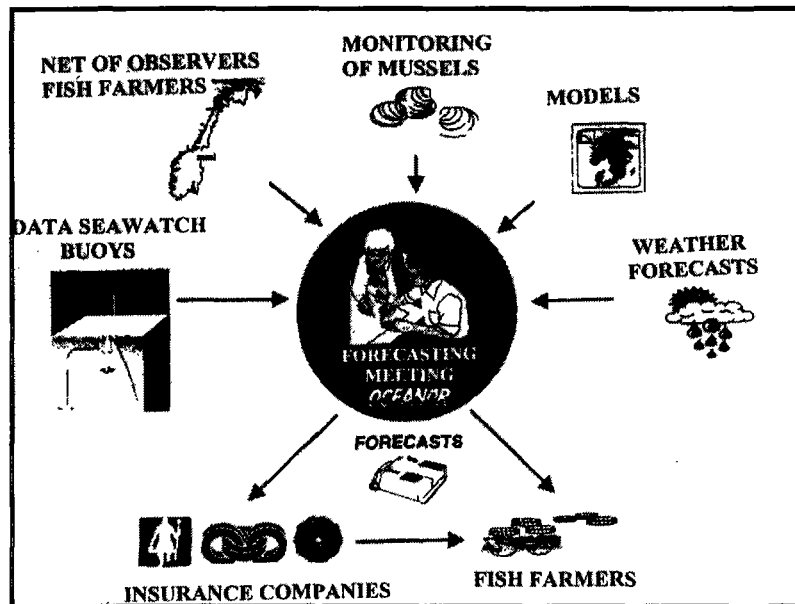
FIGURE 4.2. Scenario showing how information about HAB situations is collected, evaluated and communicated to fish farmers and insurance companies in Norway. (Source: Andersen 1996.)

- The Norwegian ALGEINFO www-page is updated frequently and summarizes the current monitoring data on HABs in the Norwegian waters. That is, it gives information on the risk of algal toxins in shellfish as well as the current occurrences of phytoplankton that can kill fish. It can be accessed at the address: **http://algeinfo.imr.no/**

Costs and benefits. The monitoring program for fish farming in Norway is primarily financed by the fish farmers together with insurance companies. Additional information is provided by the State Food Control Authority. The total cost of the monitoring is ~ US$300,000 per year, including salaries. This cost is based upon information in Andersen (1996) and may be an underestimate at present. The production value of salmon from fish farms is US$1,000 million per year. It is estimated that the value of the average annual loss of fish due to HABs is US$3 million per year, and the estimated reduction in economic loss due to HAB monitoring is US$2 million per year.

4.1.2   Pacific Northwest (North America)

Mariculture of salmon in net pens in Canada on both east and west coasts is rapidly becoming an important national product with 1999 production of Atlantic salmon of about 75,000 tons round weight (DFO 1999). By comparison, US production is less, about 24,000 tons round weight in the same year, with most production in Maine and the balance in Washington State.

4.1.2.1   Background and causative species

Routine monitoring of harmful microalgae in the Pacific Northwest by fish mariculturists began in 1987, and has been inextricably linked to differing types of fish kills that have occurred. Coastal and most inland marine waters of this region are very productive, with variable succession of species from rich spring blooms of diatoms to dinoflagellates or microflagellates in the summer and often a return to diatom dominance in the fall in general, depending on location and weather conditions. Plankton productivity is a mixed benefit, however, because the algal crop has included noxious diatoms and harmful microflagellates. This may result in occasional, large losses of net-pen reared salmon and sometimes wild fish too. Compared to other salmon growing regions of the world, the Pacific Northwest has certainly had its share of phytoplankton caused mariculture losses, according to industry insiders. Yet, known losses of wild fish are considered infrequent and rare compared to other North American waters.

A superficial review of Pacific Northwest mariculture and fish kills might conclude that the frequency of phytoplankton-related fish kills has increased in the past decade. This may be true, but probably not on a fish production-prorated basis. Rather, it more likely is related to the rapid growth in numbers of farms and changes in siting characteristics. There were relatively few mariculture ventures in the region in the 1970's and early 1980's, and they primarily cultured Pacific salmon. Atlantic salmon were imported to government net pens in Puget Sound in the early 1970's, in an attempt to build brood stock for restocking east coast US rivers. As market acceptability and culture characteristics of Atlantic salmon in mariculture became more widely known, Pacific Northwest growers began to switch their production away from Pacific salmon. A concurrent worldwide boom in demand for net pen salmon and interest in the industry brought foreign and domestic investors to the region in the late 1980's and encouraged local producers to expand. The net effect has been a large increase in the number of mariculture net pen sites in the Pacific Northwest, mostly in British Columbia which has larger, undeveloped coastline areas compared to Washington State.

The rapid growth of the industry in the 1980's resulted in mariculture spreading to new areas, previously unused by the industry. Fish-killing phytoplankton blooms may have been given some attention in siting studies, but other factors are often more pressing in mariculture siting. Only limited sampling or analysis was conducted at best, and, in many cases, no sampling was conducted at all. By trial and error siting, some fish farming locations were found to be unsuitable or less desirable for mariculture for a variety of reasons, sometimes due to repeated phytoplankton-caused fish kills. Prime examples of these areas that are no longer used are Sechelt Inlet in British Columbia due to *Heterosigma akashiwo* blooms and northern Hood Canal in Washington State due to *Chaetoceros* spp.

The primary phytoplankton species associated with mariculture fish mortality in the Pacific Northwest have been the raphidophyte microflagellate *Heterosigma akashiwo*, and diatoms of the genus *Chaetoceros*, subgroup *Phaeoceros* (Gaines and Taylor 1986, Rensel et al. 1989). *Ceratium fusus* is known to have killed fish and other marine life in southern Puget Sound. Some details of suspected and known fish-killing phytoplankton from Washington State on the west coast of North America are shown in Table 4.3. The purposeful omission of other species in Table 4.3 is for brevity.

TABLE 4.3. Fish-killing phytoplankton species known to be present in Puget Sound, Washington State, US. (Modified from Rensel 1995.)

Category and Species	Harmful Concentrations and Etiology	References
DIATOMS		
Chaetoceros concavicornis* and C. convolutus* and possibly others of the subgenus Phaeoceros such as C. danicus	> 2-5 cells ml^{-1} for salmonids, depends on chain length. Cells lodge between gill lamellae causing mucus production, irritation and leading to blood-hypoxia/anoxia	Bell 1961; Rensel 1992; 1993 Albright et al. 1993;
Skeletonema costatum*, Thalassiosira spp. * Corethron criophilum	Blooms of non-toxic diatoms caused mortality of juvenile salmon	Kent and Poppe 1998 Same Speare et al. 1989
DINOFLAGELLATES		
Alexandrium catenella	unknown; acute mortality to farmed fish not well documented or prevalent; chronic, food web problem with wild fish	White 1980; Mortenson 1985 (for A. tamarense); Eriksson 1988
Ceratium fusus* Noctiluca miliaris (= N. scintillans)	unknown; gill irritation, poorly understood variable; un-ionized ammonia causes gill damage and other problems for fish	Rensel and Prentice 1980 Okaichi and Nishio 1976
PRYMNESIOPHYTE FLAGELLATES		
Chrysochromulina polylepis	unknown; causes gill damage and osmoregulatory problems	Estep and MacIntyre 1989
Phaeocystis pouchetii	unknown; irritant substances and the alga's mucus can clog gills	Gaines and Taylor 1986 Smayda 1989
RAPHIDOPHYTE FLAGELLATES		
Heterosigma akashiwo	Probably variable, in most cases >750 to 1,000 cells ml^{-1}; cause of fish death unknown, may be similar to Chattonella	Black et al. 1991; Taylor and Haigh 1993; Tanaka et al. 1994
SILICOFLAGELLATES		
Dictyocha speculum	Unknown; siliceous skeleton may irritate gills, also possible toxin action	Larsen and Moestrup 1989
UNKNOWN ALGAL SPECIES		
Net pen Liver disease*	Unknown; chronic losses possibly caused by a microcystin-producing alga.	Kent 1990
Unidentified dinoflagellate* (Heterocapsa triquetra ?)	Mortality of delayed-release net-pen salmon in Hood Canal in summer, 1993	Horner and Rensel, unpublished data

* Have caused documented fish losses in Puget Sound or in British Columbia.

This case history deals with the types of fish killing phytoplankton in the Pacific Northwest, how they kill fish, siting of fish farms and monitoring practices that have co-evolved as a result.

4.1.2.2 *Chaetoceros* subgroup *Phaeoceros*

Causes of fish mortality. The first reported case of *Chaetoceros*-caused fish mortality involved wild lingcod (*Ophiodon elongatus*) that were captured and held temporarily in fishermen's cages in British Columbia (Bell 1961). Dead and dying fish had discolored gills and detritus including *Ch. convolutus* in gill mucus. Later, in an un-replicated bioassay, three lingcod were exposed to approximately 4 - 20 x 10^5 cells L^{-1} of *Ch. convolutus* cells for an unspecified period of time. Only one fish died and four diatom setae were reportedly embedded in a few mm^2 of gill tissue. Although the author stated that "the spines [setae] had actually penetrated the tissue and were not merely trapped in the mucus", no explanation about how this determination was made was included. Bell (1961) suggested that *Ch. convolutus* setae must break off the cells and enter the gill tissue butt-end (i.e., the former proximal end) first because of the apically pointed secondary spines on the primary setae. From that he reasoned that death could be caused by "capillary hemorrhage, by anoxia from the stimulation of mucus, or by debilitation of the tissue allowing microbial invasion." Bell's note finished by observing that it was unknown if the penetration of gills by diatom setae was a laboratory oddity or a significant natural phenomenon.

Histological and scanning electron microscopy by Rensel (1992) did not support the initial contention of Bell (1961) that *Ch. convolutus* setae break off the main cell frustule and enter the gill tissue butt end first. Bell's observations were based on one wet-mount of whole gill tissue viewed with a compound microscope (G. Bell, pers. comm.). He only reported a group of four setae in that observation that likely were grouped together because of their normal attachment in that number on *Ch. concavicornis* and *Ch. convolutus* cells. Many authors have cited Bell's initial hypothesis as the cause of *Ch. concavicornis* or *Ch. convolutus* induced stress and mortality and that the spines of the alga actually penetrate the gills. However, subsequent studies showed the importance of mucus and lamellar degeneration that was not associated with penetration by the setae, but rather associated with irritation by setae and possibly the secondary spines (Rensel 1992; Albright et al. 1993; Rensel 1993).

In the laboratory, salmon respond to *Chaetoceros* (subgroup Phaeoceros) exposure by an immediate and measured cough reaction (Rensel 1993). The coughing diminished slightly over time, probably due to physiological acclimation, as reported for other environmental irritants or chemicals (Heath 1995). Fish coughing in laboratory exposure experiments was prevalent only at moderate and higher concentrations of the diatom (e.g. 100 cells ml^{-1}), although after 2 days exposure to 10 cell ml^{-1} caused similar rates of coughing. There was a positive correlation between cough rate and exposure concentration and inverse linear relationship with blood oxygen concentration. Other experiments showed that higher cough rates were experienced from cultures with longer chains, grown in quiescent conditions. Besides coughing, the most notable behavior was inclination of some of the fish into a tail down attitude. It is possible that these fish were seeking waters of higher oxygen content near the surface, but prior studies discount that type of behavior for salmonids.

Species dynamics, example occurrences and monitoring. The *Chaetoceros* species involved in fish kills on the North American west coast usually do not bloom in high densities, but affect fish in mariculture facilities at very low concentrations (> 2-5 cells ml^{-1}). Hence the term "occurrence" rather than bloom is applicable. These occurrences can happen any time of the year, but may be more prevalent in spring and fall. As they are free-floating diatoms, their vertical distribution is controlled by vertical mixing, cell condition, and perhaps physiological factors. Chloroplasts are distributed throughout the long setae, which may be a light gathering adaptation. In several cases, researchers have found cells distributed throughout the water column, to relatively great depths (Rensel et al. 1989). However, in at least one occurrence, in a slowly flushed, vertically stratified bay, they were restricted mostly to the surface layer in unusually high density (Horner et al. 1997).

In southern Puget Sound, a State Government and Indian Tribal Co-operative fish rearing and release program suffered large fish losses in 1974, probably due to *Chaetoceros* spp. (Fraser 1975) but there were

Ceratium fusus blooms in the vicinity at the same time. The latter species is known to cause mortality or deformity of shellfish larvae (Cardwell et al. 1979), but has also been linked to mortality of pen-reared salmon and prawns, the latter also in 1974 in another bay in southern Puget Sound (Rensel and Prentice 1980). The co-operative farm is located in a shallow but relatively well-flushed channel, and has been used for commercial and public "delayed-release" culture of salmon for nearly three decades (WDF 1990). It has also been a study area regarding the environmental effects of salmon mariculture (Weston and Gowen 1989, Rensel 1989). No bloom mitigation techniques were attempted during the 1974 blooms, and very little monitoring occurred. Interestingly, the site has subsequently been used successfully for 15 years and escaped any further fish kills until 1997, when an apparent plankton-caused fish kill of unidentified origin occurred (K. Amos, pers. comm).

Both *Ch. concavicornis* and *Ch. convolutus* were present for several weeks during a net-pen fish kill in Deepwater Bay near the east coast of Cypress Island, Washington State in late October 1987 (Rensel et al. 1989). Later in the bloom, *Chaetoceros* spp. generally comprised about 30% of the phytoplankton population. The cells of these diatoms did not appear to be healthy and often had shrunken chloroplasts, suggesting that the bloom was ending. The concentration of *Ch. concavicornis* and *Ch. convolutus* decreased with depth and these species were not present at 25-m, offshore from the site. However the greatest concentration of *Ch. concavicornis* and *Ch. convolutus* at the site was at 15 m (2 x 10^4 cells L^{-1}). Other genera present included *Detonula*, *Nitzschia*, and *Thalassiosira* plus unidentified microflagellates, < 10-μm in size.

The water column at Cypress Island during the 1987 fish kill exhibited well-mixed characteristics to at least 25 m. Concentrations of *Ch. concavicornis* and *Ch. convolutus* counted toward the end of the bloom, however, indicated 1.4 x 10^4, 0.7 x 10^4 and 2.0 x 10^4 cells L^{-1} at depths of 2, 7.5 and 15-m, respectively. Chlorophyll *a* concentrations were low, less than 1.7 μg L^{-1} at all depths, and only 1.0 μg L^{-1} at 2 m. Water transparency was reportedly not reduced prior to, or during any period of fish loss. The lack of a noticeable bloom presents a particular problem for mariculturists dealing with these fish-killing species, as there is no visual cue that a harmful *Ch. concavicornis* or *Ch. convolutus* event is happening. Moreover, it is difficult to rapidly find a possible refuge area for moving the pens as monitoring by cell counting takes considerable time. The *Chaetoceros* occurrence in 1987 galvanized fish farmers in the area into learning more about fish-killing phytoplankton and supporting local research and monitoring, as described later in this report. The deadly action of the large-bodied and spiny *Chaetoceros* species was not readily accepted by other fish farming communities around the world. In Norway, for example, these species were not considered a threat to mariculture in the 1980's (Rensel, unpublished interviews, 1987). They are, however, now considered to be a threat (Andersen 1996).

4.1.2.3 *Heterosigma akashiwo*

The raphidophyte flagellate *Heterosigma akashiwo* is known worldwide as a dangerous fish killer in mariculture. The species has caused significant mortality of net-pen salmon in Puget Sound, Washington, British Columbia, Scotland, Chile, New Zealand, Japan, and other areas. Connell (2000) demonstrated with PCR studies of nuclear ribosomal DNA that 19 isolates from Atlantic and Pacific basins were virtually identical in an important internal transcribed spacer (ITS) region. This is evidence that all the isolates are one species and the high degree of ITS sequence identity implies that the organism has spread between oceanic basins in recent geographic time. This raises the possibility of spread by human means such as ballast water transfer.

Causes of fish mortality. The physiological cause of fish mortality from *H. akashiwo* exposure is uncertain despite several laboratory studies that used cultures isolated from local fish kills. Histopathology of gills of moribund subadult salmon from net-pen kills usually show major damage to the epithelium and mucus buildup, but not in one case with juvenile fish where a labile icthyotoxin was suspected as the cause

of mortality (Black et al. 1991). However, no toxin has been detected in the edible tissues (or any other tissues) of affected fish. Although *H. akashiwo* grows well in culture, axenic laboratory bioassays are non-toxic to fish (K. Banse, University of Washington, unpublished reports). Cultures with bacteria have been reported to be toxic, but results are sometimes unpredictable. Several authors have suggested that the fish-killing mechanism may be similar to that of the related alga *Chattonella antiqua* that occurs in Japan. Superoxide anion radical and hydrogen peroxide produced by the alga reportedly strip the fish gill of mucus and lead to fatal osmoregulatory stress (Tanaka et al. 1994). This does not, however, fit with current knowledge of salmonid physiology because the normal, unstressed condition is to have little or no mucus on the gills (Handy and Eddy 1991). Black et al. (1991) found no histological damage to gill and other tissue of juvenile salmon killed during *in situ* live cage studies. Conversely, gill tissue from moribund adult salmon killed in 1990 in Puget Sound showed major damage (Rensel, unpublished data from Global Aqua Inc. sites).

Heterosigma akashiwo blooms may extirpate or out-compete virtually all other algal species in the upper water column (Taylor and Haigh 1993). Their effect on larval or other fish frequenting shallow depths has not been studied, but there is evidence that wild fish or hatchery-released fish have been harmed. There were several reports of wild fish kills and wild fish acting in a distressed manner in Port Townsend Bay and the Strait of Juan de Fuca during the 1990 bloom. Dead adult chinook salmon were seen by local boaters in Bellingham Channel during the 1989 bloom, but no tissue samples were collected.

Bloom dynamics, examples and monitoring. *Heterosigma akashiwo* was present for many years in the region before the initiation of fish mariculture. A few experimental or limited net pen facilities began operation in the early 1970's, but did not gain momentum until the 1980's. Taylor and Haigh (1993) note the species has appeared with great regularity in late spring in the Strait of Georgia since 1967, when surveys were first initiated. Appearance there coincides with a rise in water temperature above 15°C and a decline in surface salinity to less than 15 ppt. There may have been a fish kill at the Lummi Indian Tribal mariculture "Seapond" near the US and Canadian border in 1976. Until 1989, mariculturists using net cages in Puget Sound were not subject to large-scale fish-killing blooms, as had occurred in British Columbia in prior years. Accordingly, there was not much interest in monitoring of phytoplankton by fish farmers.

Heterosigma blooms are somewhat predictable in the Pacific Northwest, as they apparently require quiescent weather conditions, as were experienced immediately prior to and during very large-scale blooms in Puget Sound and British Columbia in 1989 and 1990. Conceptual bloom models for subregions have been developed by Taylor (1993) and Rensel (1995). The models account for strong vertical stratification of the water column due to freshwater runoff and perhaps solar heating as prerequisites for bloom development. On the US East Coast, Li and Smayda (2000) found that *Heterosigma* blooms occurred with great regularity of timing, with a major peak in early summer and a minor peak later in the fall. Blooms of this species in the western Atlantic occur over a broad range from New England to Florida, but interestingly have not been linked to fish mortality (Tomas 1980, 1998). It is interesting to note that very large blooms that resulted in large fish losses occurred at similar timings in Puget Sound in 1989 and 1990.

The September 1989 bloom in Puget Sound was apparently associated with the Fraser River plume in Northern Puget Sound. At its peak, it covered a broad area of northern Puget Sound and the Strait of Juan de Fuca. The July 1990 bloom was an extremely large-scale event, covering virtually all areas of Puget Sound and the Strait of Juan de Fuca into the Pacific Ocean. It occurred in an extremely calm, warm period in early July following a June when Puget Sound lowland precipitation was 51% more than normal (NOAA, 1990). Moreover, routine monitoring in Admiralty Inlet approximately 10 days prior to the 1990 bloom confirmed that the surface layer was warm (near 15°C) and of reduced salinity (19.5 ppt) (Janzen 1992). This is significant given the mid-Puget Sound location of this sampling station, remote from creeks and riverine sources of freshwater. Other regional stations had similar values. Macronutrient content in the

surface layer was undetectable, but *H. akashiwo* is capable of vertical migration, moving up to 1 m/hr to obtain nutrients from subsurface depths. However, there is evidence that it remains near the surface during several of the fish kills in the region (R. Horner, unpublished data, assembled by J. Rensel).

In 1993, a bloom of *Heterosigma akashiwo* was recorded in Budd Inlet, the southern-most extreme embayment of Puget Sound and in central Puget Sound. No fish are cultured in Budd Inlet, but in central Puget in mid-July 1993, a bloom of *H. akashiwo* occurred in the Port Orchard/Brownsville area east of Bainbridge Island. This is a comparatively poorly flushed area and the soft bottom sediments may be a seedbed for some of the blooms, after inoculation during the 1989 or 1990 blooms. Antecedent rainfall and quiescent weather conditions were similar to those of the 1990 bloom. The bloom was detected by mariculturists from a nearby farm (Global Aqua Inc.), and was carefully tracked in its development by collection of frequent cell counts, hydrographic data including Secchi disk measurements and aerial observations. The bloom appeared to increase in size for several days but an abrupt weather change, including strong south winds, correlated with its rapid termination. After this bloom, the fish farming company in the area began using live cages placed in the bloom development area, as another means of fish kill early detection.

In the fall of 1994, naturally-occurring (wild) chinook, coho and chum salmon as well as several marine fish species were killed by a *Heterosigma* bloom in upper Case Inlet of southern Puget Sound (Hershberger et al. 1997). It is a shallow area where several streams enter Puget Sound and the fish were apparently unable to escape the bloom. The full extent of fish loss was unknown, but numbered at least in the hundreds. This was the first published documentation of wild fish having been killed by *Heterosigma*, but as noted above, it probably has occurred previously. The bloom occurrence fit previously-determined conceptual models of bloom development in terms of weather and timing. Additional blooms have occurred, (e.g., Connell and Jacobs 1999) but none have matched the extent and intensity of the July 1990 bloom, which was truly massive in scale. There have also been many *Heterosigma* blooms in British Columbia. However, this case history focuses on blooms in Puget Sound due to the author's involvement with those events.

Recent laboratory studies suggest that reduced salinity plays an important role in bloom formation of *Heterosigma akashiwo*. By simply adding a small amount of distilled water to the surface of vertical columns containing the alga, large quantities of the cells accumulated near the surface (Hershberger et al. 1997). The importance of freshwater-induced vertical stratification in field studies of *H. akashiwo* has been observed by Taylor and Haigh (1993). There are possible bloom-mitigation implications of these findings, discussed in the mitigation section of this report.

4.1.2.4   *Ceratium fusus*

In 1974, a dense, stratified bloom of *Ceratium fusus* was related to losses of coho salmon (*Oncorynchus kisutch*) and prawns (*Pandalus platyceros*) in a small inlet of southern Puget Sound (Rensel and Prentice 1980). This occurred at a pilot-scale mariculture farm operated by the Weyerhaeuser Company and the University of Washington in a shallow (< 7 m) inlet with very poor flushing rates (Pease 1975). The bloom was restricted to the top 4 or 5 m of water, above a sharp thermocline.

Prawns in cages moved to the sea bottom, below the bloom, survived at high rates compared to complete losses experienced by those remaining in surface waters. Large-sized sewage lagoon aerators (fountain style) and tugboat propeller wash were used in attempts to mitigate the bloom, but neither was judged effective as they just moved the bloom about the surface and did not displace the bloom with subsurface water. *Ceratium fusus* blooms in great density in southern Puget Sound inlets and bays during the summer. Although wild salmon migrate through these areas in the spring (juveniles) and fall (adults) the fish are generally not present in the summer months due to extremes of water temperature and possibly the normal assemblages of plankton. Although the cause of fish losses by *C. fusus* was not established, it may have

involved direct damage to the gills as the species has two sharp-pointed spines (unlike *C. furca*, which appears as a more typically-shaped, round-like dinoflagellate).

4.1.2.5   British Columbia (Canada)

Marine fish farming was initiated in British Columbia in the early 1970's with the culture of Pacific salmon. The industry began slowly, but increased in size rapidly in the 1980's along with expansion in other countries. At that time, the British Columbia Ministry of Agriculture and Fisheries funded a phytoplankton-monitoring program known as "Phytoplankton Watch." A consultant was hired who was responsible for training farm technicians, identifying and enumerating phytoplankton in routinely collected samples and co-ordinating communications. Some academic assistance was also provided in training and research by faculty and staff of the University of British Columbia and Simon Frasier University. A toll-free telephone hotline was established and weekly updates were made by the co-ordinator. The system relied on several "data farms" that sampled and reported regularly from differing geographic areas of the coast (Stockner 1990). British Columbia has an immensely large coastline, with hundreds of kilometers of coastline that vary greatly in hydrographic and algal bloom dynamics.

The Phytoplankton Watch program costs involved co-ordinator's salary, travel and telephone expense. The system was operated only six months of the year, during the main phytoplankton growing season. Costs were estimated to be approximately US$ 30,000 per year (E. Stockner, pers. comm.). No capital costs were associated with the program; the consultant provided her own microscope and ancillary equipment.

In the later years of the 1980 decade, a series of plankton-caused fish kills occurred in the inshore areas north of Vancouver, British Columbia. The industry opinion developed that these areas (e.g., the Sunshine Coast) were too poorly flushed or vertically mixed, and hence must be subject to repeated fish kills from algal blooms. Over time, most of the British Columbia mariculture industry moved completely out of these areas and concentrated in more remote areas that had stronger currents and higher apparent rates of vertical mixing in an attempt to avoid algal blooms. These areas included Johnstone Strait, the Broughton Archipelago and areas along the outer coast of Vancouver Island. As this move was underway, it became apparent that each fish-farming district was typically being affected differently by blooms. The need to communicate among districts appeared less important than previously thought. At some point, the government was no longer interested in funding the Plankton Watch Program. As the industry did not support it either, it was abandoned.

In the early 1990's, mariculturists in British Columbia conducted their own phytoplankton monitoring on a seasonal basis using techniques that evolved over the prior 20 years of experience. At least one consultant conducts training programs for individual fish farming companies. There are also one-day training courses for fish farm technicians offered periodically at Malaspina College in Nanaimo. College and local fisheries agency staff provide the training (I. Whyte, pers. comm.). Most recently, in 1999, a coordinated monitoring program known as the Harmful Algae Monitoring Program was initiated by Fisheries and Oceans, Canada, Pacific Biological Station that included many fish farms from different areas (Whyte and Haigh 2001). Twenty-seven sites were monitored in 2000, using standarized sampling and analysis techniques with centralized taxonomic, technical and information dissemination support. The program is funded directly by industry contributions and includes other ancillary efforts such as water quality and nutrient monitoring, special studies of new species, on site training, site selection/risk assessments.

As in most other salmon-growing areas of the world, there has been a shift from locating rearing pens in sheltered, poorly flushed waters to less-sheltered, more actively flushed areas. In British Columbia, many of the initial farms were in fjords with semi-blocking sills at their entries. These areas are now considered more environmentally sensitive as well as being generally prone to harmful microalgal blooms (SAR

1997). Concurrent with the move to higher energy areas, there has been a shift from small company-owned farms to large, corporate farms with multinational ownership, which is not unique to this area.

The shift to open channels and more actively mixing fish culture areas has not entirely solved the fish kill problems in British Columbia. Taylor (1993) noted this and concluded that it depended on the origin of the blooms relative to the fish farm location. If the blooms originate at the farm location, strong mixing was viewed as a particular advantage. If blooms were advected from outside, locating in a sheltered inlet might be an advantage. There have been several major losses due mostly to *Heterosigma* blooms in the 1990's. The reason for this is not clear, but it may be due to the combined effects of calm, warm weather with neap tides. It may also relate to interdecadal shifts in climate that influences flushing rates, vertical mixing and many other physical and biological parameters including wild salmon survival (Ebbesmeyer et al. 1989; Beamish and Bouillon 1993; Mantua et al. 2000). One particularly satisfying explanation involves optimum mixing theory. One manager presently believes that areas subject to vertical stratification, previously thought to be unacceptable for mariculture, may in fact be more suited to avoiding fish kills if mitigation techniques are used (G. Robinson, Stolt Sea Farm BC, pers. comm.). Methods such as perimeter skirts, subsurface open diffusers and airlift pumping may be quite effective in displacing surface-oriented blooms (see Section 6.4).

4.1.2.6   Washington State (US)

In Washington state, a similar pattern of response to fish killing phytoplankton occurred as was discussed for British Columbia. Net-pen culture of salmonids in Puget Sound began in 1969 with small-scale experiments at the Manchester Laboratory of the National Marine Fisheries Service, near Port Orchard (Mahnken 1975). By 1974, losses of cultivated fish that were related to the adverse effects of phytoplankton had been documented at several locations in southern Puget Sound (Fraser 1975; Cardwell et al. 1977; Rensel and Prentice 1980), in northern Hood Canal (R. Burr, pers. comm.), and Shoal Bay on Lopez Island (L. Harrell, National Marine Fisheries Service, pers. comm.). In many cases, the actual causative HAB species was not confirmed conclusively, but diatoms including *Chaetoceros* spp. and dinoflagellates or microflagellates were implicated in some cases.

Prior to the late 1980's, there were incidents of plankton-caused mariculture losses, but the documentation was sketchy at best and the incidents were geographically isolated. In part this was due to the belief that only certain parts of the region were subject to recurring mariculture fish kills. As in British Columbia, commercial fish farmers quickly moved out of the affected areas and relocated, if possible, to other regions. Some details of the sequence of events are summarized here, in part from Rensel (1992, 1995).

Federal funds from Washington Sea Grant were used to study the effects of fish killing phytoplankton on fish and to provide training and monitoring co-ordination among fish farmers. University of Washington staff and faculty were involved, as were other agency and tribal groups. A telephone hotline operated for several years, using information from the mariculturists. As the industry consolidated into just a few large companies and growing areas clustered in a few locations, the need for central co-ordination diminished and the co-operative monitoring was ended. The costs of this monitoring program are difficult to evaluate. That is because most of the budget and goals were focused on academic and applied research that was not directly related to the monitoring.

Presently there is only one remaining, large fishfarm company in Puget Sound with about 12 sites clustered in four geographic regions. The number of sites is similar to past decades, despite the consolidation in ownership. If fish-killing phytoplankton are observed at one site, there is often direct communication between staff members responsible for monitoring at different sites. There has been stiff opposition to fish farming in Washington State by shoreline residents concerned with aesthetics and water

quality. Several state agencies have sponsored comprehensive water and sediment quality reviews and regulatory efforts in response to these concerns (Rensel 2001). Combined with shrinking profitability as a result of international competition, the result has been an industry that has not expanded. Fish kills from phytoplankton have hurt profitability or contributed to losses, but it has not been an overwhelming factor.

Phytoplankton sampling at fish farms in Washington State is conducted during the spring, summer and fall, which is the normal algal growing season in that area. Most farms collect water samples or net tows at the farm sites for microscopic examination on a weekly basis, but on daily or more frequent timing during critical risk periods. Water from discrete sampling is placed in counting chambers or settled quantitatively onto microscope slides to enumerate cells. *Heterosigma* cells are counted live, but while compressed between the microscope slide and its glass cover. By introducing 0.1 ml of sample beneath a cover slip, a quantitative estimate may be made by using the more easily identified shape and movement of live cells that are slowed down by the pressure of the cover slip. For *Chaetoceros*, water is sometimes concentrated on filters, wetted with oil, and inspected for chains of the diatom.

Some farms use small fixed wing aircraft to survey the presence of blooms, particularly for *Heterosigma*, which form somewhat distinctive colored surface blooms. Often this is done in the mid-morning, before land breezes begin mixing the surface waters. "Ground-truth" surveys are conducted occasionally to verify the presence of suspected species and to note their concentrations. An observer in the aircraft uses a sampling form and map to sketch the visually apparent distribution of blooms by subareas. Many years of observations have shown that blooms in North Puget Sound orginate far from the fish farming sites, but may be advected towards the farms during fair weather that usually includes north winds.

For *Heterosigma* bloom prediction, some mariculturists have maintained live cages of salmon in suspected source areas of blooms from the late spring through fall in backwater channels of central Puget Sound. Combined with monitoring of weather (general wind and air temperature conditions), vertical stratification, cell counts and Secchi disk reading from the bloom source areas, approximate prediction of major bloom events, within a week or two, has been successful. In part this works because *Heterosigma* conceptual models of bloom intensity and duration were developed for specific subareas, based on empirical observations (e.g., Taylor and Haigh 1993; Rensel 1995). It also is possible because *Heterosigma* appears to have repeating patterns of bloom occurrence, at least in Narragansett Bay, Rhode Island (Li and Smayda 2000) but probably in other areas too. The pattern of occurrence in Puget Sound compared to Li and Smayda's (2000) study is highly similar, at least for the first peak in July, but the second annual peak seems to be earlier in the fall, based on unpublished fish farm counts and observations.

4.1.3 Japan

4.3.5.1 Background and Causative Species

Algae causing HABs in Japan are divided into two groups according to their harmful effects: 1) those that cause mass mortalities of marine organisms, and 2) species that can result in accumulation of toxins in shellfish and cause human intoxication. Only monitoring of algae causing mass mortalities among marine organisms such as fish and invertebrates will be dealt with here. During the 1970's, the frequency of HABs increased in the coastal waters of Japan due to eutrophication (Okaichi 1989). This was especially the case in the Seto Inland Sea, which is an important area for fish mariculture.

The increased frequency of HABs also increased the economic loss due to fish mortality of caged fish. The Seto Inland Sea Environmental Law was implemented in 1973 to counteract the increasing frequency of HABs. This followed massive fish-killing blooms of *Chatonella antiqua* in 1972, in which the fishermen lost 14 million yellowtail, worth 71 billion yen (US$0.5 billion). A result of the law was a

decrease in the eutrophication of the Inland Sea, which apparently has led to a decrease in the frequency of red tides. The frequency of red tides in the Seto Inland Sea was still approximately 10 per year in 1991. The economic loss caused by red tides between 1972 and 1991 was estimated to be US$165 million.

Dinoflagellates and raphidophyceae species, which are known to cause mass mortality of marine organisms in Japanese coastal waters, are summarized in Table 4.4.

TABLE 4.4. HAB species known to cause mass fish mortalities in Japanese coastal waters. (Source: Fukuyo 1992.)

Dinoflagellates	Raphidophyceans
Cochlodinium polykrikoides *Gymnodinium mikimotoi/nagasakiense*	*Chatonella antiqua* *Chatonella marina* *Fibrocapsa japonica* *Heterosigma akashiwo*

Monitoring Programs

The Japanese HAB monitoring program was reviewed by Fukuyo (1992) and Okaichi (1989). Phytoplankton cells are monitored through regular qualitative and quantitative sampling; analysis includes inspection of the whole phytoplankton community including toxic species at fixed stations. Samples are contributed by prefectures and fishermen's cooperative unions. Information from fishermen is collected at the Seto Inland Sea Fisheries Coordination Office, which then routes it to the Fisheries Agency and other national institutions and prefectural authorities. Aerial surveillance is conducted using light aircraft to determine the extent and movement of blooms. Within an hour after the flight is completed, data is compiled and faxed to the appropriate authorities (Figure 4.3).

In the Kagawa Prefecture, the fishermen participate in the monitoring program in cooperation with the Kawaga Prefecture Government (Ono et al. 1996). The Prefectural Government cannot afford to monitor all the waters used by the nurseries every day. For that reason the Government monitoring program is focused on the offshore waters, while the fishermen concentrate their monitoring on the fish culture areas (Figure 4.4).

This monitoring program was implemented in 1978. The fishermen involved in the monitoring program have been trained in phytoplankton monitoring at Akashiwo Research Institution. Training courses have been held every year since 1978. The fishermen who have attended the training courses can identify not only HAB species, but also other phytoplankton including toxic species (Ono et al. 1996).

Fishermen collect samples from 0, 10 and at some stations, 25 m depths. During the primary HAB season, the fishermen monitor the phytoplankton on a daily basis. The information obtained is used for early warning and when needed, to implement mitigation measures to prevent or minimize damage to the fish by harmful algae (Ono et al. 1996).
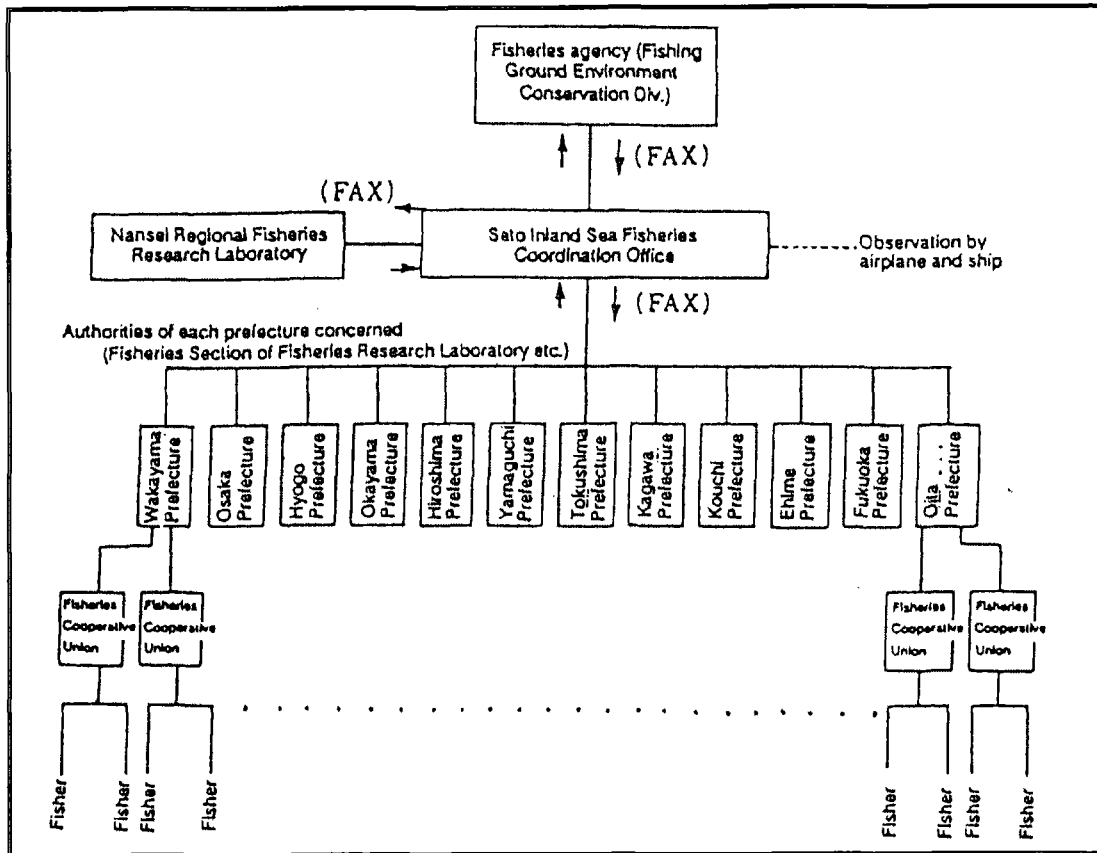
FIGURE 4.3. Information collection and distribution system for red tide/HAB information in the Seto Inland Sea, Japan. (Source: Fukuyo 1992.)
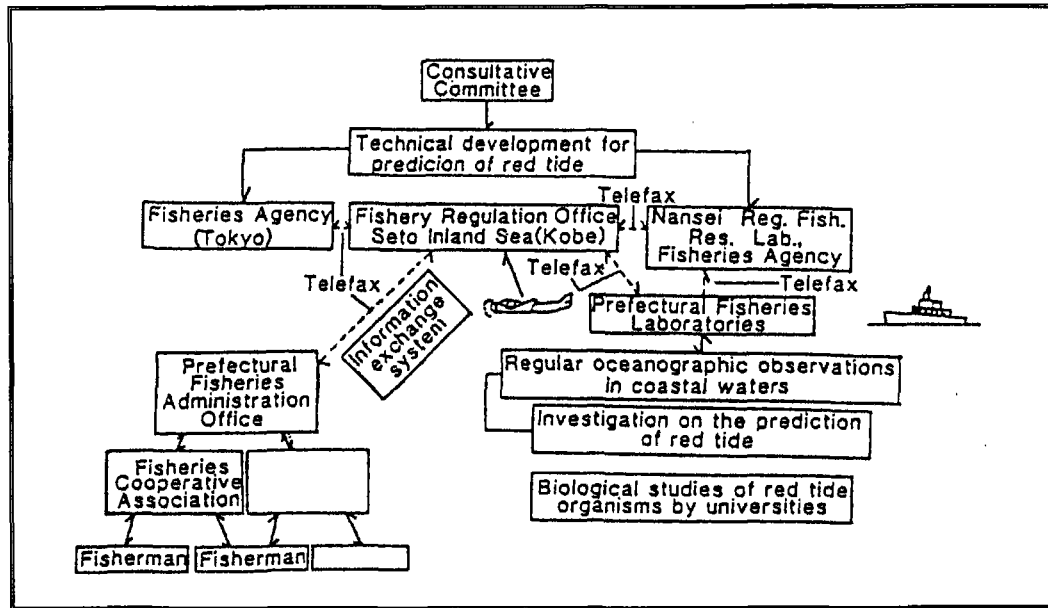
FIGURE 4.4. Information exchange and red HAB investigations in the Seto Inland Sea, Japan. (Source: Okaichi 1989.)

4.1.4 Chile

4.1.4.1 Background and causative species

Chile is the second largest producer of farmed salmon in the world. In 1995, the gross production of farmed salmon and trout was approximately 141,000 tons, following a period of rapid growth in recent years from a combined production level of 8,600 tons ten years ago. In 1998, gross production exceeded 200,000 tons. The primary species raised are Atlantic salmon, coho salmon and sea rainbow trout, in that order.

The fish farming industry there has been rapidly expanding and prospering for several reasons. Fish culture conditions are generally very good for salmon and trout. For example, water temperatures are very suitable for salmon, and current velocity at most sites is reportedly good. There are extensive, semi-protected coastal areas with little pollution. Feed costs, that are often approximately half of a salmon farmer's costs, are minimized due to the close proximity to major fishmeal production facilities in Peru, Ecuador and Chile.

As of 1998, there were approximately 60 companies operating salmon and trout farms in Chile, with approximately 361 farming concessions (leases) authorized by the fishing and maritime authorities. There are approximately 100 pending resolutions that have not been authorized yet by the authorities. There are 185 authorized (licensed) fish farms, approximately 80 of which are operating. The farms cover an area of approximately 4,700 hectares. The main markets for Chilean farmed salmon are Japan and the United States. As elsewhere, the price of farmed salmon and trout raised in Chile has declined sharply over the recent years due to increasing production (SAR 1997).

Fish killing phytoplankton have been monitored in Chile since at least 1983 by the mariculture industry, but in 1989 a systematic monitoring program was initiated and currently over 27 farm sites are monitored (INTESAL 2001) There have only been two very large-scale plankton blooms that adversely affected the mariculture industry in Chile. The first was the 1988 bloom of *Heterosigma akashiwo* that caught many of the farms unprepared for a fish-killing event of that magnitude. Details of how this species affects fish are discussed in the section on monitoring in the Pacific Northwest. The other large-scale bloom causing fish kills was from *Gymnodinium* spp. in the late 1990's, although the details have not been published. In addition to marine species in Table 4.5, there are reports of fish kills from blue-green algae (cyanobacteria) in unidentified lagoons (lakes, INTESAL 2001).

4.1.4.2 *Heterosigma akashiwo*

The major *Heterosigma akashiwo* bloom of 1988 helped define the need for phytoplankton monitoring as an early warning and management practice. The bloom was first noticed in mid-August near the Pacific side of Chiloe Island (Murphy 1988), although there may not be sufficient information to reach that conclusion (A. Clément, pers. comm.). No losses were reported immediately and no sampling was conducted. By late August, large-scale losses were reported in a few farms. The bloom spread and killed mariculture fish in a number of areas throughout most of September. At least 24 farms were affected, resulting in estimated losses of 2,000 tons of salmon and trout valued at US$16 million. Some limited water sampling at different depths and locations was conducted to try to determine how to mitigate the losses. By mid-September, some aerial observations of the bloom were made showing that large areas appeared to be unaffected. One experienced observer reported that the initial response to the bloom was "panic," and that in most cases the farmers were ill-prepared to mitigate the losses.

TABLE 4.5. Fish-killing phytoplankton species known to be present in coastal waters of Chile.
(Data source: Alejandro Clément, Instituto Tecnológico del Salmon.)

Category and Species	Comments
Diatoms	
Chaetoceros convolutus	At least three separate occurrences have killed salmon in mariculture pens. Most recent occurrence in 1998 with few fish losses. Similar, but unidentified species observed to cause abnormal fish behavior.
Leptocylindrus minimus and *L. danicus*	First occurrence of these harmful diatoms that killed salmon in Chilean pens. At least seven separate mariculture fish kill events reported, including an unusual winter bloom in 1998. Other possibly harmful diatoms might include *Asterionella, Rhizosolenia,* and *Corethron.*
Dinoflagellates	
Alexandrium catenella	Maximum cell numbers about 800 ml⁻¹. Affected mariculture fish and wild fish larvae. Cause of fish death not determined (A. Clément, pers. comm.).
Ceratium hirundinella	One reported freshwater net pen aquaculture fish kill possibly caused by this freshwater species. Some doubt involved, possibly a cyanobacteria (blue-green alga) involved.
Prorocentrum micans	One reported mariculture fish kill, physical action on fish.
Raphidophyte flagellates	
Heterosigma akashiwo	One very large-scale bloom in 1988 caused massive losses of mariculture fish. A subsequent bloom about 1996 caused smolt mortality at one farm, but in different area.
Silicoflagellates	
Dictyocha speculum	Losses of trout in mariculture facility reported once.

Murphy (1988) made some important observations regarding the bloom. Analytical tests of fish flesh for PSP were conducted in Santiago, with negative results. Murphy reported that marketing of salmon continued during the bloom, and prices were not affected (much of the product is exported). However, most of the fish were coho salmon and at that time of the year were below market size so the actual amount of fish marketed is unclear (A. Clément, pers. comm.). It is also unknown if some of the algal-killed fish were marketed, as has occurred elsewhere in the world for *Heterosigma*-killed salmon.

The vertical distribution of the bloom was monitored in some cases. It varied considerably from near the surface down to 20 m, but no data have been published. It is likely that most of the bloom was concentrated near the surface, and that much lower cell numbers were found at depth. Only one farmer

towed his cages away from the bloom, and had no losses for his efforts. Other mitigation methods were tried such as lowering of the pens and covering them with dark covers, but losses were incurred with these, in part due to damage from abrasion of the nets on the fish. As fish kills from harmful algal blooms have been relatively rare in Chile, and losses normally only average about 2 to 5% of the standing crop (A. Clément, pers. comm.), not much attention has been paid to mitigation systems subsequent to the 1988 massive losses.

4.1.4.3 Chilean fish farms and phytoplankton monitoring

Chile is only one of two countries worldwide that has a coordinated phytoplankton monitoring program for fish mariculture. It may be the only country in the world that has a phytoplankton monitoring program funded entirely by the fish farmers, with no government support. This is accomplished through the Chilean Salmon Farmers Association, in association with the Salmon Technological Institute (i.e., INTESAL Instituto Tecnológico del Salmón, S.A.). As of 1998, the program costs approximately US$ 31,000 per year, including operational costs, labor, computers, travel and other expenses (A. Clément, pers. comm.).

Routine sampling is conducted by fish farm technicians who send the samples to the program co-ordinator and technician for analysis. During normal conditions, a report is generated every 10 days and distributed to the farms. During fish kills, or when harmful algal species are present, the results are faxed immediately to the farmers as special reports including a discussion of distribution, concentrations, fish behavior and mortality, etc.

Samples are not preserved, but kept fresh for analysis. All species are counted, not just potentially harmful ones. Counting techniques involve a modified Utermol methodology using an inverted microscope. Rather than developing advanced warning mechanisms for harmful blooms, the Chileans have developed a contingency plan for dealing with the threat of fish kills (Clément 1997). This plan specifies that monitoring is a cornerstone of dealing with the problem, but also details how training, communications, co-ordination and new technology can be applied. The plan gives explicit examples of mitigation and how they can be applied to particular fish-killing algal species.

4.2 Ciguatera

Ciguatera toxins originate in benthic dinoflagellates that are grazed by herbivorous reef fish. The fat-soluble ciguatera toxins are then transferred to carnivorous fish. Ciguatera fish poisoning (CFP) is therefore not an HAB phenomenon, but it is nevertheless a serious source of seafood poisoning, and therefore deserves attention in monitoring programs for algal toxins.

Ciguatera problems are serious in the Pacific, and much can be learned from the policies of countries in that region. Unfortunately, routine monitoring of fish at the market or at the harvest site for ciguatera is presently not conducted in any country, to our knowledge. Instead, restrictions are placed on the sale or consumption of certain species of fish that are known to be frequently contaminated with ciguatera toxins. For example, in Queensland, Australia, fish species such as red bass, "chinaman fish", and paddletail are not commercially exploited due to ciguatera (Lewis 1994). On islands like St. Barthelemy in the Caribbean, fish are not harvested from areas of the island where history has shown the fish to be frequently contaminated with ciguatera toxins (Lobel et al. 1988).

Perhaps the best experience with detecting ciguatoxins and diagnosing CFP is in Hawaii. There, the Department of Health (DOH) maintains a database to track the occurrence of ciguatera cases. Physicians

and hospitals are required to report suspected cases of ciguatera poisoning. DOH attempts to obtain samples of the fish that were consumed, and arrange for analytical testing. The analytical testing is done at the University of Hawaii using a membrane immunobead assay (MIA) or a new test kit known as Cigua-Check[TM1] (described in Section 3.1.6). Some of the prior test kits utilized a solid phase immunobead assay (SPIA), and were derived from tests developed and subsequently modified over the years by Professor Hokama (Lewis 1995; Hokama et al. 1998a, b).

In past years, the State of Hawaii helped fund production of 10,000 to 15,000 SPIA analytical test kits per year that were distributed free to fishermen. The kits were simple to use and included a request for voluntary return of data regarding the fish species tested, weight, and area of catch. In one survey 1,067 fish of various species were tested for ciguatoxin, 510 were from Oahu, 402 from Hawaii, 75 from Maui and 50 from other islands (Hokama et al. 1993). Fully 20% of the fish tested positive for ciguatera, 41% were borderline, and 39% were negative. Importantly, no false negatives were noted (i.e., individuals eating fish analyzed as negative by the test kit did not report CFP symptoms). The kit protected the public when only fish assaying "negative" with the kit were eaten. Fish that tested "borderline" or "positive" were generally unsafe, especially the latter. The data indicated that the probability of getting ciguatera poisoning with a test-kit positive fish was 1 out of 3.

Clearly, the absence of a rapid, reliable, cost-effective assay for ciguatoxin has prevented countries from implementing monitoring programs. If the Cigua-Check[TM] test kit or a related product lives up to its claims and provides acceptable precision and reliability, we can expect to see fish monitoring programs to define areas at risk for ciguatera, much as is presently done to detect toxins in shellfish.

4.3 Shellfish Monitoring

Molluscs, bivalves and gastropods are typically the primary vectors of algal biotoxins to human consumers, although crustaceans (e.g. crabs and lobsters) can also transfer algal biotoxins through the food chain (reviewed by Shumway 1990). The sources of shellfish samples for biotoxin monitoring programs and their main advantages and constraints are summarized in Fig. 4.5. Clearly, the optimum, safest and most commonly used practice involves sampling of wild or cultured product directly from the natural environment, as this allows unequivocal tracking of toxins to their site of origin and targeted regulatory action. The following section provides brief descriptions of selected shellfish monitoring programs globally, which vary in scale, the number of toxins and type of resource (cultured vs. wild product) monitored, the time since their implementation and thus maturity of the program, etc. They serve to illustrate common features of monitoring strategies used worldwide, while also emphasizing their distinctive features. Other countries with well established, excellent monitoring programs are not included here due to space considerations.

4.3.1 United States

4.3.1.1 Atlantic US: State of Maine

The State of Maine has developed a comprehensive program for monitoring of PSP toxins in shellfish, in accordance with requirements of the National Shellfish Sanitation Program (NSSP). This program is selected as a case study because it is well established, very effective in meeting its stated objectives, and is

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[1] Oceanit Test Systems, Inc. 1100 Alakea Building, 1100 Alakea Street, Honolulu Hawaii. 96813.

| 1. Stocks collected from the
natural environment
(PRE - HARVEST) | • Most common practice
• Traditionally used to
  monitor wild fisheries
• Reduces economic losses,
  risk and animosity from industry |

POST - HARVEST:

| 2. Harvested product at market
and distribution centers. | • Requires rapid response
• May be an option
  for offshore fisheries
• Is usually a complement to 1 |

| 3. Commercial product supplied
by industry (eg. aquaculture) | • Applied when routine monitoring
  is limited or unavailable
• Often by special agreement
  and with cost-sharing by industry |

| 4. Imports | • Used as an occasional check
• Increasingly, the burden of monitoring
  is being shifted to the exporting nation |

FIGURE 4.5. Sources of shellfish for routine biotoxin monitoring.

one of the largest PSP monitoring programs in the US. It has been traditionally aimed at surveillance of harvest areas by individual species. The description of the program below is based on the following publications: Hurst and Gilfillan 1977, Shumway et al. 1988, Shumway et al. 1995, Hurst 1997, as well as updated information presented at the Toxin Workshop held in September 1998 in Boothbay Harbor, Maine and personal communications with Department of Marine Resources (DMR) personnel (L. Bean and J. Hurst). Other toxins have not yet posed a public health concern in this region. However, there have been occasional incidents of unexplained gastroenteritis associated with consumption of shellfish, and one lot of shellfish exported to the Netherlands was rejected on the basis of testing for DSP toxins (Morton et al. 1999). Recent, extensive sampling of mussels for DSP toxins along the coast of Maine revealed the presence of low levels of OA-like activity (6.4 to 20 ng g^{-1}, measured by the protein phosphatase inhibition assay), i.e. 100-fold below the Canadian and European safety limit, only in Frenchman Bay and Eastern Bay (Morton et al. 1999). Phytoplankton samples containing *Dinophysis* spp. yielded no activity using this assay, whereas the epiphytic community on brown macroalgae at all sites where mussels tested positive, contained *Prorocentrum lima* with positive activity. LC-MS/MS analysis confirmed the presence of DTX1 in *P. lima*.

Purpose/Objectives. The primary purpose of the shellfish PSP toxin surveillance program is "to protect ... public health, while providing for the harvest of susceptible species of marine molluscs in areas not affected by contamination" (Maine Marine Resources Law, Section 6076). Thus, an important secondary objective is to allow optimum utilization of local shellfish resources.

History and general description of the program. The biotoxin monitoring program for the State of Maine was initiated in 1958 in response to an outbreak of PSP in New Brunswick, Canada in 1957, and has been modeled to a large extent on the Canadian program. It focuses mainly on PSP toxins, the principal toxin problem in the region, but has recently also included limited monitoring for domoic acid (DA) in shellfish, which is analyzed by the USFDA. Testing for DSP toxins in shellfish has also begun recently, but is not conducted routinely, and only when phytoplankton monitoring points to the presence of *Dinophysis* spp. cells in the water column. DMR is presently developing the capability to test for DSP by mouse bioassay. There have been no outbreaks of DSP or ASP in Maine so far, although DA levels below the regulatory level have been found in mussels in late summer, and mussels from the Lamoine State Park area tested positive for DSP toxins in 1998, using both mouse and phosphatase activity assays (S. Morgan, unpubl. results).

The Maine biotoxin monitoring program has been highly effective in safeguarding public health, since no illnesses or deaths have been attributed to PSP from shellfish commercially harvested in the State of Maine. All illnesses documented have been linked to illegal recreational harvesting in closed areas. Historical data collected over more than 20 years have produced a fairly detailed knowledge of the rates of uptake and elimination of PSP toxins by the principal bivalve species tested, and thus allowed some success in predicting the annual timing and location of PSP outbreaks. More detailed knowledge gained over the years on spatial and temporal toxicity patterns has also allowed reduction in the size of areas than need to be closed to ensure public safety during toxic events.

The initial monitoring program included only five monitoring sites, but was expanded in 1961 and again in 1975, following a severe PSP outbreak in 1972 which affected 2800 acres of shellfish harvesting grounds and in response to a new influx of funding from the New England Regional Commission. Expansion of the program also reflected the rising value of the local shellfish resource: both mussel and softshell clams experienced a marked increase in landings in the 1970's (Shumway et al. 1988). At present it includes coast-wide sampling of shellfish and analysis of about 3500 samples per year for PSP toxins. This intense sampling effort allows harvesting of some species in localized areas during a toxic outbreak. Maine's shellfish resource is based largely upon a public, bottom fishery, and aquaculture activity is relatively limited. Therefore, toxicity testing is mainly based on collection of shellfish from wild populations. However, the aquaculture industry (e.g., Great Eastern Mussel Farm, Inc.) routinely submits mussels from its bottom culture operation to DMR for toxicity testing. DMR personnel located coast-wide report any unusual occurrences indicative of a toxic event, such as bird or fish kills, water discoloration and abnormal behavior of shellfish, to the Marine Sciences Laboratory in Boothbay Harbor, Maine.

Agency responsibilities with respect to implementation and enforcement of the monitoring program are outlined in Figure 4.6. A Memorandum of Understanding (MOU) between the Department of Marine Resources (DMR), the Department of Human Services and the Department of Agriculture, Food and Rural Resources designates DMR as the state agency responsible for marine biotoxin monitoring. John W. Hurst, Jr. is DMR's contact person in events involving marine biotoxins. DMR is also the agency responsible for bacteriological analysis of Maine shellfish-growing areas. This MOU also establishes the responsibilities of the three state departments in the event of a marine biotoxin emergency. Decisions on shellfish closures and product embargo made by DMR personnel require written authorization by the DMR Commissioner or his authorized designee. Enforcement of closures of harvest grounds is the responsibility of Marine Patrol officers from the Division of Marine Patrol (DMP).

AGENCY	RESPONSIBILITY / FUNCTION
Bureau of Resource Management in State of ME Department of Marine Resources (DMR)	Implements shellfish toxin monitoring program: • Sample collection • PSP toxin analysis • Establishes closures & re-opening of harvest areas • Embargoes toxic product from wholesalers/seafood dealers/ processors • Maintains toxicity records • Participates as a member of ISSC
Patrol officers; Division of Marine Patrol (DMR)	Enforces closures • Patrols harvest areas • Posts warning signs • Arrests illegal harvesters
State of ME Department of Human Services	Investigates reported biotoxin-related illnesses & informs DMR
State of ME Agriculture, Food & Rural Resources	Removes contaminated shellfish from retail establishments

FIGURE 4.6. Structure and responsibilities for the shellfish biotoxin monitoring program in the State of Maine (ME), Atlantic US. (Based on consultation with L. Bean, State of Maine DMR.)

There is close communication between DMR in Maine and government agencies (CFIA and DFA) in Atlantic Canada involved in monitoring of toxic shellfish and harmful phytoplankton, the USFDA, and officials involved in toxin monitoring from other Atlantic states in the US, such as New Hampshire and Massachusetts. Information on the status of toxicity in their respective regions is freely exchanged, especially when toxicity in an area may progress to neighboring states or Canada. Annual Toxin Workshops provide an informal forum for this exchange.

Principal shellfish species tested. PSP toxins are monitored routinely in the blue mussel, *Mytilus edulis,* and the softshell clam, *Mya arenaria,* two important commercial species in the region. Historical toxicity records for these two bivalves at some sites date back several decades. Other nearshore species are tested occasionally, such as the European oyster, *Ostrea edulis, Mercenaria mercenaria* (collected subtidally), *Ensis directus* and *Modiolus modiolus.* The Atlantic surfclam, *Spisula solidissima,* has been routinely tested since 1975 at eight stations in southern Maine waters. This species is characterized by prolonged retention of PSP toxins (Bricelj and Shumway 1998). Offshore surfclam populations from Georges Banks were first found to be contaminated with PSP toxins in 1989, and have remained closed to harvesting through 1998 (White et al. 1993). Testing of these populations was recently discontinued, since given the high toxin levels found when they were last tested, there is no incentive for fishermen to renew harvesting at this site. The ocean quahog, *Arctica islandica,* a deep-water, offshore species, has been tested since 1985. Sampling is conducted routinely from established offshore stations located by Loran, by DMR personnel via use of contract fishing boats, rather than directly from the fishermen's catch (L. Bean, DMR, pers. comm.). The sea scallop, *Placopecten magellanicus,* another offshore species, has been occasionally tested, but there is at present no consideration for lot testing of wild sea scallops, since the harvest area of scallops landed by commercial fishermen cannot be readily determined.

Sites and frequency of shellfish sampling. For the purpose of shellfish sampling, the coast of Maine is now divided into 18 areas. Each contains 4 to 20 sampling stations (Hungerford and Wekell 1993), including a primary or key station, located adjacent to a shellfish harvesting area, which historically showed high toxin levels, thus providing a reliable and early indication of the presence of shellfish toxicity (Figure 4.7). Softshell clams and mussels are collected intertidally from a total of approximately 18 primary stations, 35 secondary stations and 63 tertiary stations, which were established based on historical data. Sampling takes place weekly in primary stations, regardless of the toxicity pattern observed, between early April and October. Sampling in the southern half of the state, historically the first area to show toxicity, is initiated in the last two weeks of March to obtain baseline data. The occurrence of shellfish toxin levels exceeding the regulatory level (RL) is highly seasonal in Maine, generally occurring in the spring and summer, between early May and late October. Once toxicity is established in primary stations, sampling is extended to secondary and then tertiary stations to better localize the distribution of toxin, and samples are collected more frequently. Areas such as Casco Bay and Cobscook Bay, which contain much of the state's clam resources are closely monitored, and portions kept open when at all possible to allow safe harvesting of clams and reduce the economic impact of red tides.

Shumway et al. (1988) suggested that expanded offshore sampling might aid in early warning of toxic events, since mussels from offshore islands often become toxic before those from the mainland. This was recently confirmed by a research study conducted in the Gulf of Maine, in which mussels moored offshore showed detectable toxicity levels before those that were sampled nearshore as part of the routine monitoring program (D.M. Anderson, unpubl. data).

Surfclams, *S. solidissima,* are sampled during the spring and summer each year and also during the fall and winter during years of toxicity, because they are known to detoxify very slowly. Domoic acid is analyzed by FDA from monthly samples collected at primary stations during the PSP season.

Phytoplankton monitoring. Maine started a phytoplankton monitoring program in 1997, which is partly funded by US FDA, but is still in its infancy compared to that in Canada, Europe or Japan. Sampling is conducted between April and November, at 40 to 60 collection sites, which do not correspond to PSP sampling stations. The algal species are monitored qualitatively, and include *Alexandrium* spp., *Dinophysis* spp., *Pseudo-nitzschia* spp. and *Prorocentrum* spp. The program is being conducted by DMR and the University of Maine Cooperative Extension, using 20 volunteer groups working with equipment furnished by the program and supervised by personnel from the Bigelow Laboratory of Ocean Science or DMR. Discussions are also underway with the Maine Aquaculture Association to interest fish farmers in plankton monitoring at their leases.

Toxin analysis. Shellfish for toxicity testing are collected by DMR personnel and returned to DMR's Marine Sciences Laboratory under refrigeration, where PSP toxins are analyzed by the standard AOAC mouse bioassay. Mice (Charles River strain) are purchased from Massachusetts, and yield a conversion factor of 0.2 µg STXeq MU^{-1}. Three female mice are used per assay. Saxitoxin standard from FDA is used for calibration. Results of toxicity testing are available within a day or less of sample collection, since rapid implementation of closures is considered important in avoiding the costly problems and negative press associated with seizing of commercial catches. Two to three individuals are involved in sample collection, and five to six in toxin analysis (three at the Boothbay Laboratory and two to three at the Lamoine Laboratory).
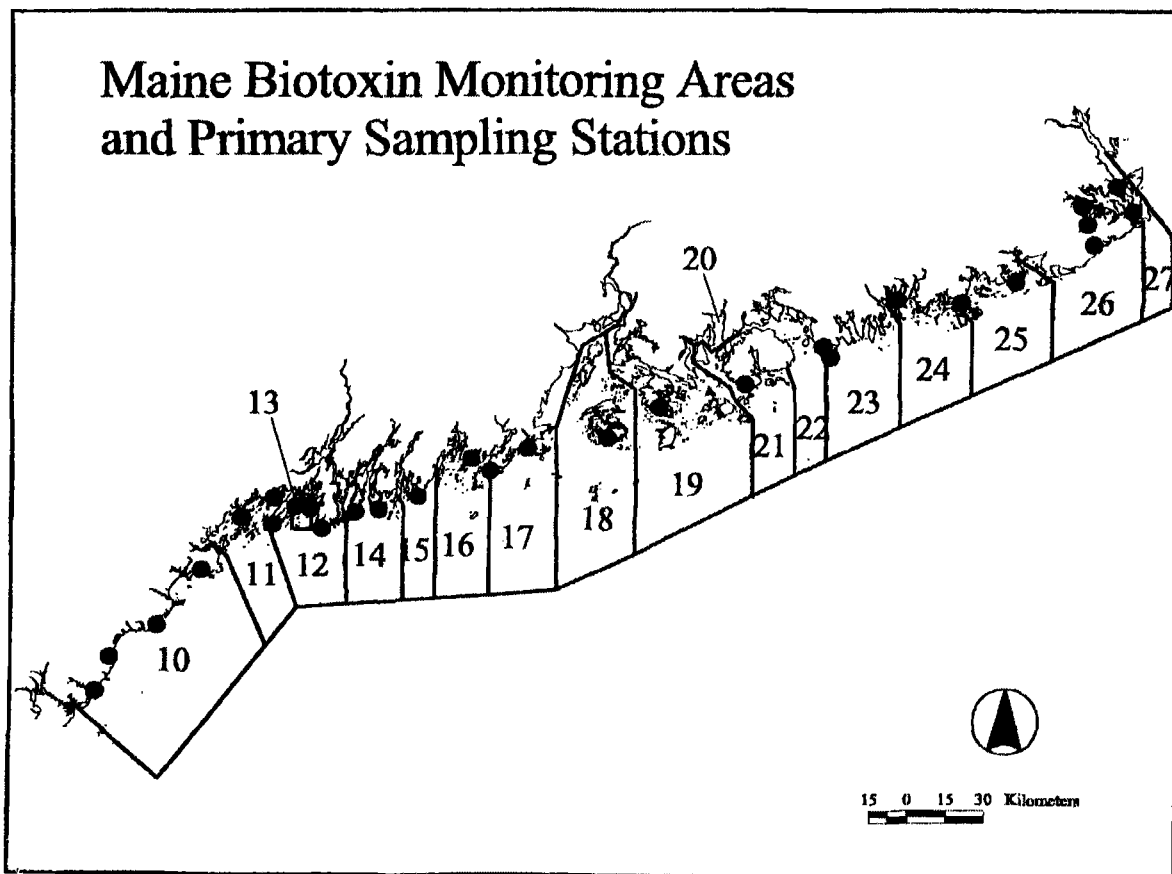
FIGURE 4.7. Distribution of primary sampling stations for shellfish biotoxin monitoring within 18 coastal regions in Maine, Atlantic, US. (Source: Maine Department of Marine Resources.)

Regulatory action. The Maine shellfish toxin monitoring program was mandated by the state legislature, and therefore its purpose and specific responsibilities are detailed in state regulations (Shumway et al. 1995). **Toxin levels in molluscan shellfish provide the basis for regulatory action, not the presence of potentially toxic algae in the water column**, although the latter may prompt increased shellfish sampling. Under state legislation, shellfish harvesting must be closed immediately in an area if PSP toxins in the tissues of shellfish from that area attain the action level of 80 µg STXeq $100g^{-1}$ or contain concentrations of other toxins known to be harmful to consumer health. However, closures are recommended at the discretion of DMR personnel (John Hurst), when toxicity is observed to gradually approach, but has not yet reached the regulatory level (J. Hurst, pers. comm.). This strategy has been adopted because PSP toxicity in *Mytilus edulis* may rise extremely rapidly, at a rate of up to 181 µg STXeq day^{-1} in Maine waters (Hurst and Gilfillan 1977). Furthermore, laboratory studies show that *M. edulis* can exceed the RL within < 1 hr of continuous exposure to a highly toxic *Alexandrium* strain (Bricelj et al. 1990). Closures become effective immediately upon signature by the Commissioner or his authorized designee. They are made by species, based on the toxin levels of the most sensitive species (e.g. *Mytilus edulis* is used as the alert or early warning species in Maine). Closures are made with safety zones surrounding the area where toxicities have exceeded the RL to provide a safety margin in the event of rapidly rising toxicity levels. State regulations also allow closures if sufficient current information is not available to adequately safeguard public health. Thus, if new toxins are suspected, DMR is authorized to recommend closures as necessary.

Precautionary closures for domoic acid were made when testing of shellfish from New Brunswick indicated that the toxin was present in Maine's adjacent shellfish harvest areas. DMR's policy is that in the case of suspect DSP illnesses, the shellfish harvest areas in question will be closed to harvesting until its safety can be established.

It is well established that although both *Mytilus edulis* and *Mya arenaria* accumulate and eliminate PSP toxins relatively quickly, mussels become toxic earlier (about one to two weeks; range = 5 to 22 days in Maine) than softshell clams, and in Maine attain peak toxicities 2 to 4x greater than *Mya* from the same site (reviewed by Bricelj and Shumway 1998). These differences in toxin kinetics among species have been used to advantage in species-specific management of stocks. For example, in 1979 a portion of Casco Bay remained open for digging of *M. arenaria* after the area was closed for mussel harvesting. This resulted in considerable savings to the clam industry, i.e., a landed value of US$ 426K, and an estimated consumer value of US$ 2.77 million. Species-specific closures have also been implemented in Cobscook Bay. Areas selected for harvesting, however, are sampled more intensively, i.e., twice a week, during the toxic season.

A schematic of the state's action plan is shown in Figure 4.8. Maine's toxin monitoring program is designed to allow for reclaiming of toxin contaminated areas. Reopening of closed shellfish harvesting grounds requires a waiting period, i.e., until toxicity levels below the regulatory level are registered for at least two consecutive weeks, and then only if historical records indicate that several rises of toxicity are not expected in the affected area. The rationale for this approach is that multiple closures and openings undermine the credibility of the monitoring program. In addition to harvest closures, embargo/confiscation and destruction of already harvested contaminated or potentially contaminated product is permitted by State law (Section 6856) and has occasionally been implemented. Toxic product is disposed in landfills. Dealer shellfish from suspect areas, which were tagged at the time of purchase with the harvester's name, location, quantity harvested, etc. are put under embargo immediately, until their safety is established. In the event of distribution to market of a toxic product, the state of Maine Department of Human Services, Division of Health Engineering, and the Department of Agriculture, Food and Rural Resources, Division of Regulations, receiving states and the FDA are notified of the fact, in order to initiate proper action. Harvest closures are more commonly implemented than embargo of harvested product, and are the

preferred strategy when possible because they reduce economic losses and are less likely to antagonize the industry. Shellfish harvesting licenses and permits may be suspended when harvesters are non-compliant.
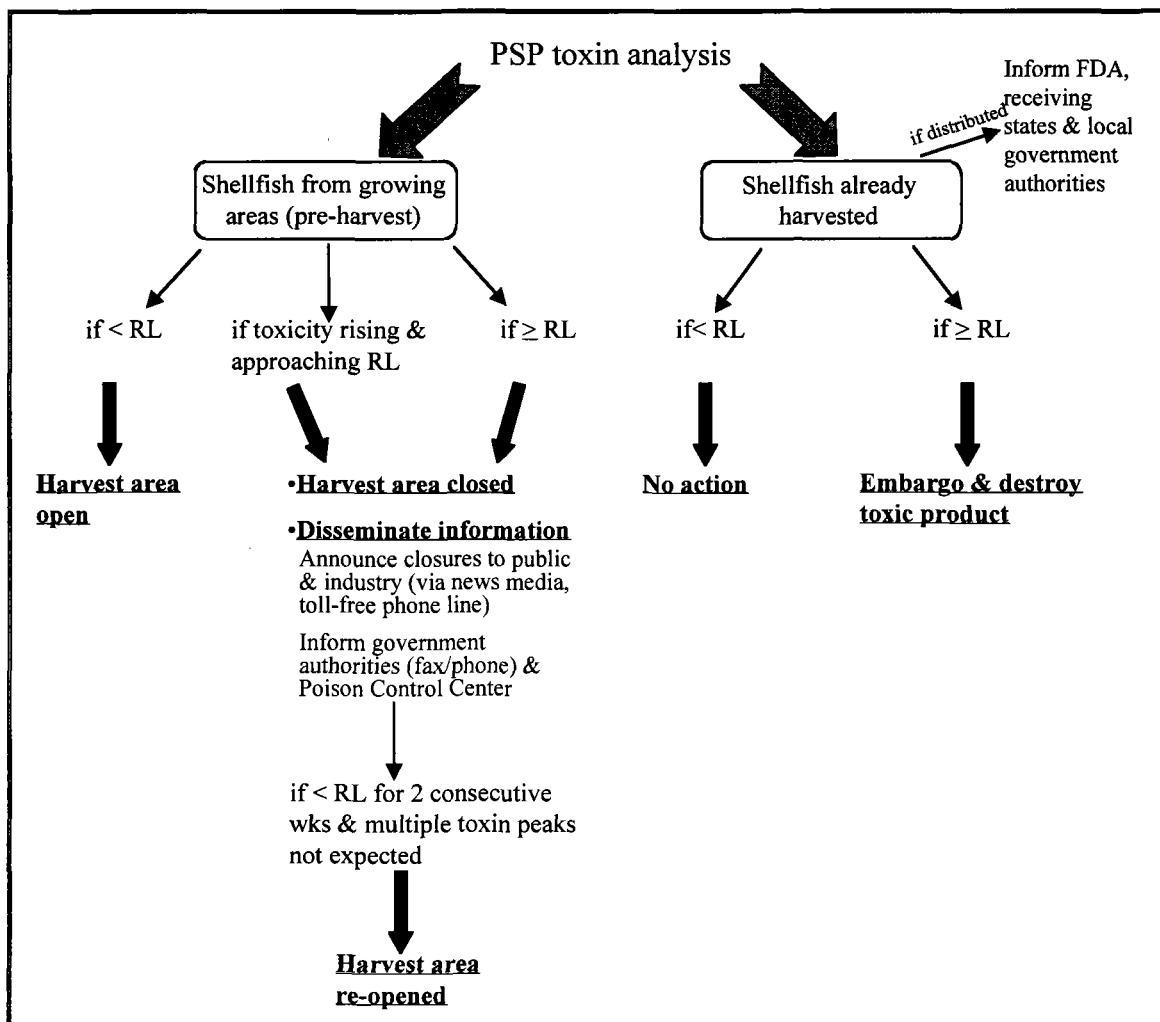
FIGURE 4.8. Action plan for the shellfish monitoring program in the State of Maine, Atlantic, US.
RL = regulatory level. (Based on consultation with L. Bean, State of Maine DMR.)

Dissemination of information. Closures are announced via the NOAA radio weather broadcasting station, TV broadcasts, and published in a local newspaper circulated in the affected area. Shellfish dealers and local government authorities are also notified of all closures. Local municipal officials are notified by phone/fax and receive copies of the legal closures. Closure notices that can be seen from land access are posted immediately at shellfish harvesting sites by Marine Patrol officers, who patrol contaminated areas and make arrests if necessary, in order to prevent further taking of shellfish from that area. The Maine Poison Control Center is informed of toxin closures, and in turn notifies local hospitals to be aware of the possibility of toxin-related illnesses. Maine hospitals, in suspect cases, submit stomach contents to DMR for analysis. The Maine Poison Control Center and the Department of Human Services investigate all reported cases of marine toxin-related illnesses and inform DMR of their findings.

DMR has established a toll-free phone line to inform the public of toxic shellfish closures. Occasional articles and TV accounts on shellfish biotoxins serve to foster public awareness of the problem. DMR maintains a computer database of shellfish toxicity, which is made available upon request, and can be accessed by date, species, sampling station and general area of harvest. Reports including tabulated raw toxicity data are produced on an annual basis. Records are also kept of DA analyses conducted by USFDA on shellfish collected in Maine. Meetings between DMR, and the Departments of Human Services and Agriculture are held every year in March to plan for that year's response to PSP.

Identified bottlenecks. Funding constraints that limit the availability of personnel were identified as the major constraints of the monitoring program.

4.3.1.2 Pacific US

The Pacific US is affected by two biotoxin problems: PSP, caused mainly by *Alexandrium catenella* and *A. tamarense*, and ASP, attributed to *Pseudo-nitzschia australis* and other *Pseudo-nitzschia* species. Outbreaks of PSP have been documented on the west coast since the 1700's and affect the states of Alaska, Washington (WA), California (CA), and to a lesser extent the state of Oregon (OR). Alaska and WA have the largest toxin monitoring programs and also support the largest shellfish production. A detailed account of PSP monitoring programs in various states by Nishitani and Chew (1988) provides the main source for this report. A report by RaLonde (University of Alaska Marine Advisory Program 2001) provided updated information on the PSP monitoring program for Alaskan shellfisheries. Due to an expansion in the harvest of wild and cultured shellfish, which continues to date, the number of shellfish samples analyzed increased markedly in the late 1970's in all four Pacific States, and reached about 1400 samples in WA and Alaska in the 1980's. At present, a total of about 3000 to 4000 shellfish samples are analyzed per year for toxins (including PSP toxins and domoic acid) in WA state (F. Cox, WA State Dept. of Health, pers. comm.).

There are several unique features to the Pacific PSP toxin monitoring programs, which are highlighted and contrasted with those of the Atlantic US. On the West Coast, PSP affects a much more extensive coastline (e.g. 5000 km of shoreline in Alaska and > 2700 km in WA), and affects a more varied, multiple-species shellfish resource. The population on the West Coast is more diverse in terms of its ethnic background. The large influx of Asian and Filipino immigrants introduces communication barriers that reduce the effectiveness of warnings posted at closed harvest sites, and has greatly increased the interest in consumption of non-traditional products (e.g. moonsnails, whelks, barnacles). Native Indian tribal communities rely heavily on shellfish harvesting for subsistence and as a primary source of income. Recreational and subsistence harvesting is a large component of the west coast shellfisheries, so monitoring of commercial harvest areas is not sufficient to protect public health. Routine phytoplankton monitoring programs have not been implemented in the past, except in a few areas (e.g. Monterey Bay, CA), but were initiated in the late 1990's by state health departments in CA and WA and by the Alaska Sea Grant Program. On the Pacific coast (WA to CA), in contrast to the Atlantic, the topography of the coastline is relatively straight; large-scale wind systems and oceanic conditions prevail. HABs appear to originate on the continental shelf and are transported to coastal beaches, and tend to be less spatially and temporally predictable than on the East Coast.

State of Alaska

PSP is a persistent problem along much of the Alaska coast south of 60° latitude, where it prevents maximal economic development of the existing shellfish industry and is a major obstacle to its future expansion. Commercial fishing follows oil as the second most valuable industry in the State, and fishing and seafood processing supply a major source of emplyment. Large commercial shellfisheries, mainly for

the butter clam, *Saxidomus giganteus*, have been permanently closed since the 1940's due to prolonged retention of PSP toxins. The value of this unexploited fishery is estimated at over $US 5M. The potential threat of PSP and requirement for lot testing to comply with state regulations also prevents the development of the Arctic sufclam (*Spisula polynyma*) fishery north of the Aleutian chain, even though PSP levels are consistently below the RL in this region. Due to the prohibitive cost of monitoring Alaska's extensive coastline (about 57% of the total US coastline) and its vast shellfish resources, routine site monitoring of PSP toxins is restricted to localized areas that still sustain commercial shellfish operations.

Principal shellfish species tested. Commercially exploited molluscan shellfish in Alaska primarily include: littleneck clams, *Protothaca staminea*, razor clams, *Siliqua patula*, and geoducks, *Panope abrupta*. Although toxic outbreaks are most frequent in spring and summer (May, June, July), they can occur year-round. This has been largely attrributed to the persistence of toxins in some bivalve species. Between 1973 and 1992, PSP outbreaks have involved the following species, ranked in order of importance: *S. giganteus* (58% of cases), musssels, *M. edulis* or *M. californianus* (22%), razor clams, *S. patula* (2%), littleneck clams, *P. staminea* (2%), unknown source (11%) (Gessner and Middaugh 1995).

The Pacific razor clam, *Siliqua patula*, is found throughout Alaska but is harvested commercialy only in Lower Cook Inlet. This species poses a low risk of PSP because it accumulates relatively low levels of PSP toxins, and most of these occur in the viscera, which are discarded prior to consumption or processing. Littlenecks rarely pose a risk of PSP because commercial harvesting is concentrated in Kachemak Bay, an area where PSP episodes are rare, although toxin levels found in 1997 resulted in suspended harvests. A diver geoduck fishery began in SE Alaska in 1989; the product is sold live or processed (eviscerated and cleaned meats separated into neck and steak portions) but the former commands a much higher price. Only the viscera of geoducks are analyzed for PSP toxins when these are shipped live, but siphons and mantles are tested in product destined for processing. Record toxicity levels (up to 1818 µg 100g^{-1} in viscera) were recorded in 1999 (Red Tides Newsletter, NWFSC and WA Sea Grant Program). In contrast to testing in WA State (see below) which pools 3 geoduck visceral samples prior to testing, individual testing for this species is conducted in Alaska. Three viscera per harvest day are required for testing, and if one of these exceeds the RL, all product must be processed.

PSP also affects Alaska's major crab fisheries, including the Dungeness crab (*Cancer magister*), tanner (*Chionoecetes bairdi*) and snow or opiolio crab (*Chionoecetes oilio*), king crab (*Paralithodes* spp. and *Lithodes aequispina*) and hair crabs (*Erimaccrus isembeckii*). PSP toxins in these crabs are confined to the viscera, but evisceration results in sectioning of product which greatly reduces its value. For example, processing of whole Dungeness crabs into sectioned product caused a $200K loss to the Kodiak/Aleutian fishery in 1977.

Toxin analysis. The Alaska Department of Environmental Conservation (ADEC) is responsible for PSP toxin analysis (by mouse bioassay) in commercially harvested shellfish and aquaculture product at its one testing laboratory in Palmer. Lot testing is required throughout the harvest season, and lot sampling requirements (number of samples for various harvest sizes) are established by ADEC. Shellfish fisheries and aquaculture operations are responsible for collection and shipping of samples to ADEC, although the actual cost of the mouse bioassay is currently borne by ADEC. These industries also have to cover the cost of storage of harvested product until test results become available. The aquaculture industry is concerned that future funding reductions will result in user fees for toxin testing by state agencies, which may be prohibitive for this industry. Alaska has no agency responsible for monitoring of recreational/subsistence fisheries. Therefore, recreational harvest beaches are considered at risk at all times of the year rather than subject to seasonal closures. Monitoring costs of biotoxin testing of recreational/subsistence harvests must be paid by the harvester (at $125 fee per sample). ADEC has a statutory responsibility to test commercially harvested products first, thus leading to delays of several days in reporting of test results to subsistence harvesters (Red Tides Newsletter, autumn 2000). Certified beaches approved for recreational

harvesting are only located in Kachemak Bay and lower Cook Inlet where commercial fisheries and the aquaculture industry regularly test shellfish. Lower Cook Inlet sustains the largest recreational fishery (for razor clams) in the state and has not experienced PSP to date.

Shellfish samples are collected by personnel from ADEC, the Division of Public Health, and shellfish harvesters. For all species except razor clams, annual site monitoring is followed by sampling of each lot prior to marketing. Alaska was temporarily removed from the NSSP between 1954 and 1975 due to non-compliance with the program's requirements, and was therefore precluded from export of shellfish to other states. ADEC has also established a citizen-monitoring program called Sea-Watch, which provides a toll-free phone number for reporting of PSP illnesses, discoloration of water, fishkills and unusual behavior of marine seabirds and mammals.

Domoic acid has not yet posed a problem in Alaskan shellfish, although it has been detected in crabs. About 3000 samples of commercially valuable fish and shellfish have been tested for DA since 1992, but the highest value reported is 11 μg g^{-1} in *S. patula* from Cook Inlet (Horner et al. 1997).

Regulatory action and dissemination of information. The Division of Environmental Health, in Alaska's Department of Environmental Conservation (ADEC) is responsible for administering the shellfish toxin monitoring program and toxicity testing. Prior to 1977 these activities were the responsibility of the Department of Health and Welfare (later named the Dept. of Health and Social Services). Warning signs are posted on some beaches, in southeast Alaskan cities and on ferries. Radio and newspaper communications are issued in Spanish, Tagalog and Laotian, as well as English. Due to the lack of control of recreational areas, a great deal of emphasis is placed on consumer education programs. Warnings about the risks of PSP are also included in the Department of Fish and Game regulations, which are distributed at the time of purchase of recreational fishing licenses. Twenty percent of all reported PSP cases have occurred among fishing industry employers, which has led to dissemination of information on PSP to new employees at these facilities.

However, despite considerable efforts directed at consumer education and the long history of PSP in Alaska, PSP outbreaks, even though under-reported, continue to the present day. Sixty-six outbreaks, involving 143 illnesses (including 2 deaths) were reported between 1973 and 1994 throughout the state. Alaska has one of the highest incidences of reported PSP in the world, attaining up to 1.5% per year at some sites (Old Harbor) (Gessner and Schloss 1996). This is mainly attributed to the widespread indifference of recreational and subsistence harvesters to PSP warnings.

Washington State

Paralytic shellfish poisoning. The PSP surveillance program was established in 1957. In contrast to Alaska, both commercial and recreational harvesting areas are routinely monitored in Washington State. Monitoring for PSP toxins and setting closures for commercial harvest areas is the responsibility of the state Department of Health (DOH) in the Department of Social and Health Services (DSHS). This agency also advises the county health departments, which are responsible for monitoring and regulating closures of recreational harvest areas. Recreational harvest areas are also sampled by volunteers and by tribal governments in tribal owned or harvested beaches (F. Cox, WA DOH, pers. comm.). The sampling frequency of commercial species varies, but is conducted biweekly in most areas during the harvesting period. When toxicities approach the safety limit, lot sampling after harvesting and before marketing may also be required. Counties sample recreational areas biweekly between April and October and also sample intermittently in the winter.

The main commercial shellfish species included in toxicity testing are the Pacific oyster (*Crassostrea gigas*), mussels (*Mytilus edulis*), razor clams (*S. patula*), littlenecks (*P. staminea*), Manila clams (*Tapes*

philipinarum), and geoducks (*P. abrupta*). The latter is an economically valuable resource (annnual value = $5-7M), and an increase in overseas demand in recent years has raised its price to $12 per pound (Curtis et al. 2000). This species was generally considered not to pose a risk to public health because viscera, the only geoduck tissue which has exceeded the PSP safety limit in the state, is removed prior to processsing. However, the viscera are consumed in soup by some tribal and immigrant communities, and evisceration can lead to reduced price, as the main market demand is for whole, live geoducks. The current method for testing for PSP toxins in this large bivalve by DOH involves pooling of 3 clams per sample, which does not take into account the large variability in toxin levels (coefficient of variation CV = 20 to 98%) documented among individual clams in shallow, nearshore waters (Curtis et al. 2000).

Since 1977, PSP also regularly affects inland waters of Puget Sound. Mussels (the bay mussel, *Mytilus edulis* and Californian mussel, *M. californianus*) are used as the sentinel organism. Mussels (*M. edulis*) are deployed in suspended cages to provide an early warning for PSP in Puget Sound and in coastal estuaries, since 1989. Commercial growers have their product tested biweekly during the winter and spring, and weekly during the summer and fall, as part of their requirements for certification. PSP levels exceeding the safety limit have also been found in predatory gastropods, such as the moonsnail, *Polinices lewissi* (Wekell et al. 1996), where toxin accumulation is restricted to the viscera. Moonsnails and other predatory gastropods are not yet included in routine monitoring, although they are a popular consumer product among the Asian community. Accumulation of PSP toxins in moonsnails is restricted to the viscera.

Some closures are applied to all species over large areas because they are impractical to monitor routinely. In other cases closures may apply to specific bays and are applied selectively by species. Dissemination of recreational harvest closures occurs via the news media and by a toll-free PSP hotline provided by DOH. Multilingual signs are posted at some beaches. A Red Tide Newsletter is published jointly by the Northwest Fisheries Science Center and the WA Sea Grant Program to provide information on HAB problems, as well as biotoxin hotlines and contacts. The increase in shellfish samples tested which was documented in the late 1970's was primarily due to an increase in monitoring of recreational harvest areas.

Domoic acid. The first outbreak of domoic acid poisoning in WA State occurred in 1991 and has had a major impact on toxin monitoring in the region. Following the deaths of seabirds (pelicans and cormorants) which were traced to the consumption of DA-contaminated anchovies in Monterey Bay, California, both razor clams (*Siliqua patula*) and Dungeness crabs (*Cancer magister*) in WA were found to be contaminated with DA. Although the source of toxin in clams was presumed to be *Pseudo-nitzschia*, this was not confirmed for the 1991 WA outbreak, although *P. pseudodelicatissima* was responsible for the 1998 clam toxicities. The toxin source in Dungeness crabs is unknown, but they are opportunistic predator-scavengers and potential predators of razor clams and other benthic invertebrates. The 1991 event resulted in immediate closure of razor clam digging areas, and of the commercial Dungeness crab fishery by emergency order. Based on the 1987 experience in Canada, a domoic acid monitoring program was rapidly established on the West Coast in 1991. Sampling of razor clams from recreational beaches was conducted by the WA Department of Fisheries and Wildlife, and domoic acid analysis (by HPLC) in shellfish was carried out by the Northwest Fisheries Center of the National Marine Fisheries Service. Domoic acid analysis in bivalves and Dungeness crabs is routinely performed by the WA State Department of Health. A certified standard DACS-1 from the Canadian National Research Council is used for calibration purposes. Analysis of canned razor clams archived by local residents showed that detectable DA levels were present in razor clams as early as 1985.

No fatalities or confirmed illnesses attributed to DA have occurred on the West Coast, although losses to the local economy were estimated at US$ 15-20M. Effective communication between Canadian and US authorities allowed early warning and rapid response to the initial outbreak. The 1991 DA crisis in the US Pacific differed considerably however, from that experienced in Atlantic Canada in 1987. In the former,

DA affected a much wider geographic area (both open ocean and estuarine environments from WA to CA) and a greater number of species, lasted longer and identification of the toxigenic source (for clams and crabs) was not available. Blooms of DA-producing species, contaminated shellfish and vertebrate mortalities have occurred again since the initial 1991 event, but there have been no confirmed cases of ASP. A major bloom occurred in 1998, associated with sea lion mortalities in central CA (Scholin et al. 2000), and record DA levels in razor clams (peak toxicity = 300 μg g^{-1}), which resulted in closure of harvesting grounds throughout the WA coast. No detectable levels of DA or trace levels were found in *M. edulis* from CA during this outbreak, while planktivorous fish contained 30-110 μg DA g^{-1}. A 2000 episode caused mortalities of gray whales during their migration from Baja California to Alaska, and of sea lions and sea otters in central CA (Ch'ng et al. 2000). Faunal mortalities in Monterey Bay, central CA in 1998 and 2000 were causd by *P. australis*.

The razor clam, *S. patula*, lives in the surf zone in Pacific beaches, and contributes to a valuable, > $5M fishery in WA State; it is the bivalve species most affected by domoic acid in the NW Pacific. Razor clam recreational areas, which were infrequently closed due to PSP prior to 1991, are now subject to annual delays of the harvesting season and closures in the spring and fall due to DA. In contrast to mussels, *Mytilus edulis*, which eliminate DA fairly rapidly and concentrate it primarily in the viscera, razor clams show prolonged retention of DA (Horner et al. 1993; Drum et al. 1993) and maximum toxin concentrations (up to 230 ppm) in the foot, which is considered a delicacy on the west coast and often consumed to the exclusion of other tissues (Wekell et al. 1994). This difference between species led Horner et al. (1997) to suggest that mussels are an inappropriate sentinel species for DA. Mussels were never found to exceed the DA safety level in WA State, where the highest toxicity (10 ppm) was documented in 1994. Furthermore, toxicity data for Pacific offshore fisheries (crabs, anchovies) are not reliable indicators of bivalve toxicity in nearshore waters and vice versa. An alternate species, the intertidal mole crab, *Emerita analoga*, is being evaluated as a sentinel species for DA in California (Monterey Bay), as this crab, which is widely distributed in the region, accumulates measurable amounts of DA during *Pseudo-nitzschia* blooms when mussels show no detectable toxin levels (Powell et al. 2000). Domoic acid was not found in oysters from WA state. The presence of DA in Dungeness crab is restricted to the viscera and undetectable in edible tissues (Wekell et al. 1994). However, toxin levels in viscera can exceed the regulatory limit, and a significant portion of the population consumes this tissue directly or indirectly as soup stock. In 1992 the FDA raised the permissible level of DA in cooked Dungeness crab viscera to 30 ppm after evaluating consumer utilization and consumption patterns.

The Olympic Region Harmful Algal Blooms (ORHAB) 5-year project, sponsored by NOAA's Coastal Ocean Program, was established in 2000 (Horner and Trainer 2001). This multidisciplinary, multi-institutional initiative, represents a partnership between federal, state and local government agencies, academic institutions, native tribes, industry and public interest groups. It is intended to foster HAB research, i.e. investigate the origins of offshore, open ocean toxic blooms and assess conditions under which they are transported to coastal shellfish, and enhance monitoring and management capabilities for HABs and contaminated shellfish. It also serves to test and validate the utility of new technologies for the identification of HAB species and rapid detection of their toxins. The main focus is on DA-producing *Pseudo-nitzschia* spp. and razor clams. The long-term goal is to achieve a sustained cost-effective state monitoring program without reliance on federal support.

Active participation of native tribes (e.g. the Quinault Indian Nation, QIN) is a unique feature of this program and is intended to foster communications between government agencies and the local tribal community, thus leading to greater trust and compliance of local harvesting bans. Tribal participation is vital as the Quinault people are the highest per capita consumers of razor clams in the state and are thus the group at highest risk. Samples for DA toxin analysis by the state DOH laboratory in Seattle are currently collected by WDFW staff and native tribes. The DOH mainains a web page with the latest levels

of DA toxicity in razor clams sampled on recreational beaches. Emergency roadblocks have also been implemented in some cases to protect the public.

Identified bottlenecks. Lack of public compliance with closures and inadequate coverage of the shoreline due to funding constraints are perceived as the two main limitations of the toxin monitoring programs in both Alaska and Washington State. Better understanding is required of the physical, oceanographic processes controlling the timing and spatial occurrence of HABs, and of offshore bloom initiation sites, in order to predict shellfish contamination in nearshore waters. The vectors and pathways of food web transfer of DA to benthic and pelagic communities need to be better established.

4.3.2 Canada

In Canada, shellfish toxin monitoring used to be the responsibility of the regional inspection branch of the Department of Fisheries and Oceans (DFO). Considerable restructuring of government administration has occurred recently, such that shellfish toxin monitoring is now (since 1997) the responsibility of the Canadian Food Inspection Agency (CFIA) through its headquarters in Ottawa and regional branches. Due to the recent date of these changes, it is difficult to evaluate their significance and overall impacts on the effectiveness of the toxin monitoring program.

Canada adopted the NSSP and developed the Canadian Shellfish Sanitation Program (CSSP) in 1948. A Memorandum of Understanding (MOU) between CFIA, DFO and Environment Canada (EC) defines the shared responsibilities of the three government agencies in the administration of the Canadian Seafood Safety Program (CSSP). Regarding biotoxins, DFO is the lead agency responsible for: a) opening and closing of shellfish grounds on the basis of recommendations from CFIA on marine biotoxin levels, b) posting, patrolling and enforcing shellfish closures; c) managing and reporting annually on conditionally approved shellfish growing areas, d) providing notification to CFIA, EC and stakeholders on location, boundaries and timing of harvesting closures and openings. CFIA is the lead agency with regards to: a) implementing the marine biotoxin monitoring program, b) recommending to DFO the opening and closing of harvestable areas due to unacceptable marine biotoxins, and c) the handling, processing, and inspection of imported and exported shellfish.

PSP and ASP are the main human health issues in Canada. For PSP toxins, an action limit of 80 μg STXeq 100g^{-1} is used for raw shellfish tissues, and 160 μg STXeq 100g^{-1} is used for canned shellfish. For DA the action limit is 20 μg DA g^{-1}. Despite the existence of a very effective monitoring program, incidents of PSP continue to occur throughout Canada (e.g., 3 fatalities and 100 illnesses between 1980 and 1989). However, most cases have involved recreational harvesters who ignored closure notices.

Canada has established an electronic communication network accessible through the Internet, or *Phycotoxins Mailing List*, presently maintained from the Bedford Institute of Oceanography, DFO, Dartmouth, NS. Subscription is open to all interested participants within and outside Canada. This vehicle allows rapid communication of events and research results related to algal toxins, as well as rapid response to queries posed by participants worldwide.

Atlantic Canada

The east coast of Canada has been historically affected by PSP and recently by ASP and DSP (Figure 4.9). Toxin monitoring in shellfish was first established in the Bay of Fundy, but in response to new outbreaks, it has been extended to Newfoundland (in 1982), SE Nova Scotia, NS and the Laurentian region, Quebec (1984), the Magdalen Islands, Quebec (1987), Prince Edward Island, PEI (1988) and east-central NS (1992). ASP was first identified in Atlantic Canada (eastern Prince Edward Island) in 1987. Despite the
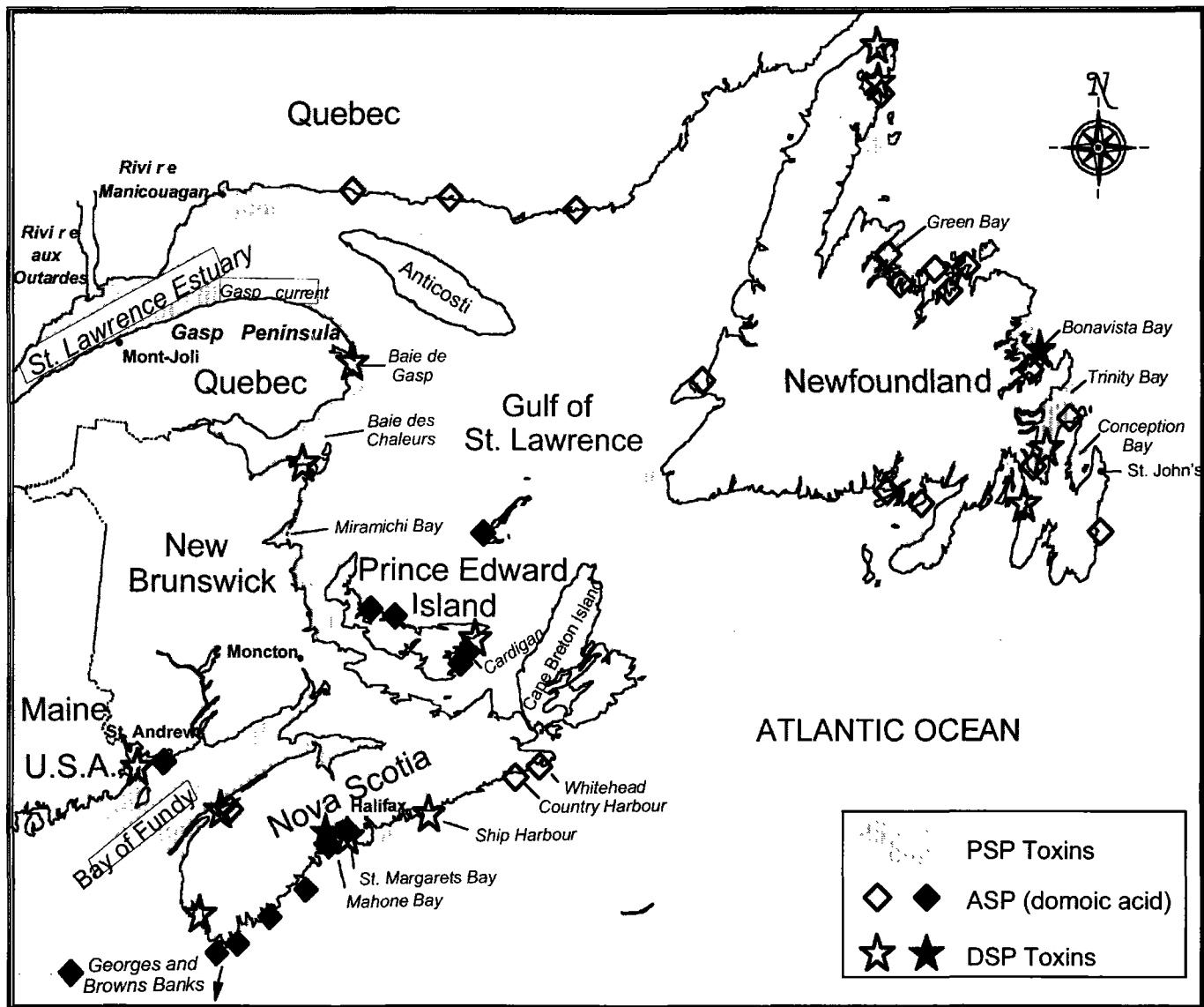
FIGURE 4.9. Regions on the Atlantic coast of Canada in which PSP, ASP and DSP toxins have been identified in molluscan shellfish.
(Source: S. Bates, DFO, updated from Bates 1997). Open symbols: toxins detected in shellfish tissue; closed symbols: shellfish harvesting closed due to levels exceeding the regulatory limit. Shaded areas: locations where PSP levels exceeded the detection limit of the assay.

recall of all bivalve products from PEI, this outbreak resulted in 107 illnesses (including 13 fatalities) attributed to ASP. Rapid development of a new analytical method (see methods below) allowed monitoring of DA in shellfish in Atlantic Canada to start in the spring of 1988, within a year of the first documented outbreak. Since 1987, the levels of DA in PEI shellfish have dropped considerably (J. White, CFIA Charlottetown, PEI, unpubl. data), and no further incidents of ASP have been documented in Atlantic Canada. It is noteworthy, however, that since 1998 the incidence of domoic acid in shellfish has extended its range and is found in detectable levels in the Magdalen Islands and the Gulf of St. Lawrence. In 1995 very high DA levels (exceeding 1300 μg g^{-1} digestive gland) were reported for offshore sea scallops, $P.$ $magellanicus$, from Georges Bank and Brown Bank, where the maximum attained was 4300 μg g^{-1} digestive gland (Stewart et al. 1998). DSP was first identified unequivocally in southern NS in 1990 when 17 cases of gastroenteritis were traced to the consumption of cultured mussels from Mahone Bay and the presence of DTX1 in mussel tissues (Quilliam et al. 1993). Although the source of DSP toxins in Nova Scotian shellfish remains uncertain, the epiphytic alga, $Prorocentrum$ $lima$, isolated from aquaculture lines at this site, was found to produce OA and DTX1 and suggested as the potential source of DSP toxins in contaminated mussels (Lawrence et al. 1998). A routine monitoring program for DSP based on the mouse bioassay has been operational in Atlantic Canada since 1990. The presence of a new group of toxins, spirolides, has been recently confirmed in bivalves throughout the region [Newfoundland, New Brunswick, Magdalen Islands and Nova Scotia (Quilliam et al., unpubl. data)] and in phytoplankton ($Alexandrium$ $ostenfeldii$) from Nova Scotia (see Section 3.1.6.) Pectenotoxins (PTX2 and PTX2-seco acid) were also found in Nova Scotian mussels, and $Dinophysis$ $acuminata$ was identified as the planktonic source of PTX2. These toxins (spirolides and PTXs) which have not yet been clearly linked to human sickness are not presently included in the Canadian biotoxin monitoring program.

A chronology of the 1987 ASP outbreak and its resolution is provided in Table 4.6 to illustrate rapid, successful response to a crisis situation involving a previously unknown algal toxin. Detailed accounts of this incident are given by Quilliam and Wright (1989) and Bates et al. (1989). The mouse bioassay routinely used for PSP toxins provided valuable information by indicating symptoms different from those of previously known toxins. Localization of domoic acid in the digestive gland of mussels pointed to a dietary origin of the toxin. Assembly of a collaborative research group in the region, a multi-agency Analytical Working Group involving personnel from DFO, NRC and Health Canada, was also critical in rapid resolution of the problem. Rapid identification of the toxin was attributed to the following factors: its water solubility which allowed discarding of the lipid-soluble fraction, the high concentration in contaminated mussels, the availability of rapid and fairly precise on-site bioassay, and the fact that the compound could be found in the chemical literature, since input of experimental data into a computer database greatly reduced the number of possible compounds involved (Quilliam and Wright 1989).

The 1987 DA crisis led to increased cooperation between industry and government, in that some shellfish growers routinely provide samples from their aquaculture sites for toxin analysis, especially in PEI, where mussel aquaculture is highly developed (1995 production of 7500 tons with an export value of US$ 11M). Testing costs for wild ocean quahogs, $Arctica$ $islandica$, south of Grand Manan, NB, were covered by the fishing industry (S. Eddy, CFIA Moncton, NB, pers. comm.).

The traditional market for scallops in Canada is for the adductor muscle, a tissue that does not accumulate significant levels of PSP toxins. However, the offshore wild scallop industry, which markets whole scallops, pays for additional PSP testing of its product conducted by a private company (MDS Environmental Services). In the 1990's interest developed in marketing of whole scallops cultured or wild-harvested from nearshore waters, which led to development of a monitoring protocol specifically targeted for this product. In the province of Nova Scotia, since 1992, scallop companies wishing to market whole product must sign a Memorandum of Understanding (MOU) with the CFIA and submit samples for toxicity testing. This MOU includes the following requirements: a) the grower must notify CFIA prior to

TABLE 4.6. A chronology of events for the 1987 ASP/domoic acid crisis in Atlantic Canada.
(Source, M. Quilliam, NRC Canada, pers. comm.)

<u>**1987**</u>

<u>November 22-25</u>:   Human poisonings reported in Quebec and New Brunswick

- > 150 people seriously ill
- > 30 people hospitalized
- 3 elderly people died

- Symptoms: vomiting, diarrhea, confusion, memory loss, and coma

<u>November 29</u>:   Toxic mussels implicated

- Health and Welfare Canada (HWC) epidemiologists correlate illness with mussels
- Neurotoxic symptoms observed in the AOAC PSP mouse bioassay
- Department of Fisheries and Oceans (DFO) inspectors trace mussels to Cardigan Bay, PEI, site of a major aquaculture industry

<u>December 11</u>:   Atlantic shellfishery closed

- Economic loss in the millions of dollars
- Political pressure is generated to solve the problem
- Federal laboratories are mobilized

<u>December 12</u>:   NRC begins investigations (team of 40 assembled, including NRC chemists and biologists and DFO personnel experienced with the mouse bioassay)

<u>December 16</u>:   Toxin (domoic acid) identified using a bioassay-directed strategy of separations and analyses.

<u>December 20</u>:   Analytical method (HPLC) developed at NRC

<u>**1988**</u>

<u>January 7</u>:   Shellfishery re-opened

<u>April 18</u>:   Source of toxin identified (diatom later named *Pseudo-nitzschia* spp.)

harvest, b) all scallop product must be tagged according to CSSO guidelines (i.e. clearly labeled with date harvested, harvest location, etc.) c) each lot must be randomly sampled for scallop toxicity and cannot be held in a processing plant at the same time as wild-caught scallops, nor can wild scallops be shucked in the plant (Watson-Wright et al. 1993, S. Hancock, CFIA Dartmouth, pers. comm.). Although enforcement action is for the whole product, growers voluntarily hold product off the market if the digestive gland exceeds the regulatory safety level. Domoic acid, PSP and DSP toxins are analyzed from high-risk areas. The nearshore industry is less developed and thus, unlike the offshore industry, unable to cover the costs of extra testing. Although its members are required to pay for toxin analysis, this is done on a cost-recovery basis. Phycotoxin monitoring is also required for roe-on scallops since the gonad of scallops can attain toxicities exceeding the safety level.

Phytoplankton monitoring and research programs on marine biotoxins have been reduced in scope in response to recent cutbacks in federal government funding (Bates 1997). This is in marked contrast with activities in this field in the US, where a multi-agency funded National Program on the Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) was initiated in 1996. On the Atlantic coast of Canada the most intensive phytoplankton monitoring program exists in the Laurentian region, Quebec. No program exists in Newfoundland. Limited phytoplankton monitoring is maintained in NS, coordinated by the Aquaculture Association of NS in conjunction with DFO, but its long-term viability due to funding constraints is questionable. The shellfish industry in Atlantic Canada has expressed a strong interest in maintaining phytoplankton monitoring in the region and a willingness to cost-share the program.

Toxin monitoring in the Bay of Fundy region

History and general description of the program: This monitoring program is selected as a case study because it is the first comprehensive shellfish toxin testing program in the world. It was initiated in 1943, coinciding with the expansion of the shellfish canning industry. Analysis of PSP toxins by mouse bioassay was initially conducted by the Department of Natural Health and Welfare, Ottawa, subsequently (after 1983) by the regional DFO laboratory in Blacks Harbor, NB, and presently by the CFIA laboratory in Moncton, NB. The area, once included in the Scotia-Fundy region, is now classified as the western New Brunswick (WNB) region. There have been no outbreaks of ASP and DSP in this region, although DA levels above the RL have been found occasionally since 1987. For example, DA, first detected in 1988 in *Mya arenaria* from Passamaquoddy Bay, was responsible for shellfish closures in 1995 (Martin et al. 1998).

Purpose/objectives. The primary purpose of shellfish monitoring in Canada, including the east coast, is to provide public health protection (Cembella and Todd 1993). A secondary objective is to enhance the utilization of seafood resources for domestic and export markets by ensuring product safety.

Principal shellfish species tested: Blue mussels, *Mytilus edulis,* and softshell clams, *Mya arenaria,* are the two main species tested. Long-term toxicity records for these two species show, as observed in Maine, that toxicity maxima for mussels are consistently higher than those for softshell clams. Additional species, e.g. sea scallops, *Placopecten magellanicus,* and lobsters, *Homarus americanus,* are also occasionally tested although not on a routine basis.

Sites and frequency of shellfish sampling: The 1987 DA crisis led to greatly enhanced monitoring of shellfish toxins in the Atlantic Maritimes, including the Bay of Fundy, which was reflected in an increase in the spatial coverage of sampling and the number of samples tested (Richard et al. 1998). This overwhelmed available government resources within the DFO Inspection Branch. The resulting need to contract outside the agency for sample collection proved too expensive, and eventually the program was downsized again.

At present, shellfish for PSP toxin testing are collected on a weekly basis from areas with detectable toxin levels and biweekly from areas that are toxin free or permanently closed to harvesting. Depending on the site, sampling is conducted throughout the year, or seasonally, between May and November. The number of sampling sites and shellfish samples between 1988 and 1997 are shown in Figure 4.10. Sampling is initiated at about the same time of the year at all sites. Since 1988, there has been no further classification of sampling sites to reflect their toxin history, as is the case in the United States. Historical data have allowed regional characterization of shellfish toxicities within SWNB. Similarities are observed within each of three areas: 1) the area between Lepreau and the mouth of Passamaquoddy Bay, characterized by high toxicities and earlier toxicity peaks, 2) Passamaquoddy Bay, characterized by low toxicities, and 3) the islands of Grand Manan and Campobello, with intermediate toxicities, and later development of toxicity peaks (Martin and Richard 1996). Continuous, long-term toxicity records of mussels and softshell clams are available for some sites, e.g., Lepreau Basin and Lepreau Harbor, which have been monitored since 1943.

For domoic acid, some shellfish sites in NB are monitored throughout the year, weekly, bimonthly or monthly depending on the season and site. Other sites in NB are monitored between May and November, either bimonthly or monthly, except for October which is monitored weekly. Samples for DSP toxins are analyzed in response to consumer complaints. Once their presence has been established at a new site, it is added to the monitoring program.

Phytoplankton monitoring: Routine phytoplankton monitoring, including identification and enumeration of HAB species, was initiated in 1987 in response to the ASP outbreak in PEI. Its main goal was to act as early warning of harmful algae for the shellfish and salmonid industries (Bates and Keizer 1996; Martin et al. 1998). It was also used to establish baseline data and long-term trends in the occurrence of HABs, and to assess the environmental effects of salmonid aquaculture, a rapidly expanding activity in the Bay of Fundy region. It is undertaken by DFO, St. Andrews Biological Station, NB. At present it is only conducted at four sites in SWNB, weekly between June and September, biweekly during May and October, and monthly from December through April. The species monitored include *Alexandrium* spp, *Pseudo-nitzschia* spp., *Chaetoceros convolutus*, *Gyrodinium aureolum* and the ciliate *Mesodinium rubrum*. *A. fundyense* and *P. pseudodelicatissima* are the toxigenic species of concern in the Bay of Fundy (Bates 1997). Phytoplankton monitoring in other Maritime regions is conducted by CFIA, although the number of sampling sites has declined markedly since 1988.

In the Bay of Fundy shellfish toxicity and phytoplankton are sampled by different agencies (CFIA and DFO respectively) often with different frequencies and locations. Where these overlap, maximum *Alexandrium* cell densities occurred 10 to 14 days prior to the peak shellfish toxicity, but there was poor correlation between *Alexandrium* concentrations and shellfish toxicity. In contrast, cell densities of *P. delicatissima* and DA levels in softshell clams were highly correlated (Martin et al. 1998). DA was only detected in shellfish once *Pseudo-nitzschia* concentrations exceeded 10^6 cells L^{-1}.

Phytoplankton monitoring has revealed that *A. fundyense* originates offshore and is advected to inshore harvesting areas with a time lag of 2-3 weeks, and has also been used as an indicator to increase the frequency of shellfish sampling.
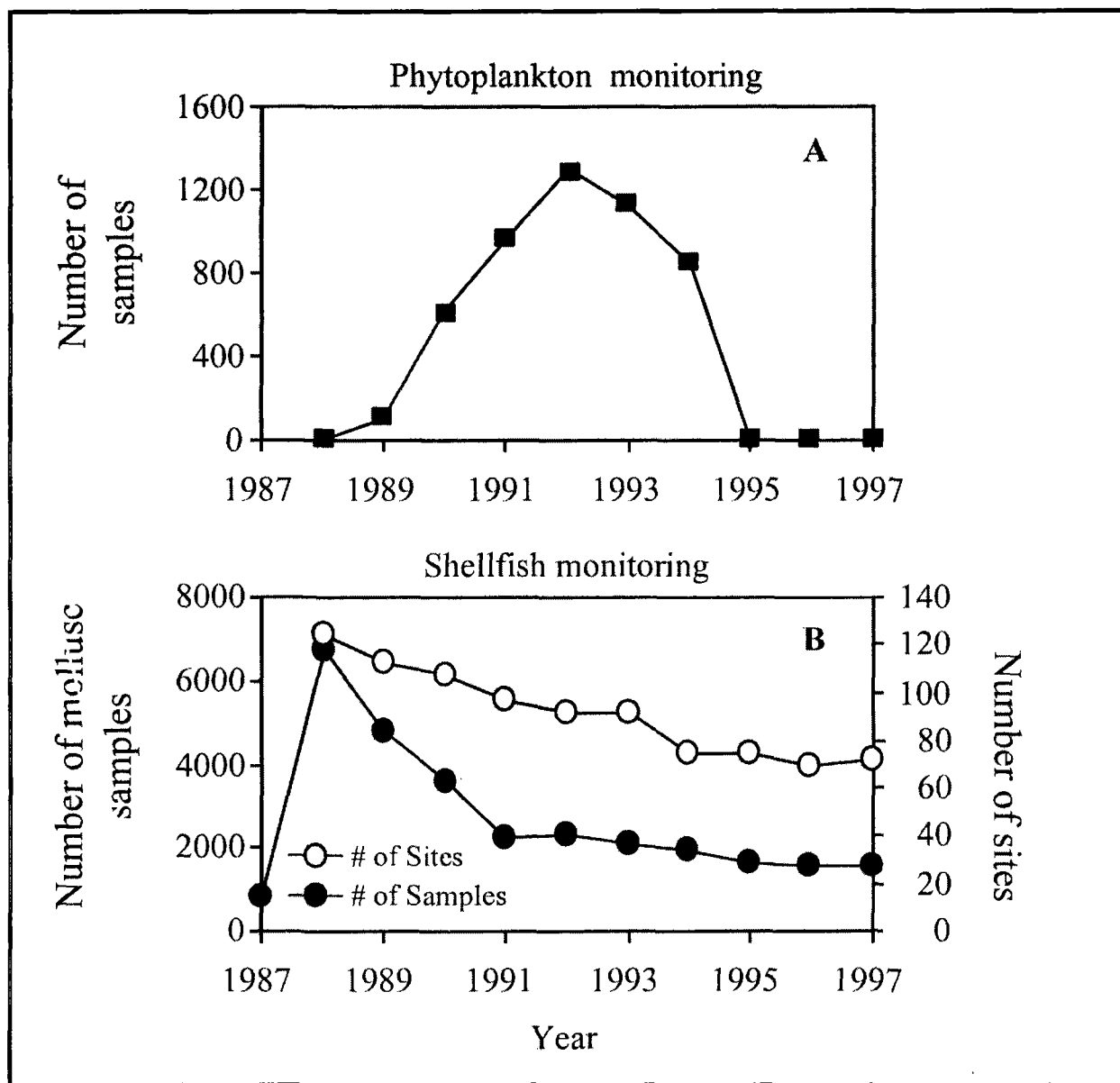
FIGURE 4.10. Temporal changes in the phytoplankton and shellfish toxin monitoring programs in the Maritimes, Atlantic Canada (New Brunswick, Prince Edward Island and eastern Nova Scotia), 1987/88-1997.

A. Number of phytoplankton samples collected by CFIA;
B. Number of shellfish samples and shellfish sampling sites

(Source: D. Richard, Canadian Food Inspection Agency, CFIA)

Toxin analysis: In the Maritimes region, all toxin analysis is conducted by the CFIA (Biotoxin Section) at two laboratories located in Moncton, NB and Halifax, NS. The AOAC mouse bioassay is used for routine analysis of PSP toxins, and has also been used to screen for unusual symptoms indicative of the presence of unknown toxins. Mice (CD-1 strain) are shipped from Montreal. An individual conversion factor is used as it is generally found to vary between 0.20 and 0.23 μg STXeq MU^{-1} among analysts. US FDA saxitoxin standard is used. In Canada, DA was initially analyzed using the mouse bioassay and a more extended observation period, but was subsequently replaced by analytical (HPLC) methods (Quilliam et al. 1989). Both PSP toxins and DA, which are water soluble, are analyzed from the same AOAC extract. If trace amounts of DA are detected, an aqueous methanol extract is used to improve the recovery and stability of DA (D. Richard, CFIA, pers. comm.).

Regulatory action. Regulations on the control of contaminated shellfish were established by DFO in 1990 under the Fisheries Law. They authorized the DFO Regional Director to prohibit harvesting of fish or shellfish species in an area where these pose a risk of contamination. **Regulatory action is dictated by toxin levels in shellfish, not by results of phytoplankton monitoring.** In the Bay of Fundy, a year-round ban on the harvesting of mussels has been imposed, due to their high risk for accumulation of PSP toxins. Temporary closures to harvesting of softshell clams, *Mya arenaria*, occur annually, generally in the summer. Permanent closure of some clam harvesting sites is also imposed due to year-round retention of PSP toxins: for example Crow Harbor has been closed since the 1940's. Canning of softshell clams with toxicities < 160 μg STXeq 100g^{-1} is allowed, but only if the clams were harvested prior to closure. No harvesting is permitted in closed areas. DFO also verifies registered processing plants for compliance. If violations are identified, compliance action is taken against the plant.

Dissemination of information: If toxin levels exceed the safety limit, CFIA contacts DFO's Chief of Conservation and Protection (C and P) Branch and a request for closure is made, which has never been denied. All local clam processors are contacted by phone, as well as the diggers' associations, the local radio stations and the provincial fisheries office. A toll-free phone line is available for recording of specific area status. So far, notification has not been included in CFIA's web site.

Identified bottlenecks in the Maritimes region: Funding cutbacks, which have greatly reduced the capabilities of phytoplankton monitoring, are perceived as a major limitation in the region. Reduced government funding and downsizing of personnel have increased the need for industry participation in contributing to the cost of toxin monitoring, yet the shellfish industry is, with a few exceptions, not sufficiently developed to sustain the costs.

Laurentian region, Quebec: estuary and Gulf of St. Lawrence

History and general description of the program: The following account is largely based on a recent review of the Quebec monitoring program by Blasco et al. (1998). The Laurentian region in Quebec comprises the estuary and Gulf of St. Lawrence and the Gaspé Peninsula. Only PSP has posed a serious problem in Quebec, where there have been more than 300 documented cases of PSP, including 25 fatalities, between 1880 and 1994. None have occurred since the establishment of the monitoring program. Although monitoring of shellfish toxins started in 1949 at some stations, a comprehensive shellfish toxin monitoring program was not developed in the region until 1984. At this time DFO Inspection Branch took on the responsibility for shellfish monitoring from the Quebec Ministry of Agriculture, Fisheries and Food (MAPAQ), a task which was transferred to the CFIA in 1997. Although recent, the program is intensive and includes both shellfish and phytoplankton monitoring. Compared to the Bay of Fundy, PSP toxicity outbreaks in the St. Lawrence region are less regular, less predictable and are therefore more difficult to monitor adequately without intense spatial and temporal sampling coverage. In 1998, domoic acid was found for the first time in sea scallop digestive glands off the Magdalen Islands at levels of up to 550 ppm

(Figure 4.9, Gilbert Sauvé, CFIA, pers. comm.) and trace levels of domoic acid were found in shellfish throughout the Gulf region in 2000. *Pseudo-nitzschia seriata* isolated from the Gulf of St. Lawrence estuary was found to produce DA in culture. Low levels of DSP toxins (maximum = 0.4 g g^{-1} in digestive gland) were also recently recorded in cultured mussels from Quebec (M. Levasseur et al. 2001).

Principal shellfish species tested. As in the Bay of Fundy, *Mytilus edulis* and *Mya arenaria* are the two main shellfish species monitored. A highly significant linear correlation was found between the toxicity of these two species (Figure 4.11A) and the slope of this correlation indicates that mussels generally become 5x more toxic than softshell clams at the same site. Other mollusc species occasionally tested, ranked by their importance as vectors of PSP toxins, are *Mesodesma arctatum*, *Buccinum undatum*, *Ensis directus*, *Lunatia heros*, *Thais lapillus* and *Colus stimsoni*.

Sites and frequency of shellfish sampling: Molluscs are routinely monitored at 85 sites from March to November. Highest toxicities occur on the south shore of the St. Lawrence Estuary and north shore of the Gaspé Peninsula. The spatial distribution of toxicity in bivalves corresponds well with that of *Alexandrium* cells.

Phytoplankton monitoring: Phytoplankton monitoring is carried out weekly at 11 coastal stations from April to November. *Alexandrium tamarense* and *A. ostenfeldii* are the two toxigenic species responsible for PSP in the region, but 18 potentially harmful species are enumerated. Phytoplankton sampling can be used with some success as an indicator of the toxicity in shellfish. For example, significant correlation was found between the time each year when mussels exceed the RL and the time when *Alexandrium* concentrations attain 1000 cells L^{-1} (Figure 4.11B). Phytoplankton and toxicity data are published in annual technical reports. This information is used by the CFIA to interpret unusual mouse bioassay deaths and by industry to establish sites for depuration plants and new harvesting sites.

Regulatory action: When several species are collected from the same area, the first species that exceeds the RL causes immediate closure of the area. Three consecutive results below the RL showing a pattern of decreasing toxicity over a 15 day period are required for re-opening of an area, in order to counter sudden toxicity rises and multiple toxin peaks.

4.3.3 Galicia, NW Spain

General description of the program: The Red Tide Monitoring Program was established in 1977 in response to a 1976 PSP outbreak in several western European countries which was traced to the consumption of mussels cultured in Galicia. At this time, 176 individuals from five countries were affected by PSP but there were no fatalities. Since 1977, PSP has only affected a few individuals who ignored warnings of government authorities and ate wild mussels, which are not routinely monitored or depurated. A toxin monitoring program is required to fulfill the EC requirement for quality control of shellfish growing areas (91/492/CEE).

In contrast to the North American programs, the monitoring program in the Rías Baixas of Galicia, NW Spain, has two unique features: it is primarily designed to monitor a high-value, aquaculture product (Galicia is the world's largest producer of mussels), and the program is comprehensive, including monitoring of shellfish toxins, phytoplankton and the collection of oceanographic and meteorological data. The main biotoxin problems in the region are PSP, caused by *Alexandrium minutum* and *Gymnodinium catenatum*, DSP, caused by *Dinophysis acuminata* and *D. acuta*, and to a lesser extent *D. caudata* and *D. sacculus*. Okadaic acid and DTX1 have been detected in *Prorocentrum lima* cultures from Ría de Vigo, but their implication in shellfish poisoning has not been demonstrated. DSP was first
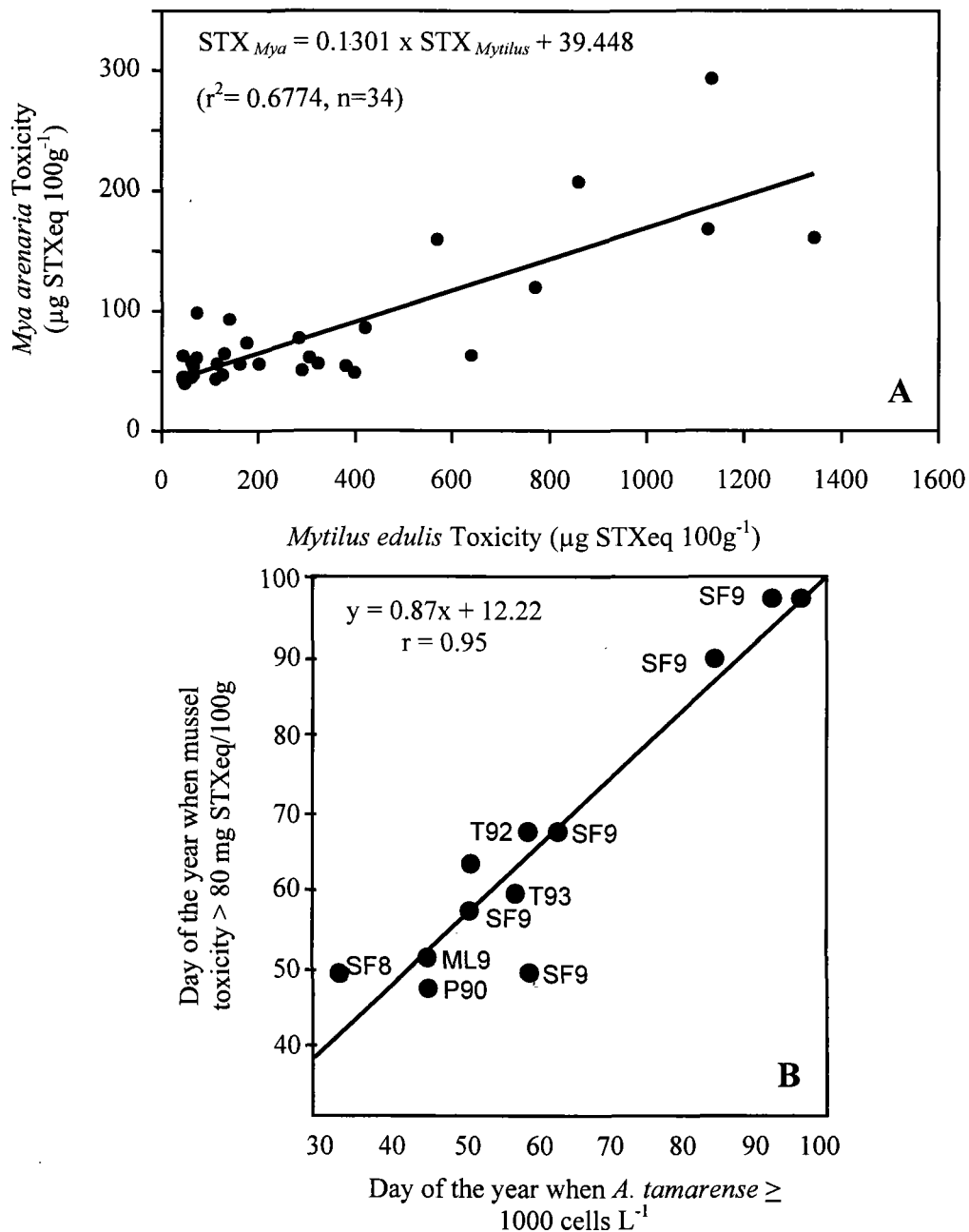
FIGURE 4.11. Predictive relationships established from HAB monitoring data from the Gulf of St. Lawrence region, Quebec, Canada (1986 to 1994).

A. Relationship between the toxicity of mussels (*M. edilus*) and softshell clams (*M. arenaria*) at Tadoussac (data plotted from Annex 6.1 in Blasco et al. 1998).

B. Relationship between the first day of the year when the concentration of *Alexandrium* spp. $\geq$ 80 µg STXeq 100g^{-1} (year and station initials indicated: SF = St. Flavie, P = Penouille, T = Tadoussac, ML = Mont Louis) (Source: Blasco et al. 1998).

documented in 1978 due to the consumption of cultured mussels, which had been depurated in chlorinated water, thus ruling out the possibility of bacterial pathogens.

Routine bioassays of DSP toxins were added to the monitoring program in 1981. Domoic acid (DA) was first documented in Galician shellfish (mussels) in 1994, in association with the presence of *Pseudo-nitzschia* spp., and although toxin levels were below the 20 μg g^{-1} safety level, routine analysis of domoic acid was included in the monitoring program in 1995 (Arévalo et al. 1998). Since then, DA levels above the safety level have been measured several times in Galician scallops (*Pecten maximus*).

Since 1986, shellfish biotoxin monitoring has been conducted regionally, by the Autonomous Government of Galicia (Xunta de Galicia). The Department of Biotoxins of the Center for Quality Control of the Marine Environment (Centro de Control de Calidade do Medio Mariño) (Consellería de Pesca, Marisqueo e Acuicultura), run by the Xunta de Galicia, is the agency involved in toxin monitoring of local molluscs. The National Health Department (Consellería de Sanidad Exterior) within the Ministry of Health is responsible for control of exported seafood product. The overall strategy for management of local stocks is to differentiate exploited species based on an understanding of their toxin kinetics (uptake and detoxification rates), and harvesting schedules (continuous vs. intermittent). The description of the program is based mainly on Campos et al. 1982, Mariño et al. 1998, and Reguera et al. 1991.

Purpose/objectives: Although food safety is the primary objective of the Galician biotoxin monitoring program, it is also designed to identify the causative agents of different toxic events, and therefore includes routine collection of phytoplankton and oceanographic data.

Principal shellfish species tested: The mussel, *Mytilus galloprovincialis*, is used as the main indicator species for toxin monitoring. Mussels are the mainstay of aquaculture production in Galicia, which has been conducted on a large scale since the 1950's. An annual production of 200,000 metric tons is achieved in intensive, suspended culture (in rafts), concentrated mainly in the Rías Bajas: Muros, Pontevedra, Vigo and especially in the Ría de Arosa which contributes 75% of total mussel production in Galicia. Other bivalves exploited from natural beds and tested for biotoxins are clams (*Venerupis* spp.), cockles (*Cerastoderma edule*) and scallops (*Pecten maximus*). PSP toxins above the safety level have also been found in the foot of the browsing gastropod *Haliotis tuberculata* (Martínez et al. 1991).

Sites and frequency of shellfish sampling: For the purpose of shellfish toxin monitoring, the region is divided into administrative zones and subzones, which discriminate between suspended culture sites (primarily mussel culture) from natural or extensively cultured natural beds, where epifauna and infauna are differentiated. For shellfish collection, there are a total of 49 primary stations, which are known to be the most prone to toxic events and use mussels as the indicator species, and 189 secondary stations (128 involve sampling of cultured mussels, 40 of infaunal molluscs, and 19 of epifaunal molluscs; Figure 4.11). Toxic events in this region are possible at any time of the year. Therefore, bivalves at primary stations are sampled once a week throughout the year, in collaboration with producers who provide their vessels for this purpose. The sampling schedule in secondary stations depends on the appearance of toxic events. Samples are taken weekly when neither toxic species, nor toxicity of bivalves is detected by mouse bioassay. Sampling is more frequent during a toxic outbreak: two to three times a week when toxin levels are below the safety level, and even daily when the levels approach the safety level and in closed areas, in order to allow for their reopening. Mussel samples are taken from two opposite ropes of the raft, at 3 depths (the ropes extend to 15 m), since *G. catenatum* shows strong vertical segregation in the water column, and this is reflected in important differences in toxicity of mussels with depth.

Phytoplankton monitoring: In addition to shellfish toxicity testing, phytoplankton monitoring is required as part of the quality control established by European regulations. Qualitative and quantitative

phytoplankton analysis is conducted at 35 primary oceanographic stations, located near areas that support mussel culture, and 14 secondary oceanographic stations distributed along the coast (Figure 4.12). Phytoplankton and hydrographic data (temperature, dissolved oxygen, fluorescence, etc.) are collected at these sites. Sampling at primary stations is carried out weekly all year-round by the Center for Quality Control of the Marine Environment, Department of Oceanographic Conditions and Phytoplankton (staff of 5), and secondary stations are sampled in collaboration with other institutions, e.g. the Spanish Oceanographic Institutes (IEO) in La Coruña and Vigo. Phytoplankton counts are available 24 hrs after sampling, and if potentially toxic species are found, this information is sent the same day by fax to the health and fisheries authorities, and the industry.

Toxin analysis: The Department of Biotoxins, with a staff of 11, carries out PSP and DSP analyses using the AOAC mouse bioassay, and Yasumoto et al.'s (1980) mouse bioassay respectively. Analysis by HPLC is used as a supplementary technique. Domoic acid is determined by HPLC-UV detection using the standard AOAC extraction protocol. Domoic acid calibration solution (DACS-1B) is obtained from NRC, Canada. A maximum of about 50 samples can be handled per day for analysis of PSP and DSP toxins. The total number of samples processed per year varies depending on the characteristics of the toxic outbreaks, but the maximum capability is high, i.e., 13,000 samples yr^{-1}, estimated at a cost of US$52 sample including sample collection and analysis.

Mussels can vary greatly in the PSP toxin composition reflecting differences in the toxigenic source. *Alexandrium minutum* produces exclusively gonyautoxins ($GTX_{1,2,3,4}$), whereas sulfocarbamoyl toxins are dominant in *G. catenatum*.

Regulatory action: All shellfish toxicity data are processed by computer by staff from the Department of Coordination and Management, which also includes a technical director and the senior staff from all other government departments. The action plan (Figure 4.13) allows for closure of shellfish harvesting areas when toxin levels exceed the safety level, as well as their reopening to reduce economic losses, and is updated by computer twice a day. Any change in the action plan is validated by the Dept. of Coordination and Management, which immediately notifies all administrative organizations, regional fisheries authorities, industry (shellfish producers, depuration plants, canners' association) and research centers by fax and e-mail. Information obtained from the monitoring program is sent by the regional government to the National Health Department. Precautionary closures of shellfish areas are also implemented when toxin levels are below the safety level. Toxic PSP-producing cells are often concentrated at the mouth of the Rías, while the middle and inner sectors remain toxin-free. Therefore, strict control has allowed selective closures to take place, in which harvest is allowed in unaffected inner portions of the Rías, while outer areas remain closed. This spatial distribution of mussel toxicity is consistent with the hypothesis which attributes the origin of *G. catenatum* cells in the Rías to horizontal advection of populations established offshore (Fraga 1996).

When precautionary closures are implemented, additional toxicity testing is carried out after mussels come out of bacterial depuration plants, where they are held in sterile seawater for 2-3 days (B. Reguera, IEO, Vigo, pers. comm.). This analysis is conducted by the Aquaculture Institute, University of Santiago de Compostela. The canning industry also has every canned lot produced at its facilities analyzed for toxins. However, these two additional controls are not legally required and are paid by private funds.
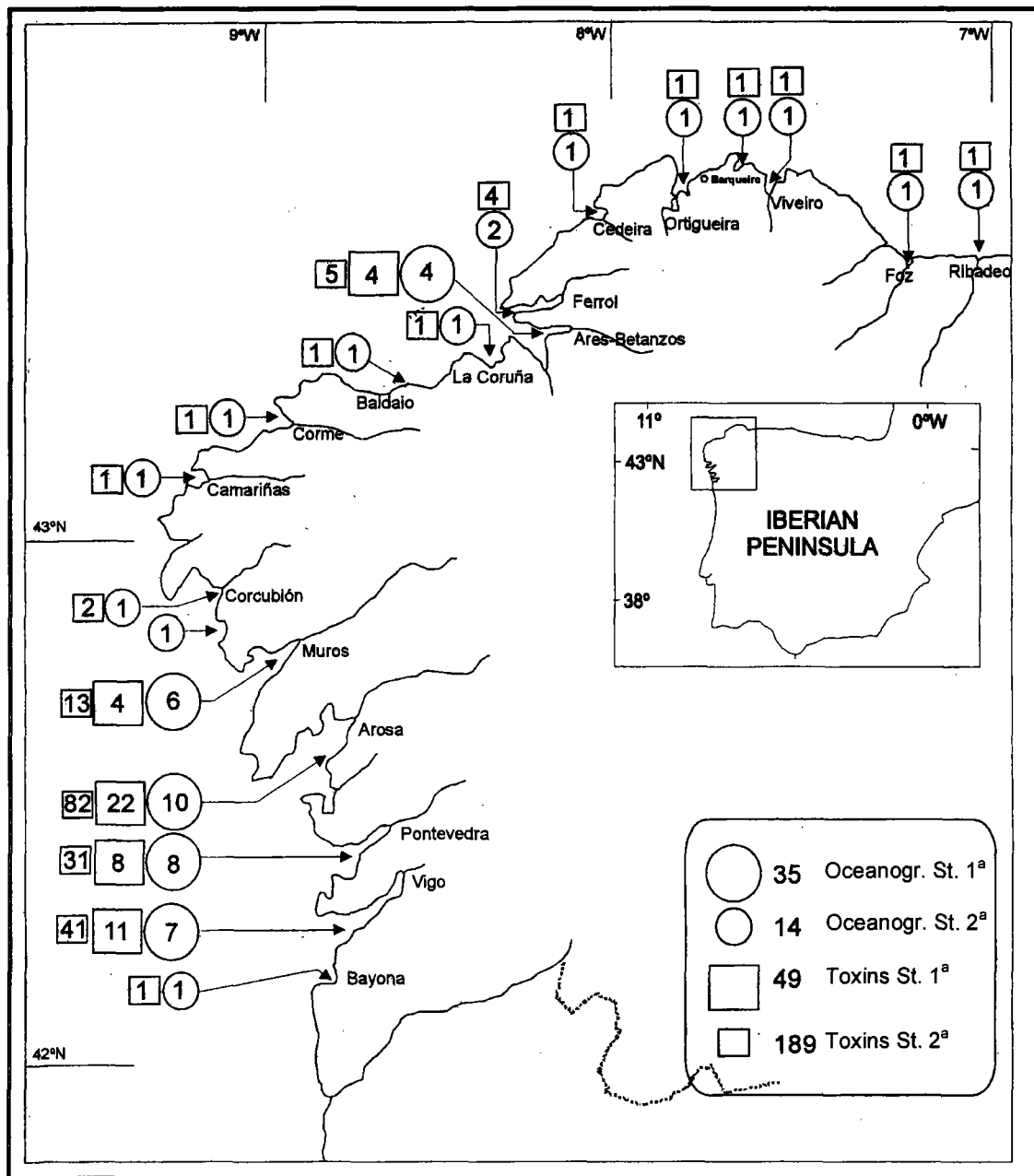
FIGURE 4.12. Location of sampling sites in Galicia, Spain. 35 primary (large circle) and 14 secondary (small circle) oceanographic stations, 49 primary (large square) and 189 secondary (small square) shellfish toxin stations. (Source: Mariño et al. 1998.)
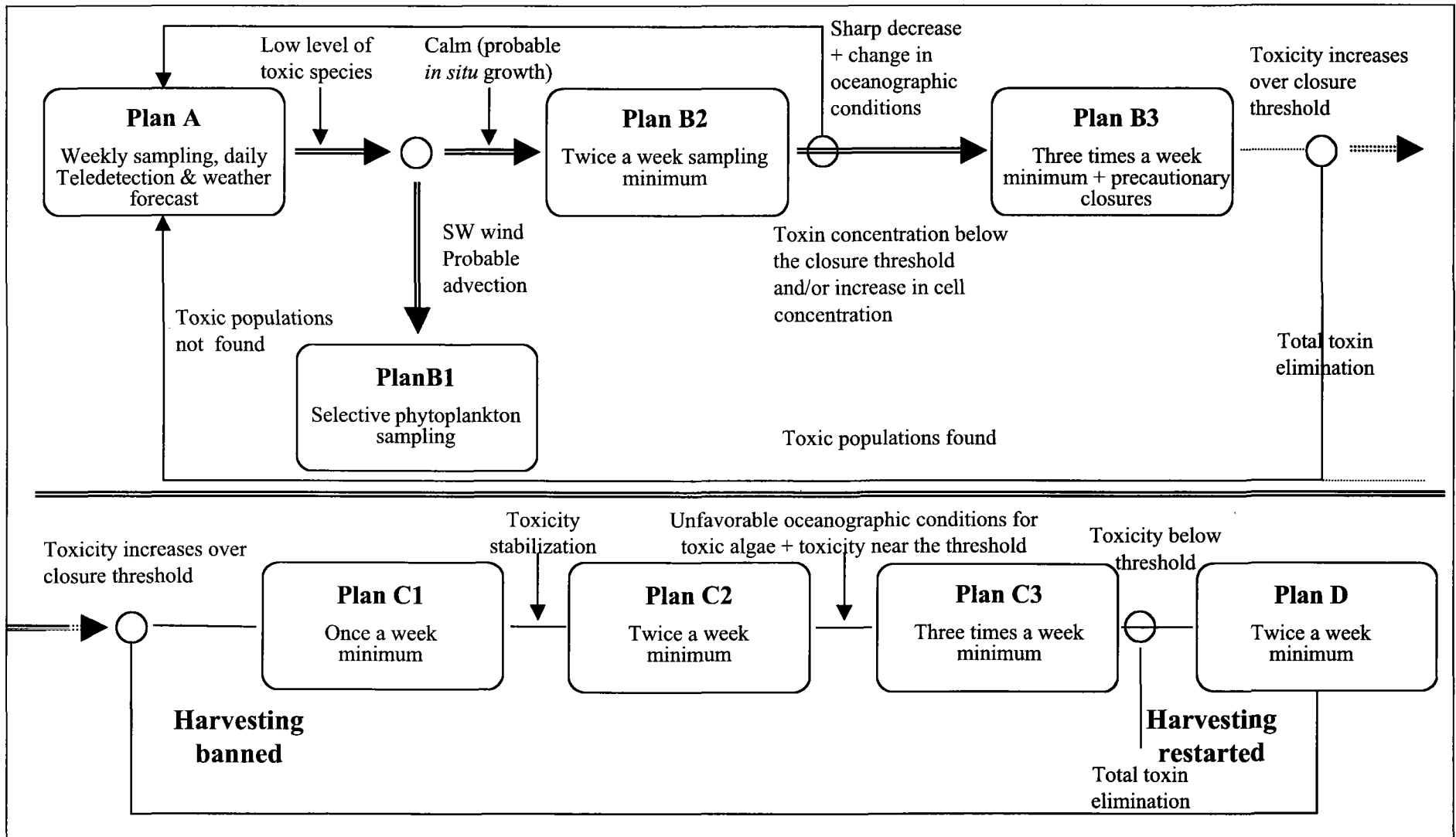
FIGURE 4.13. Action plan for the biotoxin monitoring program in Galicia, NW Spain. (Source: Mariño et al. 1998).

4.3.4 Denmark

Since 1991 an intensive monitoring program for detection of toxic algae and algae toxins in mussels in Danish coastal waters and fjords has been carried out. The primary motivation for the Danish fishers and the mussel industry to initiate a monitoring program was the occurrence of DSP-toxins in mussels for export in the late 1980's. Those events led to a dramatic decrease in the export of Danish mussels and resulted in a crisis in the Danish mussel industry because more than 90% of the harvest is exported. Before 1991, shellfish toxicity monitoring relied on random sampling conducted by the Ministry of Fisheries as well as the monitoring of phytoplankton carried out by the different Danish councils for more general environmental management purposes.

The Danish shellfishery is unique in the respect that almost all shellfish (blue mussels) are harvested on natural banks distributed in the shallow coastal areas. At present there is only one commercial culture (rope culture) site of blue mussels in Denmark, situated in Mariager Fjord.

Every year since 1991, more than 2000 samples of plankton and mussels have been collected annually (Figure 4.14). Of these more than 1000 samples were analyzed each year. During that period there have been several closings or restrictions because of the occurrence of: *Dinophysis acuminata, D. norvegica, Alexandrium tamarense, A. ostenfeldii, A. minutum, Pseudo-nitzschia seriata*-group and *Pseudo-nitzschia delicatissima*-group. *Prorocentrum lima, P. micans, P. minimum* and *Prorocentrum balticum* have also caused problems.

Because of these closures, no cases of shellfish intoxication due to consumption of Danish mussels have been reported since 1991. PSP toxins have only been reported twice in shellfish since 1990. DSP-toxins (okadaic acid) were found almost every year in Danish mussels in the period from 1991 to 2000. ASP-toxin (domoic acid) has only been observed once in the period from 1991-2000. The program has previously been presented and discussed by Andersen (1996, 1998) and Emsholm et al. (1995).

The Danish monitoring program is one of the very few monitoring programs worldwide which operates with the possibility of imposing intensified monitoring or closure of a shellfish harvesting area based either upon 1) high concentrations of toxic or potential toxic algae in the water or 2) concentrations of algal toxins in the shellfish exceeding specified action limits. In this respect the program has been used as the model for other programs, e.g. the Norwegian shellfish monitoring system (Einar Dahl, pers. comm.).

The monitoring program and the management of the shellfisheries is evaluated in a yearly status report compiled by the consultancy company Bio/consult. This report is presented at the yearly meeting of the Danish task force on HABs. Members of the Danish task force include staff from the Veterinary Service responsible for the management of the Danish mussel fisheries, researchers from universities, environmental staff from the councils, staff from the National Environmental Research Institute as well as consultants involved in the monitoring program. At this meeting, the monitoring program and management procedures are discussed in detail. Changes in the monitoring program as well as the management procedures recommended by the task force, have been approved by the Veterinary Service and have been adapted into the monitoring program and the management procedures in the following year. The task force has proven to be a valuable tool in the evaluation and further development and improvement of the Danish monitoring program. Over the years, the task force has discussed and recommended changes in, for example, the number of stations sampled, sampling frequency, sampling methods, analysis methods, the use of intensified monitoring, action limits in relation to the observed concentrations of HAB species, as well as research projects on topics such as the toxicity of species in the genus *Dinophysis*.
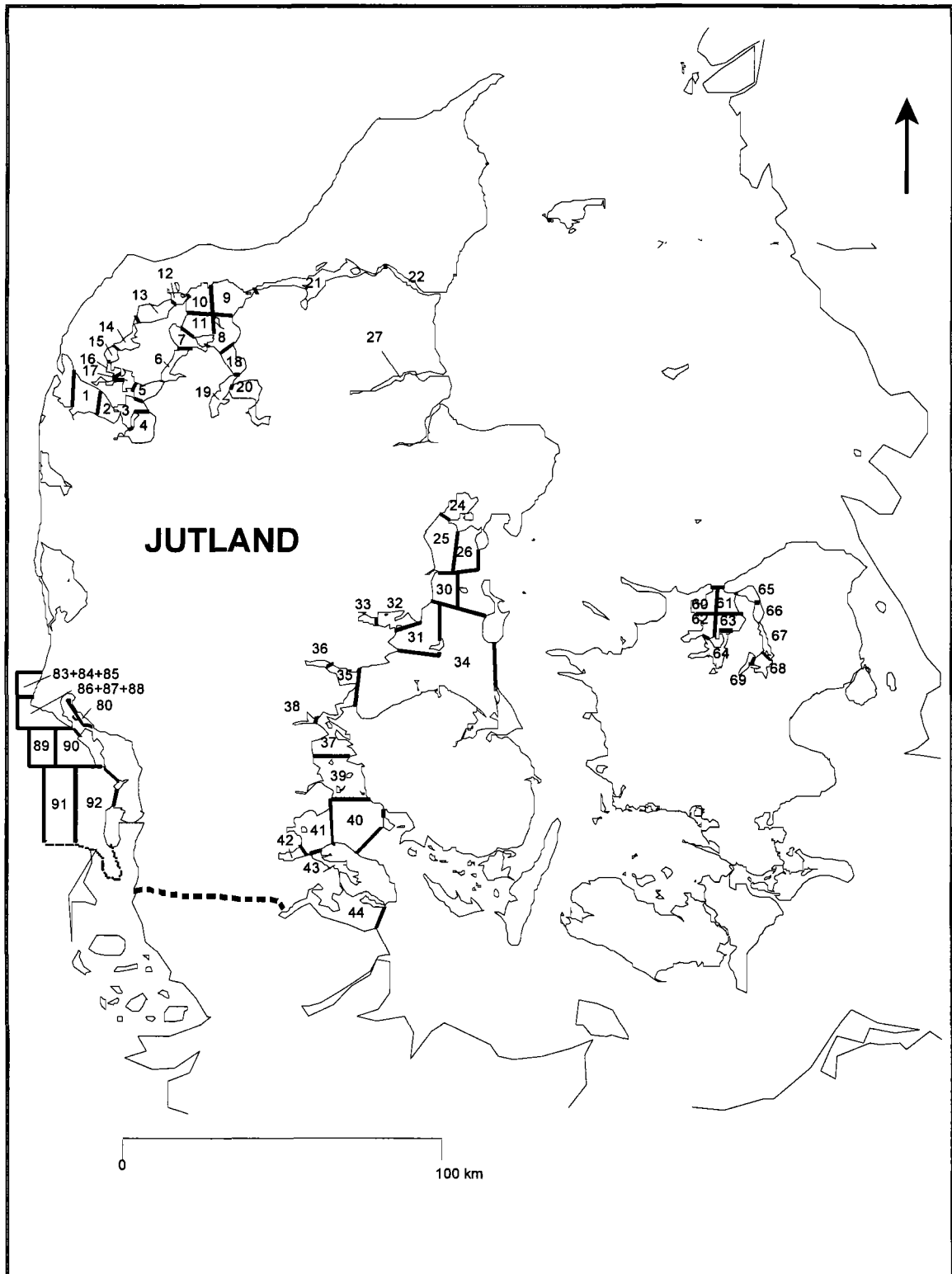
FIGURE 4.14. Areas of the Danish coastal waters and fjords where monitoring of harmful algae and algal toxins in mussels is conducted. (Source: Andersen 1998.)

Purpose/Objectives. The primary goals of the program are:

* to prevent toxic mussels from reaching the consumer

* to ensure that the effort of the mussel fishery is optimized by guiding the boats to areas with a low risk to harvest toxic mussels

Furthermore, it is the purpose of the monitoring program to provide information as the basis to improve the monitoring methods and management procedures. For this purpose plans are underway to use the monitoring data obtained within the system during the period 1991-2001 for basic risk analysis and to carry out further investigations to validate the monitoring system.

Principal shellfish species tested. The most important shellfish to be harvested in Danish waters are the blue mussel (*Mytilus edulis*), cockles *(Cardium edule)* and surfclam (*Spisula* spp.). Furthermore, a very limited commercial harvest of the European oyster (*Ostrea edulis*) is carried out in the Limfjord area.

Sampling and analysis of samples. The Danish monitoring program tests for the occurrence of toxic algae in the water and algal toxins in mussels at weekly intervals in the fishing areas. Danish coastal waters where mussels are harvested are divided into areas; e.g. the Limfjord is divided into 22 areas. Before harvesting for mussels is initiated in a given area, qualitative and quantitative algae samples as well as samples of mussels are collected by the fishermen in that location the week before, and sent to approved laboratories for analysis of the concentrations of toxic algae and algal toxins. Once harvesting begins, each boat must collect plankton samples as well as mussel samples to be analyzed on the first fishing day of every subsequent week in an area.

The plankton and mussel samples are collected by the fishers, who have been instructed in a training course taught by the consultants responsible for analysis of the samples. Qualitative, concentrated, algal samples are collected by the fishermen using a plankton net (mesh size 20 μm). Quantitative algal samples are collected using a water sampler, representing a mixture of water sampled at the surface, in the middle of the water column as well as ~ 1 m above the bottom. Both types of samples are preserved using neutral Lugol's and are kept in plastic bottles. Plastic bottles are preferred because glass bottles often break during transport to the consultancy company. Samples are sent by mail and are received the day after mailing.

Toxin analysis. Algal toxins: Mussels are examined for DSP by a modification of Yasumoto's mouse bioassay (Yasumoto et al. 1995). Throughout the year, DSP acetone extraction is used for normal monitoring of blue mussels, and ether extraction is used as the official method for all other bivalve molluscs. Examination for PSP is carried out, as a minimum, in the months April-September, by mouse bioassay using a modification of AOAC's methodology (AOAC, 1995), (pH = 2 - 2.5 instead of 3). Mussels are only examined for domoic acid by HPLC (Lawrence et al. 1989) during blooms of *Pseudo-nitzschia*. Verification for DSP and PSP is done by HPLC (Lee et al. 1987; Franco and Fernández-Vila 1993). The methodologies used for monitoring of algal toxins are summarized in Table 4.7.

Phytoplankton analysis. The qualitative investigation of the concentrated net-samples is carried out using interference light microscopy in combination with epifluorescence microscopy using the fluorochrome Calco-Fluor White, according to Andersen and Kristensen (1995), modified from the procedure described by Lawrence and Triemer (1985). Quantitative investigations of plankton samples (25-200 ml) are carried out using a combination of inverted microscopy (according to Utermöhl (1958); see also Hallegraeff et al. 1996), and quantitative epifluorescence microscopy. The toxic and potentially toxic algae reported from Danish waters are listed in Table 4.8.

TABLE 4.7. Summary of guidelines for monitoring algal toxins in the Danish mussel-fisheries.

Algal toxin syndrome	Period	Method
DSP	All year	Mouse bioassay -ether extraction (official method) -acetone extraction (used for *M. edulis* under normal surveillance) Verification by HPLC
PSP	Minimum April-September	Mouse bioassay Verification by HPLC
ASP	When blooms of *Pseudo-nitzschia* spp. occur	HPLC

TABLE 4.8. Toxic and potentially toxic algae reported from Danish waters.

DSP	PSP	Domoic acid	Other toxins
Dinophysis acuminata[1] *Dinophysis acuta*[1] *Dinophysis norvegica*[1] *Dinophysis spp.*[1] *Dinophysis rotundata*[1] *Prorocentrum lima*[1]	*Alexandrium pseudogonyaulax* *Alexandrium tamarense*[1] *Alexandrium ostenfeldii*[1] *Alexandrium minutum*[1] *Gymnodinium catenatum*[2]	*Pseudo-nitzschia seriata*-group *Pseudo-nitzschia delicatissima*-group	*Prorocentrum balticum*[1] *Prorocentrum minimum*[1] *Prorocentrum micans*[1] *Nodularia spumigena* *Aphanizomenon flos-aquae* *Microcystis aeruginosa* *Microcystis viridis*

[1]Quantified using epifluorescence according to Andersen and Kristensen, 1995
[2]Not observed in Danish waters but documented in adjacent waters

Communication of results. All new results of the different analyses are currently distributed from the respective consultant companies to the Danish Fish Inspection Service, Ministry of Fisheries as well as to the individual mussel industries and the secretariat of the Danish Association of Musselfisheries, on a daily basis, using phone and fax. The Danish authorities, in cooperation with the Danish Musselfisheries, are currently working on presentation of the monitoring program as well as the current data on web pages on the Internet. This will be operational in 1999.

Action limits on algal toxins. The action limits for algal toxins in mussels follow the guidelines outlined by EU Council directive No. L268, of 15 July 1991. That is, management of the Danish shellfish harvest is based upon the following action limits:

• DSP toxins must not be detectable using the mouse bioassay.

• PSP toxins, detected by the mouse bioassay must be below 80 μg 100 g^{-1}.

• ASP-toxins, detected by HPLC must be below 2 mg 100 g^{-1}.

If the concentrations of algal toxins are below these limits, the shellfish harvest is open in the respective areas. If the concentrations are above or equal to these limits, the harvest of shellfish is closed in the respective areas.

Action limits on algal concentrations. The recommended action limits of the toxic and potentially toxic algae are shown in Table 4.9. The action limits were originally based upon information from the literature combined with educated guesses. The action limits are continuously evaluated and revised as necessary. Over the years, the monitoring has shown that even extreme concentrations ($>1 \times 10^6$ cells L^{-1}) of species from the genus *Prorocentrum* do not result in accumulation of toxic substances in mussels. These experiences have resulted in a revision of the guidelines for the *Prorocentrum* species which at present state that there is no fixed action limit. In situations with high concentrations of *Prorocentrum*-species, restrictions on the mussel fishery are only imposed based upon results of the mouse bioassay. During the period 1991-1995, the concentration limit of the species *Dinophysis norvegica* was changed from 500 to 10^3 cells L^{-1}. This change was based upon the fact that accumulation of DSP toxins has never been detected in mussels, even in situations with very high concentrations ($>10^3$ cells L^{-1}) of *Dinophysis norvegica*.

TABLE 4.9. Recommended action limits for toxic and potentially toxic algae in relation to the Danish mussel fisheries.

Species	Closed/intensified monitoring (cells L^{-1})
Dinoflagellates	
Dinophysis acuminata	500
Dinophysis acuta	500
Dinophysis norvegica	10^3
Dinophysis rotundata	10^3
Total Dinophysis spp.	1.2×10^3
Prorocentrum lima	500
Prorocentrum balticum	No specific limit
Prorocentrum micans	No specific limit
Prorocentrum minimum	No specific limit
Alexandrium ostenfeldii	500
Alexandrium tamarense	500
Alexandrium spp.	500
Diatoms	
Pseudo-nitzschia seriata-group	2×10^5
Pseudo-nitzschia delicatissima-group	5×10^5
Pseudo-nitzschia spp.	2×10^5
Cyanobacteria	
Nodularia spumigena	$1 - 2 \times 10^5$ (colonies)

Dissemination of information. Based upon the combined results of the occurrence of toxic algae and algal toxins, the Fish Inspection Service decides whether the fishing areas are declared open, closed or under intensified surveillance. If an area is closed or under intensified surveillance, the industries are informed by the Fish Inspection Service. The fishermen and the industries can be continuously informed about the status of the different fishing areas via a recorded message on a telephone answering machine located at the Danish Fish Inspection Service. This system is depicted in Figure 4.15.
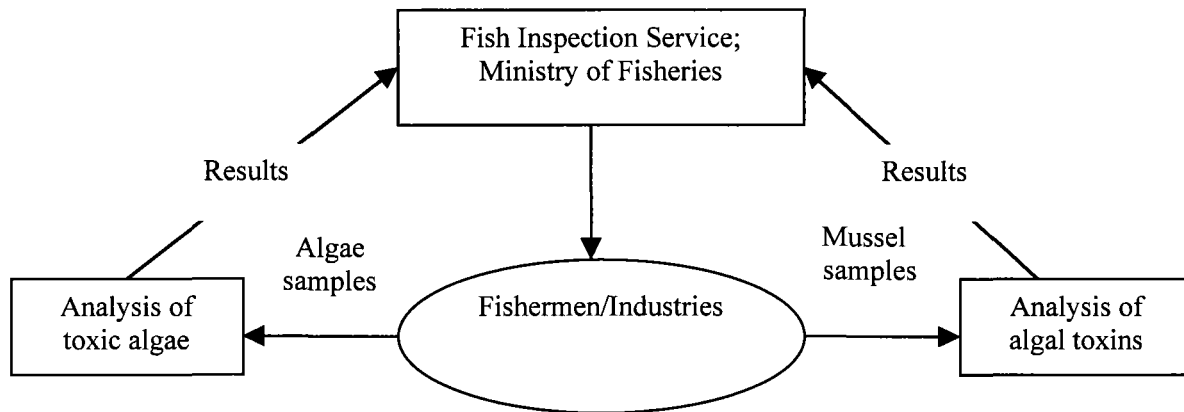
FIGURE 4.15. Flow of communication through the Danish monitoring program for toxic algae and algal toxins in mussels. (Source: Andersen 1996.)

Data management. All data on the occurrence of algae are stored in digital form using the databases TOX-sys and/or PLANKTON-sys developed by Bio/consult.

Costs and Benefits. The expenses of the Danish monitoring program are paid by the mussel industry and the fishermen. In 1997, for example, this totaled around 2.5 mill. dkr. ($\sim$ US $400,000), which constitutes $\sim$ 1% of the total export income of the mussel industry. The total cost can be broken down to $\sim$ 0.7 mill. dkr for the algae analysis and 1.8 mill. dkr. for the analysis of algal toxins in the shellfish. Sampling of phytoplankton and shellfish is carried out by the fishermen and there is no cost specified for this activity.

4.3.5 New Zealand

The following text is more extensive than is the case for the other countries presented in this section. This is because the New Zealand monitoring program is an excellent example of a comprehensive and logical program, one that was recently updated to reflect changes in technology and general knowledge about HAB toxins. The text has been edited from material provided by Catherine Seamer, Ministry of Agriculture and Forestry (MAF) Food Assurance Authority, Wellington, New Zealand, April 2001.

History and general description of the biotoxin management program. Harmful algal blooms came to the forefront in New Zealand in the summer of 1992/93, when microalgae capable of producing toxins that cause ASP, NSP, DSP and PSP were found in the coastal environment and associated with NSP-like symptoms were reported. Regulatory authorities were not adequately prepared to cope with toxic blooms, since none had been reported in New Zealand prior to that date. Regulatory decisions were difficult to make due to the involvement of multiple toxins and the observation of several different clinical syndromes. The commercial shellfish industry sustained severe economic damage. Other toxic events have followed since this initial incident: NSP recurred in 1995, DSP in 1993, PSP in several years and ASP in 1994 (Trusewich 1996). In 1998 a bloom of *Gymnodimium brevisulcatum* in Wellington Harbor was reported to cause respiratory illness (Chang 1999), but did not affect commercial shellfish growing areas. In 2000 *Gymnodinium catenatum* was reported for the first time in New Zealand, resulting in extensive closures of non-commercial shellfish areas along the North Island coastline due to contamination with PSP toxins, whereas commercial areas remained largely unaffected. Maximum toxicities reached 4027 µg STXeq 100g^{-1} in green mussels, *Perna canaliculus*, yet the *G. catenatum* bloom was not associated with

human fatalities (Mackenzie 2001). The species diversity and geographic extent of the HAB threat to New Zealand is shown in Figure 4.16.

The New Zealand case study offers a number of unique features: it very rapidly developed a sophisticated biotoxin monitoring program (including the integration of molecular probes for phytoplankton monitoring) since the first documented occurrence of toxic outbreaks in the early 1990's, and has to monitor for multiple toxins, as ASP, DSP, PSP and NSP toxins have all occurred in shellfish above the safety limit. YTX and PTX toxins and gymnodimine are also found in New Zealand shellfish. The program is also characterized by a high degree of involvement of the shellfish industry, which reflects the importance of the shellfish resource in New Zealand. Shellfish (primarily mussels) contributed ~86% of exported aquaculture product in 2000. Whereas early on the commercial biotoxin program was entirely subsidized by the government, currently all costs are covered by the shellfish industry, although the industry is paid by the Ministry of Health for sharing their information with this agency.

As a response to the 1992/93 toxic events, the New Zealand Marine Biotoxin Management Board (MBMB) was formed under a memorandum of understanding (MOU) between the agencies involved, to manage and develop the New Zealand Marine Biotoxin Management Program. It was comprised of senior representatives from the Ministry of Agriculture, Ministry of Health and the New Zealand Fishing Industry Board. The Marine Biotoxin Management Board was responsible for the implementation of "The New Zealand National Marine Biotoxin Management Plan". The objectives of the MBMB were:

- To enable the different parties in the management of biotoxins to fulfill their relevant statutory duties, obligations and responsibilities;
- To minimize the risk from hazardous levels of marine biotoxins in New Zealand shellfish and fish products to consumers;
- To develop procedures to achieve an efficient and cost-effective marine biotoxin surveillance program;
- To collect and analyze information and data to enable the MBMB and the parties to the MOU to give the public and the New Zealand fishing industry early warnings of marine biotoxin events, their prevalence and their persistence;
- To investigate alternative options for monitoring marine biotoxins, testing and information gathering;
- To collect and analyze sufficient information and data with a view to developing a safe but less intensive method of monitoring

The MBMB had a standing Technical Committee comprised of representatives from the member agencies and the New Zealand Fishing Industry Board to deal with the following matters:

- Amendments to the National Marine Biotoxin Management Plan;
- Approval of changes to sampling site location, sampling species and sample frequency as well as opening and closure criteria;
- Laboratory specifications and approval of analytical methodology;
- Act as a focal point for research information exchange, both in New Zealand and overseas.

The National Marine Biotoxin Management Plan covered both commercial and non-commercial harvesting of shellfish. The plan met the requirements of the 1980 Shellfish MOU between the USFDA and the New Zealand Ministry of Agriculture and Fisheries. The MOU is presented in detail in the "National Marine Biotoxin Management Plan" which is available from the Marine Biotoxin Management Board, Wellington, New Zealand (1996). Additional information can be obtained from Wilson (1995) and Trusewich et al. (1996). Under the MOU bivalve shellfish exported to the USA are harvested, transported,
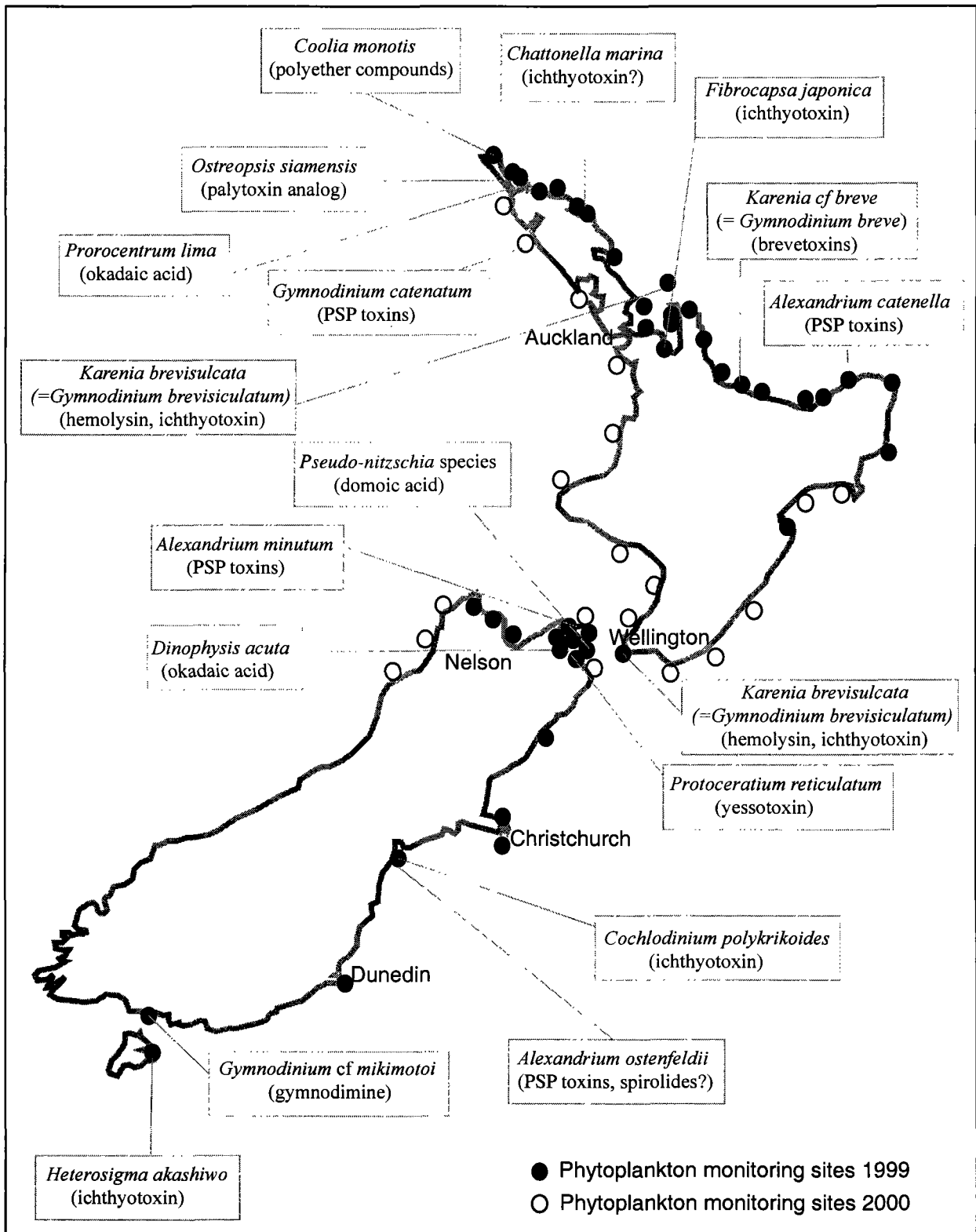
FIGURE 4.16. Map showing the many different toxic or potentially harmful phytoplankton species in New Zealand waters. (Modified from a map provided by Kirsten Todd, Cawthron Institute, Nelson; with additional information from H. Chang, NIWA, pers. comm.)

processed and labeled in accordance with the New Zealand Fishing Industry Agreed Implementation Standards 005, Shellfish Quality Assurance Circular 1995. The latter is equivalent to the USFDA NSSP manuals. This circular is updated and amended by the Ministry of Agriculture and Forestry in consultation with the New Zealand Seafood Industry Council. The Shellfish Quality Assurance Circular 1995 is pursuant to Regulation 19 of the Fish Export Processing Regulations 1995 and these regulations are pursuant to the Meat Act 1981. The National Marine Biotoxin Management Plan is pursuant to the Shellfish Quality Assurance Circular 1995.

The 1980 MOU contains an understanding that the Ministry of Health (MoH) will be responsible for the sanitary survey, classification and monitoring of shellfish growing areas from which shellfish are harvested. The MoH is also responsible for ensuring that all shellfish for sale on the domestic market comply with the requirements of the Food Act 1981, and the Food Regulations 1984. These were amended in 1993 so that shellfish grown and harvested for sale on the domestic market also meet the requirements of the USFDA NSSP manuals.

In each regional commercial growing area a shellfish quality assurance program delivery center (SQAPDC), composed of shellfish industry representatives, authorized personnel from the authorities, and any other person who may be required, was formed. The SQAPDCs (containing from one to many growing areas) were set up to ensure that all the requirements of the National Marine Biotoxin Management Plan were met in a cost-effective and efficient manner. In non-commercial growing areas, District Health Boards have sole responsibility for management of marine biotoxins. Each SQAPDC and/or District Health Board public health unit is responsible for developing a regional or local marine biotoxin monitoring plan that contains:

- Agency and personnel contact details at local and national levels;
- Definition of the marine biotoxin sampling sites for each growing area;
- Sampling procedures for phytoplankton and shellfish, including frequency of sampling for each growing area;
- Early warning indicators;
- Contingency plans;
- Procedures for notification of results to industry and others;
- Procedures and draft letters for growing area closure and re-opening;
- Locations for signs to be posted;
- Draft media statements and wording signage;
- Procedures for detention and recall of harvested product in accordance with the standards and MoH recall protocols for domestic market product;
- Surveillance procedures for closed areas.

A single marine biotoxin management plan covers both commercial and non-commercial shellfish growing areas in areas where both activities co-occur.

Current management of marine biotoxins in New Zealand. In October 1996, the MBMB disbanded, as the different parties involved in the management of marine biotoxins were able to fulfill their relevant statutory duties, obligations and responsibilities without its presence. The MOU between the USFDA and MAF is still in effect. At this time separate marine biotoxin programs for commercial and non-commercial shellfish harvesting areas were established. The safety of the non-commercial shellfish became the Ministry of Health's responsibility and is maintained in accordance with the Food Administration Manual Section 27: Marine Biotoxin Control through contacts with the District Health Boards for sampling and management services and contracts with science providers which provide analytical services. Section 27 of the Food Administration Manual is consistent with the National Marine Biotoxin Management Plan.

Funding for this program, including support of sampling, laboratory and District Health Board costs, is provided by the Ministry of Health.

The commercial program became MAF's responsibility, managed through the previously established SQAPDCs and funded by the shellfish industry. All costs incurred that relate to the Shellfish Quality Assurance Program such as District Health Board and MAF inspectors costs and sampling and laboratory tests, are charged to and paid for by the shellfish industry. Local marine biotoxin management plans are still required, based upon the National Marine Biotoxin Management Plan as before.

MAF Food Assurance Authority now publishes and maintains the National Marine Biotoxin Management Plan pursuant to the New Zealand Fishing Industry Agreed Implementation Standards 005.1, Shellfish Quality Assurance Circular, 1995. Industry participation is an integral part of the Shellfish Quality Assurance Program and MAF works with the New Zealand Seafood Industry Council, Ministry of Health and the Marine Biotoxin Technical Committee in setting standards and altering the National Marine Biotoxin Management Plan.

Under the revised monitoring program, the Marine Biotoxin Technical Committee was retained with representatives from The Ministry of Agriculture and Forestry, Ministry of Health and the New Zealand Seafood Industry Council. The committee carries out the same functions as under the MBMB, and took on the additional task of running six monthly marine biotoxin science workshops. These are informal forums where regulators, health protection officers, industry members, research scientists, and analytical scientists meet and discuss pertinent issues. These workshops are invaluable in directing research to aid the needs of the marine biotoxin sector and are a good example of the collaborative nature of the New Zealand Shellfish Quality Assurance Program. The Marine Biotoxin Technical Committee aims to achieve a unified approach to marine biotoxin control between industry, MAF and MoH, in New Zealand. Harmonization between the non-commercial and commercial programs with respect to lab methodology, research initiatives, opening and closing criteria and general amendments to the National Marine Biotoxin Management Plan is attempted and, on the whole, achieved.

Non-commercial Marine Biotoxin Control

Purpose: To provide information and procedures for management of shellfish toxicity arising from toxic phytoplankton in order to prevent illness through issue of public warnings to non-commercial (recreational) shellfish gatherers.

Shellfish species tested. Bivalve species are preferable to non-bivalve species. The program utilizes green-shell mussels (*Perna canaliculus*) whenever possible because this species generally indicates the presence of marine biotoxins before other species. The preferred species to be sampled are (in order): *Perna canaliculus,* blue mussels (*Mytilus edulis*), tuatua (*Paphies subtriangulata*) and pipi (*Paphies australis*). Scallops must be considered separately from other shellfish species because generally only the muscle and roe are consumed and the tissues with potentially higher toxicities (viscera and mantle) are discarded. Non-commercial scallops normally have only the muscle and roe analyzed; however, where toxin levels are rising, whole scallop samples may be analyzed as the toxin level is likely to be significantly higher in the whole animal. Results from adductor muscle and roe combined are used to close and open areas for domestic commercial as well as non-commercial harvesting.

Some non-bivalve species may be examined for the presence of marine biotoxins; e.g., paua (*Haliotis iris*) have been sampled on a few long stretches of coast where there are no bivalves and found to contain PSP and DSP toxins. While sea urchin (kina) (*Evechinus chloroticus*) sampling is not specifically included in the program, this species is covered by monitoring of bivalves and is included in the list of shellfish species that should not be consumed as a precaution when public warnings are issued. Crabs and crayfish

are not usually sampled; however, they may be analyzed if toxicity in sentinel species such as green-shell mussels is recorded. As a precaution, the Ministry of Health also issues public health warnings advising that crabs and crayfish be gutted before cooking and consumption.

Sampling frequency and location. Areas of the coastline are classified according to the occurrence of toxicity and perceived human health risk. The amount of sampling that occurs depends on the classification. In areas where testing of shellfish samples has shown persistent or recurrent biotoxins, weekly sampling of shellfish for biotoxins along with phytoplankton monitoring occurs. In areas of low risk, weekly phytoplankton samples are taken along with monthly monitoring of shellfish samples.

Non-routine sampling is undertaken during toxic events. Such sampling may be necessary to:

- Further investigate areas where toxin or toxigenic phytoplankton levels are found to be rising towards or have exceeded a regulatory limit;
- Define affected areas and species;
- Ensure that toxicity is no longer present in affected areas. This may require additional samples from other representative sites or shellfish species in the affected area. Shellfish and phytoplankton samples may need to be taken more frequently than weekly, e.g. twice weekly, from areas adjacent to a known affected area to allow early detection of spread of toxicity to these areas;
- In areas where closure periods are likely to be lengthy, the sampling may be less frequent than weekly until toxicity declines.

Sampling locations in the Public Health program are specified in District Health Board contracts. These may be varied and/or added to when events occur. In selecting or relocating marine biotoxin shellfish sampling sites, the following factors are considered:

- The history of marine biotoxin and phytoplankton activity in each area;
- Coverage of major shellfish harvesting areas;
- Common water bodies;
- Shellfish species available;
- Amount of harvesting by non-commercial harvesters;
- Open seasons for seasonal fisheries;
- Areas closed due to "rahui" (prohibited areas suggested by local aboriginal tribes for historic/cultural reasons), Ministry of Fisheries closures, marine reserves and sign-posted sewage/faecally contaminated areas;
- Accessibility in all weather conditions;
- Major current flows;
- Retention zones and circular flow patterns;
- Areas where rivers have a major impact on salinity;
- Any other factors considered relevant;

The routine monitoring sites are subject to review to meet the needs of the monitoring program.

Toxic Shellfish Poisoning. An integral component of the non-commercial marine biotoxin program is the surveillance of Toxic Shellfish Poisoning (TSP, which includes all known shellfish-related biotoxin syndromes). All suspected cases of TSP are legally notifiable and are investigated by local public health staff. Confirmed cases are defined as being those where TSP symptoms occur within relevant time frames and which are associated with toxicity in leftover shellfish sufficient to account for those symptoms.

Regulatory action. To ensure shellfish are safe to eat, the following conditions must be met:

- Consecutive samples from representative sites and species must be below the regulatory limits over the specified periods in Table 4.10.
- Once below the regulatory limit, toxin levels should be decreasing or static in consecutive samples;
- No TSP cases reported from the consumption of shellfish harvested since the date of collection of the first clearance sample from within or adjacent to the closed area;
- Analysis of other available species of shellfish from adjacent areas shows toxins below regulatory levels.

Commercial Marine Biotoxin Control

Purpose: To ensure public health control related to biotoxins produced by harmful phytoplankton in commercial shellfish harvest.

TABLE 4.10. Conditions required for determination of safe consumption of non-commercial shellfish in New Zealand.

Toxin	Regulatory Limit	Number of consecutive samples	Period (days)
PSP	80 μg 100g^{-1}	3	14
NSP	20 MU 100g^{-1}	2	2
ASP	20 ppm	3	14
DSP	20 μg 100g^{-1}	2	7

Shellfish species tested. Species tested in the commercial program depend on what product industry is targeting for export. Generally this includes green-shell mussels (*Perna canaliculus*), dredge oysters (*Tiostrea lutaria*), rock oysters (*Saccostrea glomerata*), Pacific oysters (*Crassostrea gigas*), cockles (*Austrovenus stuchburyi*), pipis (*Paphies australis*), scallops (*Pecten novaezelandiae*) and queen scallops (*Chlamys delicatula*). Testing generally occurs weekly but is detailed in the local marine biotoxin management plan and determined by the authorized health officer.

Local Marine Biotoxin Management Plans. The local marine biotoxin management plan will be developed by the authorized health officer in consultation with the shellfish industry and the inspector. An authorized health officer is a person employed by the district health board who is a health protection officer or has other acceptable public health expertise. The local plan is developed so that it adheres to the National Marine Biotoxin Management Plan managed by MAF. In addition to the requirements for local marine biotoxin management plans listed earlier, the local plan is now also required to include a map of the growing area(s) which shows:

- Boundaries of the classified growing areas identified by their number;
- The species and location of commercial shellfish;
- The location of the shellfish and phytoplankton sampling sites;
- The predominant current flow(s).

In addition to these requirements, the management plan for scallops, apart from whole scallops, will address the following:

- The sampling management plan at the commencement of the scallop season;
- The procedures to be followed as vessels move from one scallop bed to another;
- The parts of the scallop to be analyzed for biotoxins and the intended markets;
- Scallop samples from commercial areas are analyzed in two fractions. Results from adductor muscle and roe combined are used to close and open areas for export (excluding the EU) and domestic commercial as well as non-commercial harvesting. The roe is also analyzed separately and results are used to control export certification to the EU.

Regulatory action/re-opening requirements. In re-opening an area after a marine biotoxin event has occurred an authorized health officer should determine that all the requirements stated below have been addressed:

- All growing areas in the area to be opened shall have been adequately covered by the reopening sampling plan;
- The types of shellfish sampled from the area shall be representative of those species normally harvested from the area;
- Once below the regulatory limit, toxin levels shall be decreasing or static in consecutive clearance samples in order for the area to be re-opened;
- No cases of human illness, notified to a Medical Officer of Health and consistent with the accepted case definitions for PSP, NSP, ASP or DSP shall have resulted from the consumption of shellfish harvested since the date of collection of the first clearance sample from within or adjacent to the closed area;
- Toxin levels shall be decreasing or static in adjacent areas;
- The cell counts of toxigenic phytoplankton identified shall be decreasing or static;
- The information available shall be sufficient to make an informed and reasoned food safety decision;
- Results from samples of the edible portion of shellfish flesh from representative sites/species in the closed area shall meet the same sampling criteria as for non-commercial species (Table 4.8).

Toxin analysis (commercial and non-commercial): All shellfish samples to be analyzed for the presence of marine biotoxins must comprise a minimum of 12 individuals and the sample must weigh a minimum of 400 g of edible flesh. Shellfish samples should arrive at the testing laboratory within 24 hrs of collection, alive, and in good condition. Wherever possible the samples should not be frozen. Commercial scallop samples must be split into two portions: 1) for analysis of roe alone (400 g), and 2) for analysis of adductor and roe combined (400 g).

Testing for NSP, PSP, DSP and ASP is carried out at several laboratories within New Zealand which are accredited by MAF and IANZ International Accreditation New Zealand, the national authority for the accreditation of laboratories, inspection bodies and other technical, competence-based activities. The laboratories are annually evaluated by a MAF laboratory evaluation officer (LEO), who is certified by the FDA, and receive written approval to allow them to test for marine biotoxins for the New Zealand Shellfish Quality Assurance Program. Table 4.11 summarizes the methods used for marine biotoxin detection in New Zealand by the regulatory laboratories.

TABLE 4.11. Methods used for HAB toxin detection in New Zealand.

Toxin	Method
PSP	3 mouse bioassay
ASP	HPLC (for DA)
NSP/DSP (screen test)	Acetone extraction, 3 mouse bioassay
NSP (confirmation)	Ether extraction, 5 mouse bioassay
DSP (confirmation)	• DSP-Check ELISA or Protein phosphatase inhibition (DTX-3, OA) • LC-MS (DTX-1 and OA) • LC-MS or HPLC (YTX) • LC-MS (PTX)

Analysis for PSP is carried out by mouse bioassay according to Irwin (1970). Shellfish are examined for domoic acid by HPLC following the extraction procedures described in Lawrence and Menard (1991) and the HPLC procedure described in Wright et al. (1989). Shellfish are screened for the presence of DSP and NSP toxins by a modification of the Yasumoto mouse bioassay method (Yasumoto et al. 1978). When 2 or more mouse deaths occur in the DSP/NSP screen test within 24 hrs, DSP confirmatory tests are required. When any mouse death occurs in less than six hrs, NSP is confirmed by ether extraction and five-mouse bioassay by the procedures described in Irwin (1970). Until 2001 the DSP check used in New Zealand was the DSP-CHECK ELISA kit. The DSP/NSP screen test regime was developed in 1993/94 before DTX-3, OA diol ester, pectenotoxin and yessotoxin were detected in New Zealand shellfish. While the screen test is still appropriate for screening of the DSP and NSP groups and the APHA ether test method is still appropriate for confirming NSP toxins, the DSP CHECK ELISA is no longer appropriate as the only confirmatory test for DSP toxins (which here include OA, OA diol ester, all DTXs, all YTXs, and all PTXs). When an anomaly occurs between the DSP/NSP screen test and the confirmatory DSP CHECK ELISA/APHA ether test, then the following analyses are conducted to identify or confirm detectable, DTX-3, OA diol ester, PTXs and YTXs:

- Hydrolysis of the sample, then analysis by the DSP CHECK ELISA, Protein Phosphatase Inhibition Assay or LC-MS. This addresses the DTX-3, DTX-1, OA and OA diol ester toxins;
- LC-MS or HPLC for YTXs
- LC-MS for PTXs

Validation work has now been undertaken for the detection of OA and DTX-1 by LC-MS, eliminating the need for the DSP CHECK ELISA. The LC-MS detection method of OA and DTX-1 is based on the methodology developed by Goto et al. (2001). The HPLC detection of YTXs is based on methods of Yasumoto and Takizawa (1997). Validation work for the analysis of PTXs and YTXs by LC-MS is ongoing.

There are 55 commercial and 85 non-commercial shellfish sampling sites from which samples were collected for marine biotoxin analyses during the period June 30th 1999 – 1 July 2000. Table 4.12 shows the number and type of analyses carried out for samples obtained from non-commercial and commercial sites during this period.

TABLE 4.12. Number of shellfish samples tested for toxicity in New Zealand for 1999-2000.

Test Method	Non-commercial samples	Commercial samples
DSP/NSP Screen	664	1248
ASP - HPLC	829	869
PSP mouse bioassay	947	837
DSP ELISA CHECK	92	106
NSP Ether mouse bioassay	21	56

Analytical methods used by the marine biotoxin laboratories must be approved for use by the Marine Biotoxin Technical Committee and MAF. Laboratories performing marine biotoxin analyses are evaluated by a USDA-certified New Zealand Laboratory Evaluation Officer annually and receive written approval from MAF prior to performing these analyses.

A guide to the validation of new test methods has been written by MAF to aid in the validation of new marine biotoxin analyses. The guide was the product of a collaborative effort between researchers, analysts, regulators and industry. It describes the process that must be followed by development teams seeking approval to use new test methods, and has been developed in accordance with several internationally accepted validation guides [e.g. Eurachem Guide (1998), ISO guide 17025, the draft ISSC Process for Acceptance and Approval of Analytical Methods for the NSSP and Joint FAO/IAEA Expert Consultation (1999) Guidelines for single laboratory validation of analytical methods for trace-level concentrations of organic chemicals]. Currently there is a big drive within New Zealand to introduce LC-MS technologies into the shellfish quality assurance program. Research on the development of alternative methods for toxin detection has been driven by: 1) the need to satisfy an increasing demand for elimination of live animal bioassays, 2) the discovery of novel toxins which require development and rigorous testing of new methods for monitoring and regulatory purposes, and 3) the potential of using a single method for all relevant toxins.

Phytoplankton Monitoring. In 1995, phytoplankton monitoring was initiated in a few areas where biotoxin activity had occurred. By 1997, phytoplankto monitoring was extended to many more areas as a part of the routine biotoxin monitoring efforts. Trained personnel carry out phytoplankton sampling at commercial and non-commercial sites. The Cawthron Institute conducts training and monitoring of compliance by the staff. Quantitative samples are taken either with a hose sampler or a van Dorn bottle. Qualitative samples are taken by vertical towing of a plankton net (20 μm mesh size), but these are rarely used for monitoring purposes. Samples packed in polystyrene cooler or cardboard boxes filled with newspaper must be delivered to the laboratory within 1-3 days. For each site, a live as well as a sample preserved in Lugol's Solution is required. A sample submission sheet should be included with each sample. Phytoplankton monitoring sites spread throughout the country have been established in collaboration with scientists. Selected sites must be:

- Representative of common water bodies and located at points where blooms are likely to persist;
- Located as close as possible to a current flesh testing site;
- Accessible in most weather conditions;
- Relatively free of land runoff;
- Clear of the surf zone.

During the period June 30th 1999 – 1 July 2000 there were approximately 64 commercial and non-commercial phytoplankton sites where routine weekly samples were taken. There are 33 non-commercial

phytoplankton sites from which weekly samples are collected at present. All proven toxin producing species and possible toxin producing species present in New Zealand coastal waters, as well as proven toxin producing species that occur worldwide, are detected in this monitoring program. A list of such species is contained in both the National Marine Biotoxin Management Plan and the Food Administration Manual. Phytoplankton samples are collected and analyzed weekly. If toxic species are present in numbers above the appropriate trigger level, then flesh testing of shellfish will occur. When samples are received, they are pooled for analysis. When the trigger levels in Table 4.13 are exceeded in these composites, discrete depth samples are analyzed to enable a targeted approach to sampling of shellfish. The following levels have been determined in consultation with scientists. The detection level of toxic phytoplankton in water samples is 100 cells L^{-1}.

The Marine Biotoxin Technical Committee agreed that there should be a single system for use of phytoplankton results that are used to trigger sampling of shellfish flesh for commercial and non-commercial marine biotoxin programs. Phytoplankton monitoring is used as an early warning indicator of toxicity appearing in shellfish. Any detection of toxic *Alexandrium* spp. or *Gymnodinium catenatum* is considered a high hazard and will trigger shellfish sampling for PSP. *Gymnodinium cf breve* commonly occurs at low levels but the risk of toxicity is only likely at higher concentrations, so the trigger level is higher for this species. The shellfish industry is notified when phytoplankton in commercial areas reach levels specified in the third column of Table 4.13 as voluntary closures may be made. When toxic shellfish events are occurring, routine weekly phytoplankton sampling should continue as this can give an indication of the progress of the event.

TABLE 4.13. New Zealand National Marine Biotoxin Plan – phytoplankton action levels.

Phytoplankton species	Toxin	Concentration in composite sample used to trigger testing of shellfish flesh (cells L^{-1})	Industry voluntary closure level (cells L^{-1})	Concentration which triggers public health warnings (cells L^{-1})
Alexandrium minutum	PSP	100	500	5,000
Alexandrium ostenfeldii	PSP	100	500	5,000
Alexandrium catenella	PSP	100	500	5,000
Alexandrium tamarense	PSP	100	500	5,000
Pseudo-nitzschia spp. (>50% of total phytoplankton)	ASP	50,000	200,000	N/A
Pseudo-nitzschia spp. (<50% of total phytoplankton)	ASP	100,000	500,000	N/A
Gymnodinium cf breve	NSP	1,000	5,000	5,000
Prorocentrum lima	DSP	500	1,000	N/A
Dinophysis acuminata	DSP	1,000	2,000	N/A
Dinophysis acuta	DSP	500	1,000	N/A
Gymnodinium catenatum	PSP	100	N/A	N/A

In the non-commercial program, only gene probe technology is used to differentiate *Pseudo-nitszchia* spp., which are ubiquitous in New Zealand. This genus is of particular concern to the scallop industry. The gene probe can rapidly identify toxic species of *Pseudo-nitszchia* and determine their cell densities in the water column. It has been developed by researchers in New Zealand and is used by the Ministry of Health to trigger flesh testing. This method is likely to be incorporated into the commercial program when full validation work has occurred. Gene probes are also being developed in New Zealand for many other species, in particular *Gymnodinium* spp. and other NSP-producing species.

Regulatory Action/Management. A public health warning is issued and commercial harvest in an area is closed when the toxin levels in shellfish exceed the following regulatory limits: PSP: 80 μg 100g^{-1}; NSP: 20 MU 100g^{-1}; ASP: 20 ppm; and DSP: 20 μg 100g^{-1}. Furthermore, a public health warning is issued if:

- Levels of toxic *Alexandrium and G. breve* species exceed 5000 cells L^{-1} (Table 4.13).
- Two or more cases of human illness, reported by a medical officer of health, have resulted from consumption of shellfish from an area, and are consistent with the clinical case definitions for PSP, NSP, ASP or DSP.

Furthermore, closures of commercial areas will occur when:

- The local marine biotoxin management plan has not been adhered to;
- If any marine biotoxin-producing organism is determined to be potentially hazardous to health for which criteria, either cell counts in the water column or biotoxin meat concentrations have not been established;
- Where cell counts of toxic phytoplankton may produce a level of toxin that will cause a health risk industry may close an area voluntarily based on phytoplankton results alone (Table 4.13).

Generally areas subject to public health warnings should extend to the next sample site below the regulatory limit unless there are geographical, hydrographical or historical reasons for closing smaller or larger areas. If there is enough data to exempt certain shellfish species from a closure the closed status may be applied to a particular species.

Dissemination of information. Results of shellfish toxicity above the regulatory limit are immediately communicated by the laboratory to the Health Protection Officer or Authorized Health Officer by phone, followed within an hour by fax notification. The Health Protection Officer/ Authorized Health Officer is responsible for communicating results to the relevant agencies, including the local shellfish quality assurance program delivery center in accordance with the local plans. Results are also entered into a central database (Food Net) held by the Ministry of Health. The SQAPDC will coordinate the procedures for detention and recall of harvested product and closure procedures if this is required. Phytoplankton results are also reported to the Health Protection Officer/Authorized Health Officer and the SQAPDC within 24 hrs of receipt of the sample. Results at or above the trigger levels are reported immediately by phone. The notification is confirmed by fax within an hour.

Future needs: The development of "animal friendly", rapid and cost-effective toxin analysis methods has been identified as a high-priority need for the future, given the number of toxins identified in the region. Other identified needs include information on the toxicity of yessotoxins and pectenotoxins which have both been detected in New Zealand shellfish, provision of real-time information on toxin-producing phytoplankton species, and improved characterization of the number, distance between sampling locations and water column depth of samples in mussel growing areas.

4.3.6 France

France has a long tradition of shellfish mariculture. The estimated annual production of cultured shellfish is 200,000 – 250,000 tons, produced by ~ 140 shellfish farmers. The French HAB monitoring program was initiated after extensive blooms of *Dinophysis* which led to poisoning of shellfish consumers during the summers of 1983 and 1984. The present HAB monitoring program includes monitoring of phytoplankton and phycotoxins in shellfish and is carried out by the French Phytoplankton Monitoring Network (REPHY; Figure 4.17). REPHY is a national program covering the entire French coast, although additional monitoring is performed by universities for research purposes. The institution responsible for REPHY is IFREMER (Institut Français pour la Recherche et l'Exploitation de la Mer). Mitigation measures/action plans to reduce acute problems are a part of the program.

The REPHY monitoring strategy is primarily based upon detection of toxic algal species in water, which subsequently determines the initiation of toxicity tests in shellfish. This strategy avoids a permanent monitoring program for toxins in shellfish. However, management actions and decisions concerning the harvest of shellfish are based upon the toxicity level in shellfish only, and not upon the concentration of toxic algal species.

In practice, intensive monitoring is performed in periods and areas which are known by experience to be affected by blooms of toxic algal species. That is, some areas are monitored intensively throughout most of the year while others are only monitored intensively during shorter periods of the year.

In France, most toxic episodes are DSP related, but some PSP episodes as well as fish kills caused by other HABs are also observed. Toxic and potential toxic algae registered in French waters are listed in Table 4.14. The main areas affected by DSP are the coastal waters of Normandy, south Brittany (since 1988), the Atlantic coast and the west coast of the Mediterranean Sea. During DSP episodes, occurrences of toxic species of the genus Dinophysis were observed. Okadaic acid is the major DSP-toxin. Since 1988 PSP-toxicity in shellfish has been observed in northern Brittany. The species responsible for the toxicity was the dinoflagellate *Alexandrium minutum*. The maximum toxicity detected to date in mussels and oysters was 400 µg STX eq. $100g^{-1}$ meat. *Gymnodinium nagasakiense* caused scallop mortality and/or growth inhibition from 1976 to 1987 in Western Brittany. During the summer of 1995, a very extensive bloom of this species led to considerable marine animal kills, including fish, shellfish, worms, urchins, etc. along the Southern Brittany coast. Another species of *Gymnodinium* was responsible for fish mortality in 1993 on the coast of Corsica. A bloom of *Heterosigma akashiwo* was also responsible for fish mortality in Western Brittany in 1994. In late 1992 and 1993 unknown toxins (neither DSP nor PSP toxins) were found in mussels from the Atlantic Coast without any registered occurrence of toxic algae in the water.

The French Phytoplankton Monitoring Program has recently been discussed by Belin and Berthome (1991) and by Belin (1993).

Purpose/Objectives. REPHY has three complementary objectives:

- acquisition of information on French coast phytoplankton populations, discolored waters and exceptional blooms
- human health protection, through monitoring of species producing toxins which accumulate in shellfish
- marine animal health protection, through monitoring of species toxic to fish and shellfish.
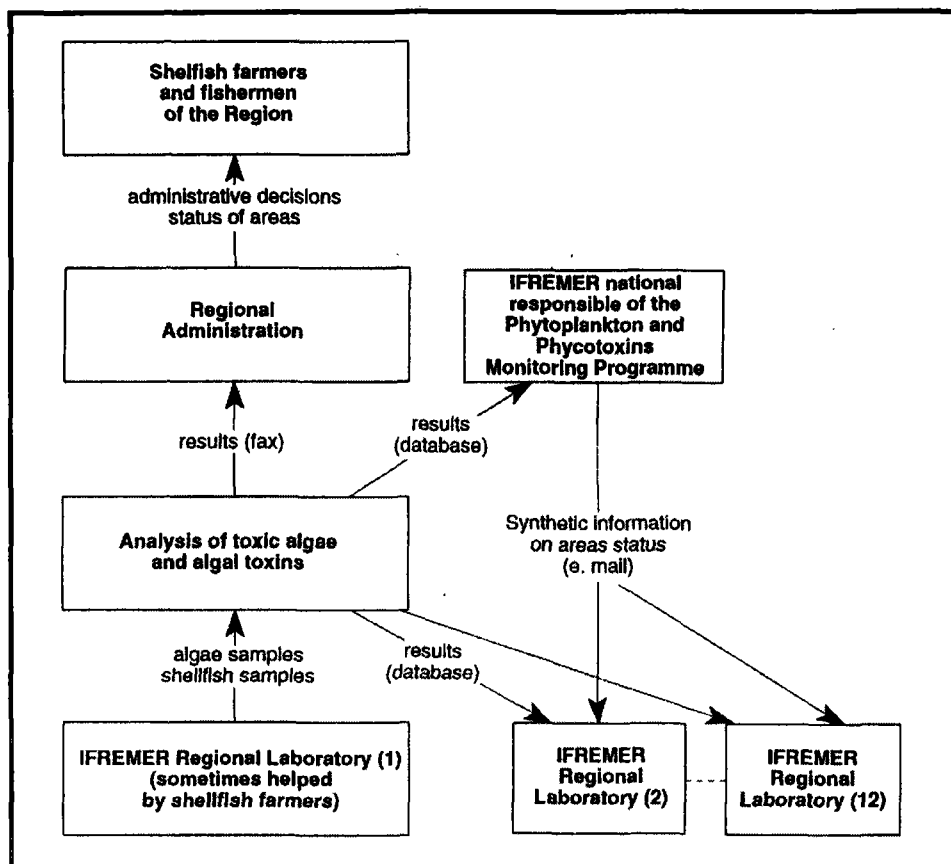
FIGURE 4.17. Network used for shellfish poisoning monitoring in French coastal waters. (Source: Andersen 1996.)

TABLE 4.14. Toxic and potential toxic algae recorded in French waters.

DSP	PSP	Other toxins
Dinophysis cf. sacculus	*Alexandrium*	*Gymnodinium cf. nagasakiense = G. cf. mikimotoi =*
Dinophysis cf. acuminata	*minutum*	*Gyrodinium aureolum*
Dinophysis cf. norvegica	*Alexandrium*	*Gymnodinium sp.*
Dinophysis caudata	*tamarense*	*Prorocentrum minimum**
Dinophysis tripos	*Alexandrium*	*Heterosigma akashiwo*
Dinophysis rotundata	*catenella*	*Dictyocha speculum*
Dinophysis spp.		*Pseudo-nitzschia pseudodelicatissima**
Prorocentrum lima		

*Species which are present in French waters but which have not resulted in accumulation of toxins in mussels, even though they are sometimes present in high concentrations.

Principal shellfish species tested: Different species of mussels, clams, cockles and oysters are the major shellfish produced and analyzed for presence of algal toxins in France.

Sample collection and analysis: Twelve coastal laboratories perform sampling, observations, analysis and data acquisition from more than 100 sampling stations distributed along the entire French coast (Figure 4.18). The qualitative and quantitative analysis of phytoplankton covers the total phytoplankton community, including harmful and potentially harmful phytoplankton species. Samples from a few stations are analyzed twice each month, simultaneously with measurements of physical-chemical parameters and chlorophyll. Other stations are also sampled throughout the year, but the samples are only analyzed for the occurrence of harmful and potentially harmful species. On the remaining stations, water and shellfish are sampled when harmful species are detected in the area in general.

Toxin analysis: Algal toxins: The analysis of algal toxins in shellfish in France is carried out using well established methods. ASP is analyzed using HPLC, whereas PSP and DSP toxins are analyzed using mouse bioassay. Information about the methodology used for detection of algal toxins in shellfish is compiled in Table 4.15.

Phytoplankton analysis: Toxic algae: water samples are collected from surface water and fixed using Lugol's. The quantitative investigation of the algae samples (10-25 ml) is carried out using inverted microscopy, according to Utermöhl (1958).

Regulatory action/management: The regulatory action for shellfish harvesting is, as mentioned earlier, based upon the results of the analysis of algal toxins in the shellfish following the action limits summarized in Table 4.15. The regulation of the shellfish harvest is carried out by the local administration in the different regions. All data from the coastal laboratories are entered and stored in a national database. The data can be consulted and extracted in real time. Data are stored on a national server in the SYBASE database, together with all other results from IFREMER monitoring programs on coastal waters (hydrography, chemistry, bacteriology and biology). Data acquisition and updating are performed by a number of laboratories, through the TCPIP network, on 'client' PC's using WINDOWS. Complementary software can be used for statistical analysis of the results as well as cartographic presentation.

Results of shellfish toxicity are disseminated to the local fisheries administration by fax, which takes official measures to prohibit the marketing of shellfish from the incriminated sector. The other concerned administrations (health, veterinary, etc.) and the local and regional media are informed. The public is informed by radio, television and newspapers and/or notice boards.

Costs and benefits: Approximately 50 people are involved in the monitoring program on a part-time basis, which is equivalent to 16 full-time persons a year. The total cost of running REPHY is approximately US $1,200,000. This estimated annual budget includes the personnel cost which is about $800,000.

TABLE 4.15. Methods used for detection of algal toxins as well as the action limits of toxins and the resulting action.

Toxin Type	Method	Action limit	Regulation status
ASP	HPLC	2 mg 100 g^{-1}	Closed
DSP	mouse test	2 or 3 mice dead before 5 hours (equivalent to between 2 and 4 µg OA per g. of digestive gland)	Closed
PSP	mouse test	80 µg STXeq 100 g^{-1}	Closed
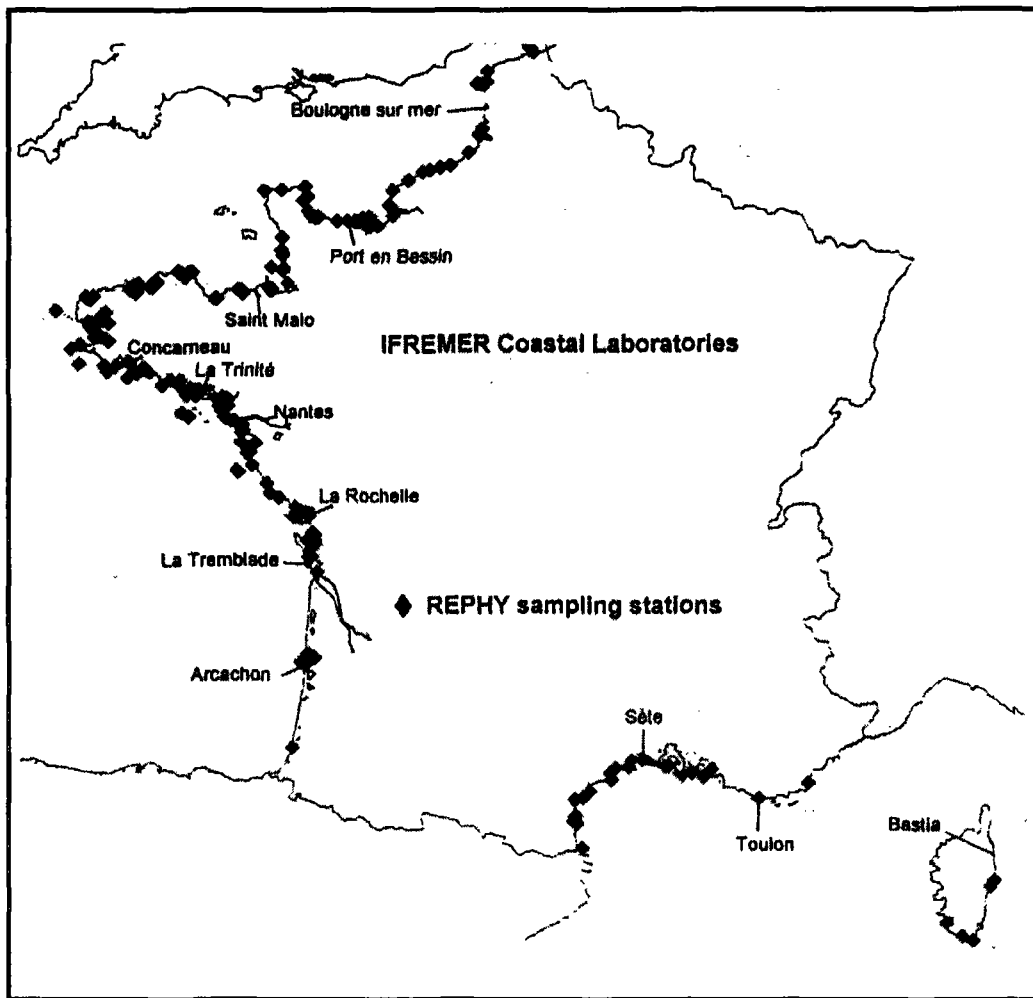
FIGURE 4.18. Location of IFREMER laboratories and REPHY sampling stations. (Source Andersen 1996.)

4.3.7 Control of Imported and Exported Seafood Products

This section illustrates procedures implemented to ensure import/export of shellfish that are safe for human consumption. Several generalizations can be made about importation procedures in North America and the EU (detailed below):

- The exporting nation is responsible for determining the safety of the shellfish being exported.
- Tagging is required for identification of the source of origin (growing waters) of imported product. This allows tracking of contaminated product;
- Although health certification of product by a competent authority in the country of origin is required, spot-testing of product is also implemented by the receiving country in order to verify compliance with its sanitation requirements;

- Conditions and requirements of the receiving country cover all steps, from harvesting to market delivery, including transport, storage/holding, depuration (for shellfish) and processing (e.g. cooking, freezing) and packaging, of fishery product.

4.3.7.1   US: the NSSP/ISSC program

In the US the Food and Drug Administration (FDA) is charged with the responsibility of assuring that all food items shipped in interstate commerce are safe. The US National Shellfish Sanitation Program (NSSP) is a cooperative program between the federal government and individual states, established in 1925 and recognized by the US FDA and the Interstate Shellfish Sanitation Conference (ISSC), for the sanitary control of shellfish produced and sold for human consumption. Its purpose is to promote and improve the sanitation of shellfish moving in interstate commerce and the uniformity of state shellfish programs. In the context of this report only aspects relating to shellfish biotoxins will be described, although the program is broader in scope and includes control of bacterial pathogens, anthropogenic contaminants, etc. The NSSP requires that state laws and regulations provide an adequate legal basis for the sanitary control of all interstate phases of the shellfish industry. It recognizes that immediate emergency action to halt harvesting and processing of shellfish requires proper legal authority, and should not be hampered by lengthy administrative procedures.

The ISSC, formed in 1982, includes as members the FDA, the shellfish industry, control agencies from shellfish producing and receiving states, the National Marine Fisheries Service (NMFS, NOAA, Dept. of Commerce), the US Environmental Protection Agency (EPA) and the academic community, all as voluntary participants. A non-voting representative from each of three task forces within ISSC (growing areas, processing and distribution, and administration) is included in the ISSC Executive Board. A memorandum of understanding (MOU) established in 1984 between FDA and ISSC recognizes the ISSC as the primary national organization of state regulatory officials that provides guidance on matters related to shellfish sanitation, including harvesting, processing and shipping of fresh and frozen shellfish (oysters, clams, mussels, whole or roe-on scallops). Scallops are specifically excluded when the final product is the shucked adductor muscle only. The ISSC, which holds an annual meeting, also provides a forum for participants to resolve major issues concerning sanitation, and disseminates information to interested parties via news media, publications, regional and national meetings, and the internet.

The US Hazard Analysis Critical Point Control Program (HACCP) is a seafood safety program estabished by the US FDA in 1995, which focuses on preventive rather than reactive measures. The US Department of Agriculture has established a parallel HACCP for the meat and poultry industry. HACCP establishes the requirement for shellfish dealers to implement a written HACCP Plan, which includes: a) a list of most likely hazards associated with the product (including natural toxins), b) identification of critical control points where potential hazards can be controlled or eliminated in all phases from harvest to delivery of product to the market, c) establishment of preventive measures and a list of critical limits which must be met at each critical point, d) procedures to monitor the critical control points, e) corrective action plans to ensure product safety at each of these critical stages, f) verification procedures to ensure that the HACCP is effectively implemented and adequate to ensure food safety, and g) a description of a record-keeping system to document the HACCP system. HACCP regulations apply to both live and processed shellfish.

Under international agreement with the US FDA, foreign governments also participate in the NSSP. Therefore, any country/economy that wishes to export shellfish product into the US must comply with NSSP/ISSC guidelines, and an MOU between FDA and the national agency responsible for shellfish safety is required. The MOUs may restrict harvest areas and selected shellfish species. Importers are required to verify that their overseas suppliers follow HACCP. At present, modifications are being considered that would allow foreign counterparts with well established shellfish sanitation programs

differing in some respects with that established in the US (e.g. EU countries), to achieve equivalency status through agreement with the FDA. A list of shellfish dealers/shippers, domestic and foreign, who have been certified by state and foreign authority as meeting the public health control measures specified by the Ordinance, is published monthly by FDA (Interstate Certified Shellfish Shippers List, September 1, 1998). Shippers are defined as anyone who grows, harvests, buys, or repacks and sells shellfish. Certification is for one year, and repeated non-compliance results in removal from this list. Compliance is verified by lot testing of shipped product by US federal or state laboratories. Currently, the following foreign countries/regions have MOUs with FDA and are included in the certification list: Canada (BC, NB, PEI, Newfoundland, Quebec), Chile, Korea, Mexico and New Zealand.

The NSSP Manual, developed through the ISSC, consists of a Model Ordinance and supporting documents (ISSC 1997). The Ordinance provides readily adoptable standards and administrative practices necessary for sanitary control of molluscan shellfish. The NSSP/ISSC guidelines establish a requirement for monitoring and certification of shellfish growing waters, licensing of commercial harvesters of wild and cultured product, and certification of shellfish dealers. Harvesters are allowed to sell only to dealers listed in the Interstate Certified Shellfish Shippers List. Safety of harvested product is thus achieved by monitoring at its source of origin rather than upon arrival at the market. Based on monitoring results, growing areas are classified as approved, restricted, conditionally approved or prohibited. Areas classified as restricted can only be harvested by special license. Conditionally approved areas experience intermittent, predictable contamination events. Prohibited status requires closure of shellfish harvesting waters, and is applied if conditions are such that they pose a public health risk, as follows:

   a) the concentration of PSP toxins in raw edible tissues is > 80 µg STXeq 100g^{-1};
   b) any NSP toxin is detected in shellfish meats and/or counts of *Gymnodinium breve* > 5000 cells L^{-1};
   c) the concentration of domoic acid is > 20 ppm in the edible portion of raw shellfish

Closed status may be applied selectively to some shellfish and not others occurring in the same growing area. Each state or shellfish control agency within the country which has signed an MOU, must establish a marine biotoxin contingency plan which defines administrative procedures, laboratory support, monitoring strategies and patrol procedures needed to provide public health control. All laboratory toxin analyses must be performed by a laboratory that is found to conform or provisionally conform by FDA with NSSP requirements. Methods of biotoxin analysis must be the current AOAC and APHA methods for PSP and NSP toxins respectively. For any biotoxin-producing organisms for which criteria have not been established under the NSSP Ordinance, either cell concentrations in the water column or biotoxin concentrations in tissues may be used as criteria for closure [see item b) above]. The presence of biotoxins in shellfish requires investigation on the harvesting, distribution and processing of shellfish and corrective action by state authorities. Harvesters are required to tag each shellfish container, such that the tag is in place while shellfish are being transported to the dealer. Both a harvester's and dealer's tag must be provided, unless the harvester is also the dealer. Thus, tagging allows tracing of the harvest product to its dealer and the harvesting area of origin, when a health problem occurs after the product is placed in the market.

Export of shellfish product to EU countries requires that the dealer/nation of origin meet EU sanitation requirements and be issued a Health Certificate from a competent authority, e.g. the FDA in the US (Shumway et al. 1995). If dealers are in compliance with NSSP regulations, they will meet the EU requirements. There is no evidence as yet of the occurrence of DSP cases in the US. The FDA cannot certify that a product is DSP-free because there is, at present, no FDA approved method for DSP. However, documented absence of DSP-producing phytoplankton by the dealer may be sufficient to meet EU importation requirements (Shumway et al. 1995).

4.3.7.2 Canadian import program

Canada adopted the National Shellfish Sanitation Program (NSSP) in 1948, and developed the Canadian Shellfish Sanitation Program (CSSP), whose main aim is to ensure that all bivalve molluscan shellfish growing areas meet approved federal water quality criteria, that pollution sources are identified and that all shellfish sold commercially are harvested, transported and processed in an approved manner. Prior to April 1997, the departments of Fisheries and Oceans (DFO) and Environment Canada (EC) were in charge of administering the CSSP. The Fish Inspection Directorate of the Canadian Food Inspection Agency (CFIA) is presently the agency responsible for administration of the CSSP with respect to the control and inspection of exported and imported molluscan shellfish to ensure their safety with respect to biotoxins and other contaminants. Legal authority is provided under the Fish Inspection Act and Regulations. An MOU is currently being revised to define the specific roles of the three organizations (CFIA, DFO and EU) with respect to the CSSP. Importers are required to obtain an import license issued by the CFIA and in order to do so, they must comply with requirements established by a Quality Management Program for Importers (QMPI). Compliance is verified by the CFIA, which performs standard analyses at pre-established frequencies. Importers may choose to obtain a Shared or Enhanced QMPI Importer status, which allows them to demonstrate compliance by suppliers through their own testing program (reviewed and approved by the CFIA), but still requires them to submit to product testing in Canada at a minimum frequency of 15%. Biotoxins in imported product must not exceed Canadian tolerance levels.

Importers are required to notify the CFIA prior to or within 48 hrs of importation, and shipments are detained pending the results of inspection and can only be released for sale with consent from the CFIA. If entry of shipments in Canada is denied, the importer is notified and, subject to the appeal process provided by the Fish Inspection Regulations, the shipment must be destroyed or removed from Canada within 45 days of notification. Fresh and frozen bivalve molluscs are not permitted entry from any country except those which have sanitary control programs that have been approved by the CFIA. Product labeling requirements are specified. In cases of violations, the CFIA may take enforcement actions, which include seizure of product and license sanctions. Other seafood product (including fish, crustaceans and other marine animals or parts) also fall into CFIA's jurisdiction. Entry prohibitions are specified for some species, such as puffer fish of the family Tetraodontidae (due to the risk of tetrodotoxin).

4.3.7.3 The European Economic Community (EEC)

Council Directive 91/268/EEC establishes uniform conditions for the production, depuration and transfer to the market of live bivalve molluscs intended for human consumption, thus ensuring movement of safe product among member nations. The provisions also apply to gastropods. Bivalves must originate from production areas, which comply with EU requirements. Therefore, as in North America, control is initiated at the growout area from which shellfish were harvested. One of the main differences with procedures implemented in North America is that in the EU, live bivalves for human consumption can only be placed in the market after purification treatment, which is intended for depuration of bacterial pathogens. All consignments entering the EU must be accompanied by a Health Certificate that identifies the production areas of origin and any depuration treatment conducted after arrival to the EU. Conditions are also described for transport from the growout area to the dealer, purification plant, relaying area and processing plant. Harvesting techniques must not cause excessive damage of shells, and animals must not be exposed to extreme temperatures after harvesting, or re-immersed in water which could cause additional contamination. A registration document must identify bivalves during transport and distribution until retail sale; i.e. identify the harvester, the date of harvesting and the location of the production area. The establishment of dispatch (distribution) must also be identified. Detailed conditions are also specified for treatment of bivalves in the purification/depuration plant and packaging. Experts appointed by the

Commission are allowed, in cooperation with competent authorities of the importing EU nation, to carry out spot-checks as required to verify compliance with EU requirements.

A reference laboratory must be designated in each EU member nation to coordinate the analysis of biotoxins, and a EU central reference laboratory (the "Ministerio de Sanidad y Consumo" laboratory in Vigo, Spain) is responsible for coordinating biotoxin monitoring carried out by the individual national laboratories (Directive 1993/166/EEC).

Provisions applied to imports of live bivalves from other, non-EU nations, should be at least equivalent to those governing EU products. Inspection of product to verify equivalence is carried out by experts appointed by the Commission from member nations. In the establishment of equivalency of third world countries with respect to the EU, consideration is given to a) the legislation in that country, b) the organization of the competent authority and of its inspection services, as well as the ability to monitor the implementation of legislation, c) health conditions during production and transfer to the market and the biotoxin monitoring programs of these production areas, d) the regularity and rapidity of the information provided by the third country on the presence of toxic plankton in production areas, e) the assurance provided on the compliance with EU standards.

Consignments of live bivalves imported into the EU from a third country must be tagged to identify the country of dispatch, the species of bivalve mollusc (both common name and scientific name), and identify the authorized dispatch center. Detailed tagging specifications are provided (91/268/EEC Annex). Live molluscs must be harvested from production areas approved and inspected by a competent authority in the third country, originate from an approved establishment and accompanied by a Health Certificate (Annex 1), as required for EU member nations. This Certificate must be drawn up in at least one of the official languages of the country of entry, and, if necessary, in one of the languages of the country of destination. Comparable procedures to the ones described above for bivalves are laid out for the importation of fish product (Directive 91/493/EEC). A list of third-world countries, which have been approved for export of molluscs to the EU is, published (97/20/EC). Conditions are specified for holding of fish in vessels and freezing of product at processing plants. Certain products are prohibited from placement in the market: poisonous fish of the families Tetraodontidae, Molidae, Diodontidae and Canthigasteridae, and fishery products containing biotoxins such as ciguatera toxins.

4.4 Monitoring for *Pfiesteria*-like Organisms

Several US mid-Atlantic and south-Atlantic coastal states maintain monitoring programs for *Pfiesteria*-like organisms. In an effort to coordinate these activities, a workshop was held and a report written (NOAA 2000) in which participants proposed a suite of standard parameters for monitoring conditions at sites at risk from toxic strains of *Pfiesteria*. Details of these protocols are given in the report (available at website: **http://www.redtide.whoi.edu/pfiesteria/NOAAworkshops/Pfiesteria_Monitoring_Rept.pdf**.

There are three elements to these monitoring programs: 1) monitoring conducted during fish kill or lesion events (rapid event response), 2) monitoring in areas known or considered at risk for toxic *Pfiesteria* outbreaks (comprehensive surveys and assessments), and 3) monitoring of areas that could support toxic *Pfiesteria* strains (routine monitoring). For each of these elements, sampling protocols for water quality, fish health, and phytoplankton composition are recommended. The level of detail in the report is too great to be presented here, but a brief summary follows. Routine water quality monitoring is conducted to measure physical and chemical parameters, including temperature, salinity, pH, dissolved oxygen, dissolved ammonia, and chlorophyll *a*. (During a rapid response event, additional measurements are taken, including DON, DOC, and DOP.) Fish populations are sampled with cast nets to capture ten to twenty 1-2 inch fish. Lesion and other abnormalities are noted, and necropsies performed on subsamples.

Integrated water column samples are taken in duplicate and examined for phytoplankton community composition. Scanning electron microscopy is used to confirm species identification. During a fish lesion or fish kill event, water column samples are taken to conduct presumptive counts of *Pfiesteria*-like organisms. Protective clothing and respirators should be used when responding to a potential ongoing toxic *Pfiesteria* outbreak. It is also emphasized that toxicity be verified using fish bioassays because that is the only technique at present that can be used to verify the presence of actively toxic strains of *Pfiesteria*-like species.

4.5   HAB Impacts on Beaches and Recreational Waters

HABs can have impacts that go far beyond the more obvious effects of shellfish poisonings or fish and shellfish mortalities. One important category involves changes in the aesthetics of the coastal environment. Included in this category are effects on beaches, which have been closed due to high biomass HABs in some areas, such as in Hong Kong. From a management perspective, there are very few policies about this type of impact, and even fewer about other aesthetic or ecosystem impacts, which are typically subtle and hard to detect.

4.5.1   Recreational use of beaches/coastal waters

A request to the worldwide Internet mailing list for workers on red tides and HABs called PHYCOTOXINS, asking for examples of HAB species which cause problems related to recreational use of beaches and nearshore marine waters gave only few responses with exact names of HAB species and the references to problems they have caused. There were, however, many requests from list subscribers who wanted information on this topic. These responses clearly show that official guidelines and/or regulations on beach safety during HABs are scarce and only exist in a few countries. This section is based upon information to be published by World Health Organization (WHO) (compiled by Sørensen, pers. comm.)

Recreational use of beaches includes many different activities. Some, such as swimming or bathing, involve direct skin contact with the surrounding water; whereas others, such as surfing, sailing, sun bathing etc., may only involve exposure to sea spray. A third means of exposure to HAB species in seawater can be by oral intake/swallowing of water. In the following sections, intoxications through inhalation of sea spray and direct skin contact with water will be presented separately. A list of species known to cause human intoxications is compiled in Table 4.16. It is important to recognize that most of the non-toxic HAB forming species are harmless to humans.

As a precaution, the following guidelines are recommended for all marine and fresh water-based recreation and should be included in information disseminated to the public:

- Avoid areas with visible algal concentrations and/or algal scums in the sea or on the shore. Direct contact or swallowing appreciable amounts of water is associated with the highest health risk.

- On the beach, avoid sitting downwind of any algal material drying on the shore, which could form an aerosol, and be inhaled (particularly in areas with *Gymnodinium breve* blooms).

- If sailing, windsurfing or undertaking any other activity likely to involve accidental water immersion in the presence of dense algal blooms, wear clothing which is close fitting in the openings. The use of wet-suits for watersports may result in a greater risk of rashes, because algal material in the water trapped inside the wet-suit will be in contact with the skin for long periods of time.

After coming ashore, shower or wash yourself down to remove any algal material. Wash and dry all clothing and equipment after any contact with algal blooms and scum. If any health effects are subsequently experienced, whatever the nature of the exposure, medical advice should be promptly sought.

TABLE 4.16. Summary of toxic phytoplankton species involved in human intoxications through inhalation or direct contact.

Exposure routes	Toxic species
Inhalation of sea spray	*Gymnodinium breve*
	Gymnodinium brevisulcatum
	Pfiesteria piscicida
	Pfiesteria shumwayae
Dermal contact	Cyanobacteria spp.
	Lyngbya majuscula
	Oscillatoria nigroviridis
	Schizothrix calcicol
	Trichodesmium spp.
	Pfiesteria piscicida
	Pfiesteria shumwayae
Oral intake	*Nodularia spumigena*
	Pfiesteria piscicida
	Pfiesteria shumwayae

4.5.2 Species toxic to humans through inhalation of sea spray, etc.

Brevetoxins: Neurotoxic shellfish poisoning (NSP) is caused by consumption of toxic seafood contaminated by brevetoxins produced by marine dinoflagellates. The toxins are polyethers and so far nine derivatives are known (Schulman et al. 1990; Baden and Trainer 1993). The symptoms include nausea, vomiting, diarrhea, chills, dizziness, numbness and tingling of the face, hands and feet occurring 3-4 hrs following ingestion. However, humans can also be exposed to brevetoxins by inhalation of a sea spray aerosol containing fragments of algal cells and/or brevetoxins released into the surf by lysed algae (Baden et al. 1984; Scoging 1991; Bates et al. 1993). The signs and symptoms here are severe irritation of conjunctivae and mucus membranes (particularly of the nose) followed by persistent coughing and sneezing and tingling of the lips. The effects are not usually observed more than a few kilometers inland (Pierce 1986). In addition to causing NSP, brevetoxins can kill fish, invertebrates and seabirds and possibly lead to mortalities in manatees and dolphins (Abbott et al. 1975; Forrester et al. 1977; O'Shea et al. 1991).

Species: Brevetoxins are produced by the unarmored marine dinoflagellate (*Gymnodinium breve*). Until 1993 blooms had only been reported in the southeastern United States and eastern Mexico (Steidinger 1993), but NSP was also detected in New Zealand in 1993 (Fernandez and Cembella 1995) and in 1998 (Chang, pers. comm). In the 1998 incident in New Zealand, the causative species was *Gymnodinium brevisulcatum* (H. Chang, pers. comm.).

Recommended maximum values: The state of Florida has run a general control program for *Gymnodinium breve* since the 1970's. Since 1984, areas are closed for shellfish harvesting when cell counts exceed 5000 cells L^{-1} (Hungerford and Wekell 1993). Harvesting areas are re-opened when mouse bioassay results show that shellfish from the closed areas contain less than 20 Mouse Units (MU) 100 g^{-1} of shellfish meat

(Steidinger 1993). No beaches are closed as a result of aerosolized toxin, although concentrations as low as 10,000 cells L^{-1} are sufficient to cause discomfort in beachgoers (B. Roberts, pers. comm.).

Pfiesteria piscicida: *Pfiesteria*, known as the "ambush fishkiller," can also intoxicate humans through inhalation of the toxins, either by exposure to blooms in coastal waters or to cultures while working in the laboratory. The toxins are not well described at present, but it is thought that they are neurotoxins, which can cause severe but reversible symptoms in humans, such as learning disorders, memory loss and change in behavior (Burkholder and Glasgow 1997). *Pfiesteria*-like organisms have not yet been found anywhere other than in the eastern and southeastern US.

The following is extracted from the NCSU Aquatic Botany Laboratory *Pfiesteria piscicida* Page (**http://www.pfiesteria.org**). "Thirteen researchers who worked with dilute toxic cultures of *Pfiesteria* sustained mild to serious adverse health impacts through water contact or by inhaling toxic aerosols from laboratory cultures. These people generally worked with the toxic cultures for 1-2 hours per day over a 5-6 week period. The effects include a suite of symptoms such as narcosis (a "drugged" effect), development of sores (in areas that directly contact water containing toxic cultures of *P. piscicida*, and also on the chest and face), uniform reddening of the eyes, severe headaches, blurred vision, nausea/vomiting, sustained difficulty breathing (asthma-like effects), kidney and liver dysfunction, acute short-term memory loss, and severe cognitive impairment (= serious difficulty in being able to read, remember one's name, dial a telephone number, or do simple arithmetic beyond 1 + 2 = 3). Most of the acute symptoms proved reversible over time, provided that the affected people were not allowed near the toxic cultures again. Some of these effects have recurred (relapsed) in people following strenuous exercise, thus far up to six years after exposure to these toxic fish-killing cultures. Moreover, subcutaneous injection of crude toxin preparations from fish-killing cultures has induced serious learning impairment and memory loss in experimental laboratory rats (work by Drs. Levin and Schmechel at Duke University). The discovery, the "hard way," that *Pfiesteria* is unusual in its ability to produce toxins which can aerosolize, led to requirements by state and federal officials that all further work with toxic fish-killing cultures of this dinoflagellate be conducted in biohazard level III containment systems in a limited-access facility. These precautions must be followed for any research with live toxic cultures of *Pfiesteria*."

4.5.3   Species toxic to humans through dermal contact

Health hazards: Marine cyanobacterial dermatitis ("swimmers' itch" or "seaweed dermatitis") is a severe contact dermatitis that may occur after swimming in seas containing blooms of certain species of marine cyanobacteria (Grauer and Arnold 1961). The symptoms are itching and burning within a few minutes to a few hours after swimming in the sea where fragments of the cyanobacteria are suspended. Visible dermatitis and redness develops after 3-8 hrs, followed by blisters and deep desquamation (Grauer and Arnold 1961). Two toxic components, debromoaplysiatoxin and lyngbyatoxin A, have been isolated from marine cyanobacteria (Mynderse et al. 1977). These toxins are highly inflammatory and are known to be potent tumor-producing compounds (Gorham et al. 1988; Fujiki et al. 1991). The toxins have only been tested by skin application so nothing is known of their oral toxicity. More research is needed on possible tumor promotion risks on human populations. To date no studies have demonstrated cancer initiation by cyanobacterial toxins (Carmichael and Falconer 1993). Thus far outbreaks have only been reported from Japan and Hawaii (WHO 1984; Grauer and Arnold 1961).

Species: The cyanobacterium *Lyngbya majuscula* is known to produce debromoaplysiatoxin and lyngbyatoxin A and the cyanobacteria *Oscillatoria nigroviridis* and *Schizothrix calcicola* are known to produce debromoaplysiatoxin (Mynderse et al. 1977). Furthermore, cyanobacteria from the genus *Trichodesmium,* which is a frequently occurring genus in sub-tropical areas, can cause swimmers itch.

Another species to add to this list is *Pfiesteria piscicida*, which may be linked to skin disorders in those who have contacted water during the time when toxic *P. piscicida* zoospores are present (Morris, 1999)

Recommended maximum values: At present there are no international criteria for assessing the threat of cyanobacteria in recreational waters. The general guidelines for algal blooms should be followed.

4.5.4 Species toxic to animals (including humans) through oral intake while swimming

Health hazards: *Nodularia spumigena* is a regular bloom-forming species in brackish water environments e.g. the Baltic Sea and Australian estuaries. *N. spumigena* was the first cyanobacterium recognized to cause animal deaths from drinking water (Francis 1878). The toxin produced by *N. spumigena* (nodularin) is a cyclic pentapeptide, which acts as a hepatotoxin. It induces massive hemorrhage in the liver of animals, and also has effects on the kidneys (Eriksson et al. 1988; Sandström et al. 1990).

In the 19th century, several toxic blooms and accumulations of *N. spumigena* were recorded. There are reports of blooms of *N. spumigena* associated with poisoning of ducks (Kalbe and Tiess 1964), dogs (Edler et al. 1985; Nehring 1993), young cattle (Gussmann et al. 1985) and sheep (Main et al. 1977). So far there have been no official reports of human poisoning by *N. spumigena*, but humans may be as susceptible to the toxins as other mammals. It is therefore reasonable to believe that small children might accidentally ingest toxic material in amounts, which could have serious consequences, e.g. not immediately diagnosable liver damage.

A recent report showed toxin present in edible mussels harvested in an estuary containing toxic *Nodularia* (Falconer et al. 1992). There is no further information regarding the risk from consumption of shellfish and fish from areas affected by *N. spumigena,* so there is a need for additional research.

Recommended maximum values: At present there are no international criteria for assessing the threat of toxic cyanobacteria in recreational waters. In Australia, a no-adverse effect guideline level has been published by Jones et al. (1993) and corroborated by Falconer et al. (1994). It is recommended that 1 μg toxin (microcystin or nodularin) L^{-1} of drinking water, or 5,000 cells ml^{-1} water, should be regarded as the upper limit for safe consumption. These unofficial guidelines are based on toxicity calculations from mouse dosing and pig feeding trials using the freshwater cyanobacteria *Microcystis.*

4.5.5 Non-toxic phytoplankton

Some of the most common non-toxic HAB species are the heterotrophic flagellate *Noctiluca scintillans* (Ryther 1955), the autotrophic ciliate *Mesodinium rubrum* (Taylor et al. 1971) and the haptophycean flagellate *Phaeocystis globosa* and *P. pouchetii* (Lancelot et al. 1998). No direct human health impacts have been identified in connection with the occurrence of these species, though *Noctiluca* has been associated with fish kills, possibly due to high concentrations of ammonia (Okaichi and Nishio 1976). No direct human health impacts have been reported from exposure to *Aureococcus anophagefferens.* However dense blooms can constrain the use of recreational waters by causing discolored water, reduced transparency and foam or scum formation due to the production of mucilage. The degradation of the blooms is also associated with bad odors.

4.5.6 Mitigation/precautionary measures

For the general public, it is recommended that a health information brochure be prepared and published about algal blooms and toxic algae, their possible health effects, reporting procedures for any health

problems thought to be linked with water-based recreation and recommended protective measures. A good example of such a booklet is the Swedish publication "Algal Blooms - some questions and answers" published by Informationscentralen för Egentliga Östersjön (in Swedish). The booklet contains "frequently asked questions" such as "Can we swim in the sea during a red tide with no health risk?" or "Can we eat fish caught during a red tide?" The booklet attempts to provide clear and simple answers to these questions. Another example of material for the public about how to use coastal waters during HABs is a poster used to warn the Danish public as well as foreign tourists in Denmark about the risk of swimming during certain kinds of blooms, typically of *Nodularia*.

This general information should be followed by a more specific warning during algal blooms or periods with toxic shellfish and fish. Press statements (sent to newspapers, television and radio), warning notices, leaflets and posters at the beach and camping sites, etc. should be used to inform the public of the potential danger, and conversely, of the lack of danger from harmless, visible algal blooms. In some areas information on HABs is distributed instantly to the users of the monitoring system by telephone, telephone answering machine, fax, e-mail and the Internet; e.g., The Baltic Sea Algaline at **http://www.fimr.fi**.

The hazard or risk of injury from algal toxins will depend upon the degree of contact with the toxins, and can be reduced by avoiding contact with high densities of algae and algal scums, and by preventing access of livestock and pets. In the following, examples of monitoring programs in relation to recreational use of coastal waters, extracted from Andersen (1996), are presented.

Denmark: In Denmark, the counties are responsible for monitoring water quality in the coastal areas. Each county has several monitoring stations at which a range of parameters is monitored at least once every month, including phytoplankton. If a bloom occurs, the sampling program can be expanded to involve more stations and more frequent sampling. In addition, if a bloom is identified by the public, the local county officials can be contacted via a special environmental emergency phone line. The counties have an emergency routine, which involves collection of samples, which are analyzed either at a county laboratory or a private consultancy firm. If the bloom is dominated by a harmful species; e.g., the cyanobacterium *Nodularia spumigena,* warnings are posted on beaches and the local news media are notified and advised to inform the public. The National Environmental Research Institute (NERI) is currently in the process of establishing an Internet webpage with general information about HABs as well as on the current HAB situation in Danish coastal waters.

Italy: In the Adriatic coastal waters, increasing algal problems have occurred during recent years due to accelerated eutrophication from nutrients originating in the Po River. The major problems are an uncontrolled growth of planktonic algae causing enormous build up of biomass. This can be a constraint to the recreational use of coastal waters, but it also eventually causes oxygen deficiency, killing benthic organisms and forming enormous masses of mucilage (gel), assumed to be produced by the algal blooms. Some of these mucilage events covered up to 10,000 km^2 of the coastal area in 1988 and 1989.

Blooms in regions with hundreds of millions of cells L^{-1} leading to colored water (yellowish, green or wine red) and as thick as vegetable soup are not an entirely new phenomenon. What is new about the problem is that it has progressed from being occasional to becoming chronic. The diatoms and dinoflagellates responsible for building up the large biomass along the upper Adriatic coast are listed in Table 4.17.

TABLE 4.17. Diatoms and dinoflagellates responsible for HABs on the upper Adriatic coast.
(Source: Marchetti 1992.)

Diatoms	Dinoflagellates
Asterionella japonica	Gonyaulax polyedra
Chaetoceros laciniosus	Gymnodinium spp.
Nitzschia longissima	Protoperidinium pellucidum
Skeletonema costatum	Prorocentrum micans
Thalassiosira spp.	Scrippsiella trochoidea

4.6 HAB Impacts on Ecosystems

One of the least understood impacts of HABs is on ecosystems. Negative effects can be obvious, such as with mass mortalities of fish or marine mammals (e.g. Scholin et al. 2000), but they can also be subtle and difficult to discern, as would be the case if the feeding or survival of larvae or other life stages of different species were affected during a bloom.

Several toxic marine dinoflagellates and flagellates have been associated with the death of fish and invertebrates. The most common include the dinoflagellate species: *Gymnodinium breve* (Steidinger et al. 1998a), *Gyrodinium cf. aureolum* (= *Gymnodinium mikimotoi*; Tangen 1977; Potts and Edwards 1987), *Gyrodinium galatheanum* (Nielsen 1993), *Cocchlodinium polykrikoides* (Na et al. 1996), and *Pfiesteria piscicida* (Burkholder and Glasgow 1997), the raphidophycean flagellates: *Heterosigma akashiwo* (White 1988), *Chattonella antiqua* and *Chattonella marina* (Endo et al. 1985) and the prymnesiophycean flagellates: *Prymnesium parvum* (Shilo 1969), *Chrysochromulina polylepis* (Rosenberg et al. 1988) and other *Chrysochromulina* species (Moestrup 1994). This list is far from complete, as there are many other HAB species, which also cause mortalities of various types within ecosystems.

The toxins produced by these species can have strong ichthyotoxic effects and for some species, such as *Chrysochromulina polylepis* and *Prymnesium parvum*, severe cytotoxic effects have also been demonstrated (Underdal et al. 1989; Yasumoto et al. 1990). During the 1988 bloom of *Chrysochromulina polylepis* in Scandinavian waters, not only dieoffs of invertebrates and fish were detected but also death of macroalgae. *Gymnodinium mikimotoi* and *Gyrodinium galatheanum* can cause severe necrotizing degeneration of the gills and produce toxins with hemolytic effects (Jones et al. 1982; Yasumoto et al. 1990; Nielsen 1993). *Pfiesteria piscicida* has been linked to massive fishkills in the mid-Atlantic US, though a toxin has yet to be fully characterized (Burkholder et al. 1992). Furthermore, it has recently been shown that the toxin produced by *Pfiesteria* can have harmful effects on marine invertebrates under field conditions (active fishkill events) or in culture bioassays (containing toxic stages of *Pfiesteria* spp. >1,000 cells ml^{-1}. Effects have been observed on *Argopecten irradians* (bay scallop), *Callinectes sapidus* (blue crab), *Crassostrea virginica* (eastern oyster), *Mercenaria mercenaria* (northern quahoq), *Mytilus edulis* (blue mussel) and *Venus cancellata* (Venus clam). (See: http://www.pfiesteria.org.)

Another HAB toxin with significant ecosystem impacts is domoic acid (DA) which is also responsible for ASP events. As exemplified in a study by Scholin et al. (2000), DA can move through the food web from its diatom source to top predators, including sea lions, sea otters, and possibly whales. This same toxin has been linked to seabird deaths (Work et al. 1993; Sierra Beltrán et al. 1997) as well.

Non-toxic HABs can also have dramatic ecosystem effects; for example, those caused by brown tide species such as *Aureococcus anophagefferens* in Long Island, New York, Narragansett Bay, Rhode Island and New Jersey waters and *Aureoumbra lagunensis* in Laguna Madre, Texas. Blooms of these species

have caused death to benthic suspension feeders such as mussels, and die off of eelgrass, the latter due to decreased light penetration (Boesch et al. 1997, Bricelj and Lonsdale 1997). (See also section 1.1.)

It might be possible to reduce the negative impacts of HABs on species or ecosystems using the same approaches suggested for aquaculture areas in Section 6 of this report. For example, applications of clay to flocculate and remove toxic cells might be considered environmentally acceptable relative to the damage that will occur in a sensitive ecosystem without treatment. This might occur when a particularly important resource is threatened, such as an endangered species, or a special marine reserve. Very little is known at present about bloom mitigation methods, so care must be taken that no strategy is undertaken without first assessing the possible negative impacts of the treatment itself.

4.7 Monitoring Program Costs

Most shellfish biomonitoring is financed by government agencies (Andersen 1996). For finfish mariculture and capture fisheries, however, most fish farming companies or their associations pay for monitoring of phytoplankton or toxins. In Finland, Norway and Portugal, part of the monitoring programs are financed by research institutions and in the US, federal and state agencies have sponsored regional monitoring programs that may be temporary or permanent. In Norway, Netherlands, US, and Canada, private users of monitoring data sometimes pay part of the monitoring costs, depending on the resource being monitored. The contribution of the shellfish industry to toxin monitoring costs in Atlantic Canada, for example, remains small, but there is increasing pressure due to cutbacks in government funding to increase this contribution in the future.

Detailed cost evaluation of toxin monitoring programs is difficult to obtain and variable from year to year. A compilation of available data is shown in Table 4.18. The relative cost of monitoring as a percent of production value is higher for shellfish (0.1 to 5%) than for finfish (0.02-0.05%). This is because shellfish monitoring includes costly analysis of algal toxins, and in some cases phytoplankton monitoring, whereas finfish monitoring generally only involves phytoplankton analysis. Note that the cost of biotoxin monitoring can vary considerably from year to year, in response to the magnitude of toxic outbreaks, the development of new fisheries industries, and changes in budgets. The scale of a biotoxin monitoring program will often depend on the value of the affected resource relative to the cost of the monitoring program. In regions or countries where shellfish resources are well developed, intensive monitoring has proved cost-effective (e.g. Maine, Galicia, New Zealand). In contrast, when shellfish resources are limited or underexploited, reduced monitoring effort or blanket closures may be more cost-effective.

Increased participation of industry in cost-sharing of monitoring is possible only when the industry is highly developed and the value of the resource and profit-margins are high. In New Zealand the total value of exported aquaculture product (primarily salmon, mussels and oysters) has risen steadily since the 1980s, and was estimated at NZ$ 210M (~US $87M) in 2000 (Helen Smale, Marlborough Shellfish Quality Assurance Program, pers. comm.). Industry currently pays fully for biotoxin testing of commercial product and thus owns the resulting data. The current annual budget for New Zealand government authorities to conduct HAB and biotoxin monitoring for non-commerical product is ~NZ $1.45M (~US $0.6M), with ~9% of this amount allocated to phytoplankton analysis and 26% for shellfish analysis (C. Seamer, MAF Food Assurance Authority, Wellington). About 16% of this budget (NZ $230,000 ~ US $95K) is paid to industry for access to monitoring results for commercial product from 80 classified commercial growing areas, and a reserve fund is included for additional sampling and analytical cotss when marine biotoxin evens occur.

One of the more detailed documentations of the cost of a monitoring program was prepared by Mariño et al. (1998). They suggested that the cost-effectiveness of a monitoring program is a function of its goals,

and tends to be higher if longer-term goals are established. The biotoxin monitoring program in Galicia provides a long-term environmental database that yields benefits far beyond those of ensuring seafood safety. A detailed breakdown of the costs associated with monitoring of environmental quality (including monitoring of biotoxins and HABs, as well as microbial pathogens and pollutants) was provided for Galicia, NW Spain (Mariño et al. 1998; Figure 4.19). The costs of biotoxin/HAB monitoring comprised 70% of the annual operating budget for environmental monitoring, and out of this, a large portion (60%) was allocated to personnel costs. It was estimated that the cost per sample (for shellfish collection and analysis of PSP and DSP toxins) was US$52. Similarly, personnel costs account for 71% of the annual cost of the shellfish toxin monitoring program in the state of Maine, US (L. Bean, ME DMR, pers. comm.).

TABLE 4.18. Approximate annual production value for finfish (F) and shellfish (S) versus the approximate cost of monitoring HABs (in US$). (Modified from Andersen 1996 and other sources.)

Country - Region	Total cost (US$)	Production value (US$)	Cost/Production (Percent)	Source
Chile (F)	20K	400M	0.05	(1)
Canada - BC (F)	448K	167M	0.27	(4)
USA - Washington (F)	30K	24M	1.3	(5)
Norway (F)	300K	1000M	0.03	(1)
Denmark (F)	4K	25 - 30M	0.02	(1)
Denmark (S)	500K	46M	1.10	(1)
France (S)	800K	-	-	(1)
Portugal (S)	425K	200M	2.1	(1)
Spain-Balearic Is. (S)	11K	225K	5.0	(1)
Spain-Catalonia (S)	200K	-	-	(1)
Spain-Galicia (S+F)	[a]1114K	160M	0.07	(2)
UK-Scotland (S)	280K	22M	1.2	(1)
Uruguay (S)	35K	3M	1.2	(1)
Canada-Maritimes (S)	135K	10M	1.4	(1)
USA-Washington (S)	660K	50M	0.1	(1)
USA-Maine (S)	281K	-	-	(3)

(1) Andersen 1996, response to IOC survey; (2) calculated from Mariño et. al. 1998; (3) L. Bean, ME DMR, pers. comm. (1998); (4) our estimates and SAR 1998; (5) our estimates, Rensel unpublished
[a]Cost of HAB monitoring includes monitoring of shellfish biotoxins, phytoplankton and oceanography, coordination and management.

Phytoplankton monitoring costs for finfish farming are difficult to determine, as financing is often by the individual farmer or company. Some companies have a staff member to coordinate sampling at different sites, but often sampling and analysis are performed by the staff of an individual farm site or cluster of farms. As described in other sections of this report, coordinated monitoring on state or province-wide basis has been dropped in favor of individually operated programs in the US Pacific Northwest. On the Mediterranean coast of Spain, Greece, and Italy, and in Scotland, our brief surveys of insurance companies and fish farmers suggest that HAB problems for fish mariculture are relatively minor or non-existent. Accordingly, no HAB monitoring occurs for fish farms in those areas. However, in Chile and Norway, monitoring is coordinated on a national basis.
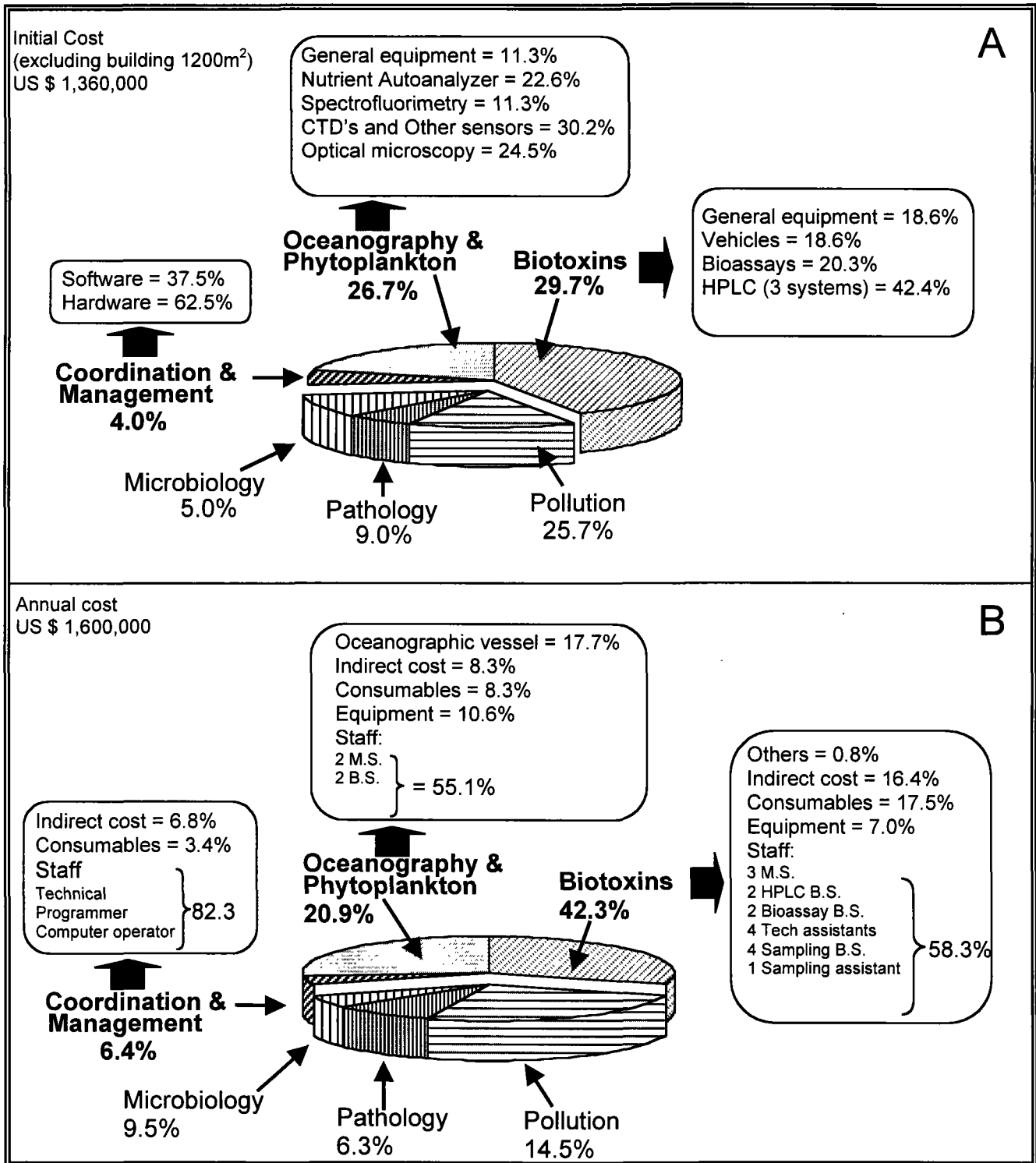
FIGURE 4.19. Cost of the harmful algae monitoring program in Galicia, NW Spain within the context of overall environmental quality monitoring. A) Initial cost; B) annual operating cost. (Redrawn from Mariño et al. 1998.)

Monitoring in Chile is funded by the Chilean Salmon Farmers Association. In Norway, it is funded in association with the Salmon Technological Institute. The program involves co-operative monitoring at individual fish farming sites, with centralized analysis of samples and reporting. At present (1998) the program has relatively modest costs of approximately US$ 31,000 per year, including operational costs, labor, computers, travel and other expenses (A. Clément, pers. comm.). Norwegian monitoring for HABs is funded by the fish farms and their association, together with insurance companies and the State Food Control Authority (Andersen 1996). Insurance companies pay Oceanor A/s, (the consultant that provides SeaWatch buoy systems), approximately US$150,000 per year to provide services for fish farmers (Stel and Mannix 1996). Total cost of the Norwegian monitoring program was approximately US$ 300,000 in 1995 (Andersen 1996).

Although high, the cost of monitoring must be weighed against the economic losses of harvestable product, as well as the losses associated with public health care in the absence of adequate monitoring. An attempt to estimate the total cost of PSP, DSP and CFP in Canada, in terms of medical and lost productivity costs, and loss of harvested product was made by Todd (1995) (Table 4.19). In his study costs were broken into three categories. Medical or societal costs include medical care, hospitalization, emergency transportation, laboratory testing [chemical or mouse bioassay estimated at Can.$200 to 400 (ca. US$133 to 267) and illness investigation. Private costs include productivity losses through unearned wages (borne by employees or insurance companies), and industry-related costs comprise lost revenue through bans on harvesting, and lost business to related industries due to public concern for the safety of seafood ("halo" effect). It should be emphasized that there is a great deal of uncertainty around the reported figures, since multipliers were used to account for the number of unreported cases. PSP was found to incur relatively high private costs (79% of total), that were largely accounted for by fatalities, estimated at 3 per year. Private costs were also the dominant cost component for CFP, and were accounted for by lost productivity associated with the long-term effects of CFP toxins. In Canada, exposure to CFP is largely through fish consumption in tropical countries frequented by tourists.

TABLE 4.19. Estimated annual cost of PSP, DSP and CFP outbreaks in Canada, broken into societal, private and industry-related. (See text, data from Todd 1995.)

Type of cost			Cost (in US $)			
	PSP	%	DSP	%	CFP	%
Societal	25,583	17	18,333	29.7	49,990	21.3
Private	118,633	78.6	10,133	16.4	179,883	73.0
Industry-related	6,667	4.4	33,333	53.9	13,333	5.7
*Total	150,883		61,800		234,207	
*Cost/case	1,000		1,267		733	
# of cases/yr approx. estimate)	[a] 150		[b] 50		[c] 325	

a. 10 reported cases plus 140 unreported (estimated)
b. Not all cases clearly linked to DSP toxins, but are based on epidemiology associated with consumption of pathogen-free shellfish.
c. 325 illnesses reported per year. Used a multiplication factor of 100 to account for under-reporting and under-diagnosis of cases.

*Original data converted to US$ using an exchange rate of 1.5 Can$ per US$.

5 ADMINISTRATION OF MONITORING PROGRAMS

On a worldwide basis, diverse arrays of HAB monitoring programs are operative. The overall goal of most of these programs is to protect the human consumer from intoxication when eating shellfish and to protect aquaculture and fisheries activities against losses due to harmful blooms. However, even though the goals of the monitoring programs are quite similar, their structure can vary greatly because of differences in administrative policies, available technology, economic constraints, and in the magnitude of the resources or activities being protected. The structure of HAB monitoring programs was the issue investigated by an IOC/ICES questionnaire circulated in 1995. The major results of this investigation are published in Andersen (1996). A second questionnaire on the structure of HAB monitoring programs was launched in 1999. Compilation and presentation of the information is in progress.

5.1 National/regional HAB Monitoring Programs

Andersen (1996), referring to different questionnaire responses, concluded that of a total of 76 countries and regions where information is available, 45 had on-going HAB monitoring programs whereas 31 had none as of 1995 (Figure 5.1). Some countries/regions, which had no HAB monitoring programs in 1995, have now implemented programs (e.g. China, Vietnam Greenland)
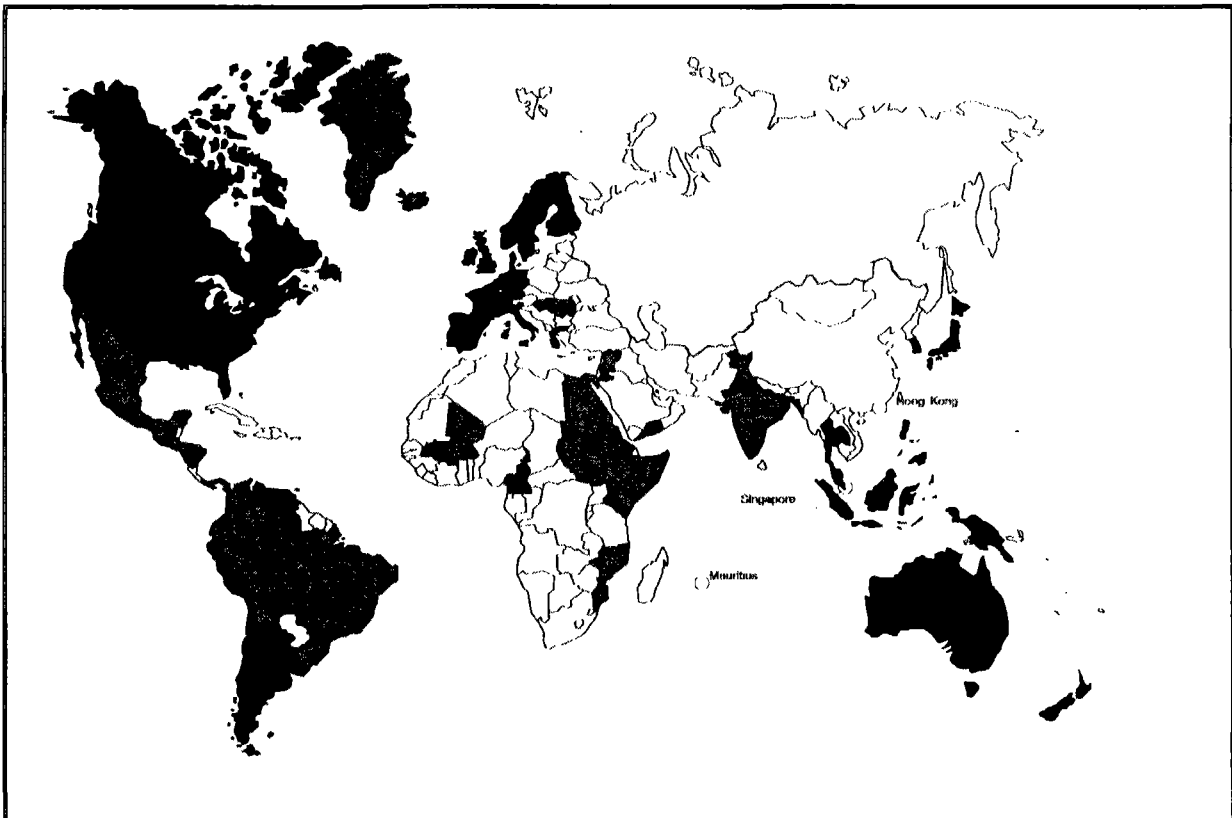
FIGURE 5.1. Worldwide status of HAB monitoring programs in 1966. (Source: Andersen 1996.)
White: no information, Black: HAB monitoring program exists; Gray: No HAB monitoring.

HAB monitoring programs fell into 2 major categories:

- Focused programs devoted to monitoring and management of HABs in relation to shellfish harvesting and/or fish farming.

- Programs run as integrated parts of general environmental monitoring, with no specific focus on the detection of HABs for management use.

The questionnaire showed that HAB monitoring programs can be either national or regional, covering relevant geographic regions in terms of harvest areas for shellfish and aquaculture sites. National programs can be broad, monitoring for toxic algae, and algal toxins in shellfish; e.g., France; or narrow, only monitoring toxins in shellfish. The narrowly focused programs might only monitor toxic algae in relation to mariculture, as in Chile or the northwest US. In some countries several HAB monitoring programs, with different purposes, are in operation. This is the case in the US where different states have monitoring programs running with different purposes (i.e., programs concerning either shellfisheries or aquaculture or both), and in Japan, where there are different programs for shellfish and aquaculture. Likewise, Denmark has separate programs for shellfish, fishfarming and environmental quality. In most cases, government authorities fund HAB monitoring programs.

Most (70%) of the HAB monitoring programs were initiated for management of shellfish either cultured or wild stocks. Most (82%) of the countries/regions responded that the HAB monitoring programs were initiated and planned by governmental authorities. Only 4 countries, Canada (West Coast), Chile, Norway and Denmark, have HAB monitoring initiated by private organizations, and these programs were to protect aquaculture activities, primarily salmon.

Operation/ practical monitoring: In most cases (85%) the institution/organization which initiated the HAB monitoring program was also responsible for carrying out monitoring operations. In a few cases, (Denmark, Finland, part of Spain and part of Sweden), monitoring was carried out as a collaboration between organizations. In the case of Denmark, sampling of algae and mussels in relation to the mussel fisheries is carried out by the fishermen on location, investigation of samples is carried out by private consultancy laboratories and the data are collected by government authorities. In some cases the management structure as well as the conduct of the HAB monitoring programs is advised by or has to refer to a task force if the program is to be changed. In some situations; e.g., New Zealand, the Marine Biotoxin Management Board must confirm and approve any changes in the HAB monitoring system, whereas in Denmark the ALGAL GROUP will discuss different issues and suggest changes in the monitoring and management procedures which then must be approved by the Veterinary Service. Furthermore, if an unusual HAB occurs in Danish waters the ALGAL GROUP will be summoned to discuss the current situation and give advice to the Veterinary Service on what to do. This kind of administrative unit for technical advice is also currently part of the Japanese and Philippine management system. It is called the "National Red Tide Task Force" in the Philippines (Figure 5.2).

5.2 Public Education and Communication

Public education can be a major tool in efforts to minimize the impacts of HABs and algal toxins. In some cases, the process is gradual, but the end result is still worth the effort. For example, in Hawaii, there were approximately 100 cases per year of ciguatera poisoning in the late 1970's and early 1980's, (Y. Hokama, pers. comm.). At the present time (1998) there are less than a dozen clinical cases each year. The occurrence of ciguatera-affected fish has probably not declined, but rather public education efforts are
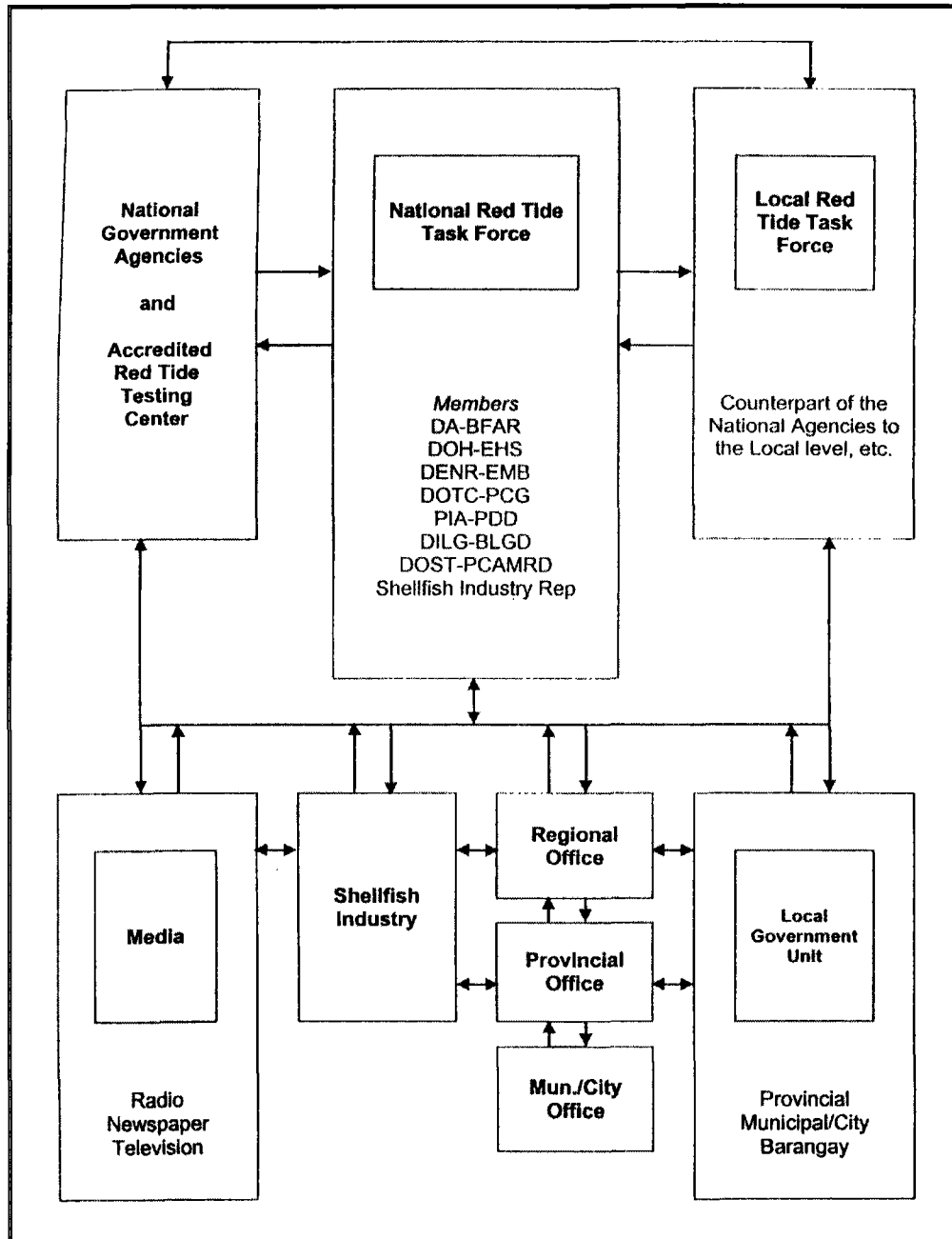
FIGURE 5.2. Organization of red tide/HAB monitoring in the Philippines. (Source: National Red Tide Task Force 1999)

being successful. At present, 80 to 90% of the population of Hawaii surveyed are aware of the problem of ciguatera poisoning.

There may also have been a shift in fish consumed by people in Hawaii in the past two decades that could account in part for the reduced number of clinical cases. As the public awareness of the problem grew, consumption of reef fish may have declined in favor of offshore, pelagic fish not known to contain CFP

toxins (Y. Hokama, pers. comm.). Some reef fish are still caught alongshore and in remote areas, tested (by the fishermen using analytical test kits described in Section 3.1.7), and sold into local markets. There is no testing of these fish by the state or other agencies.

General information

Public education and proper communication are the tools to be used to prevent "over reaction" from the public during a HAB event, such as the "halo effect" on the fisheries and fisheries products. A communication program should be an integral part of the overall management program for HABs. During an event, all official communication from the monitoring and management program to the public and the press must go via the communication center. Members of the different teams involved in HAB monitoring and management must refer to the communication center when they are asked questions from the public or the press. Such a routine would minimize the risk of contradictory information from the official monitoring and management program, and thus will avoid confusing the public and those directly impacted by HABs. It is recommended that booklets about health problems associated with algal blooms and toxic algae, the diagnosis and treatment of poisonings, the surveillance of groups who could be at risk as well as reporting procedures to the Public Health Authorities be prepared and published for general practitioners and medical clinics.

As discussed earlier, it is also recommended that health information brochures be published and distributed about algal blooms and toxic algae, their possible health effects, reporting procedures for any health problems, and so forth. The Danish Veterinary Service has published a brief "folder" about the risk of collecting and eating shellfish (Figure 5.3). It is distributed by the local libraries and tourist offices and can be obtained by mail by request to the Danish Veterinary Service. Another Danish poster discusses the risks of swimming in waters with algal blooms (Figure 5.4). In the Philippines the public is informed using colorful posters telling people how to handle seafood and which kinds of seafood are safe to eat and which are not during a HAB (Figure 5.5).

Using the Internet is an obvious way of distributing general information on HABs to the public. The information can be easily updated. Local webpages can be linked to other pages with additional information. At present general information on HABs is available on a number of webpages; e.g.:

http://www.redtide.whoi.edu/hab/

A page which contains general information on a wide range of issues in relation to HABs such as:

- What are Harmful Algal Blooms (HABs)?
- Introduction to Algal Blooms and "Red Tides"
- Photo Gallery, Visible Algal Blooms
- Photomicrographs
- Satellite imagery
- The algae species
- Which algae are responsible for the harmful effects?
- US finfish, shellfish and wildlife affected by toxic or harmful microalgal species
- Adverse Impacts
- What effects do Harmful Algal Blooms have on higher trophic levels?
- Human Illness
- Food poisoning associated with Harmful Algal Blooms
- Information on diagnosis and treatment
- HAB Distribution Maps
- Where and how frequent are HABs along the US coast?

- Pertinent Information
- National Plan; Announcements; US Researchers Directory; Other
- Links to other Internet web pages
- Harmful Algal Bloom Events in the US and around the world

http://www.tripprep.com/quicksum/qscigu.html and

http://vm.cfsan.fda.gov/~MOW/chap36.html

Both of these web pages containing specific information on ciguatera fish poisoning (CFP).

http://www.pfiesteria.org/

A web page containing information devoted to the fish killing dinoflagellate *Pfiesteria*.

Sometimes toxic algae appear among the algal food of mussels.

Mussels are good to eat -but be careful if you collect them at the coast. They can be toxic!

The toxins produced by the algae can accumulate in the mussels, which, for that reason, can be harmful to eat by humans. As a result the human consumer can get diarrhea or paralysis, and may exhibit other symptoms of intoxication.

The Fish inspection service is responsible for monitoring mussels in areas where mussels are harvested commercially. If toxins are detected in mussels, the area is closed to harvesting, until a new sample shows that mussels in the area are safe for consumption

Please note that the monitoring only covers the deeper waters distant from the shoreline. The conditions can be different on the shore

When you collect mussels please note:

- don't collect close to the outlet of rivers or sewage outfalls

- if you are close to an area which is closed for commercial mussel harvesting because of the pre-scence of toxic algae, check. Information can be obtained at the local fish inspection service or The Danish Ministry of Fisheries

- and remember, when you cook the mussels: Throw away all mussels not completely closed before they are boiled, they must close immidiately when you tick the shells. Throw away all mussels which have not opened their shells during boiling.

FIGURE 5.3. A Danish brochure presenting information on the risk of collecting and eating shellfish in relation to algal toxins. (Source: The Danish Veterinary Service, pers. comm.)

ÅRHUS COUNTY COUNCIL
Office of the Environment

WARNING to holidaymakers - lakes in Mid Jutland!

Every summer aquatic flora of blue-green algae make an appearance in the lakes of Mid Jutland. The water becomes turbid and green with an appearance similar to vegetable soup or paint. Blue-green borders of rotting algae collect along the shore.

In extremely rare cases a dangerous poison may develop in connection with this algae bloom.

As it is not possible to give prior warning of this the following rules should be observed to avoid accidents:

1. Never bathe in places where the water is so cloudy that you are unable to see your feet when wading.

2. Never bathe close to areas where algae have collected on the lake.

3. Never allow small children to paddle or play in the algae which have been washed onto the shore.

4. Never allow pets to drink or dogs to bathe in lake water which is coloured green by algae.

FIGURE 5.4. Danish information material for the public about the risk of swimming during algal blooms/red tides. (Source: the County of Aarhus, pers. comm.)

FIGURE 5.5. Philippine poster informing the public about safe handling of seafood, and which seafoods are safe to eat during a red tide. (Source: R. A. Corrales, pers. comm.)

Warnings

This general information should be followed up by a more specific warning during algal blooms or periods with toxic shellfish and fish. Press statements (sent to newspapers, television and radio), warning notices, leaflets and posters at the beach and camping sites etc. should be used to inform the public of potential hazards. In some areas information on HABs is distributed instantly to the users of the monitoring system by telephone, telephone answering machines, fax, e-mail and the Internet. Hot line phones with updated information on the current algal toxin situation in shellfish are at present available in Denmark, Norway, Sweden, and the US, whereas the same kind of information is available on the Internet from Norway and Ireland.

6 MITIGATION AND CONTROL

The goal of HAB research and monitoring is to protect public health, fisheries resources, ecosystem health, and coastal aesthetics. This requires an understanding of the many factors that regulate the dynamics of HABs and the manner in which they cause harm, but by itself, that knowledge does not provide protection. Management and mitigation strategies are needed that reduce impacts by avoiding the blooms or minimizing their effects (termed *mitigation* or *impact prevention*) or by actions which directly target the bloom population (*control*). Examples of impact prevention strategies include moving fish cages from the path of an HAB, reducing the quantity of fish food to reduce their susceptibility to a bloom, or reducing pollution inputs to a region in an effort to decrease the number or size of bloom events. Examples of control efforts would be direct application of chemicals or other biological control agents that kill or disrupt HAB cells during blooms.

The approaches which are more general in nature - i.e., those which are useful over a large area and which address the general HAB problem are discussed first. Thereafter, strategies to reduce impacts are discussed in the context of those which specifically address the problems of fish farms, shellfish toxicity, and ciguatera. The list of topics discussed includes strategies used throughout the world against HABs.

6.1 Impact Prevention

There are a variety of impact prevention strategies that can be employed which indirectly affect the size of a HAB population or its impacts.

6.1.1 Monitoring Programs

Detection of HABs and their associated toxins in algae or fish and shellfish is an essential element of any management strategy since it prevents toxic fish and shellfish from reaching the market or provides early warning to fishermen and producers. As a result, numerous monitoring programs have been established in coastal waters throughout the world in an effort to provide either advance warning of outbreaks or to delineate areas that require harvest restrictions. As described in Sections 3 and 4, this monitoring is conducted for HAB species and/or for their toxins. The latter has become quite expensive in recent times due to the proliferation of toxins and potentially affected fisheries resources. Concurrent monitoring programs for PSP, DSP, ASP, and even AZP are now in place in some countries (e.g., Ireland); each test being conducted on different shellfish species. The costs of such programs are thus significant and growing in parallel with the proliferation of HAB toxins. This general approach to impact prevention is to be pro-active, monitoring shellfish and fish with sufficient temporal and spatial coverage to permit selective closures of discrete sections of the coast (e.g., Shumway et al. 1988).

Monitoring programs thus fall under the impact prevention category of mitigation. They will not be discussed further here other than to point out that the "safety net" created by these monitoring programs has been very effective in most countries. Illnesses and deaths from HAB toxins are rare in areas where effective and sustained programs have been established. There remains, however, an urgent need to improve on monitoring procedures, especially through the development of new assay technologies that can reduce costs and increase throughput.

6.1.2 Nutrient Reductions

General Description

HAB-algae, just like all plants, require certain major and minor nutrients for their nutrition. These can be supplied either naturally from marine and freshwater biogeochemical processes, or through human activities, such as pollution. One of the explanations given for the increased incidence of HAB outbreaks worldwide is that these events are a reflection of increased pollution and nutrient loading in coastal waters (e.g., Smayda 1990). At the simplest level, HAB species may increase in abundance due to nutrient enrichment but remain as the same relative fraction of the total phytoplankton biomass (i.e. all phytoplankton species are stimulated proportionally by the enrichment). More often, this enrichment results in the dominance of particular groups of algae that are best able to capitalize on the enrichment. Indeed, some contend that there has been a *selective* stimulation of HAB species due to the changes in nutrient supply ratios from human activities (Smayda 1990). Even natural changes in supply ratios are thought to influence dominance of various types of non-harmful algae (e.g., Tilman 1977). Regardless of the mechanism, there is no doubt that HABs have increased in certain areas of the world where pollution has also increased. Conversely, there are many affected areas that have little or no anthropogenic inputs of nutrient, yet have ongoing HAB problems (e.g., PSP in shellfish in remote regions of Alaska).

Effectiveness and limitations

It follows from the above discussion that a reduction in pollution, or a change in the ratios in which major nutrients are supplied to coastal waters may lead to a decrease in HAB frequency or severity. This therefore represents another approach to impact prevention. A classic example of this type of mitigation strategy was seen in the Seto Inland Sea of Japan, where pollution increased nutrient loadings dramatically in the 1960's and early '70's, during which time visible red tides more than tripled. Legislation was passed in 1973 that mandated a reduction in industrial and domestic effluents, and several years later, the number of red tides began to decrease (Okaichi 1997). They eventually fell to 30% of peak levels, where they have held to this day.

Another prominent example is from the Long Island picoplanktonic "green tides" of the 1950's. During that time, Great South Bay and Moriches Bay, located on the south shore of Long Island, New York, were subject to extremely dense blooms of algae, sometimes exceeding 10 million cells L^{-1}. The resulting turbidity turned the water a vivid green color. This not only altered the aesthetic quality of that region as a recreational area, but these blooms were also thought to be the principal cause of the failure of the local oyster industry. Research (reviewed in Ryther 1989) correlated the green tides with the development of a duck farm industry located along the tributary streams and coves of these bays. Inevitably, tons of nitrogen and phosphorous found their way into the receiving waters. The connection between the green tides and pollution from the duck farms was established through a series of surveys and laboratory experiments. The dense green tides which occurred in the 1950's diminished during the 1960's as the flushing characteristics of Moriches Bay were increased by opening a channel to the ocean and by the gradual demise of the duck farming businesses. Pollution control measures were also imposed on existing duck farms, and there have not been any recurrences of the green tide blooms since.

These are but two examples of several where reductions in the pollution loading of coastal waters resulted in a decrease in the incidence of algal blooms. Others could be cited as well, such as the Black Sea (Bodeanu 1993) or Tolo Harbor in Hong Kong (Hodgkiss and Ho 1997). Several other examples could be cited of areas of the world such as the German Bight or the Baltic Sea where increases in pollution loadings through the years have been accompanied by a change in algal species composition and an increase in algal biomass and HAB incidence (Radach et al. 1990, Smayda 1990). Unlike Tolo Harbor or the Inland Sea, these areas have not seen a decrease in HAB phenomena, either because there has been no

reduction in pollution, or because the time frame since pollution control policies were implemented has been too short to reveal trends.

As coastal communities and countries struggle with pollution and eutrophication issues, the implications of these studies are profound. Increasingly, the possible stimulation of HAB species by domestic or industrial effluent is being raised by those in opposition to the construction or relocation of sewage treatment facilities or discharges. One example is a new sewage outfall in Massachusetts Bay (US). Opponents of the project cited the time series described above and argued that an adverse impact of the outfall would be a change in phytoplankton species composition and an increase in HABs within the Bay. The outfall has just begun operation, so it will be a few years before we will know if these concerns were justified.

The stakes in this and related controversies are large, and the scientific uncertainty significant. The public, the press, and regulatory officials expect scientists to provide predictions and answers, yet their expectations exceed present capabilities. Competitive outcomes in phytoplankton species selection and succession cannot yet be predicted in natural waters, nor can the relative effects of natural versus anthropogenic factors in HAB development be resolved. To address the concern that the phytoplankton species composition will change with the different quantities and ratios of nutrients in the effluent from a new outfall, ecosystem-level models are required that may be a decade or more away. Even when the focus is narrowed to a few key HAB species, their responses within the Bay ecosystem cannot be modeled or estimated with any accuracy because their nutrient requirements have not been well characterized in laboratory studies.

Practicality

It is exceedingly difficult to predict with any certainty what the effect of pollution control strategies will be on HAB incidence, except in situations where the pollution loading is massive (e.g., in the Inland Sea of Japan in the late 1970's). Moderate levels of pollution will likely be associated with high algal biomass, and perhaps with numerous red tides, but those will not necessarily be harmful or toxic. In recent years, some have even used the term "favorable algal bloom" or FAB (J. Hodgkiss, University of Hong Kong, pers. comm.) to highlight the fact that elevated biomass can be beneficial to coastal productivity and fisheries, as long as bloom levels do not reach levels where damage will occur due to anoxia, toxins, or other effects. What is needed from a management perspective is a quantitative relationship between particular loading parameters and toxic or harmful effects from algal blooms. This has not been accomplished as yet, but some efforts lead in that direction, such as the relationship between N:P ratios and specific types and species of harmful dinoflagellate abundance (Hodgkiss and Ho 1997). However, these types of analyses need to be evaluated more thoroughly and expanded to include more years of data (e.g., Yung et al., 1997) before they can be used to justify major policy decisions on water quality options.

To summarize the status of nutrient reduction as a mitigation strategy, the argument can be made that to reduce HAB incidence in certain areas, strict pollution control regulations would have to be instituted. **However, before control strategies based on reduction of nutrient inputs are implemented, it is essential that the case be proven that human pollution is in fact responsible for the proliferation of HAB algae in that area.** To accomplish this, the stimulatory influence of anthropogenic nutrient inputs on HAB incidence must be determined, and this is one of the more pressing and intractable unknowns facing regulatory agencies charged with water quality control. The analysis required is extensive and expensive, and in most cases, exceeds the capacity of the scientific community to provide predictions of outcomes under different loading scenarios. Considerable money and effort could be expended in attempts to reduce blooms by implementing pollution control strategies, only to find some years into the future that HABs have not abated, or that species have been replaced by another assemblage that is equally harmful. On the other hand, if adequate field, laboratory and modeling studies of specific situations are conducted, the risks

of misdiagnosis are limited. The recourse of no action may lead to profound and long-term ecological damage in cases where nutrients are indeed the problem.

6.1.3 Ballast Water Introductions

General Description

Ships have long been recognized as a major vector for the introduction of non-indigenous and harmful organisms (Rosenthal 1981). The intensity of shipping and the structure of the fleets, however, have since undergone drastic changes, in particular during the past decade. Invasions and population explosions of exotic species in various parts of the world are causing significant ecological and economical damage, some from introduced HAB species (e.g., Hallegraeff et al. 1997).

The reason for growing concern about ballast water introductions relates to the rapidly changing scenarios of human use of coastal habitats, with increasing pressure on natural coastal resources. Scenarios affecting the likelihood of ballast water-mediated changes in coastal habitats that can result in the introduction of HAB species include: 1) Increasing density of mariculture units near shipping routes (more chances for transfer); 2) Increasing sea traffic (number of ships and routes; larger critical mass); 3) Increasing speed of ships (shortened transfer time, higher survival chance); 4) Increasing size of ships (larger ballast volumes, more oxygen available); and 5) Changing human population density in the coastal zone (more activities).

A recent estimate indicates that 80% of the world's cargo is transported via ships and the volume of ballast water released into coastal waters may be on the order of 10 billion tons per year (Rigby and Hallegraeff 1996). A minimum of about 3000 aquatic species is transferred by ballast water intercontinentally every day. The transfer of microalgae through ballast water (including HAB species) is no exception. Ballast water is mainly released in harbors or inshore areas, which is where mariculture facilities are located. Native and wild species as well as mariculture species are at particular risk and suffer when HAB species are released in their vicinity.

Effectiveness and limitations

A number of strategies have been investigated that could eliminate the threat from viable cysts or cells of HAB species being introduced to a region via ballast water. These include ballast exchange at sea (Rigby et al. 1993), sterilization with hydrogen peroxide, chlorine, or sodium azide (Ichikawa et al. 1992; Montani et al. 1995), heat treatment at 40-45°C (Hallegraeff et al. 1997), and electric shock (Montani et al. 1995). Rigby et al. (1993) document the extremely high cost of peroxide treatments at nearly US$500,000 per trip, and chlorine at $50,000 per trip. Of the many options that have been considered, heat treatment using waste heat from the vessel's engines is perhaps one of the most feasible and environmentally friendly, with a one-time cost (unspecified) of modifying engineering designs (Hallegraeff et al. 1997). It may be that the cost of this approach will be too high for most ships, but it is an option that should be considered. An additional concern, however, is the need to install a treatment system that controls for all types of potential invaders – not just HAB cells or cysts. A treatment option like ballast water heating may not be effective against resistant organisms, like bacterial pathogens.

Reballasting in the open sea is another attractive option, but it has been criticized because it does not remove HAB cysts that have accumulated in sediments at the bottom of the ballast tanks. Nevertheless, the International Maritime Organization (IMO) has prepared voluntary guidelines for this procedure which were adopted in 1993.

6.1.4 Species Introductions via Mariculture Operations

There are documented cases of unintentional introductions and transfers of non-indigenous species (macroalgae and associated fauna) through mariculture activities in coastal and marine waters (Rosenthal 1980; Carlton 1985). Few scientific studies have been undertaken to assess the risk of red tide/HAB species transfer with live transport of seed oysters, market shellfish, and live fish (Honjo et al. 1998; Scarratt et al. 1993), but these demonstrate the potential for transfer of live dinoflagellate cells in aquaculture shipments. Laboratory studies also show that viable cells of thecate dinoflagellates can be released in large quantities from feces of bivalves exposed to toxic blooms (see Section 6.5.3). These may serve as an algal inoculum and pose a risk if shellfish are relayed from toxic to unaffected areas for toxin depuration. Holding of animals in contained, land-based systems for a period (a few days) sufficient for gut evacuation is therefore recommended prior to transfer to the field.

During the 2000 bloom of the PSP-producer *Gymnodinium catenatum* in New Zealand, the aquaculture industry was severely affected by widespread prohibitions on the movement of shellfish (e.g. mussel and oyster seed) from affected to unaffected areas to prevent translocation of cysts (Mackenzie 2001). A method was subsequently developed for separation of mussel seed from *G. catenatum* resting cysts associated with drift algae, which allowed transfer of clean seed to unaffected growing areas.

6.1.5 Prediction

6.1.5.1 Models

One aspect of impact prevention would derive from accurate predictions of the timing or transport pathway of HAB outbreaks. This would give fish farmers and other affected parties the opportunity to take actions that can minimize impacts, such as selling the fish before they are killed by the HAB, or moving fish cages to refuge sites. Prediction of HABs is an important and useful goal, yet prediction can only come from a detailed understanding of the factors controlling bloom dynamics. Our level of knowledge about each of the many HAB species varies significantly and even the best studied remain poorly characterized with respect to bloom or population dynamics. The end result is that despite the proven utility of models in so many oceanographic disciplines, there are no truly predictive models of population development, transport, and toxin accumulation for any of the major HAB species worldwide. Furthermore, given that a feature common to most HABs is a strong association with physical factors - in particular, meteorological forcings such as wind, sunlight, and rainfall, our predictive capability can only be as good as our ability to predict the weather. This is nevertheless a worthy goal, as even warnings on time scales of a few hours to a few days can be of great value.

A variety of models have been developed for HABs (reviewed in Franks 1997), ranging from formulations that simulate life cycle dynamics (Eilertsen and Wyatt 2000) to physical/biological coupled models that simulate small-scale features such as population accumulations at fronts (e.g., Franks 1992) or the large-scale population dynamics of a particular HAB species in a given region (e.g., *Alexandrium fundyense* in the Gulf of Maine; McGillicuddy et al., unpub. data). Many other models are under development, some of which aim to be predictive, but these are largely in the developmental stage at present, and there are few publications that can be sited at present.

6.1.5.2 Remote sensing

Another approach to impact prevention would be early warning based on remote sensing of HABs. Satellite remote sensing has long been considered a tool with great potential for detecting and tracking

HAB populations, but this technology has not yet fully lived up to this promise (see Section 3.4.4.5). The situation is changing, however, and the advent of new satellite sensors such as SeaWiFS and better algorithms for resolving chlorophyll levels in coastal waters is leading to more attempts to use this approach in HAB research and management. It has also been demonstrated (e.g., Anderson and Keafer 1993; Tester and Steidinger 1997) that sea surface temperatures can be used to track water masses that are associated with certain HAB species. Nevertheless, the present situation remains one of potential rather than actual application of remote sensing as a forecasting or prediction tool. Studies are needed that obtain satellite images of ocean color and SST concurrent with field measurements on bloom distribution or toxicity under a variety of meteorological conditions. With sufficient background information of this type, development of conceptual models will be possible, allowing remote sensing images to be used in the future for forecasts of impending outbreaks along specific sections of a coast. Progress in this area should be accelerated by the launch of several more satellites designed to collect ocean color data. Remote sensing from aircraft can also provide useful information with less reliance on clear weather conditions, but the expenses are significant, and development efforts are again needed to determine the types of sensors and the oceanographic features that should be monitored in this fashion. In this context, it should not be forgotten that human observers on airplanes are very effective in detecting some HAB outbreaks. These observations can be very useful in a management program, especially if a regular surveillance system is established, such as with commercial passenger aircraft, a local flying club or a military flying service. Coupled with a rapid response capability, in which a small vessel could be dispatched to sample and characterize the observed bloom, this can be an effective management tool.

6.2 Bloom Control

The direct control of blooms has been attempted or proposed using a variety of chemicals or additives designed to kill HAB cells in the water column. Direct control can also involve the physical removal of the cells, either through skimming or some other mechanical means, the addition of a flocculant that scavenges cells and transports them to bottom sediments, or even biological control through the introduction of a predator or other pathogenic agent that can destroy HAB cells.

6.2.1 Chemical Control

General Description

This category of mitigation relies on chemicals to inhibit or destroy HAB organisms. Many chemicals have been proposed for use as HAB mitigation agents. Some are supported by claims that the chemicals are able to mitigate HABs. However, these claims are generally unsupported by independently produced data.

There are many chemicals capable of destroying algal cells, but only those which show some degree of specificity towards the HAB algae should be considered. Many are used for algal control in freshwater reservoirs, but then the objective is control of all algal growth, not just one or several selected species. In the sections below, only chemical strategies that have been used or considered in the HAB context are discussed.

Copper: The first, (and probably the most extensive) attempt to control an HAB on a major scale took place in Florida in 1957 (Rounsefell and Evans 1958). Based on observations that low concentrations of copper ions killed *Gymnodinium breve* and that copper is a natural constituent of seawater, copper sulfate in powder form was dropped by crop-dusting aircraft over more than 10,000 acres (about 16 square miles) along 32 miles of shoreline. The rate of application was 20 pounds per acre (6.5 tons per square mile) at a

cost of about US$4 per acre or US$1,000 per mile of beach (1957 dollars), assuming the spraying covered a half-mile width. A minor attempt to add copper by dragging burlap sacks containing copper ore was also attempted, but was only effective in a small area and was too slow to be used on a major outbreak.

At the beginning of the dusting operation, *G. breve* cell concentrations were high (1-10 million cells per liter) at a number of locations, well above the threshold for fish mortality for that species. Application of the copper powder quickly reduced these concentrations to near zero, but the effects were not long lasting. Within 2 weeks, two of the five localities that were treated had *G. breve* concentrations that were once again high enough to kill fish. It is now clear that cells from waters outside the treatment zone were carried by tides and currents towards shore, re-establishing the red tide. This is consistent with the concept that blooms of this species in nearshore waters originate offshore (Tester and Steidinger 1997) and are delivered to the coast by hydrographic forcings, although that was not known at the time.

The conclusions of the copper dusting can be summarized as follows (Rounsefell and Evans 1958).

1. For areas close to land (up to about 3 miles offshore) and in shallow water (up to at least 30 feet), the dusting was effective in destroying *G. breve* cells;

2. Within 10-14 days, some treated areas had high *G. breve* cell concentrations again;

3. The spraying was effective against the *G. breve* cells, but the beaches were still littered by dead fish killed elsewhere and brought to shore by tides and winds. (It is not clear whether these fish were killed by the copper treatment (and its associated toxin release from ruptured cells) or by bloom populations in other locations.)

4. Residual effects from the treatment were minimal, as copper concentrations dropped to background levels within a few days of the additions.

5. The chief benefit from this type of unrestricted spraying was the temporary relief from the choking and coughing caused by airborne toxin from *G. breve* cells.

Rounsefell and Evans (1958) concluded that dusting with copper sulfate was too expensive as a control method for red tide/HAB outbreaks of major size. The authors concluded "Until a cheaper and more effective means of control is discovered, the application of copper could serve in local situations to give immediate but temporary relief from airborne toxin."

One of the issues that remains unresolved about the use of copper (or any chemical that breaks up cells) is based on the presumption that toxin is released into the seawater and will kill fish and other organisms, just as it does if no treatment is attempted. Steidinger (1983) states: "Any disruptive chemical ... that lyses [breaks] cells will increase the toxicity of the growth medium." A "worst-case" scenario is that the toxin released by the copper was lethal to as many fish as would have died from the bloom had no treatment been attempted. On the other hand, the released toxin might have been diluted by mixing or been degraded by intense sunlight such that the ecological impact would be minimal. The actual impact probably lies between these two extremes, but no publications are available that document fish or animal mortalities associated with the operation itself. It is thus not possible to compare the "one-time" mortalities from toxins released during the control treatment with the natural (and perhaps sustained) mortalities that would have occurred with an untreated bloom that impacts the coast for weeks or months. Any future efforts to use copper or other chemicals must be preceded by an evaluation of the stability of toxins in natural waters and an assessment of the extent to which released toxin can negatively impact the system being treated. Its widespread use is not even encouraged in lake restoration, where leading technical authorities have concluded its usefulness is outweighed by its drawbacks (Cooke et al. 1993)

Another unexplored issue is that of accompanying or "collateral" mortality of co-occurring organisms due to the treatment chemical itself. For example, copper is broad-based in its lethality, affecting a wide range of aquatic plants and animals. It is likely that a large number of planktonic and benthic animals were affected by the 1957 treatment in Florida, and thus that in areas with strict environmental regulations, a chemical with this lack of specificity might never be allowed.

Aponin and other algal chemicals: A chemical called "aponin" was suggested by Martin and co-workers (e.g., McCoy and Martin 1977) as a control agent for toxic *G. breve* blooms. Aponin is a sterol surfactant produced by the blue-green alga *Gomphosphaeria aponina*. The purified compound disrupts *G. breve* cells, and living *G. aponina* cells release aponin into the seawater and cause breakage of *G. breve* within 4-10 days. Despite these positive results, objections to the use of aponin or *G. aponina* in bloom control are many (Steidinger 1983). These problems are: 1) aponin is hydrophobic (like oil), and thus would have to be chemically modified for use in water. Any chemical added as a "carrier" could be degraded by bacteria, significantly reducing the expected life of aponin activity; 2) aponin loses 75% of its activity in the pH range of seawater; 3) aponin disrupts *G. breve* cells, and thus releases toxin into the water where it can kill fish; 4) the effects of aponin on marine algae and organisms that co-occur with *G. breve* has not been investigated; 5) the amount of purified aponin required to treat a major bloom is prohibitive with respect to the costs of purification and of growing huge quantities of cells; 6) application of aponin would require Government approval, and such approval is unlikely until all possible "side-effects" of the chemical can be evaluated and a thorough impact assessment conducted. A rational assessment of these issues leads to the conclusion that aponin does not have significant promise as a control strategy at this time. The potential use of *Gomphosphaeria aponina* (or *Nannochloris* spp.) as a biological control agent is discussed in Section 6.2.4.5.

Kakizawa et al. (1988) found that various forms of octadecatetraenoic acid extracted from brown algae (*Cladosiphon okamuranus*) had allelopathic effects on some microalgae. These substances and numerous other compounds from macroalgae and other natural aquatic species may hold promise for HAB control, but much work remains to identify and isolate them as well as to investigate their effectiveness and specificity of action.

Ozone: See Section 6.4.6.

Other Chemicals: The US Fish and Wildlife Service Bureau of Commercial Fisheries initiated a major program to screen chemicals for controlling *G. breve* blooms in 1959. Marvin (1964) reports that 4,700 compounds were evaluated, predominantly organic in nature. The initial phase of the study identified acutely toxic compounds capable of causing 100% mortality of *G. breve* within 24 hours at a concentration of 0.04 ppm in culture medium made with filtered seawater. Only 191 chemicals met this criterion. The second phase of the investigation then determined the minimum concentration of each compound needed to cause mortality of *G. breve*, and the specificity of the compound for *G. breve*. These tests were conducted in culture medium made with artificial seawater (i.e. made from distilled water and chemical salts). Four compounds were toxic at 0.0004 ppm, five at 0.001 ppm, 20 at 0.004 ppm, 32 at 0.01 ppm, and 120 at 0.04 ppm. Only those compounds toxic at < 0.01 ppm level were evaluated further. Specificity was evaluated by testing the compounds against commercially important organisms (post-larval shrimp, small fish and crabs, and other forms of marine life from the Gulf of Mexico). The criterion used was that a compound must not kill more than 50% of any of the indicator organisms at 0.01 ppm. This reduced the potential control chemicals to 6, which were then re-checked against *G. breve* using large cultures made with Gulf of Mexico seawater. Results were disappointing, as 5 of the 6 compounds were not toxic in 24 hours at 0.01 ppm, and two of those remained non-toxic after 48 hours of exposure. The compounds that were toxic at 0.01 ppm at 48 hours were: tellurium diethyldithiocarbamate; the sodium salt of dimethyldithiocarbamic acid mixed with the sodium salt of 2-mercaptobenzothiazole; sulfide, bis (2-hydroxy-3-bromo-5-chlorophenyl)-bis- dimethylaminobutyne mono salt; and sulfide, bis (2-hydroxy-3-

bromo-5-chlorophenly)-cyclohexamine mono salt. These results suggest that Gulf of Mexico seawater contained an inhibitor which reduced the effectiveness of the toxicants tested. More testing was planned using the short list of effective compounds in seawater-based culture medium, but no reports are available, suggesting that the program was terminated before further results could be published.

Effectiveness and Limitations

Attempts to use chemicals to directly control HAB cells in blooms will encounter many problems and environmental objections. The use of copper sulfate in the 1957 Florida *G. breve* control effort highlights several of these problems. As discussed above, toxins may be released from cells as a result of the treatment, which theoretically can cause fish mortalities and other impacts similar to those the control operation is attempting to prevent. A second concern is that the chemicals are likely to be non-specific and thus will kill co-occurring algae and other organisms indiscriminately. Efforts to find a "magic chemical bullet" that will somehow kill only a particular group of HAB species seem futile, as it is difficult to imagine a specific target for a chemical that is characteristic only of that one organism.

However, it may be that the tendency to require extreme specificity in mortality is unjustified, at least in comparison to agricultural control practices, which typically impact a broad range of organisms, only some of which are harmful. This is clearly an issue that regulatory agencies and various public and private interest groups must discuss and resolve if chemical control of HABs is to be attempted. Control strategies that must meet a "magic bullet" requirement may be unrealistic, whereas acceptance of a lower level of specificity might open up numerous options.

In summary, although chemicals and integrated pest management are used extensively in agriculture and insect control on land, application of this technology to the ocean is new and controversial. Environmental concerns are significant in this regard, as it seems unlikely that a chemical compound can be identified that specifically targets only HAB species and leaves other algae and marine animals unaffected. Each candidate chemical will require extensive testing for lethality, specificity, and general safety, and each must surmount significant regulatory hurdles. Although direct chemical control of HABs is not a strategy of choice given other potentially more benign alternatives (e.g. clays), the success of this approach in terrestrial systems suggests that it should not be completely ruled out.

6.2.2 Flocculants (clays and long-chain polymers)

General Description

A flocculant is a material that, when added to water, scavenges suspended particles until the "flocs" begin to become heavy and fall to the sediments below. Inorganic flocculants (e.g., aluminum sulfate or various ferric salts) are commonly used to purify fresh water in reservoirs. These compounds typically neutralize the negative charge of colloidal particles, allowing aggregation and sedimentation. Macromolecular flocculants are synthetic molecules that collect particles by means of a process called bridge formation. Huge polymer molecules are very effective in this regard, the most commonly used being polyacrylamide, which accounts for 90% of the market. There are regulations concerning residuals, however. The Japanese experience with both inorganic and macromolecular chemical flocculants is that they are generally too expensive for large scale bloom control (Shirota 1989). There is also concern about the application of metals such as ferric sulfate, as iron has the potential to stimulate blooms.

One flocculant that shows considerable potential is clay. The natural water-clearing properties of clays are often seen during and immediately after storms when the seawater near the mouth of rivers becomes turbid from clay minerals eroded from land. This turbidity decreases dramatically several days later and the

seawater can become very clear, with considerably enhanced transparency. This is a result of flocculation. The clay particles have adsorbed inorganic and organic materials, and aggregated algae and other particles to form a floc. The floc continues to grow in size as it accumulates particles, and eventually falls to the sediments when its density exceeds that of seawater.

Algae have cell walls composed of proteins, carbohydrates, and other materials, which can hydrate or ionize in seawater, giving the cell a surface charge. In the simplest terms, the addition of clay to a suspension of cells alters the charge distribution so that cells may adhere to the clays. The variable physiology of different algal species affects the degree to which they can be removed by flocculation, and the structure and charge of various types of clay can affect flocculation as well. Results from one type of clay applied to one specific organism cannot therefore be extrapolated to other clays or other organisms. Interestingly, Shirota (1989) claims that the clays not only flocculate and remove cells by sedimentation, but that some cells are killed and ruptured by the treatment. A mechanism was proposed whereby aluminum is eluted from the clay by ion exchange and is released into the seawater where it kills HAB cells. This may be true for *Chattonella*, but needs to be confirmed for other HAB species. If clay treatment ruptures HAB cells, the impacts of released toxins must be considered.

Effectiveness and Limitations

The Japanese (reviewed in Shirota 1989), Chinese (e.g. Yu et al. 1994a,b,c), Koreans (Na et al. 1996), and Americans (Sengco et al. 2001) have studied the theory behind clay as a flocculant in seawater, and have tested a variety of natural and treated clays on HAB species in culture. Depending on the treatment used, removal of 95-99% or more of cultured cells from some HAB species has been accomplished with clay additions. The Japanese consider kaolinite to be a very poor flocculant in seawater, with or without acid treatment (Shirota 1989), and propose the use of montmorillonite at 200 g m^{-2} of surface to be treated. Chinese workers claim that the coagulation of kaolinite is much greater than that of montmorillonite (Yu et al. 1994c), that no acid treatment is needed, and that 1/10th as much clay is needed for effective cell removal (e.g., 20 g m^{-2}). This perhaps relates to the type of kaolinite used. The kaolinite in the Chinese experiments had a three-layer structure, similar to montmorillonite. Japanese kaolinite has two layers. Furthermore, additional papers by Yu et al. (1994d) demonstrate that the removal capacity of kaolinite can be substantially enhanced by modifying the surface of the clay by pre-treatment with polyhydroxy aluminum chloride (PAC) to increase the bridging between surfaces. When PAC is added, more than 90% of the cells of the dinoflagellate *Prorocentrum minimum* are removed from suspension using clay at a concentration of 0.1 g L^{-1}, compared to 2 g L^{-1} with no treatment - a 20-fold improvement. The effectiveness of clay treatment varies greatly with the algal species used and the clay mineral dispersed.

In practice, the Japanese have used clay to treat natural blooms on several occasions. Starting first with laboratory tanks, they studied the effects of different clay loadings on fish survival (Shirota 1989). With clay loadings ranging from 0 to 40,000 ppm (40 g L^{-1}), 40% of the sea breams survived at 40,000 ppm, and 100% were normal at 1,500 ppm. At 2857 ppm, the fish were stressed, but did not die.

Clay was then dispersed in the vicinity of mariculture cages where fish were dying during a *Cochlodinium* bloom (Shirota 1989). Results of the clay treatment, which was applied at 110 to 400 g m^{-2} (yielding 110 to 400 ppm at a depth of 1 m) are given in Table 6.1. As can be seen from the table, the method was deemed very effective by the fishermen, as virtually no fish mortality was observed in field trials even though a dense bloom was present. Some dying fish, which were struggling to breathe at the surface of the water during the bloom even recovered vitality once the clay was applied. To make this method more acceptable to the fishermen, problems needed to be resolved with respect to the cost of the clay (reported to be US$200-300 per ton), storage of the clay on-site, and dispersion methods. No cost estimates of this clay treatment were provided by Shirota (1989).

Japanese workers also looked into dispersal of the clay from the air as a strategy for large-scale bloom treatment. A detailed analysis of this operation is given in Shirota (1989). Helicopters were used, each capable of carrying 800 kg of clay. Both powdered and liquid clay were used, and the latter proved most effective. Clays were applied in the range of 200 g m^{-2}, 20-50 x the concentrations that are now considered sufficient for good cell removal (Sengo et al. 2001). Although Japanese researchers concluded that airborne dispersal of clay is feasible as a bloom control strategy, no subsequent publications are available that describe further tests of the concept or actual control attempts on major HABs. No costs were provided for these tests, but they are sure to be significant due to the high cost of chartering helicopters.

TABLE 6.1. Evaluation of the clay flocculation method by Japanese fishermen during a *Cochlodinium* bloom. (Modified from Shirota 1989.)

Item	District			
	Hegushi	Wakizaki	Ikara	Average %
% Effectiveness of treatment				
- effective	80.0	60.0	81.8%	78.3
- somewhat	16.7	40.0	18.2%	19.6
- unclear	3.3	0	0	2.2
Effect on fish (%)				
- fish died	3.3	0	0	2.2
- Slight effect	23.3	0	45.4	26.1
- No effect	73.4	199	54.6	71.7
Application amount (kg)				
20 - 100	6.6	0	0	4.3
120 - 200	20.2	40.0	0	17.4
220 - 400	20.0	20.0	0	19.6
420 - 600	26.7	20.0	0	19.6
More than 600	26.7	200	100	43.5
Place of clay dispersion				
- into net cage	0	12.5	0	1.6
- around net cage	57.5	50	78.6	61.3
- far from net cage	42.5	37.5	21.4	37.1
Problems				
- dispersion method	12.8	14.3	0	16.9
- cost	12.8	71.4	66.7	32.3
- storage of clay	25.6	14.3	0	16.9
- damage to fish	10.3	0	0	6.2
- time to prepare clay	13.4	0	0	9.2

Chinese workers are now considering applying these methods to HABs on the Chinese coast (Z. Yu, pers. comm.). The cost of clays directly from the mines in China is about US$15 per ton. If the clay were used at about 200 g m^2 or 570 tons per square mile, the materials cost would be about US$12.50 per acre for a single treatment. Several applications through time might be necessary for effective bloom control. A coagulant added to the clay to improve its removal efficiency is made from recycled aluminum scraps and

waste acid, so it is not expensive. It is also used at a very low concentration, and thus adds only marginally to the cost of the clay, although Chinese colleagues could provide no specifics. Logistically, the clay loading is 9x the weight of the copper sulfate used in the 1957 control effort in Florida by Rounsefell and Evans (1958), but at 1/4 the cost (without adjustment for nearly 40 years of inflation).

The most recent efforts to use clay to control HABs have been conducted by the Koreans (Na et al. 1996; H.G. Kim, pers. comm.) In 1996 and 1997, workers used clay to control blooms of *Cochlodinium polykrikoides* in the vicinity of a large fish mariculture industry. The same organism had bloomed in 1995, causing over US$100 million in losses due to fish mortalities (H.K. Kim, pers. comm.). In 1996, 100,000 pounds of clay (termed yellow soil or loess) were dispersed over 100 miles^2 using water sprayed onto a mound of clay on a flat barge. The fishermen waited until mid-day to apply the clay, since the *Cochlodinium* cells were in subsurface layers, and did not kill the fish until the layers rose to the surface around mid-day. Clay was then applied in several applications for 3-4 hrs, and then stopped until the next day. The target loading for each application was 400 g m^{-2}. No attempt was made to measure the actual concentration of clay applied, but this crude application procedure undoubtedly resulted in highly variable and inefficient loadings. The operation was considered a major success, however, as HAB-associated fish mortalities were reduced to less than 1% of those experienced in 1995. In 1997, the operation was repeated during another *Cochlodinium* bloom, with over 50,000 tons applied before a storm dispersed the remainder of the bloom.

The cost of the clay treatment in 1996 was estimated to be US$1 million (H.K. Kim, pers. comm.), although it should be noted that the clay used had at least 10-fold lower efficiency for removing HAB cells compared to others that have been tested (Sengco et al. 2001), and the application procedure from a barge was highly inefficient and wasteful.

Practicality

If clay treatments are to be used to control HABs, a number of issues must first be resolved. First, a local source of inexpensive clay needs to be identified, since transportation costs would quickly render this method uneconomical. Clays are very different in their abilities to flocculate cells (Sengco et al., 2001), so a screening program of local clays would be needed as a research project.

Another issue that must be addressed relates to the effect of sedimented or resuspended particles on benthic organisms. Evidence that the clay/organic aggregates are not detrimental is found in the studies of Portmann (1970) and Howell and Shelton (1970) who investigated the effects of clay from pottery operations on the bottom fauna of two bays near Plymouth, UK. As a result of the area's pottery industry, clay was distributed over 48 km^2, accumulating in bottom sediments at the very high loading of 188 kg m^2. Fish and benthic organisms were abundant in the area, however, and many seemed to thrive with the clay substrate. Shirota (1989) reports that clay/organic flocs are an excellent food source for sea cucumbers, so it is likely that many other benthic animals would benefit from the nutrition in the material carried to the bottom with the clay. However there are also numerous studies showing detrimental effects of suspended sediments and of fine-grained substrate on benthic suspension-feeders (e.g. Bricelj and Malouf 1984; Grant and Thorpe 1991; Cranford and Gordon 1992). Additionally no studies have been conducted on the possible impacts of flocculation and sedimentation of toxin-containing organisms. Stable toxins like the brevetoxins would likely persist in the sediments for months, entering the food chain when the organic detritus is consumed by benthic organisms, and being released into the water as that material decays. Accordingly, other species such as *Heterosigma akashiwo* that have highly labile and possibly low concentrations of toxins may be more suited for the use of flocculants. Special consideration should be given to effects of clay on coral reefs and other sensitive habitats and life history stages. If clay is to be seriously considered as a bloom control strategy, an area for future study is clearly the fate and effects of sedimented and resuspended toxins.

Depending on the amount of clay that is dispersed in a HAB control effort, the floc loadings to bottom sediments could be significant, so, there is reason to be concerned about oxygen depletion caused by the sedimented biomass. However, Shirota (1989) reports that clay deposited on bottom sediments can actually reduce the risk of low dissolved oxygen in bottom waters, presumably because the clay layer seals off organic-rich sediments from the water column. This is clearly another area where research is needed.

6.2.3 Physical Control

There are a number of possible bloom control strategies that involve physically destroying the cells or removing them from the water.

6.2.3.1 Skimming of Surface Water

Shirota (1989) describes a study conducted in Japan whereby a ship attempted to collect HAB plankton using equipment that aggregated the cells and then separated them from the seawater. This equipment was called "pressure flotation separation equipment", used predominantly in the treatment of wastewater from surimi fish-paste production. With this process, HAB algae are theoretically scavenged by fine bubbles released into the water. The cells float to the sea surface where they are removed by a skimmer. A prototype of this equipment was tested in 1973, but the results were not encouraging. Workers tried to add the flocculants PAC (alum) and acrylamide to enhance particle capture, but ran into serious problems with the filtration of the resulting cell/particulate aggregates. This method was not pursued further.

6.2.3.2 Ultrasonic destruction of HAB cells

In laboratory experiments conducted in Japan, cells of *Rhodomonas, Heterosigma, Chattonella,* and *Prorocentrum* were exposed to ultrasonic waves at frequencies of 19, 1600 and 400kHz. The high-energy treatments killed most of the algae in less than 2 minutes. However, the ultrasound was only effective over a 50 cm depth and was not useful at low cell concentrations (Shirota 1989). This concept was therefore abandoned.

6.2.4 Biological Control

6.2.4.1 Grazing by zooplankton and suspension-feeding benthos.

There are a variety of organisms that could conceivably be used to control red tide blooms, but in reality, this approach towards control has many logistical problems and is a long way from the application stage. One obvious group of organisms to consider as a biological control agent is zooplankton, small animals which co-occur with algae and eat them as food. For example, Martin et al. (1973) suggested that marine ciliates could be cultured and used for control of *G. breve* cells. Likewise, Shirota (1989) indicates that the Japanese considered the use of zooplankton such as *Acartia clausi* in controlling red tide blooms. However, both Shirota (1989) and Steidinger (1983) provide calculations that illustrate the logistical impracticality involved in growing red tide predators in the laboratory in sufficient quantity to control blooms. Both authors arrive at estimates that are completely unrealistic with respect to cost, space, and facilities. For example, Shirota calculates that a 33,000 m³ tank would be needed to hold sufficient zooplankton to treat a red tide 100m long and only 1 m deep. Furthermore, this tank would have to be

maintained constantly during the red tide season so that the zooplankton would be ready for deployment at the appropriate time. This is not an idea worth pursuing.

Japanese researchers took the predator concept one step further and examined whether bivalves such as clams or oysters could be placed in a wall of cages and used as a massive filter to clear the water of toxic algae. Bivalves vary in their ability to filter water, but some can filter 10-50 liters per hour. Here again, the calculations reached impractical levels, as a barrier of cages containing oysters would need to contain 720,000 individuals just to treat a volume of water passing through a square 50 m on a side (Shirota 1989). However, grazing by suspension-feeding bivalves is known to play an important role in controlling phytoplankton biomass in shallow, enclosed estuaries (e.g. Officer et al. 1982; Alpine and Cloern 1992) and could therefore potentially provide an effective control mechanism in shallow waters (coastal bays or aquaculture ponds). Feeding rates are generally inhibited at bloom levels of toxic species. However, it has been suggested that bivalves at historical peak abundance levels in Great South Bay, New York, could have the potential to control the initiation of brown tides at low densities ($\leq$ 20,000 cells ml^{-1}) of *Aureococcus anophagefferens*, which are below the cell density threshold that inhibits clam filtration rates (Schaffner 1999; Bricelj et al. 2001). It was estimated that a bottom stocking density of 34 clams m^{-2} would be sufficient to equal the doubling rate of *A. anophagefferens* in a 1 m water column, a typical depth in large portions of Great South Bay (Bricelj et al. 2001). Even higher grazing pressure is expected from abundant populations of small bivalves, e.g. *Gemma gemma* and *Mulinia lateralis*, as filtration rate per unit biomass is greater for small individuals. Polyculture of bivalves in shallow shrimp ponds may also allow control of algal blooms, which can cause anoxic events and shrimp mortalities in areas of extensive shrimp culture, such as the Bohai Sea region in northern China. Further studies are required to test the feasibility of this biological control mechanism in shallow ecosystems.

6.2.4.2   Viruses

Other forms of biological control are also possible, but remain poorly investigated in the context of HABs. For example, viruses have the potential to be highly specific and effective control agents. Viruses are very abundant in coastal seawater and are now recognized as being significant in the dynamics of marine ecosystems (Fuhrman and Suttle 1993). Although most of these viruses probably infect bacteria, there are viruses, which do infect algae, and some have been directly implicated in the demise of red or brown tide blooms. One example is from Norway, where the collapse of a bloom of the coccolithophorid *Emiliania huxleyi* occurred simultaneously with the appearance of many viruses in the surrounding water and inside the algal cells (Bratbak et al. 1993). Similarly, Nagasaki et al. (1994a,b) linked the collapse of a bloom of *Heterosigma* to the appearance of virus particles within the cells. Viruses have also been observed in brown tide cells on Long Island and suggested as a natural control mechanism (Sieburth et al. 1988; Milligan and Cosper 1994). There have also been reports that viruses can affect community structure by preventing a species from blooming. For example, attempts to establish fresh water cyanobacterial blooms in large containers (mesocosms) met with mixed results because of the presence of viruses which infected the cells (Dejardins and Olson 1983).

Our knowledge of viruses that infect marine phytoplankton is limited, however, and is based either on observations from a few infected cells collected during blooms or on the scattered isolates which have been cultured. Thus far, no viruses have been cultured from infected dinoflagellates.

On a theoretical level, there are a number of features which make viruses attractive as biological control agents (Suttle 1996). First, viruses replicate rapidly, releasing hundreds of viral particles when every host cell is disrupted. During a bloom, the rate of viral propagation would potentially be accelerated because infection depends upon the frequency with which the virus encounters host cells (Suttle 1996). HABs would appear to be ideal for rapid viral multiplication. Another important feature is that viruses tend to be host-specific. This means that one could potentially target a specific algal species, leaving closely related

organisms unaffected - the ultimate "magic bullet". In reality, however, viruses are sometimes so host-specific that they are unable to infect different genetic strains of the same host species. This is perhaps the reason that many viruses are able to co-exist with the species that they infect, since a bloom population of an algal species in nature is often a mixture of different genetic strains of a single species. Thus, some cells would be infected, and others not.

The foregoing highlights one of the problems that stands in the way of using viruses as biological control agents for HABs - the very existence of a bloom suggests that indigenous viruses are unable to keep the algal population in check. Control of a bloom requires that a pathogen be located and isolated that is capable of causing the lysis (rupture) of a specific bloom organism, yet the persistence of a bloom suggests that no such virulent pathogen exists at that site. This is related to the tremendous genetic diversity among phytoplankton and viral populations. Even though cells in a bloom may appear identical, and the viruses infecting that bloom may appear morphologically indistinguishable as well, there is a great deal of genetic heterogeneity in both populations. An individual virus will be able to infect only certain strains within the HAB population, so it becomes necessary to isolate a suite of viruses to obtain a broad spectrum of infectivity. Given a situation where the HAB problem has persisted for decades, one can expect a co-existence to have developed between viruses and certain HAB populations. It would thus be very difficult to isolate "local" viruses that could infect a significant portion of the HAB populations.

Viral control of an established bloom will likely require the introduction of a virus or viruses that have a broad host range and which are isolated from the location or time when the bloom was not present (Suttle 1996). This suggests that viruses obtained from HAB populations from other parts of the world might be more useful than those isolated from local waters. Whether such viruses exist is a legitimate question, and whether they could be used effectively in control remains unknown as well. Another concern is that environmental regulations concerning the release of a viral pathogen might be severe. There might well be concerns some viruses might be able to switch hosts, such that a control strategy might have unexpected consequences within the planktonic community. Some research effort could be devoted to further exploration of this avenue of biological control, but the potential limitations of this strategy should be recognized as well.

6.2.4.3 Parasites

Parasites also have potential as biological control agents for HABs. There are a variety of different parasite species which can infect marine organisms, and a number of these are dinoflagellates, which infect other dinoflagellates. For example, the dinoflagellate *Amoebophrya ceratii* is a well-known intracellular parasite of free living dinoflagellates, including toxic dinoflagellates in the genus *Alexandrium* (Taylor 1968; Nishitani et al. 1984). The highly virulent nature of parasite infection of dinoflagellates has led to the suggestion that these might be effective in controlling HAB populations (Taylor 1968).

Parasites attack dinoflagellates in much the same way that viruses do. A single dinospore attaches to the host cell wall, penetrates it, and begins to multiply inside the cell. When the cell finally bursts, it releases hundreds of new dinospores, which then move on to infect other hosts.

A key issue with respect to biological control is that of host specificity, as it would be ideal if an introduced parasite would only attack the target HAB organism and then die-off after the demise of the bloom. Nishitani et al. (1984) argue that *A. ceratii* lacks the host specificity needed if this parasite is to be used to control their target species, *A. catenella*. Their results indicate that *A. ceratii* infected numerous other co-occurring dinoflagellates as well. More recent evidence (W. Coats, pers. comm.) suggests that *A. ceratii* may have more host specificity than previously thought. Resolution of these specificity issues is clearly important, although an argument can be made that absolute host specificity should not be a

requirement - that such standards are seldom imposed in control of terrestrial pests. In agriculture or in pest management (e.g. mosquitoes), for example, applications of chemicals or other control agents are seldom species-specific. In such situations, the ecological consequences of the "collateral" mortality of non-target organisms have been an acceptable cost relative to the benefits to society or commerce from the control of the target species. These concepts have never been openly discussed or debated in the context of marine planktonic ecosystems, and they should be. Whatever the outcome, however, parasites remain a fruitful, though long-term area of investigation with respect to the control of HAB populations.

6.2.4.4   Bacteria

A body of work by Japanese scientists suggests that bacteria could play an important role in controlling HABs. An intriguing example is the *Gymnodinum mikimotoi* - killing bacterium described by Ishida 1999). A bacterial strain isolated at the end of a *G. mikimotoi* bloom exhibits strong and very specific algicidal activity against this dinoflagellate species. Cultures of *G. mikimotoi* are completely destroyed within 24-38 hrs of the time a bacterium is introduced to a culture. Further studies showed that this "killing substance" was produced in response to materials excreted from the dinoflagellate in a highly specific manner; excretion from a variety of other species did not elicit production of this substance. In turn, the compound's algicidal activity was restricted to *G. mikimotoi* and one other closely related species but had no effect on other dinoflagellates or flagellates tested. A second example of a potentially specific bacterial/algal relationship was reported by Furuki and Kobayashi (1990) who found that a *Cytophaga* species isolated from the declining phase of a *Chattonella* bloom was lethal to that alga and could be cultivated in sea water only when that sea water was spiked with disrupted cells of *Chattonella*.

More work is needed to pursue the possibility that bacteria may be important in sustaining HABs or are important in their declines. The status of studies on bacteria thus far has been confined to basic scientific investigations of the nature of the interaction. No practical efforts have yet been attempted to use bacteria to control HABs. Issues that must first be addressed include the ecological impact of such products, the specificity of the organisms, the dynamics of the algicidal process, and the logistics of delivering the bacteria over a large geographic area.

6.2.4.5   Other algae

Some workers have proposed the use of a small (2 μm) green alga called *Nannochloris* sp. as a control agent for Florida *G. breve* blooms (Taft and Martin 1986). This organism has also been described as the cyanobacterial species *Gomphosphaeria aponina* (Eng-Wilmot and Martin 1977). A chemical produced by this alga (aponin) was discussed earlier in this report as a potential control agent, but some consideration has also been given to direct seeding of a Florida bloom with *Nannochloris* itself. Although details are sketchy due to premature termination of that research project (Taft and Martin 1986), the control strategy would have involved growing huge tanks (swimming pool size) of *Nannochloris* which would then have been delivered to the bloom site and added to the water. Two different impacts were hypothesized. First, due to the very rapid intrinsic growth rate of *Nannochloris* (up to 9 times faster than *G. breve*), the introduced species might be able to out-compete the toxic cells for available nutrients and light, reducing the size of the *G. breve* population through competitive exclusion. In addition, *Nannochloris* is reported to be capable of releasing aponin into the seawater such that it ruptures *G. breve* cells in 4-10 days (Eng-Wilmot and Martin 1977), terminating the bloom. *Nannochloris* is also a very poor (indigestible) food source for commercially important bivalves and could thus have detrimental effects if it dominates the phytoplankton.

The potential for this strategy to be successful is exceedingly difficult to evaluate due to the lack of experimental data. For example, the species of algae involved is not clear, with one set of papers

describing it as a blue green alga (e.g. Eng-Wilmot and Martin 1977), and another describing it as a green alga (e.g., Taft and Martin 1986). The description of the former is that of a nearshore, benthic (bottom-dwelling) alga that may not grow in the stratified, offshore waters where *G. breve* thrives (Steidinger 1983). If *Nannochloris* is the correct designation, the growth habitat of the species is uncertain, as most *Nannochloris* species are adapted to high nutrient, nearshore waters with freshwater influence (see below; Ryther 1989). Another missing detail is the manner in which the two species grow when combined together in culture, not just in small flasks, but in larger outdoor containers under natural conditions. Many small picoplankton are actively grazed by fast-growing microzooplankton that would be too small to affect *G. breve*, so the bloom dynamics of each species in natural waters might not be a simple reflection of the difference in growth rates. In other words, the loss rates of the smaller, control alga could be much larger than for *G. breve*, and the results of a competition experiment would thus be difficult to predict. The only conclusion that can be reached at this time is that this control strategy is not well understood at present, and considerable laboratory and mesocosm work would be needed to assess its possibilities.

6.3 The Arguments Against Controlling HABs

The concept of HAB control is a politically and scientifically charged topic that has not received extensive attention from the scientific community worldwide (Anderson 1997). This is because in many cases, there is insufficient knowledge of the physiology, oceanography, and bloom dynamics of HAB species to justify plans for specific control strategies - i.e. we cannot control what we do not understand. In addition, there is a legitimate concern that human efforts to control these natural phenomena may make matters worse. Steidinger (1983) discusses the concept that HAB control, even if it were feasible, should not be pursued in Florida. One argument in support of this view is that Florida *G. breve* blooms and their associated fish kills have an important ecological function similar to natural fires or other perturbations. Some ecologists argue that fires help to establish the ecological stability and productivity of ecosystems by removing unfit species, controlling diseases, and allowing for the invasion of new species. They believe that systems that are under pulsed stress, such as those exposed to episodic HABs, are healthier and more resilient than those that are unstressed. A related issue is the claim that fishermen report higher catches of fish and crabs following Florida *G. breve* blooms (Steidinger 1983). This may relate to decreased predation on those resources, higher food availability due to the deposition of dead fish, or reduced competition.

There are, therefore, potential benefits from HABs. Nevertheless, it seems unwise to rule out discussion and research on HAB control on the basis of *a priori* assumptions that such efforts will not work or will cause unacceptable harm to coastal ecosystems. This has been the *de facto* position of many scientists and managers throughout the world since the 1957 copper experiment. Arguments that we should not interfere with natural phenomena such as HABs ignore the possibility that humans may have already interfered and made the problems worse through pollution and other modifications of the coastal zone (Anderson 1997).

Biological control, chemical control, and integrated pest management are used on land to control unwanted plants, insects, and animals, often over millions of acres. It seems timely to consider whether similar approaches might be effective in the ocean. This review has highlighted a number of promising control or mitigation strategies for HABs which are worth further evaluation. Some of these are decades from possible implementation, but others are further developed and thus are worth considering in the immediate future. Almost without exception, however, research or pilot-scale studies are needed to investigate the methods under realistic conditions. Even if the eventual conclusion from these investigations is that direct or indirect control of HABs is not possible without serious environmental or societal impacts, a great deal of fundamental knowledge will have been gained that can help us to better understand and manage these phenomena.

6.4 *In Situ* Bloom Mitigation Methods for Fish Mariculture

Mitigation of HABs has been defined *sensu stricto* as means taken to reduce HAB blooms or their effects (Jenkinson 2000). Mariculturists want to keep their stock alive and focus on near-field areas (i.e., within or near their fish farms). This discussion is limited to large-scale fish mariculture, which in almost all cases means net pens but could include lagoon culture or other types of impoundments. Shellfish culturists generally do not practice bloom control but rather aim to reduce impacts (see Section 6.5).

Mitigation or control of HABs is usually not a mariculture priority in a given region until a major fish killing bloom occurs. Such events generally cause a degree of chaos for the fishfarmers, and often for government regulators and the public too. Fish mariculture is relatively new in most coastal seas of the world, and therefore it is just a matter of time before most fish farms experience either naturally occurring or human caused/exacerbated HABs. The use of chemicals to control HABs was initiated and largely abandoned in decades before the recent rise of the mariculture industry, as previously discussed. Recently there has been a number of physical or chemical mitigation measures proposed, but most have not been evaluated with regard to collateral effects on other marine species or water and sediment quality. A few of these techniques may emerge as economically and environmentally viable approaches for near- or far-field mitigation of HABs. Here we focus on the former, beginning with relatively simple physical practices used by many fish farmers worldwide.

Fish farms may exacerbate HAB populations in some cases due to enrichment from waste discharge, but proper siting can reduce or eliminate such effects. See Rensel and Whyte (2001) for an overview of that topic, and a review of mariculture impacts edited by Black (2001).

Although much has been learned about the mechanisms responsible for fish death from HABs, there is much uncertainty and lack of definitive understanding for several important species and groups of fish-killing HABs. A variety of physiological mechanisms, singly or in combination, may lead to fish mortality. They can be generally categorized as: 1) physical damage or irritation of gill tissue leading to mucus production, blood hypoxia and possibly bacterial infection, 2) toxigenic reactions to ichthyotoxic agents, 3) blood hypoxia from environmental oxygen depletion or 4) gas bubble trauma from oxygen supersaturation (see Rensel and Whyte 2001 for additional discussion of causes). As more basic research is conducted about the underlying physiological causes of fish death from HABs, the design and implementation of mitigation methods should become easier and more effective.

6.4.1 Aeration

General Description: Aeration involves introduction of atmospheric air into the water using pumped water via aspiration of air, or directly with air-blowers or compressors associated with a wide variety of distribution systems. It is widely practiced in mariculture, usually to offset high loading density of culture organisms or low concentrations of dissolved oxygen (DO) in the supply water. Aeration is also used in transporting and treating fish along with oxygenation. Aeration may be useful in mitigating the physiological effects of non-toxic phytoplankton blooms that cause environmental hypoxia or anoxia, i.e., noxious algae. It may also be used in combination with other methods as discussed in this review, but is generally not a useful treatment for toxic HAB exposure unless ambient DO concentrations are low and the damage is restricted to minor effect on the gills. Such fish kill events have occurred, most recently in Hong Kong where only large, active cultured species were killed by a mixed *Chattonella* spp. bloom in the spring of 2001 (P.S. Wong, pers. comm.).

The DO requirements of cultured marine fishes vary greatly depending on the species and several other factors. For example, salmonids generally need greater than 7 mg L^{-1} DO for stress-free growth and fish

health considerations (Whitmore et al. 1960; Davis 1975; Piper et al. 1982). However, some semi-tropical cultured fishes can tolerate DO concentrations as low as 2.5 mg L^{-1} (Wu 1990). Water temperature, activity level, size of fish, loading density, container type, amount and quality of ration, lighting and other factors also influence DO requirements of marine fishes being cultured.

Typical Equipment: There are three broad categories of aeration equipment to consider, (see Escobal 1996) and other citations below for details of operation and integration:

a) *Pumped water systems* are principally those that utilize venturi nozzles to aspirate air into the flow of water that is directed to the fish as needed. Any type of pump (volume, pressure, submerged, others) may be used to create the water flow, but some types are more economical and efficient for differing depth of discharge. There are several designs of venturi nozzles to accommodate different and varying flow rates. Pumps may be powered by electricity or fuel-powered engines; the most dependable performance is from shore power with diesel-powered generator backups, but these are commonly not available for most mariculture installation.

b) *Pumped air systems* include air pumps, compressors and blowers to force air through distribution devices, usually diffusers such as airstones or porous pipes and hoses, using the same power sources discussed above. This category could include airlift pump systems, but this category is so important and prevalent in mariculture that they are discussed separately, below. Pumped air systems also include subsurface and hypolimnetic destratification devices that could have some application in mariculture and HAB treatment but are usually reserved for lake restoration.

c) *Surface agitators, fountains, splash aerators, vertical pumps and paddlewheels* are designed to disturb the surface of the water and to enhance the transfer rate of oxygen from the atmosphere to the water. Boyd (1991) judged paddlewheel aerators to be the most efficient in transferring oxygen and circulating water in large but shallow ponds. To date, few of these surface-oriented systems have been used in fish mariculture, probably due to their limited depth penetration and the common perception that they are better suited for aquaculture in freshwater or brackish ponds.

Effectiveness and Limitations: For anoxia or hypoxia events, caused by HABs, upwelling of low DO water or other factors, some of the available aeration devices are effective means of mitigation, and others hold promise for future development.

Turbulence: For toxic algal blooms, aeration is of very limited effectiveness in mitigating physiological damage to fish. Aeration cannot achieve supersaturated levels of dissolved oxygen that may, in some cases, help keep affected fish alive during toxic or noxious microalgal blooms. Turbulence produced by aeration or other means may kill or impede the growth of some dinoflagellates (e.g., White 1976). Aeration also reduced the harmful effects of noxious diatoms, as Rensel (1992) showed by comparing the effect on fish of long chains from gently aerated cultures of *Chaetoceros concavicornis* versus short chains of the diatom grown in much more actively aerated conditions. The use of turbulence as a mitigation agent has not been tested in a commercial application, however, and may require too much mixing energy and time of exposure to be cost effective or practical.

Mitigation of Toxic or Noxious Effects of Red Tide: Aeration does not help attain supersaturated levels of dissolved oxygen that may help mitigate the effects of exposures to certain types of noxious algae (see *Oxygenation*, below). The transfer rate of oxygen to the water is inversely proportional to the difference between ambient and desired concentrations, which is why aeration is more effective with low background DO levels such as in sewage treatment applications, rather than in aquaculture, which has much higher continuous DO requirements for survival of most cultured species.

Type of Units Suited for Net Pens: The options here narrow to either systems using pumped air from diffusers such as airstones, or aspirators (horizontal, self-powered units or nozzle types) used in conjunction with water pumps.

Diffusers are probably the most widely used means for aeration of aquaria, but they suffer from several problems when scaled up for use in commercial mariculture applications. The most primitive and ineffective are constructed of drilled plastic pipe, but these have poor oxygen transfer characteristics. Airstones (Figure 6.1, right) and micro-diffuser hoses offer approximately three times better oxygen transfer rates. Blowers (Figure 6.1, left) are often used as air sources for diffusers, as they provide large volumes of air at low pressures. Combined, these units have the ability to operate efficiently to a depth of about 2.5 to 3 metres. Compressors are less suited for this task as they operate at high pressure. Their advantage is to be able to force air to greater depths when needed. In either case, airstones are subject to extensive biofouling, plugging and damage from handling and unregulated or variably peaking pressure supplies. They therefore require periodic maintenance and replacement. An additional benefit of diffuser-type aeration is the ability of small bubbles to help strip excess carbon dioxide and ammonia from the water. Diffusers are rated as moderately effective compared to other aeration devices, with transfer efficiencies ranging from 0.6 to 2.0 kg O_2 KW^{-1} hr^{-1} (Lawson 1995).
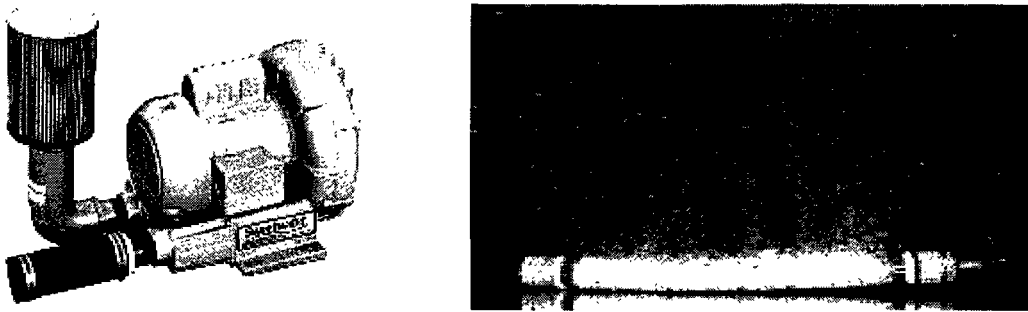
FIGURE 6.1. Electrical powered regenerative blower (left) and diffused airstone (right). (From AES, Inc.)

Wu and Lee (1989) built and tested several variations of a blower-driven aeration system for use at a Hong Kong fish net-pen mariculture facility. They tested several types of diffusers including porous tubing, airstones attached to pipe manifolds and PVC tubing with outlets. The porous tubing performed the best, and they were able to raise ambient DO from 0.5 to 2.0 mg L^{-1}, a safe level for many of the cultured species utilized in that area.

Aeration in net pens using submerged airstones has been criticized by Kils (1979) as producing a net flow of water from below the cages and into a vertical, semi-closed circular flow pattern. This transports deep water into the cages, but for subsurface hypoxia conditions, is exactly what mariculturists try to avoid. Another disadvantage is that the surface flow pattern away from the pens results in rapid dispersion of the entrained deeper water that would be best retained for use by the fish. The author designed a rotating venturi nozzle system, said to work well in mariculture cages, but no testing results were published. The Kils (1979) paper was written before the widespread use of perimeter skirts or pens (described later), which may obviate the need for specialised aeration systems by trapping pumped water within the cages.

Propeller-driven aspirators are floating or submerged motors connected to a hollow shaft and propeller that draws air in from the surface and forces the air/water mix in any subsurface direction chosen. They are a type of venturi mechanism, but not to be confused with venturi nozzles, which are discussed later. An example from a tropical lagoon (Figure 6.2) shows the electric motor mounted on pontoons with the shaft and propeller not visible beneath the water. Transfer efficiencies range from 1.3 to 1.9 kg O_2 KW^{-1} hr^{-1} (Lawson 1995).
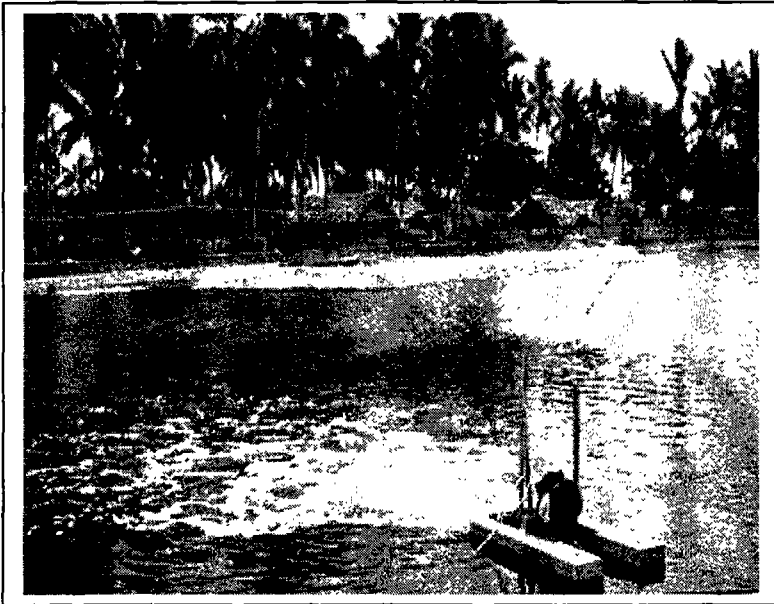
FIGURE 6.2. Typical pond application of a propeller-driven, electrically powered air aspiration system. (Source: Aeration Industries International Inc., Minneapolis, MN.)

Use of propeller-driven aerators requires expensive generators or shore-based power cables, neither of which is a reasonably priced alternative for most types of fish mariculture. Additionally, these units pose the possible risk of electrical shock injury if not installed correctly. Figure 6.3 shows an underwater view of the hollow shaft and propeller of one of these units in operation, with the bottom of the supporting pontoon in the background. These units have the ability to force air and dissolved oxygen into water to relatively great depths. In shallow areas, care is required so as to not stir up sediments from the bottom. This is easily achievable on most units by adjusting the angle of discharge in an arc from vertical to near horizontal. As with all aerators reviewed here, they could be much more effective when skirts are used around pens to retain DO-enhanced water (for pens) or if other means are used to avoid advection of treated water out of the culture unit (for lagoons or onshore farms).

FIGURE 6.3. Underwater view of a propeller-driven, electrically powered air aspiration system, showing the hollow tube and prop wash from the propeller. (Source: Aeration Industries International Inc., Minneapolis, MN.)

Venturi Nozzles: One of the most cost-effective means to aerate water, using existing pumping equipment, is the use of venturi aspiration nozzles. Their oxygen transfer efficiency is rated the highest of all available systems, from 2.0 to 3.3 kg O_2 KW^{-1} hr^{-1} (Lawson 1995). Venturi nozzles, represented schematically in Figure 6.4, introduce atmospheric gases into water by the vacuum created in a pump system, when a passive air-bleed input is allowed. There are known engineering requirements for such units, and several designs on the market with varying characteristics, efficiencies and size. Larger venturi nozzles must be mounted on rigid surfaces to counteract the jet-like force of the discharge. However, they can be mounted in opposing groups of 2 or 4 to counteract the physical thrust they produce.
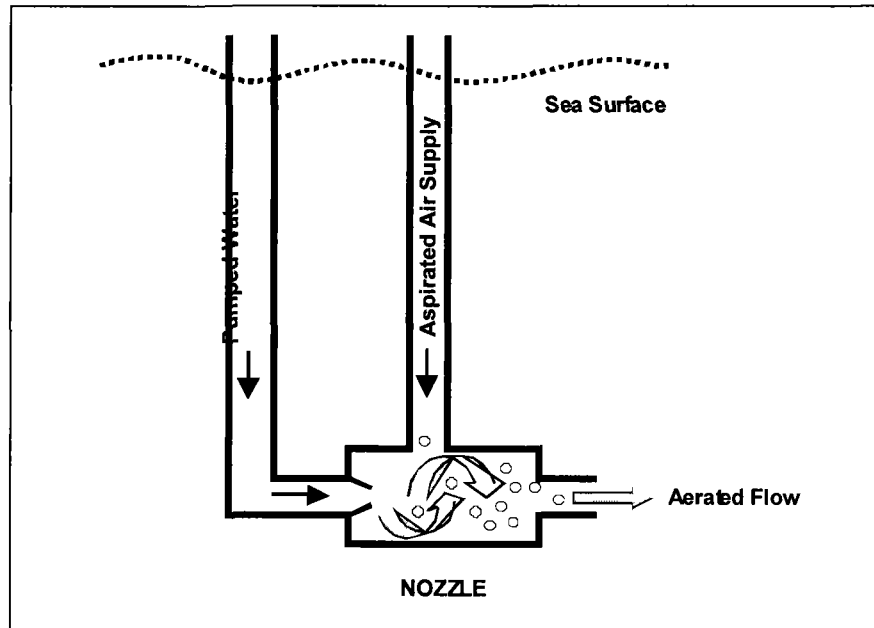
FIGURE 6.4. Schematic diagram of a venturi nozzle system using pumped seawater from any source and a rigid airline to allow suction of air.

Venturi nozzles have depth limitations; the deeper the venturi nozzle is placed in the water, the more restriction on pumped water flow and air aspiration. However, with increasing depth, aspirated air supply rate decreases, but oxygen transfer efficiency increases due to exposure time of the bubbles to seawater. The available trials (e.g., Colt and Tchobanoglous 1981) indicate that venturi systems have competitive oxygen transfer rates compared to all other types of aeration units.

Combined Uses: Aeration may be much more effective when combined with perimeter skirts around net pens, allowing the treated water to be retained. The effectiveness of clay flocculation may be increased through an initial period of aeration, followed by a quiescent period to allow settling, although this approach needs further study.

Worldwide Use: Aeration is used in freshwater aquaculture in a host of countries, too many to begin to list here. In marine aquaculture, however, its use is more limited as tidal exchange is relied on for oxygen supply in replacement water. Aeration tends to be used seasonally in areas that have naturally occurring environmental hypoxia events. Simple aeration, i.e. without any sort of airlift pipe to the surface, does not always work well to displace plankton from net pens because the aerated water plume cannot be oriented

toward the fish exactly. This could be a problem particularly in strong tidal currents. However, in some cases simple aeration has been reported to work well in displacing blooms from large net-pen arrays (G. Robinson, Stolt Sea Farm, BC, pers. comm.). Some British insurance companies hold that opinion as well (R. Morris, Roberts Morris Bray Insurance Brokers, pers. comm.). But in most cases, simple aeration alone is not viewed as an effective means, on its own, to mitigate the effects of HABs on mariculture.

Costs and Practicality: Capital costs of differing types of aeration equipment to achieve a similar result are highly variable, depending primarily on the type of pump, compressor or blower selected. Other costs, such as hoses, diffusers and nozzles, are minimal in comparison. Differing operating costs and other considerations, such as the use of used equipment and imbalances in design of system components, can result in additional cost variability.

As most methods have approximately similar transfer rates of oxygen per kilowatt of power (Colt and Tchobanoglous 1981), the choice often depends on the application and the pre-existing equipment inventory of specific mariculturists within different regions. Mitchell and Kirby (1976) compared total costs including capital, operating and maintenance costs for a ten-year life of several types of aeration systems. They found the least expensive to be paddlewheel systems, followed by diffuse aeration systems, floating aerators and finally, propeller-driven aspirator systems. Paddlewheel and fountain systems are thought not suitable for mariculture net pens because they only influence the very surface of the water column, but may be useful for small pens in some cases as they also provide water circulation benefits. A comparison of capital costs, however, indicated the highest rates were for diffuse aeration systems. Capital costs were judged by Colt and Tchobanoglous (1981) to be the most critical of all aeration costs. Operating or maintenance costs can be prorated over longer periods, but capital costs create a more adverse effect on cash flow in a start-up mariculture venture. Moreover, airstones and porous tubes tend to clog easily and require frequent maintenance. Venturi systems are generally not subject to these limitations. Most means of aeration are subject to potential supersaturation problems for the fish, as they introduce atmospheric gases under pressure including nitrogen gas.

Table 6.2 presents capital cost estimates for diffuse aeration versus venturi nozzle systems with regard to use in small-scale net pens. As transfer efficiencies of oxygen are similar among the cited systems, the analysis simply reports the amount of air introduced to 2 m depth and relative costs. Both systems become more limited in forcing air to greater depth, but both have improved transfer efficiencies as the bubbles remain in the water for longer periods before surfacing. The analysis shows the affordability of venturi nozzle systems to be better by nearly an order of magnitude, or even more if electrical power cost is considered.

Mariculture DO Requirements:

As discussed above, diffuse aeration systems have been used for mitigating the effects of environmental hypoxia caused by HABs or upwelling of naturally hypoxic water. In order to carry the analysis further, we estimated the total DO demand of fish stock at a fish farm to give insight into the required sizing and effects of aeration. This is also discussed later in relation to perimeter skirts and sediment oxygen demand.

To estimate the dissolved oxygen requirements of a small-scale semitropical mariculture net-pen farm in a hypoxia event or during a HAB event when perimeter skirts might be deployed, we provide the following analysis. Assuming an annual fish production of approximately 2 tons and overlapping brood years of two year classes of fish, fish biomass at any given time will vary from about 50% to 125% of the total annual production, or from about 1 to 2.4 tons. Fish respiration of several marine fishes cultured in Southeast Asia was reported by Wu (1990). From his Table 2 we calculated the mean oxygen consumption rate of nine species as 4.2 mg kg^{-1} min^{-1}. The base oxygen demand for our typical mariculture facility would

therefore average 0.3 to 6 kg O_2 hr^{-1}. Loss of aerated water from the pens may be considerable, as perimeter skirts (discussed later) are apparently not widely used in most areas. It is difficult to speculate what factor must be applied to the above rate functions to adjust for these losses. But it would probably be at least a factor of two, resulting in a required rate of 0.6 to 1.2 kg O_2 hr^{-1}. Dissolved solids, detritus and other DO demanding matter as well as varying temperatures result in lower actual efficiencies in the field. To adjust for these, we use the rapid solution method of Shelton and Boyd (1983), with 20°C as an average value. Applying the inverse of the solution factor of 0.55 for an ambient DO concentration of 2 mg L^{-1} @ 20C, raises the required rate of 1.1 to 2.2 kg O_2 hr^{-1}. To check this value against a literature "rule of thumb", we note that it typically takes about 1.2 KW of aeration energy to supply DO for 1 ton of fish (Beveridge 1987). It would therefore take 1.1 to 2.6 kW (1.5 to 3.6 hp) aeration systems to supply oxygen to a typical Southeast Asian mariculture net-pen facility. Allowing for non-average conditions of fish biomass, reduced ambient DO, and higher rates of fish respiration means that at least 4 kW (~5 hp) power source should be available.

To summarize, venturi nozzles may be a practical solution for the need for additional aeration in small-scale fish mariculture to deal with toxic, noxious or anoxia-producing blooms. They could be used as a backup or augmenting system for farms that presently have aerators, or could provide a primary method for farmers that presently have no aeration equipment. Together with perimeter skirts, discussed later, they constitute one of our preferred recommendations for HAB mitigation at small-scale fish mariculture facilities.

TABLE 6.2. Comparison of diffuser air systems estimated capital costs versus venturi nozzle systems, assuming depth of placement at 2 m. Costs are US dollars.

Aeration Method	Air Volume to 2m depth (CFM*)	Estimated Total Costs US$	Cost per CFM* US$
Electrical Driven Blower[1] with Porous Pipe Diffuser[2]	190	$2,182	$11.48
Gas –powered Blower with Porous Pipe Diffuser[3]	100	$2,389	$23.89
Venturi Nozzle[4] with Water Pump[5]	2,000	$2,432	$ 1.22
Venturi Nozzle[4] without Pump Costs	2,000	$1,904	$ 0.95

*Cubic feet per minute.

[1] Medium-duty Blower (Gast Co.) 190 CFM at 2 m using 5.5 hp unit = $1,382. Assumes available shore power but does not include electricity costs. .

[2] Pipe diffuser estimated at $500, plus $300 for 1 cm supply lines and couplings medium bubble

[3] Belt-driven blower with 5 hp gasoline engine and same plumbing as above, an example of a typical small scale net- pen wash down pump.

[4] Eco-aspirator using 03 gage nozzle, (AES part No. AP-1) are US$47 each, @ 25 GPM each need 32 each = $1,504 plus bracing and hoses for $400.

[5] Pedestal Mounted style (Pacer) pump, total dynamic head of 9 m (includes head and friction) with five hp integral gasoline pump yields 200 GPM for $528.

6.4.2 Oxygenation

General Description: Oxygenation is the use of bottled, compressed oxygen or oxygen generation equipment to increase dissolved oxygen concentrations in water. Such systems are used in a variety of processes, but typically in commercial mariculture are often reserved for transfer of fish, recirculating systems and development of new culture species systems, or as emergency backup systems for intensive, pumped-seawater systems. Supersaturated oxygen in fish culture water has been tried with some success in government hatcheries in the US Pacific Northwest and is often referred to as "oxygen supplementation". It has been a key factor in design of on-shore marine salmon farms that were planned to rear fish at very high densities to offset high capital and operating costs. The process is typically not used with net-pen mariculture and other forms of extensive or semi-extensive mariculture, for reasons discussed below.

Typical Equipment: Compressed oxygen is widely available in storage cylinders and tanks for medical and commercial applications such as welding. Oxygen generation equipment is widely available, and involves a variety of accessory equipment.

Effectiveness and Limitations: Oxygenation has been shown in laboratory studies to be effective in rehabilitating the blood-oxygen content of Atlantic salmon mortality when exposed to *Chaetoceros concavicornis* (Rensel 1992, 1993). Supersaturation forces oxygen into the fish through gills and other body surfaces, partly compensating for malfunction of the gills. The degree of supersaturation must be monitored carefully, as too high a level may also be toxic to fish. Moderate levels <400% of air saturation (300% when other gases are supersaturated) is considered acceptable to salmonids, although further research is needed in this area. Rensel (1992) found that 180% supersaturated oxygen was insufficient to increase the blood oxygen of Atlantic salmon exposed to 400 cells ml^{-1} of *Chaetoceros concavicornis*. Levels above 200% were sufficient to raise blood oxygen concentrations to that found in control fish (~40 mm Hg). No improvement was noted, however, in cough rates (a behavorial index of noxious phytoplankton effects) of the affected fish.

As many other types of harmful microalgae, including some toxic species, injure or kill fish by damaging the gills, it would seem reasonable that supersaturation with oxygen might improve fish survival. Yet, there are few studies in this regard. Black et al. (1991) found no improvement in survival of juvenile salmonids exposed to *Heterosigma akashiwo* in live cage bioassays when high levels (~200 to 400%) of oxygen supersaturation were applied. This is a research area, and there is presently some conflicting information.

Combined Uses: Oxygenation may be combined with tarps or skirts around the pens, allowing air to be retained and other methods such as airlift pumping.

Costs and Practicality: This method may have some application for mitigating the effects of red tides on mariculture, but only for very profitable or risk-adverse situations, such as valuable brood stock maintenance. The applicability of oxygenation becomes cost effective, compared with aeration, when the desired levels of DO exceed moderate concentrations (e.g., 5 mg L^{-1}, at STP). Oxygenation becomes truly cost effective at higher levels approaching and exceeding saturation (e.g., ~ 8 mg L^{-1}, at STP). As discussed above in the aeration section, supersaturated levels of DO have proven useful in increasing blood oxygen concentrations of fish affected by harmful microalgae. The method may also be technically feasible to offset fish losses due to toxic algal blooms. For net-pen applications, however, oxygenation is most likely a cost-prohibitive means of harmful microalgae bloom mitigation, as shown in the example in Table 6.3. The analysis is for salmon mariculture, but the fundamentals could be scaled to apply to marine fish culture.

TABLE 6.3. Hypothetical example of oxygen supersaturation as a method for harmful microalgal bloom mitigation for salmon net-pen systems. (Source: Rensel 1992, with 1998 capital costs.)

Mariculture Net Pen total volume (of one pen): 1,150 m^3 (12m x 12m x 8m deep)

Fish stocking rate: 16 kg m^{-3} (moderate for salmonids)

Fish stock biomass: 1,150 m^3 x 16 kg m^{-3} = 18,400 kg

Fish oxygen requirement: 18,400 kg x 300 mg O_2 kg^{-1} wet weight h^{-1} = 5.5 kg O_2 hr^{-1}

Loss of oxygen due to leakage from pen (to air and vertical/horizontal exchange) arbitrarily set at .25

Fish oxygen plus loss requirement = 5.5 kg O_2 hr^{-1} x 1.25 = 6.8 kg O_2 hr^{-1}

100% oxygen (air) saturation at 12°C, 30 ppt salinity is 8.9 mg L^{-1} O_2, 200% saturation would be 17.8 mg O_2 L^{-1}.

Capital costs of oxygen generation to achieve 200% saturation are approximately US\$ 10,000 per pen, without distribution system. With distribution system and accessories, total estimate is US\$ 11,200.

Assuming 25 cages/farm, total capital cost would be US\$ 280,000, adjusted to US\$320,000 to account for installation and support barge costs. (Does not include depreciation and maintenance costs).

Farm stock value (FOB dock) estimated to be US\$ 0.50 per kg. Maximum standing stock (biomass) = 18.4 tons x 25 cages = 460 tons x US\$ 0.50/kg = US\$230,000.

Analysis: Oxygenation equipment costs alone outweigh value of the product and approach the capital cost of the cages and nets. If severe HAB events are infrequent, e.g., < once per 5 to 10 years (which is typical in some regions), equipment purchase would be unreasonably expensive given the risks.

6.4.3 Airlift Pumping

General Description: Airlift pumping is a means to pump water, by utilizing the force of rising air introduced into a submerged, vertical pipe. The rapidly rising action of the air bubbles vertically entrains water, which is replaced by water sucked into the bottom of the tube. Airlift pumping is considered a highly effective means to pump water from depths to the surface, but not above the surface of the water. It is widely used in aquaculture, both in laboratory and commercial applications. Other types of pumps such as pressure or volume pumps could be used to move water in algal bloom mitigation processes, but they are much more expensive in terms of operating costs per unit water moved. They can pump water above the water surface, for additional aeration or other purposes, but are typically not used in net-pen mariculture because of cost.

Typical Equipment: For mariculture net-pen applications, typically a compressor or other type of air pump forces air through a hose and into a large diameter (e.g., 25 cm) pipe or hose to a point 2 to 4 m

beneath the surface. The hose may be terminated with a coarse airstone, to create smaller bubbles if additional aeration is required, but in most cases this is not done as it limits the efficiency of pumping.

Effectiveness and Limitations: As noted above, the method is very useful for pumping water, but has limited use in aerating water. It is also not useful for pumping water above the air/water interface. Another limitation relates to hydrographic conditions used during a bloom. If there are large density differences between the subsurface and surface water, there is a possibility that the airlift-pumped seawater could rapidly sink out of the net pens. However, when combined with perimeter skirts or tarps it has been one of the main methods used in coastal areas of the N.E. Pacific for dealing with *Heterosigma akashiwo* blooms. Although this species has well-known vertical migration characteristics, it has usually been concentrated near the surface during documented fish kills in North America. (Note however, it is not uncommon to have cells distributed to $\geq$ 10 m depth at other times in channels and passages that are well mixed or actively mixing). The airlift pumping of deep (e.g., >15 m) seawater to skirt-enclosed pens can be very effective in excluding the bloom and reducing fish mortalities. From a low flying airplane, the contrast of the often blue-green water within the pens stands out in sharp contrast to the surrounding brown/red bloom affected water.

Combined Uses: As with simple aeration, the use of perimeter skirts, discussed below, is beneficial for retaining the pumped seawater. There may also be means to combine filtration of HABs with airlift pumping, using large bag filters, if the target HAB cells are not fragile and not easily ruptured.

Costs and Practicality: Seawater pumping by airlift is by far the most cost efficient means to move water, particularly from an operation and maintenance point of view. It has been widely and successfully used along the North American west coast, which has recurring harmful algal blooms. If mariculture pens are located in areas with deep water beneath or nearby, the method may be one of the best means to avoid fish kills from HABs. There are no available cost estimates for the technique, but if compressors or air blowers are already available, the costs will be restricted to the capital and installation costs of vertical pipe and support lines and annual placement and maintenance. Often mariculturists will use corrugated plastic, non-perforated drainage pipe (e.g., Advanced Drainage Systems brand and similar) because it is inexpensive, lightweight, easily modified and durable.

6.4.4 Moving Pens from Blooms

General Description: Moving mariculture pens away from an area affected by fish-killing blooms to a known refuge area has been an effective mitigation measure practiced worldwide by mariculturists. It has major risks, however, such as loss of stock due to ripped nets and loss of cages due to wave or towing forces beyond the design tolerances of the pens. For larger pens it can be extremely expensive too, requiring a number of very large tugboats and rigging. As most of the world's mariculture currently involves the production of salmon and trout in temperate areas, this applies to them in particular.

Typical Equipment: The primary equipment needed is a suitable vessel or fleet of vessels, capable of pushing or towing the pens. Although the term towing is used for this general topic, pushing of smaller cages with small vessels is typically more effective than towing. Pushing provides more directional control than towing, and there is no propeller backwash into the cages that can add to the currents of movement to stress the fish. Towing of larger cages is typically done by inshore or coastal-going tugboats, but bridling and rigging has to be done most carefully to avoid damage to the cages.

Effectiveness and Limitations: In our survey of international mariculture companies and insurers, several authorities noted that towing could be an effective bloom mitigation technique, depending on the size of

the facility. The trend toward much larger cages, often made of flexible plastic pipe up to 100m diameter in some cases, complicates moving of the cages in some circumstances for the salmon-growing industry. Choice of a suitable refuge destination for towing is another major consideration. Aerial and boat surveys may have to be done to determine the extent of the bloom, and interference with shipping-vessel navigation, strong tidal currents, wind wave effects on pen structures and other factors have to be weighed. Benthic impact on refuge area from the fish is usually not a consideration, as the fish are often not fed during towing so fecal loading is greatly reduced and the pens are not anchored in a single location.

Harmful *Chaetoceros* events are difficult for fish farms to manage by towing because the concentration of these diatoms that harms fish is below the threshold of visible change to water transparency or effect on other easily measured hydrographic parameters such as DO or pH. Accordingly, the vertical and horizontal distribution of these cells in the water column is difficult to ascertain over large areas. Finding a true refuge area for *Chaetoceros* caused fish kills may therefore be difficult. One approach is to simply scan samples of seawater with a dissecting microscope or even by eye, as the *Chaetoceros* chains that are truly harmful are easily visible even to the naked eye.

Combined Uses: Towing of net pens has been useful for preventing salmon net pen losses due to the harmful microalgal blooms, but careful planning and a measure of luck are necessary for a successful execution. Large net-pen farms owned by the Norwegian company Global Aqua successfully avoided blooms of the microflagellate *Heterosigma akasiwo* in Puget Sound, Washington, in 1991 by towing to a nearby channel that had intense vertical mixing and little visible bloom. A subsequent bloom and towing in 1997 to a different area, through a narrow channel, led to considerable loss of fish from ripped nets. Small fixed-wing aircraft were used for bloom reconnaissance and the fish were not fed. Other salmon growers in Puget Sound have contingency plans to move their cages, and pre-arrangements with tugboat and insurance companies. Aerial surveys by small aircraft or boat have been useful in this regard, particularly when conducted in the morning before sea or land breezes have mixed cells away from the near surface.

Worldwide Use: Towing of net pens to avoid HABs has been practiced in Norway (Andersen 1996), for example during blooms of *Chrysochromulina polylepis* and *Gyrodinium aureolum* (Anonymous 1988). Similarly, *Chattonella antiqua* blooms in the Seto Inland Sea of Japan have been avoided by moving mariculture farms. The method has been used on the west coast of North America as well, in Washington State and British Columbia.

Costs and Practicality: The costs of towing net pens may be substantial, although larger fish farms' insurance companies may offset part of the expense. Smaller scale mariculturists typically do not have insurance. Towing involves the risks of structural damage to facilities or fish escape. A towing contingency plan should be devised in advance that includes protocols for dealing with anchoring systems and timing of movement with regard to tides. Practice towing exercises may be warranted too. Costs of towing would vary greatly depending on the size of the facility to be moved, the temporal duration of the harmful microalgal event, and whether the moving was contracted. In most cases, for smaller fish farms, we would expect that the individual mariculturists would use their own vessel(s) to conduct the towing. In summary, the main constraints to moving of mariculture facilities include the need to identify suitable refuge areas that do not interfere with marine navigation and the risks of facility damage or fish loss.

6.4.5 Perimeter Skirts

General Description: Perimeter skirts or tarps refers to plastic or other types of fabric that may be suspended vertically around the outside perimeter of net-pen fish farms to create a barrier between the sea

and the fish held in the net pens. The method is usually not practiced without some other mitigation technique, such as airlift pumping or aeration, but is an important component contributing to the success of those other methods.

Typical Equipment: A variety of materials may be used to fabricate perimeter skirts. The most common material is low-density polyethylene (LDPE), that is low strength but UV resistant (J. Halstead, Research Nets Inc., Bothell, WA, pers. comm.). String-reinforced LDPE is available too, for more challenging environments or extended life. Thickness of the sheets used is typically about 6 mils, and rolls with many widths are available from 1 m to 12 m or more. Grommets may be installed for hanging the skirt onto the pens. A lead-cored line may be attached to the bottom edge of the skirt, to counteract tidal currents and keep the skirt in place. The skirt may be rolled up, tied with twine and hung on the edge of the cage until deployment is necessary. The weight of the lead line helps deploy the skirt. During periods of reduced algal bloom risk, the skirt may be removed and stored to improve its useful life span.

Effectiveness and Limitations: As previously stated, perimeter skirts for floating mariculture systems are highly valuable tools to deal with algae blooms and other problems. Typically, skirts are deployed to deal with toxic microalgae located near the surface, not throughout the entire water column. It is possible in the case of small-scale mariculture to deploy them the entire depth of the water column to the bottom to isolate the fish from the environment, but this is rarely done. For environmental hypoxia events, a similar choice must be made regarding depth of skirt deployment. If leakage occurs from the top of a skirt-enclosed pen, aeration could actually draw in subsurface sources of hypoxic water due to circulation patterns known to exist in these circumstances (see previous discussion of Kils 1979).

There may be some upper limit of tidal currents that allow the use of the method, and weighting of the bottom of the skirt and flotation of the supporting raft may have to be increased at more current-swept sites. Biofouling of the skirts should not present a problem, as they are only deployed for relatively short periods and left rolled up the rest of the time. Another potential limitation is the oxygen demand of the fish and the seabottom. If the skirts were deployed all the way to the bottom, the oxygen demand of the sediments may have to be considered. Even in areas with eutrophic seabottoms, sediment oxygen demand (SOD) is insignificant compared to the fish respiration needs of mariculture.

Another limitation regarding use of perimeter skirts is possible ammonia build up. Unfed fish produced at least 3 to 4 times less ammonia (i.e., ammonium plus ammonia, Brett and Zala 1975) than fed fish. So the use of skirts must be coupled with the best management practice of "stop feeding". Nitrification of ammonia to nitrate proceeds very rapidly in any aerated marine environment, as repeated monitoring studies by Rensel (1989, and unpublished) and Washington State Department of Fisheries (1990) demonstrates. In these studies, concentrations of fish-excreted ammonia were altered to nitrate and possibly nitrogen gas a short distance "downstream" of the salmon net pens. Marine bacteria are very ubiquitous in, on, and around mariculture pen structures, and act as oxidizing agents. Aeration also is an accepted means of stripping ammonia from the water. Aeration may be conducted in any case where fish stocking density is high and perimeters are deployed.

Yet another potentially valid criticism of the use of perimeter skirts is that there may be no source of HAB-free water for the fish, if the skirts are not deployed before the onset of a bloom. Obviously, it would be best to have a sampling and warning system where surrounding fish culture zones can be warned in advance to deploy their perimeter skirts. However, if it is too late, there could be a need to use technology such as small amounts of non-toxic flocculants such as clay, discussed in Section 6.2.2 of this report. The clays used for this do not hurt the fish, and a one-time application could clear the water, until the bloom subsides and the perimeter skirts may be removed.

Combined Uses: See general description.

Use in Other Countries: Perimeter skirts are utilized in several countries, particularly those with blooms that tend to be concentrated in specific strata. In Singapore, Lim (1989) reports that both affected fish cages and toxic algal blooms are located in the upper 2 m of water. One means of mitigation used by farmers there is deployment of PVC plastic perimeter skirts to a depth of at least 2 m to isolate the fish. Some surround their entire raft, others just individual cages. In British Columbia, "tarping" around cages is practiced to depths of 12 m or more (G. Robinson, Stolt Sea Farm BC, pers. comm.). Problems with longevity of some types of materials have been reported, and in strong currents the skirts tend to lift and become contorted. In Washington State, not only have perimeter skirts been used for containment of airlifted water, but also different designs of skirts made of fine mesh are used by one company to prevent larval crabs (megalops) or the genus *Cancer* from entering the cages. The crab larvae are known to cause significant gill hemorrhaging during part of the spring.

Costs and Practicality: Perimeter skirt costs vary with the quality and weight of the fabric utilized, and with the sizing of the bottom weights. Several quotes were obtained for panels that were equipped and ready to deploy. Some minor savings could be had by mariculturists obtaining the raw materials and installing grommets and weights on their own. For small-scale net-pen mariculture, to surround an entire 230 m^2 surface area cage system to 3 m depth, the cost of inexpensive, 6 mil HDPE panel with grommets installed would be approximately US$84. A separate quote for a reinforced (110 g m^{-2}) panel to 6 m depth was US$375. The latter would include about US$260 for material and the balance for labor. The difference in materials would result in differing durability and longevity. The latter could last many years if properly cared for and only deployed a few times. To this must be added the cost of lead line or weights.

6.4.6 Ozone

General Description: Ozone is a three-atom allotrope of oxygen; it is colorless but has a distinctive odor that is noticeable to humans at low concentrations. It is a strong oxidizing agent and is dangerously toxic to humans at levels < 0.1 mg L^{-1}. It has been used in Europe, North America and elsewhere as a water disinfecting agent (Owsley 1991). In some aquaculture applications it is very effective, e.g., for removing certain pathogens such as *Giardia lamblia* and infectious fish viruses such as the infectious hematopoietic virus. It has also been used to destroy toxic microalgae or toxins (Rosenthal 1981). Although ozone is unstable and has a short half-life, it is also very toxic to fish and residues must be removed before aquaculture use.

Effectiveness and Limitations: Ozone must be produced *in situ* with an ozone generation unit due to its short half-life. The efficiency of these units is improving, and the costs decreasing, but it still remains an expensive and "high-tech" means of HAB mitigation. Due to the requirement for electrical power, pumps, residue removal mechanisms (such as heaters or packed columns), ozone use for commercial scale fish mariculture is judged not to be cost effective.

Costs and Practicality: A first order analysis of the amount of ozone required for mariculture is a function of the injection rate, amount of material being oxidized, tidal flushing rate, surface loss rate, depth, area and volume of the treated area in the fish farm. A typical fish culture zone in Southeast Asia might be 1 km long, 200 m wide and 6 m deep, with tidal flushing rate of about 0.1 per day. For this estimate, the ozone requirement would be on the order of 400 kg per day. One can argue it is not required to inject the ozone continuously (only inject for part of the day); but it seems the cost would be substantial even if 40 kg per day are used. An approximate estimate of the capital costs alone would be US$76,000 for the generation equipment, not including the distribution equipment, compressors, and power supply. The

latter can be a huge cost in marine water raft applications, if adequate safety standards are followed. The risk of overdose is very large too, and would mandate an accurate and well-maintained ozone monitoring system for the treated water. Ozone treatment holds promise for intensive, recirculating and other types of high technology mariculture use in the future, but for the present it is well beyond the practical and economically feasible reach of most fish mariculture operations.

6.4.7 Site Selection

General Description: Judicious choice of aquaculture sites based on past or predicted fish-killing microalgal occurrence is an obvious, but seldom-exercised measure to reduce the risk of fish kills. In the practical and competitive fish mariculture industries worldwide, there are many important siting and permitting considerations that may take precedence over harmful algae concerns. Mariculture farms are sometimes located by trial and error method in this regard, but there have been exceptions where prior study has been effective for avoiding unsuitable locations. General aquaculture siting references include volumes by Edwards (1978); Sedgwick (1982); Beveridge (1987); and ICES (1992) and there are many impact studies and reviews too (e.g., Black 2001). A host of papers and industry magazine reports deal with this subject as well (e.g. Landless and Edwards 1976). With the exception of Beveridge (1987), few deal with HAB considerations. The following describes general approaches to be taken in countries and areas where HABs are possibly or likely to be a problem for fish mariculture.

Hydrographic monitoring should be conducted in the general vicinity of a proposed fish mariculture project. This can be used to evaluate site suitability for the cultured fish species and to obtain some indication of HAB-fishkill risks. Variables collected in vertical profiles such as water temperature, salinity, DO, turbidity and chlorophyll *a* along with Secchi disk water transparency can be surrogate indicators to predict the possibility and risks of harmful microalgal blooms or events. Concurrently collected phytoplankton composition and biomass data are very desirable. Typically, current speed, direction, wind wave exposure and bottom substrate composition are evaluated too. An experienced and skilled analyst can use these data to evaluate site suitability using indices such as the degree of vertical stratification during neap tides and clement weather. A well-mixed and relatively strong tidal flushing are generally considered advantageous in avoidance of microflagellate and dinoflagellate blooms and turbulence actually may destroy cells or reduce growth of some dinoflagellate species (White 1976). In temperate coastal marine waters, high background levels of dissolved inorganic nitrogen often accompany such dynamic conditions in outer coastal areas so that light is typically the factor limiting microalgal production. This is an added advantage to site selection in such areas.

There is a tendency for some HABs to recur in the same areas and some species have benthic stages or cyst forms that will be deposited on the sea bottom. This can be determined through repetitive annual sampling of the water column during high-risk periods, but sediment collection for examination of dinoflagellate or microflagellate cysts may be a useful alternative in some cases. Strong tides necessary to disperse waste products from some mariculture locations may preclude the presence of a soft bottom substrate and cyst deposition. Hydrographic and phytoplankton data from prior studies by universities, government agencies or anecdotal observations of other groups frequenting the subject area may be available to help evaluate HAB risks. If these are not available, field surveys should be conducted during the appropriate algal bloom season(s). Alternatively, another approach is to operate only small scale or test facilities while hydrographic, phytoplankton, fish growth and survival data are gathered.

Strong vertical mixing may not be useful for all harmful species, however, as the diatom *Chaetoceros concavicornis* may be harmful at relatively low concentrations (Rensel 1993, Albright 1993) and be present throughout a well mixed water column to 40 m or more (Rensel Associates and PTI

Environmental Services 1991). Despite extensive site surveys and location of facilities in strong current areas, there remains the risk of blooms being advected into a usually safe area from offshore or adjacent waters. Examples include *Gyrodinium aureolum* blooms along the coast of Norway (Dahl and Tangen 1990), shellfish toxin-producing *Gymnodium catenatum* blooms on the northern Atlantic coast of Spain (Fraga et al. 1988) and the fall 1994 *Heterosigma akashiwo* blooms in active tidal channels of the northern central coast of British Columbia and again in the summer of 1997 on the outer Vancouver Island coast.

6.4.8   Alternative Fish Culture Systems

General Description: Traditional floating marine net-cage systems are easy to operate, require relatively low capital investment, rely on simple and proven technology, and allow for easy incremental changes in production capacity. When properly sited and operated, their impacts are found to be minimal. For algal bloom mitigation, however, they have specific design limitations that are difficult to surmount unless additional equipment is utilized. Alternative net-pen and other rearing technology may be viewed as a means of HAB mitigation for fish mariculture. There are many alternative mariculture systems that may be used to rear fish; although most are reiterations of prior attempts that were never broadly adopted for good reasons including high capital and maintenance costs. This section describes three relatively new and developing technologies: offshore cages, bag systems and onshore tank farms. We also present some tentative conclusions of the advantages, limitation and affordability of each type of system.

Offshore cages:

These consist of heavily reinforced net pens attached to novel cage structures that either flex with the waves, or are submerged in one fashion or another. We do not include manufactures' estimates of wave height durability, as the frequency of the waves, not the height, is often a more important parameter for offshore cage operation. Other novel systems are being introduced at the time of this writing, but are not reviewed here, such as a new Norwegian design by Storm Havbruk (Loden Laks AS, Fiskeforsyningen AS, Project and Design Services AS and the Nutreco-owned company Skretting).

Circular Polyethylene Pipe Cages: These systems involve two or more polyethylene pipes for flotation enclosed by a one-piece rotationally moulded stanchion that also supports an interior perimeter handrail and net attachment base. They are used more often in protected inshore locations, but some designs claim offshore capability. A narrow walkway of wood planking is sometimes attached above the flotation pipes. These systems are used extensively in low to moderate current speeds (i.e., to about 50 cm sec^{-1}) and in "moderate" wave heights and frequency conditions. They are often quite large, 40 to 60 m diameter and up to 100 m most recently. They are considered useful in semi-protected waters, not in more exposed and full-scale oceanic conditions. They are used to rear salmon, trout, seabream, tuna and other species. Manufacturers include Industrial Plastics Co. of Washington State and AquaSure Co. in British Columbia. A 30 m diameter cage costs about US$20,000, without nets.

Flexible Rubber Cage: These cages include the first examples of offshore cages that were used for net-pen rearing in many locations around the world. Well known names in this category include Bridgestone Hi-Seas Cage and the Dunlop Tempest Cage, both made by vehicle tire manufacturers. The strategy with these designs is to use one or more continuous, rubber hoses with pneumatic flotation. The simple design may not include walkways or other functions, thus keeping the system uncomplicated and flexible for offshore conditions. Nets are attached to the floating collar, but not hung by them, to evenly distribute the load. Fishing floats distributed around the perimeter of the cages provide for net flotation. Most are circular, but some are other shapes. The inherent flexibility of the systems is reportedly resistant to wear and can survive storm conditions at sea. There are many hundred Bridgestone Hi-Seas Cages operating in

various conditions, but these systems are suitable only for large-scale culture of fish. The costs of these cages are relatively high, perhaps three times higher than polyethylene cages. They also require support vessels of some magnitude, separate feeding systems, and other support mechanisms.

Ocean Spar^{TM} Systems: The Ocean Spar offshore cage is one of the latest offshore systems, manufactured by Ocean Spar Technologies, of Bainbridge Island, Washington. This company was founded by commercial fishing trawl manufacturers with an understanding of the extreme forces involved in offshore fishing. A novel part of the system is the use of partly-submerged, external corner braces known as "spar buoys" that are designed to interact with the net-pen assembly to dampen and minimize wave and current stress (Figure 6.5, Loverich and Croker 1993). These spar buoys, with vertical motion heavily dampened by ballast plates, have little response to short-period waves, called "chop", and become wave followers for the longer period waves, known as ocean swell. The net pen, being attached to the spar buoys at the top and the bottom, moves in phase with the spars, greatly reducing dynamic loads on the netting. In addition, since the anchors are installed with a predetermined minimum tension, the currents have a minimal effect on reducing the total rearing volume. The entire floating tension structure then becomes relatively transparent to waves passing through it and highly resistant to shape changes caused by ocean currents. These systems have been tested in rigorous oceanic-like environments in the very exposed waters off the coast of Washington State (Strait of Juan de Fuca), and are now in use near New Hampshire, Hawaii, Philippines, Ireland, Faroe Islands, Canary Island, and Cypress.
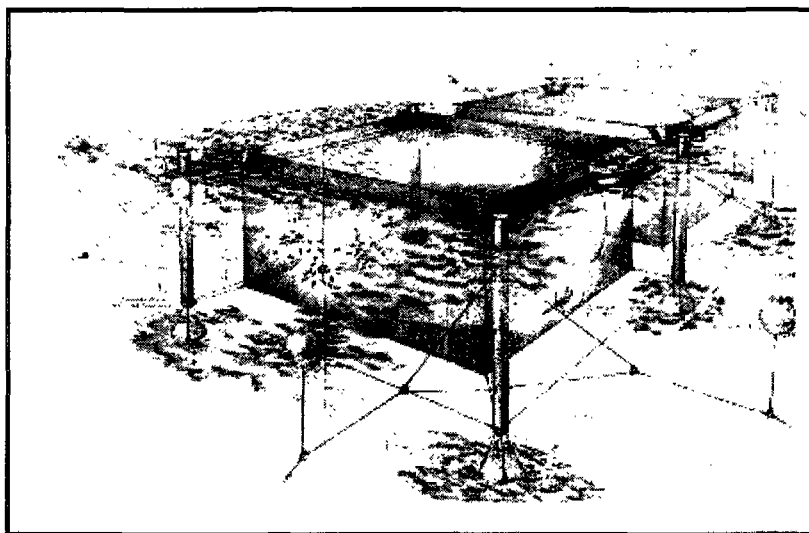
FIGURE 6.5. A series of Ocean Spar pens showing the corner spars and supporting anchoring structures. (Source: Ocean Spar Technologies LLC.)

Bag Systems:

Bag systems involve the use of cages that are constructed of impervious materials such as nylon or hypalon. Water is pumped into the cage, and solid wastes may be collected from a conically-shaped bottom sump. The idea is not a new one, and has been used in the past in Alaska and elsewhere but has had several reincarnations over the years ranging from cone shaped arrays to rectangular and square shaped enclosures. These systems are highly dependent upon a power source for pumps, aeration, or oxygenation. In some regards, they might be similar to an onshore (i.e., upland) tank farm, except they

are located in the water and use flexible but impervious-sided pens rather than fibreglass, steel or other rigid materials to contain the fish. Pumped water into the top creates a positive head pressure within the bag that helps maintain the shape of the bag assembly. Environmental restrictions in some areas and recurring phytoplankton-caused fish kills have spurred the interest by manufacturers to produce bag systems.

Onshore Tank Farms:

Onshore tank farms are similar to freshwater rearing facilities used for several species of fish such as salmon, but use saltwater sources that in most cases must be pumped from the sea into very large permanent tanks. The technology was promoted as the solution to fish farm environmental problems in the early to mid-1980's, and some large companies invested in design and sale of the technology. It involves complicated and expensive rearing systems focusing on high-density fish culture, to help offset the costs. Oxygenation of the rearing containers is usually prescribed, and huge pumps and supply pipes are necessary.

Effectiveness and Limitations: Offshore cages, bag systems and onshore tank farms are not widely used at this time anywhere, but there could be applications of these in the future. All have been touted as a solution to nearshore pollution that may be linked to some mariculture practices. As discussed below, the offshore systems fulfill this promise, depending on the site selection, but bag systems and onshore tank farms have major limitations in this regard.

Offshore Cages: These systems have proven to be useful in some applications, such as in semitropical areas with marine fish and for salmon mariculture in temperate waters, but at present they are not widely used. This may be due to a number of factors, including capital expense and lack of durability of some of the older systems. In general, true offshore waters are usually much more oligotrophic than inshore, coastal waters, so the fate of waste nitrogen from really large facilities should be considered. Some offshore areas such as the waters near the Southeast US coast and Florida are considered nutrient sensitive, and toxic *Gymnodinium breve* blooms may actually initiate there. In more extreme north or south latitudes, however, large tides and natural nutrient flux along with light limitation of phytoplankton blooms are better conditions for preventing eutrophication and effects of offshore cages.

Bag Systems: Bag systems have been used for years on a small scale in limited applications (e.g., Arlo 1991). To the best of our knowledge, they have never been used in commercial scale mariculture (except in government supported test facilities), but rather are considered appropriate for specialized situations when highly valuable fish stocks need to be reared at a specific location. For example, bag systems have been considered for use to rear endangered species of salmon in North America where the fish must be reared in their natal waters. In that case, the capital and operation costs may be outweighed by the risk of loss due to plankton blooms or other perturbations of surface water quality. The ability to pump water from relatively deep water, when available, results in an efficient means to avoid most, but not all, harmful plankton blooms. For other applications, however, the high purchase and operational costs and risk of power loss are a concern. The claims that they may be used to control pollution seem disingenuous, as there are no practical and inexpensive means to dry the waste products or remove the salt, if it is intended for use as agricultural fertilizer. Several of the authors have observed fish kills from HABs in these cages in Tasmania, despite computer-controlled and variable depth intake systems designed to sense and avoid chlorophyll rich layers of seawater. For some high value fish (e.g., live fish sales in S.E. Asia) or for threatened species (e.g., chinook salmon in parts of the N.E. Pacific), bag systems offer a means to control risk if properly sited and operated.

Onshore Tank Farms: Perhaps the most important ingredient for an onshore tank farm is a suitable site. It must be a low elevation location, with relatively deep water immediately offshore to minimize the expense of large diameter pipelines. Infrastructure support includes the need for high voltage, multiple phase power supplies. It is important to note, however, that waste treatment is not feasible with onshore tank farms, as there are no cost-effective means to remove the solids and deal with the salt that remains.

6.4.9 Filter Systems

General Description: Filter systems to remove different sized particles are commercially available and range from simple bag or sock filters that physically trap cells to elaborate mechanical process filters such as rotating screens. Sand filters are commonly used to remove particulate matter from large-scale seawater aquaria and laboratories. Stacking screens, strainers, cartridge filters, bead filters, wound poly or ceramic filters and a myriad of other variations are available too, but are usually for small-scale mariculture applications. Swirl separators are mechanical devices that rely on the use of centrifugal forces for removing particulate matter that has a density greater than water. This technology has been used in treatment of wastes from the food processing and related industries. There are many versions of this technology available, and only one has reportedly been adapted to use for removing phytoplankton cells in particular [Hydrodynamic Filtration System (HFS) by I-Tek, Inc. of Hampton, Virginia.]

Effectiveness and Limitations: Although filtration systems have proven to be useful for marine laboratories, marine aquaria, and commercial hatcheries, they are typically not economically viable for large-scale grow-out systems such as fish net-pen mariculture facilities. Tests of the HFS were reportedly conducted in Korea at the Korean Ocean Research and Development Institute (KORDI) at Sang-Ju, NamHae, KyungNam Province, with the toxic alga *Cochlodinium polykrikoides*. Evaluation included cell counts, chlorophyll *a* and coulter counter analysis. In one test, removal efficiency was about 93%. As expected, the unit was more efficient for removal of larger particles or algae in the 30 to 40 μm range for which 92% removal was achieved. Removal efficiency dropped to about 80% for the smallest particles in the 4 to 10 μm range. No third party independent testing of this unit has been made. The HFS, like all types of filter systems, suffers from the potential flaw of rupturing HAB cells, and passing the toxins or harmful substances to the fish. In particular, this may apply to *Gymnodinium* complex dinoflagellates that are easily destroyed in any handling or processing. Mechanical filtration (except reverse osmosis) could be supplemented with activated charcoal treatment to remove toxins, but the costs would be entirely prohibitive for large-scale "grow-out" systems.

Combined Uses: Filtration could be used in concert with perimeter nets and aeration, to limit the volume of water needed, but the rate of filtration required to quickly remove HABs would be cost prohibitive.

Costs and Practicality: Cost estimates for HFD are not readily available, and large-scale commercial filtration systems are typically designed and built on an individual basis. We believe that filtration systems, including the HFD, are not suited for any form of net pen or other extensive or semi-intensive mariculture systems due to the large volumes of water involved and the potential problems with release of toxins into the treated water from delicate cells. For example, a recent bloom in Wellington Harbor, New Zealand, where a seawater pump and filtration system passed *G. mikimotoi* toxin into a marine laboratory, killed a crop of cultured bivalve larvae (P. Redfearn, NIWA, Wellington New Zealand, pers. comm.). For small-scale mariculture, simple bag filters of small mesh (e.g., 10μm) could be bought or constructed from the proper materials. But filtering the water is a strategy that seems inappropriate for any HABs affecting large-scale mariculture. Toxic blooms kill the fish too fast to allow filtration to be used and other blooms that cause environmental hypoxia are better dealt with by aeration (or broader environmental assessment and cleanup), possibly with perimeter skirts.

6.4.10 Dietary or Chemical Treatments

There are no readily available or government-approved therapeutic drugs designed specifically to treat fish that have been affected by HABs, but efforts have been made to develop them (Rensel and Whyte 2001). A major killer of farmed fish is the formation of the superoxide anion radical (O_2^-) by *Chattonella antiqua* and *Heterosigma akashiwo* (Tanaka et al. 1994; Oda et al. 1997; Nakamura et al. 1998) and *Cochlodinium polykrikoides* (Kim et al. 1999). The mechanism for death is considered to be mucus stripping from the gills of the fish, which leads to osmoregulatory dysfunction and ultimately death. Reduction of the oxygen radical to the more harmful hydroxy radical is effected in the seawater. Quenching the radicals with the enzymes superoxide dismutase, catalase, and glutathione peroxidase has been demonstrated to protect fish in laboratory studies (Yang et al. 1995) and could alleviate the problem in affected waters (Colt et al. 1991). Addition of such chemicals to the water would depend on the fisheries and environmental agencies in the jurisdiction where the farms are sited, but implementation could hold promise if cost effective.

Physical gill damage from HABs is another major killer of fish (Albright et al. 1992). Consequences of the damage include hypersecretion of mucus and blood hypoxia. Chemicals that reduce mucus production could potentially provide mitigative action. Mucolytic agents such as L-cysteine ethyl ester fed to fish have reportedly reduced gill mucus production and sustained fish during exposure to harmful *Chaetoceros* in the laboratory (Yang and Albright 1994). This appears to be a useful approach to dealing with acute exposures, but extended exposures over months may not be as germane for the following reasons. Feeding fish during HABs is not recommended because the high oxygen demand needed for digestion competes with that for basal metabolic maintenance; and moderate amounts of mucus production on the gills from chronic exposure to harmful *Chaetoceros* is likely beneficial to the fish. Mucus discharge along with coughing response provides a defense mechanism for removal of the HABs from the gills (Rensel 1993). Without mucus, some species of spiny phytoplankton that lodge in the gills would likely be enveloped through lamellar fusion, as has been documented for *Corethron* spp. (Speare et al. 1989). Mucus provides a protective barrier and lubricating ability for the gills, without which the gills are more susceptible to secondary infection from bacteria, viruses and parasites. A different approach using cysteine compounds has been preliminarily tested, not by feeding it to fish, but by treating their culture water (Jenkinson and Arzul 2001). In these tests, cysteine, ethyl cysteine ester and L-acetyl cysteine were used to reduce "rheotoxicity" (thickening of the water due to mucus produced by several HABs) and cytotoxity of HABs that included two species of *Gymnodinium*. Some of these chemicals are widely used in medicine as mucolytic agents, which protect by selective reduction against damage from free oxygen radicals. This approach is both technically and economically viable, and should be tested on a pilot scale.

Other drugs such as adrenaline and acetylcholine that are vasoactive agents, regulating the distribution of blood to the gill secondary lamellae (Part et al. 1982) could possibly be of some use in treating HAB-affected fish. The need to administer these orally and the potentially high costs, however, limit their usefulness.

6.4.11 Miscellaneous Mitigation Practices

General Description: This section briefly describes other miscellaneous management practices for HAB mitigation in finfish mariculture. We treat these with less detail because some, such as feeding practices, are indisputably correct, and others, such as submerging net pens, are unproven for the most part.

Feeding and Handling Practices: An effective and low cost algal bloom mitigation practice for finfish aquaculture is to withhold feed immediately prior to, if possible, and during HAB events. This reduces the

digestive demand for oxygen leaving only oxygen required for basal metabolism. Over a period of several weeks, however, this practice causes increased stress due to low glycogen stores, catabolism of tissues, and susceptibility to chronic diseases such as bacterial kidney disease. Similarly, cessation of all fish handling and restriction of human activity nearby to reduce stress on the fish further reduces oxygen demand by the fish. However, both of these strategies are usually not adequate to deal with major HAB events.

Submerging net pens: Submersion of pens to avoid HABs is generally not recommended for standard designs of net pens because it is technically difficult for commercial-sized systems. Preliminary studies such as Rubach and Svendsen (1993) show it to be technically possible. For sites in deep water, which are the exception, the method may offer some practical advantage using systems designed for temporary or permanent submersion (e.g., Sea Station design by Net Applications Systems, Bainbridge Island, WA). For small-scale conventional net pens in protected bays, a mechanism could be designed to lower each cage beneath the surface. However, conventional mariculture cages are not equipped to deal with the different structural stresses of submerged nets in areas of moderate or stronger tidal currents.

Pre-emptive Harvest: Harvesting of fish just prior to occurrence of a major HAB event is considered by some to be a mitigation means. However, this is very undesirable as marketing large volumes of fish on short notice is difficult and some net pens, such as in S.E. Asia, are used for sale of live fish that are far more valuable than killed fish. Additionally, pre-emptive harvest would be predicated on an effective early warning system that usually does not exist in most fish mariculture areas.

Deep Net Pens: In large-scale net-pen mariculture, some companies use very large and deep net pens as a means of increasing rearing volume, lowering stocking density and avoiding HAB problems. However, it is controversial whether fish will purposely seek refuge in deeper water during blooms. Salmonids in net pens that are suffering from stress or environmental problems often orient themselves at the surface of the water and are accordingly referred to as "finners", despite having access to deeper waters beneath.

6.4.12 Survey of Mitigation Used Worldwide

This section briefly summarizes methods that are used in a number of countries to mitigate HAB events *in situ* at fish mariculture facilities. A number of leading industry experts and major fish farm insurance companies were contacted and given a questionnaire regarding mitigation.

The result of the survey in Table 6.4 show that areas with persistent HAB problems, such as British Columbia, tend to have a wider variety of measures used to combat the problem. Some areas, such as the Mediterranean coast of Spain, report virtually no problems with HABs killing mariculture fish. The most widely used method of HAB mitigation is cessation of feeding and reduction of handling stress, which is more of a best management practice than a pro-active mitigation measure. Aeration is the most widely used true mitigation measure, but in many cases it is specialized aeration such as the use of open diffusers, to move water to the surface as described above. Moving pens and perimeter skirts are widely used, and considered by many as the most effective measures. There are, however, major differences of opinion in this regard. Use of clay treatment is rapidly increasing in Korea, has been used in Japan, and is being studied in other areas. Chemicals are not widely used anywhere, although many have been tried on a trial basis. Ozone is also not used for commercial-scale mariculture at present.

TABLE 6.4. Summary of mitigation measures used in fish mariculture facilities.

E = experimental; L = limited use. Data from a representative cross-section may not be entirely comprehensive. BMPs = Best Management Practices such as stop feeding, no handling, reduce stress by any means. Alternative systems mean onshore tank farms, offshore cages or bag systems.

Country – Region	Aeration	Oxygenation	Airlift Pumping	Moving Pens	Perimeter Skirts	Ozone	Site Selection	Alternative Systems	Filter Systems	BMPs	Sink Pens	Pre-emptive Harvest	Deep Nets	Clay	Chemicals	Sources
British Columbia	X	X	X	X	X		X	E		X	E	X	X			1
Chile	X				X					X	X		X			2
Denmark	X			X			X			X		X				3
Greece																4
Hong Kong	X			E	E	E				X						5
Korea										X				X		6
New Zealand				X						X						7
Norway	X		X	X	X		X	L		X		X	X			8
Japan	X													X	E	9
Singapore				X	X					X			X			10
Spain-Med. Coast	--															11
USA-Washington State	X		X	X	X		X	E	E	X		X				12

1) B. Hicks, International Aquafoods; G. Robinson, Stolt Seafarm; I. Whyte, Fisheries and Oceans, Canada; 2) A. Clément, INTESAL, Puerto Monte; 3) Per Andersen, Bio/consult as., Denmark; 4) Y. Karakassis, Greece; 5) This document and K.C. Ho, Open University of HK for ozone; 6) Kim 1998; 7) F.H.Chang, National Institute of Water and Atmospheric Research Ltd.; 8) T. Thorsen, Vesta Insurance, Norway; J. Morris Roberts Morris Bray Insurance Brokers, London; Andersen 1996; 9) Shirota 1989; 10) Lim 1989; 11) G. Smart, Culmarex, Spain; 12) Rensel and Whyte 2001 and this document.

6.5 Impact Prevention, Mitigation and Control Strategies – Shellfish

Some of the mitigation strategies applicable for fish (sec. 6.4), namely site selection, pre-emptive harvesting, and movement of suspended culture systems to unaffected areas, are also of relevance to molluscan shellfish. In general, management of shellfish stocks in toxin-affected areas is still highly reliant on preventive strategies, especially monitoring for early toxin detection, rather than control measures. Control of toxins in marketed product by evisceration is possible in some cases (e.g. for DA in crabs and PSP toxins in crabs and geoducks in the Pacific US), but results in economic losses, as the price of whole, live product is considerably higher than that which undergoes processing.

6.5.1 Species Selection

The risk of toxin contamination can be controlled to some degree by selecting suitable shellfish species and/or tissues for marketing in regions affected by toxin outbreaks. This requires a comprehensive understanding of toxin kinetics (toxin accumulation and elimination rates) of local or imported species, which can be gained from extensive monitoring data and/or research efforts. Bivalve species can differ by up to a factor of 100 in their ability to accumulate PSP toxins even when exposed to comparable conditions of toxification (Bricelj and Shumway 1998). These differences are not related to taxonomic status but at least partially reflect differences in nerve sensitivity to the toxins and thus feeding response to toxic cells. Peak historical toxicities attained by North American species (Figure 6.6) provide an indication of this relative capacity for toxin uptake, although it is best determined from laboratory or field studies in which the different species are exposed to common conditions of toxification. Eastern oysters, *Crasosstrea virginica*, the razor clams, *Ensis directus* and *Siliqua patula*, and hard clams, *Mercenaria mercenaria*, generally accumulate much lower levels of PSP toxins than other species. In contrast, mussels, *Mytilus edulis*, butter clams, *Saxidomus giganteus*, and surfclams, *Spisula solidissima*, accumulate extremely high PSP toxin levels. In a laboratory study, the Pacific oyster, *Crassostrea gigas*, and Manila clam, *Tapes philippinarum*, attained at least an order of magnitude lower PSP toxin levels than the scallop, *Pecten maximus*. Such a ranking of species in terms of their contamination risk is available for North American species, but a similar database needs to be developed for the South Pacific. Extrapolation among taxonomically related species is unfortunately not possible. High interspecific variability in accumulated toxin has also been shown for other algal toxins (e.g. DA), but is best documented for PSP toxins.

6.5.2 Detoxification

Detoxification rate is another parameter useful in selecting suitable species for marketing in areas affected by toxic algae. Detoxification rates have been calculated from single- or two-compartment models fitted to shellfish toxicity data obtained in the field or in laboratory studies. Biphasic detoxification, characterized by a faster initial detoxification phase, followed by a slower one, has been described in several bivalve species, especially those that show prolonged retention of toxins. Species that detoxify rapidly often only experience temporary, seasonal harvesting closures in areas where toxic blooms show predictable, seasonal occurrence. Bricelj and Shumway (1998) classified bivalves as fast to moderate detoxifiers and slow detoxifiers, based on calculation of their rates of elimination of PSP toxins (% loss of toxin day^{-1}) from exponential decay functions (Table 6.5). Again, the available database is heavily biased towards North American species. In general, fast to moderate detoxification is associated with toxin loss rates of about 6 to 17% day; these species require ca. one to ten weeks to attain the safety level. All mussel species tested to date (*Mytilus edulis*, *M. galloprovincialis*, *Perna viridis*, etc.), except the South American mussel, *Aulacomya ater*, fall into this category. In contrast, slow detoxifiers take several months to years to attain the safety level, and generally exhibit toxin elimination rates $\leq 1\%$ (range = 0.3 to 4%).

Elimination of PSP toxins to levels below the safety level, even for bivalve species that detoxify fairly rapidly, typically requires a period longer than the 48 hrs adopted in several countries for bacterial depuration. Transfer of bivalves from a non-contiguous toxic to toxin-free area is often restricted by local regulations. It can also pose a risk because several thecated dinoflagellate species [e.g. *Alexandrium* spp. (Bricelj et al. 1993; Laabir and Gentien 1999) and *Prorocentrum lima* (Bauder and Cembella 2000)] are known to readily survive gut passage in bivalves and could therefore provide an inoculum in a previously unaffected area. Viable cells of *Heterocapsa circularisquama* were also found in shipments of pearl oysters from Japan (Honjo et al. 1998). In contrast, naked *Gymnodinium mikimotoi* cells did not remain intact following gut passage (Laabir and Gentien 1999). Transfer for a few days to a land-based facility in

recirculating filtered seawater would be necessary to allow gut evacuation of feces prior to safe transfer to the field. Prolonged holding of animals in a land–based facility would only be economically viable for a high-value product.
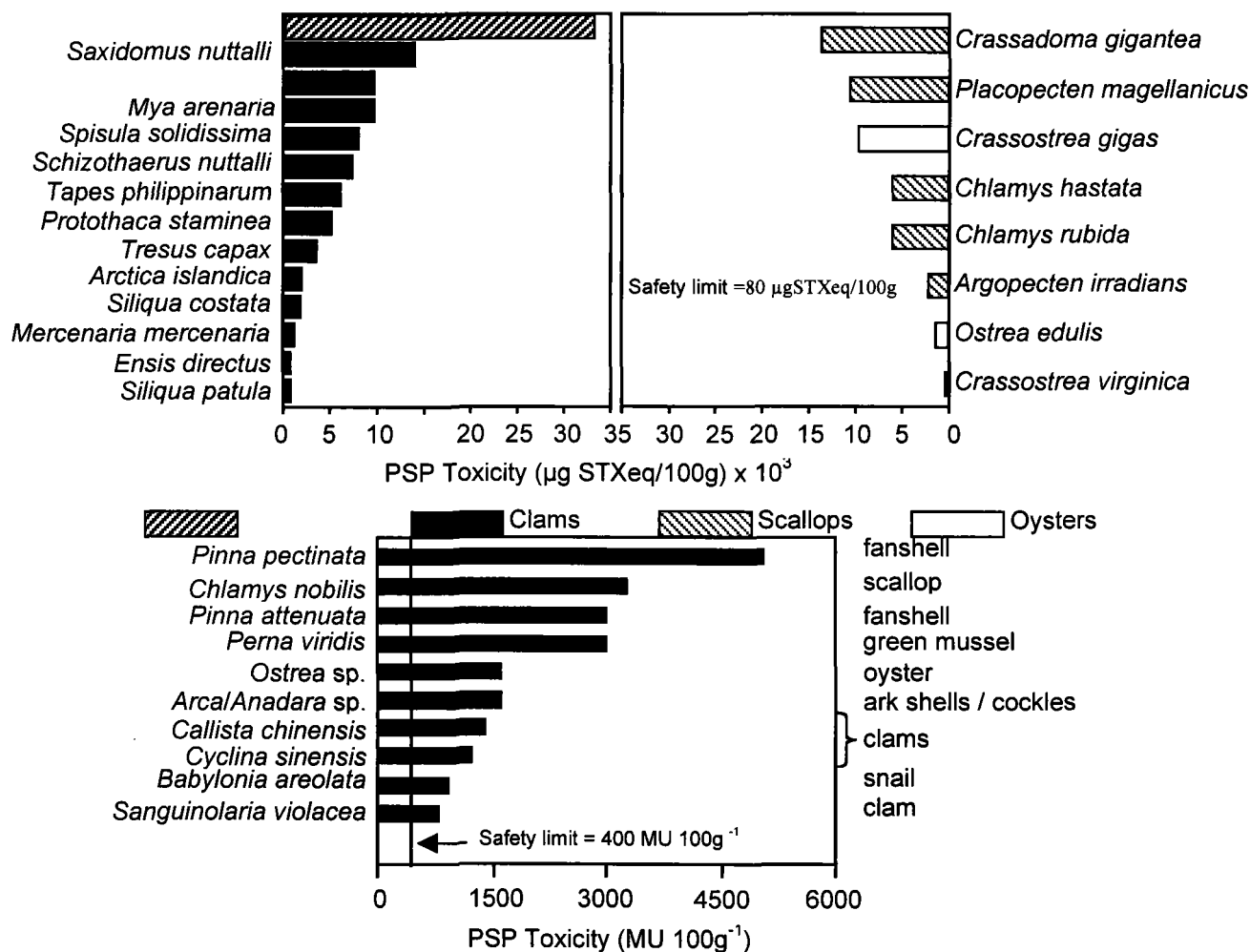
FIGURE 6.6. Upper: **Maximum PSP toxicities historically recorded in field-toxified North American bivalves.** (Plotted from Table 1 and Fig. 1 in Bricelj and Shumway 1998; see this source for common names.) Lower: **Toxicity maxima in molluscan shellfish from southern China, Guangdong, recorded in 1990-1992.** (Modified from Lin et al. 1994; only species with maxima exceeding the safety limit, out of 33 tested, are shown.)

TABLE 6.5. Detoxification of PSP toxins by bivalve molluscs (adults unless indicated), as measured by the time required to attain the regulatory level (RL = 80 µg STXeq 100g⁻¹), and detoxification rate (% loss of toxin day⁻¹), determined for whole tissues unless specified.

(Edited from Bricelj and Shumway 1998, see reference for details on calculations and original sources.) F = field; L = laboratory; Dig. = digestive gland; juv. = juveniles. Data for *Aulacomya ater* from Andrinolo et al. (1999) and % loss day⁻¹ also calculated from an exponential fit to data in Compagnon et al. (1998).

	Species	Time to RL (weeks)	Detoxification Rate (%/day)	Location
	A) Fast to moderate detoxifiers:			
CLAMS	*Tresus capax*	5.2 < t < 11.6	-	BC, Canada
	Mercenaria mercenaria	3.4 - 6.1	9.3 - 9.5	L
	Meretrix casta	4.4	-	Kumble Estuary, India
	Mya arenaria	1.0 - 4.0	9.8	Maine, USA
		3.3 - 4.0	-	Bay of Fundy, Canada
		4.7	7.7	St. Lawrence Estuary, Can.
MUSSELS	*Mytilus edulis*	0.6 - 9.6	10.7	Maine, USA
		5.1	13.8	Gaspé Bay, Canada
	Mytilus californianus	2.8 - 9.0	6.6 - 7.4	Pacific N. America
	Choromytilus palliopunctatus	1.2 < t < 7.0	17.2	Oaxaca, Mexico
	Perna viridis	1.7	9.3	L
	Modiolus modiolus	0.9 - 9.2	7.0	Maine, USA
OYSTERS	*Crassostrea gigas*	0.6 - 2.0	-	Pacific N. America
	Crassostrea iredescens	1.8 - 3.8	8.9 - 18.1	Oaxaca, Mexico
	Crassostrea cucullata	6.9	5.5	Kumble Estuary, India
	Ostrea edulis	6.4	4.0	Maine, USA
	Pecten maximus (scallop)	6.4	7.4	L

	Species	Time to RL (weeks)	Detoxification Rate (%/day)	Location
	B) Slow detoxifiers:			
CLAMS & COCKLES	*Saxidomus giganteus*	114	0.3 - 0.6	BC, Canada
		> 14	-	L
	Saxidomus nuttalli	> 73	0.9	CA, USA
	siphon	≥ 39	0.4 - 0.8	"
	Spisula solidissima	> 26	-	L
		96 - 100	0.8 - 1.9	ME,USA
		> 69	0.4 - 1.6	Georges Bank, USA
	Cardium edule	11.2	3.3	Portugal
	Soletellina diphos	> 51	1.2 - 3.6	Tungkang, Taiwan
SCALLOPS	*Placopecten* Dig.+mantle+gill	> 28	0.2 - 0.6	L
	magellanicus Dig.	> 5.2	0.6	L
	Dig.	> 8.7	-	Bay of Fundy, Canada
	Mantle	> 17	-	Bay of Fundy, Canada
	Patinopecten yessoensis Dig.	> 12	1.2 - 4.1	Funka Bay, Japan
	Dig.	> 17	3.8	Ofunato Bay, Japan
	Aulacomya ater (mussel) Dig.	17	1.7-1.8	Southern Chile, Peninsula Valdés, Argentina

Acceleration of detoxification may in some cases be possible by manipulation of environmental variables. Temperature manipulation was found to be ineffective in accelerating PSP-toxin depuration in two bivalve species characterized by prolonged retention of PSP toxins: butter clams, *Saxidomus giganteus* (Madenwald 1985), and surfclams, *Spisula solidissima* (Bricelj et al. 1998). In this study, the data suggest that an increase in temperature may only serve to increase the rate of transfer of PSP toxins from surfclam viscera (which show temperature-dependent detoxification) to other tissues that show strong binding of toxins (temperature-independent detoxification). Faster detoxification can be achieved in some cases when animals are fed during the decontamination period. For example, Dungeness crabs (*Cancer magister*) detoxify domoic acid from their viscera at a significantly higher rate (15.4% loss day^{-1}) when they are fed non-toxic clams than when starved (4.3% day^{-1}) (calculated from an exponential fit to data in Lund et al. 1997).

Much more limited information is available on the detoxification kinetics of ASP, DSP and especially NSP toxins. Detoxification rate of domoic acid is very rapid in mussels, *M. edulis*, (38 to 72 % loss day^{-1}), but occurs relatively slowly in species such as the Pacific razor clam, *Siliqua patula,* and Atlantic scallop, *Placopecten magellanicus* (Table 6.6). Laboratory studies yielded DA detoxification rates for viscera of 16.5, 14.8 and 5.8% loss day^{-1} in three Pacific scallops, *Patinopecten (Mizuhopecten) yessoensis, Chlamys rubida* and *Chalmys hastata* respectively (calculated from exponential detoxification equations provided by I. Whyte, DFO, BC, Canada, pers. comm.). Additional information on detoxification kinetics of DA in scallops is needed, especially in the context of the extensive closures of offshore scallop fisheries in Scotland and Ireland caused by DA contamination in 1999, which affected both the queen scallop, *Chlamys opercularis,* and king scallop, *Pecten maximus*. A summary of estimated detoxification rates for DSP toxins from selected literature studies is shown in Table 6.7. It is difficult, however, to compare data between studies because different methods of DSP toxin analysis were used, and different toxin metabolites were tracked. A detailed study on detoxification kinetics in bay scallops demonstrated that, in contrast to PSP toxins, DSP toxins were eliminated at a slower rate from viscera (8.4% day^{-1}, Table 6.6) than from the gonad and other tissues (50 and 74 % day^{-1} respectively) (Bauder 1997).

For NSP toxins, relatively long-term retention was demonstrated under field conditions (Sarasota Bay, Florida) in the clam *Chione cancellata* (Steidinger et al. 1998b). Clams with a peak toxicity of 95 MU 100g^{-1} required ≥ 26 wks to attain the 20 MU 100g^{-1} regulatory level (RL). In contrast, relatively rapid detoxification of NSP toxins (5 days to achieve the RL from initial levels of 49.8 MU 100g^{-1}) was found in the Pacific oyster, *Crassostrea gigas*, under laboratory conditions (20°C, ozone or UV sterilization used to inactivate live algal cells released in seawater) (Fletcher et al. 1998), again suggesting that detoxification rate is species-specific.

Additional studies are required to improve our understanding of detoxification kinetics, mechanisms and pathways of all major toxin groups once the animals are no longer exposed to the toxin source, thus allowing prediction of the timing and duration of closures and development of methods to accelerate depuration. Models of shellfish biotoxin contamination/decontamination that can predict the rate of toxin uptake and loss from phytoplankton concentrations and cell toxicity and other environmental factors (e.g. temperature) are being developed for key bivalve species and toxins (e.g. Moroño et al. 1998a,b for PSP toxins in mussels). As our understanding of toxin kinetics becomes more sophisticated, such models will become increasingly useful as a mangement and monitoring tool.

TABLE 6.6. Detoxification of domoic acid in bivalve molluscs (whole tissues unless specified). L = laboratory; F = field; regulatory level, RL = 20 µgDA g^{-1}; % loss day^{-1} calculated from an exponential fit to the data; Dig. = digestive.

Species	Initial toxicity (µg DA g^{-1})	Time to RL	% loss day^{-1}	Conditions/ Source
Mytilus edulis	21.9	-	69 – 72	11°C, L (1)
	66.5 (Dig.)	6.7	-	7 – 9°C, L (2)
	130	4	38	15°C, L (3)
	300	≈ 35	≈ 40	F, winter (4,5)
	73.1 (Dig.)	12	-	F (6)
Placopecten magellanicus	3108 (Dig.)	>> 14	10	L (7)
Siliqua patula	147	135	-	F(8)
	73 (foot, mantle, and siphon)	54	-	

(1) Novaczek et al. 1991; (2) Outerbridge 1992; (3) calculated from Scarratt et al. 1991; (4) Martin, pers. comm.; (5) Silvert and Subba Rao 1991; (6) Martin et al. 1990; (7) Douglas et al. 1997; (8) Wekell et al. 1994.

TABLE 6.7. Detoxification rate of DSP toxins from bivalve molluscs (viscera). (Modified from Bauder 1997.)

Data from cited studies were fitted to an exponential loss equation. Toxin concentrations from mouse bioassay data were converted from 1 µg DSP toxin g^{-1} = 4 MU g^{-1}; D8OA = okadaic acid-diol ester

Species	Method	Toxin	% loss/day	Location	Source
Argopecten irradians	LCMS	D80A+ OA+ DTX1	8.4	Lab	(1)
Mytilus edulis	LCMS	DTX1	15.4	Mahone Bay, NS, Canada	(2)
	HPLC	DTX1	5.7	"	(3)
	Mouse assay	OA	8.4	Lab	(4)
	HPLC	OA	3.0	Lab	(5)
	Mouse assay	OA	1.7 – 2.5	Lab	
	HPLC	OA	11.1 – 17.8	Pond	
	Mouse assay	OA	3.4 – 13.7	Pond	
M. galloprovincialis	LCMS	OA	18.9	Adriatic, Italy	(6)
Patinopecten yessoensis	Microbial inhibition	OA	3.2 – 7.8	Lab	(7)
	ELISA	OA	7.6	Japan	(8)

(1) Bauder 1997; (2) Quilliam et al. 1993; (3) Gilgan et al. 1995; (4) Yasumoto et al. 1978; (5) Marcaillou-Le Baut et al. 1993; (6) Poletti et al. 1996; (7) Kikuchi et al. 1992; (8) Tazawa et al. 1989.

6.5.3 Tissue-Compartmentalization of Toxins (product selection)

Safe harvesting in areas affected by red tides/HABs is also possible by marketing tissues that show reduced or negligible capacity for toxin accumulation. For example, the locomotory tissue (adductor muscle, and the muscular foot) of bivalves typically contains less than 3% of the PSP toxin body burden despite its large contribution to total body mass (Bricelj and Shumway 1998). The large foot of some species such as *S. patula*, and Arctic surfclam, *Mactromeris polynyma* (used in sushi) is considered a delicacy. Only the foot of the carnivorous gastropod, *Argobuccinum ranelliformes* (palo palo) is consumed in southern Chile and the PSP toxicity of this tissue was always below the safety limit and generally undetectable by HPLC (Compagnon et al. 1998). In contrast, toxin levels well above the safety limit were found in the foot muscle of another gastropod, *Concholepas concholepas* (loco) in this study. The prominent adductor muscle of scallops is the main product consumed in N. America, and typically only attains one to three orders of magnitude lower PSP toxicity than the digestive gland. It is noteworthy, however, that domoic acid accumulates at highest levels in the foot of *S. patula*, whereas it is concentrated in the viscera in mussels (*Mytilus edulis*), indicating that binding of toxins may be highly toxin-specific as well as species-specific. Anatomical partitioning of PSP toxins in bivalve molluscs is reviewed by Bricelj and Shumway (1998).

It is also noteworthy that the adductor muscle of naturally or laboratory contaminated scallop species studied to date (*Placopecten magellanicus* and *Pecten maximus*) show undetectable or only trace amounts of domoic acid, well below the 20 μg g^{-1} RL, allowing safe marketing of this product (Douglas et al. 1997; Stewart 1998; Arévalo et al. 1998). Care must be taken in processing, however, as leakage of toxin may occur from the digestive gland and may also be associated with the intestinal loop which coils around the adductor muscle. In contrast, DA can exceed the RL in the roe (gonad) when DA contamination is severe, thus affecting the roe-on market. In 1995, when offshore Atlantic sea scallops, *P. magellanicus*, reached very high DA levels in Georges Bank and Browns Bank (> 1300 and > 2500 μg g^{-1} viscera) the roe attained 150 μg g^{-1} (Stewart 1998). Similarly, Bauder et al. (2001) found that a significant portion of the total DSP body burden (12%) was associated with the gonad of bay scallops (*Argopecten irradians*).

The digestive gland (visceral mass in clams) contains the bulk (80 to 98%) of the PSP toxin body burden in all bivalve species studied to date, during toxification. PSP toxins are also restricted to the digestive tract in finfish and DA is accumulated exclusively in the hepatopancreas of Dungeness crabs (*Cancer magister*). Thus the action level for DA in the Pacific US is based on the toxin level of the viscera in this species. The bulk of accumulated DA is also concentrated in the viscera of *M. edulis* and *Crassostrea virginica* (but not in razor clams, *Siliqua patula*). Domoic acid can be incorporated at levels exceeding the RL in scallop gonads, as found in offshore Scottish scallops (Philipp Hess, Marine Institute Fisheries Research Centre, Ireland). Therefore, evisceration or removal of the viscera may in some cases reduce toxicities of remaining tissues to safe levels. It is important to note, however, that the relative distribution of toxins does not remain constant over time. During detoxification, due to the rapid exchange rate of PSP toxins between tissue compartments, non-visceral tissues (mantle and gills) typically show an increase in the relative (%) contribution to the total toxin body burden and may eventually contain a greater proportion of total toxin than the viscera over the course of detoxification. Detailed knowledge of tissue-specific toxin kinetics is therefore useful in risk assessment.

6.5.4 Vertical Placement in the Water Column

Vertical stratification of toxic algae in the water column (especially in the case of motile dinoflagellates) has suggested the possibility that suitable depth placement of bivalves could serve to minimize the risk of toxin accumulation. Field experiments in the Bay of Gaspé, Atlantic Canada, showed that offshore

mussels became 2 to 5x more toxic when suspended at the surface than near-bottom (Desbiens et al. 1990; Desbiens and Cembella 1993). However, the proliferation of *Alexandrium* throughout the water column and the high toxicities encountered in this region precluded the possibility of using this method of mitigation. Its effectiveness and practicality is likely site- and algal species-specific. However, bivalves such as *Mytilus edulis* can accumulate PSP toxin levels exceeding the safety level within less than an hour, such that even brief exposure to toxic cells could yield unacceptable toxicities.

6.5.5 Processing of Seafood

The industrial canning process has been found to significantly decrease the levels of PSP toxins present in some shellfish species, but was only shown to be practical when the initial toxin levels were relatively low. The cockle, *Acanthocardia tuberculatum*, is harvested on the Mediterranean coast of Spain and marketed exclusively as a canned product. Harvesting for this species was banned due to the presence of persistent levels of PSP toxins since the occurrence of a 1989 toxic bloom. In 1996 the Commission of European Communities (CEC) published a decision (91/492/EEC) which established conditions for harvesting and processing of certain bivalves from areas where PSP levels exceeded the 80µg STXeq 100 g^{-1} safety limit. This decision applies specifically to *A. tuberculatum* and authorizes Spain to harvest cockles when toxicities in edible tissues are > 80 but < 300 µg STXeq 100 g^{-1} (Burdaspal et al. 1998). Bivalves must undergo processing clearly defined in the Annex of this decision (Table 6.8). The decision was based on findings that this heat treatment effectively reduced the toxicity of cockles, which initially contained up to 800 µg STXeq 100 g^{-1} raw meat, to levels below the detection limit as determined by mouse bioassay (Berenguer 1993). Toxin reduction of cockles containing up to 300 µg STX eq 100 g^{-1} raw meat was > 82-88% based on the mouse bioassay, and 97% using HPLC and a fluorometric method (Burdaspal et al. 1998). The dominant toxins present in fresh tissues were dcSTX, GTX$_5$ (= B$_1$) and STX. A toxin monitoring program was established for the final processed product in 1996, in which edible tissues (foot), the distal (pigmented) portion of the foot, which is known to concentrate PSP toxins, and the associated liquid are analyzed for their toxin content.

The canning process was also shown to reduce toxicity levels in PSP-contaminated Japanese scallops (Noguchi et al. 1980; Nagashima et al. 1991). Retorting (110°C, 80 min or 122°C, 22 min) effectively reduced toxicities of scallops containing up to 2000 µg STXeq 100 g^{-1} of digestive gland below the safety limit, but heating (70°C, 20 min) was insufficient. Further testing is required to ensure that the method is applicable to PSP-contaminated species with toxin compositions differing from those of shellfish used in these studies. Additionally, unexplained variation in the residual toxin levels found in processed cockles harvested at different times of the year was noted by Burdaspal et al. (1998) and requires further investigation.

Household cooking (e.g. boiling, steaming, frying), however, is not an effective means of detoxifying shellfish to safe levels because PSP toxins are relatively heat stable, although it may reduce initial toxicity. A survey conducted by the Division of Public Health in Kodiak Island, Alaska, US, an area endemically affected by PSP, showed that the prevalent belief among the local residents that cooking is an effective treatment to prevent PSP was one of the reasons contributing to their lack of responsiveness to PSP warning signs in shellfish harvest areas (Gessner and Schloss 1996). One of the conclusions of this study was that increased effort should be directed towards educating the public on the inefficacy of this and other prevention methods to discourage illegal harvesting in contaminated areas. The Dungeness crab on the Pacific US coast can also accumulate PSP toxins but does so exclusively in the viscera. Since boiling of whole crabs introduces a risk of spreading toxins from the viscera to other tissues, health authorities in Alaska recommend removal of the viscera prior to cooking of crabs. Therefore, consumer education

TABLE 6.8. Annex to the Commission Decision 97/77/EC of 18 January 1996, published in the Official Journal of the European Communities of January 20 1996.

Molluscs have to undergo the following operations sequentially.

1. Preliminary cleaning in fresh water for a minimum of two minutes at a temperature of 20° C, plus or minus 2° C.

2. Pre-cooking in fresh water for a minimum of three minutes at a temperature of 95° C, plus or minus 5° C.

3. The separation of flesh and shells.

4. Second cleaning in running fresh water for a minimum of 30 seconds at a temperature of 20° C, plus or minus 2° C.

5. Cooking in fresh water for a minimum of nine minutes at a temperature of 98° C, plus or minus 3° C.

6. Cooling in running cold fresh water for approximately 90 seconds.

7. The separation of the edible parts (foot) from the non-edible parts (gills, viscera and mantle) mechanically with water pressure.

8. Conditioning in containers closed hermetically in a non-acidified liquid medium.

9. Sterilization in autoclave at a minimum temperature of 116° C for a time calculated according to the dimension of the containers used but which cannot be lower than 15 minutes.

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programs on the potential risks associated with various seafood handling practices are a useful management tool.

Domoic acid in Dungeness crabs is also confined exclusively to the viscera, primarily hepatopancreas. Processing of crabs contaminated with DA by cooking in either fresh or salted (3% NaCl) water can greatly reduce the DA levels of the hepatopancreas, by 67 to 71%, resulting in transfer of most of the toxin to the cooking water (Hatfield et al. 1995). A very small amount of toxin is transferred to the meats (non-visceral body and leg meats; < 2 μg DA g^{-1} for crabs initially containing 22 μg g^{-1} in the hepatopancreas). Subsequent storage of cooked crabs under conditions typically used following processing and distribution (–23°C for up to 90 days) had little effect on the total DA level in crabs, such that non-visceral tissues contained detectable but very low levels of DA.

Scallops (*Pecten maximus*) also retain DA primarily in the viscera (52 to 88%) (Leira et al. 1998). Frozen storage (for up to 180 days) of whole scallops contaminated with DA resulted in ~43% reduction of toxin levels, but there was significant transfer of the toxin from the hepatopancreas to the rest of the tissues (Leira et al. 1998). Industrial processing conducted on Galician scallops involves sterilization (at 155°C for 35 min) and packing in brine (3.2 g NaCl 100g^{-1} at pH = 6.5) or pickling (in olive oil and vinegar at pH = 2.6). Both result in high transfer of DA from the scallops to the packing media (30 to 65% of total toxin content) rather than destruction of DA, thus posing a threat to consumers of this processed product. In conclusion, processing methods must be carefully evaluated for each product and specific toxin to determine their effect on total toxicity and toxin distribution among tissues.

6.5.6 Detoxification by chemical agents

Ozonation is commonly used to depurate bivalves of bacterial pathogens, and can inactivate toxins from extracts of dinoflagellates and shellfish tissues. It was therefore promoted as a method for elimination of PSP toxins from whole organisms (Blogoslawski and Stewart 1978, Blogoslawski et al. 1979). Initial results by these authors in detoxifying softshell clams, *Mya arenaria,* showed some promise, but were invalidated in subsequent trials with the same species by White et al. (1985). One of the main problems with the use of ozonated seawater is that it does not necessarily come into contact with toxins incorporated in tissues. False expectations have been raised about the applicability of this method.

A new chemical method for decontamination of PSP toxins in shellfish was recently developed by Lagos et al. 2001) (Figure 6.7). This involves alkaline (pH = 9) immersion of contaminated product (e.g. live animals or shucked meats) followed by heating (e.g. boiling). Washing steps are required to remove toxins released into solution. This method was reported to yield 99% decontamination of Chilean mussels with an initial toxicity of 6800 µg STXeq. 100 g^{-1} (molar toxin composition ~40% $GTX_{1,4}$, 58% NEO and < 10% STX). Only one alkaline treatment was required for shucked meats rather than live mussels. The alkaline treatment was also incorporated as a step in industrial processing of canned clams (initial toxicity of whole tissues = 2943 g STXeq. 100 g^{-1}), in conjunction with the elimination of more toxic tissues (e.g. viscera, mantle and siphons), to reduce the toxicity of marketed product (the foot) to levels below the safety limit. However, the PSP toxicity of muscular tissue is generally low, and the clam data reported do not allow calculation of the relative contribution to decontamination of each processing step (alkaline treatment vs. evisceration).

6.5.7 Biological control

The presence of intestinal bacteria that can convert PSP biotoxins, from $GTX_{2,3}$ to STX has been documented in several invertebrates, including coral reef crabs, gastropods and bivalves (Kotaki 1989). These, however, would lead to an increase in net toxicity rather than providing a means of detoxification. However, some bacterial isolates from bivalve guts were able to reductively transform $GTX_{1,4}$ to GTX $_{2,3}$ *in vitro*, thus leading to a reduction in toxicity, while others apparently degraded $GTX_{1,4}$ without the appearance of GTXs (Smith et al. 2001). Bacteria isolated from the digestive system and gills of bivalve species that eliminate domoic acid relatively rapidly (*Mytilus edulis* and *Mya arenaria*) were shown to be capable of biodegrading DA and their growth was stimulated by DA (Stewart et al. 1998). Bivalve species that eliminate DA more slowly (*Placopecten magellanicus* and *Modiolus modiolus*) rarely yielded DA-degrading bacteria. Biodegradation capacity for DA was not detected in a variety of bacteria isolated from sediment and waters from the natural environment in this study. It was therefore suggested that the bacterial flora of mussels might play a role in their ability to rapidly eliminate domoic acid.

Bacteriological methods allowing toxin inactivation, degradation or enzymatic transformation to non-toxic or less toxic derivatives, or biotechnological approaches involving genetic manipulation of shellfish stocks need to be investigated in the future. For example, it was recently demonstrated that significant differences in PSP toxin accumulation rates (up to 50-fold) may occur among individuals of the same bivalve species (*Mya arenaria*) under identical conditions of toxin exposure, and preliminary work suggests that these differences may have a genetic/molecular basis at the level of the nerve sodium channel (Bricelj et al. 2000).
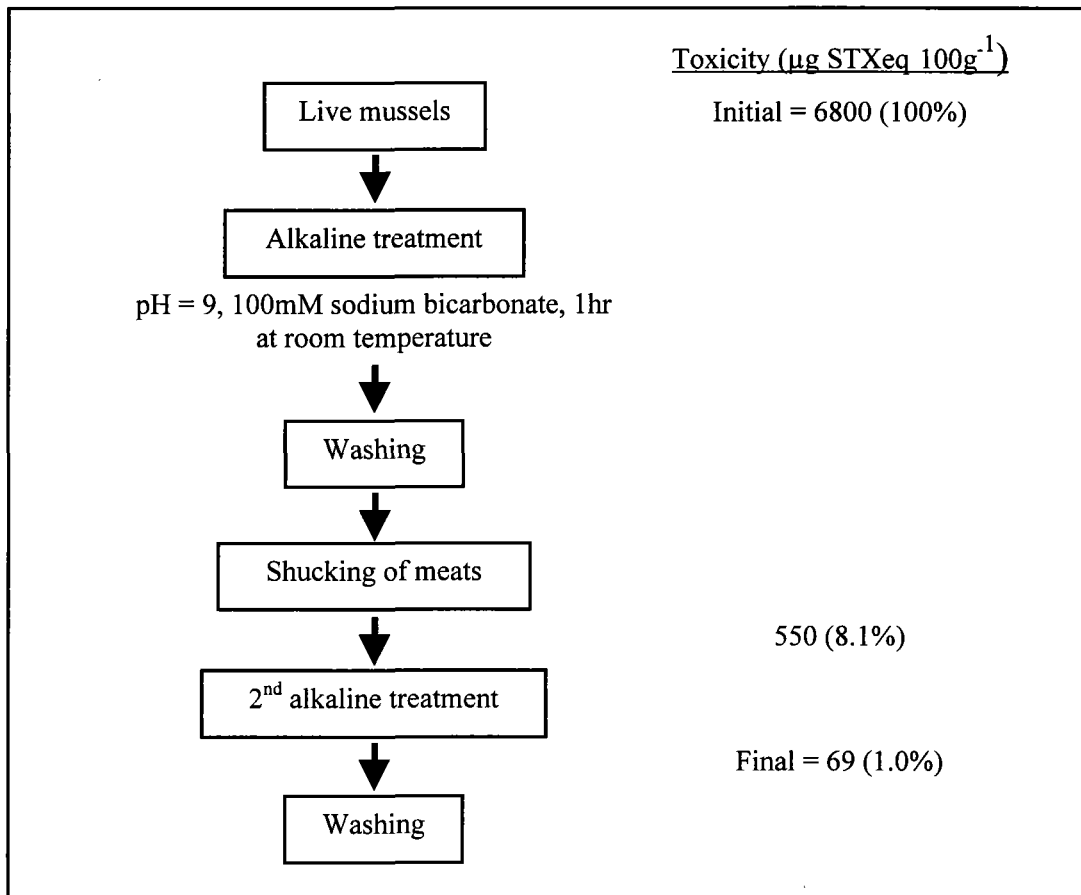
FIGURE 6.7. Flow chart of steps involved in chemical detoxification of mussels contaminated with PSP toxins and resulting decrease in toxicity. (Drawn from text in Lagos et al. 2001.)

6.6 Ciguatera Therapy

At present, it is difficult to detect ciguatera toxins in fish early enough to prevent fish from being consumed. As a result, many individuals are poisoned by CFP annually. Although this report is focused on management and mitigation strategies to detect HABs and mitigate their effects on fisheries products, it is worthwhile to highlight a medical treatment, which can be of tremendous help to victims of ciguatera fish poisoning.

The human symptoms of CFP are a direct result of the stimulation of the adrenergic and cholinergic nervous system due to the opening of sodium channels by ciguatoxin. The acute symptoms are many, and include nausea, diarrhea, vomiting, gastrointestinal cramping, paresthesias, and bradycardia. In addition to the acute symptoms, the chronic effects of CFP can persist with varying severity for months to years after the acute illness, with significant long-term disability as a result.

A variety of treatments have been tried for intervention in CFP (Blythe et al. 1994). These include antihistamines, corticosteroids, calcium supplements, amitriptyline, fluoxetine, and lidocaine derivatives.

None of these therapies have proven to be useful. In contrast, treatment with mannitol proved to be very effective, and should thus be promulgated as an important therapeutic step to be followed in all CFP cases. The treatment involves intravenous infusion of 20% mannitol (1 g kg^{-1} at a rate of 500 ml per hour) over 30 minutes, "piggy-backed" on an intravenous infusion at 30 ml per hour of 5% dextrose in saline solution. Blythe et al. (1994) describe controlled studies in which ciguatera victims were given mannitol at varying times after the initial poisoning. Mannitol therapy was effective in all ages and both sexes. There were no reported side effects. The treatment was most effective if given within 48 hours of exposure, but was still moderately effective if given 3-14 days after exposure. One individual even showed benefits from treatment 70 days after exposure, although one victim with a 1-year exposure showed no improvement. Those who reported a positive response to the mannitol did not experience the CFP symptoms again. Those who were not treated with mannitol continued to report symptoms for at least 3 years after the exposure.

This is clearly a method that should be publicized throughout the medical community, and to the general public. Prompt treatment of CFP victims will dramatically reduce the impact from this poisoning syndrome.

7 CONCLUSIONS

This report has reviewed a vast array of programs, strategies, and technologies that are used throughout the world to monitor and manage HABs. The following section provides highlights of the major concepts and approaches. Table 7.1 summarizes these technologies.

7.1 General Monitoring Issues

- As a consequence of the growing awareness and intensity of harmful blooms, HAB monitoring and management was ongoing worldwide in at least 45 countries/regions as of 1995. This number has certainly increased since then, and probably exceeds 50 at this time.

- The major goal for HAB monitoring and management programs concerning shellfisheries is to protect public health. A secondary objective is to allow fisheries development in areas affected by HABs. These programs are most often conducted by government agencies such as Fisheries Departments, Veterinary Services, Health Departments as well as different regional agencies.

- Another major goal for HAB monitoring and management programs is to protect aquaculture producers against economic losses due to harmful effects to the resource. These programs are sponsored or conducted by the aquaculture companies alone or in conjunction with government agencies, sometimes at the request of insurance companies.

- Basic parameters in these monitoring programs center on the presence of algal toxins in shellfish (and possibly fish) and the quantitative occurrence of HAB species. HAB monitoring and management programs must be well documented, organized and as simple as possible to be functional and effective.

- Communication within a HAB monitoring/management program as well as with the public is of major importance to avoid misinformation as well as mismanagement leading to public over reaction (i.e., the "halo effect").

- HAB monitoring and management programs should be reviewed at least once a year and should be flexible to respond to changes in available funds, industry demands, etc.

- It is suggested that an HAB monitoring and management program be a coordinated program including components on fish culture, shellfish harvest, beaches, and ecosystems. A "Task Force" or association is typically needed to organize, implement, respond and revise the monitoring and management procedures.

- An HAB monitoring and management manual should be prepared, describing the structure of the monitoring program in detail, the methods to be used and the management plans/actions in relation to HABs.

- Training courses should be implemented in the fields of phytoplankton taxonomy, quantitative analysis of phytoplankton including HAB species as well as handling and evaluation of results using databases.

- It is highly recommended that a geographic information system (GIS) database for storage, handling, evaluation and presentation of monitoring data should be implemented as one of the major tools for management of HABs. Output tables and graphics as well as statistical analysis from such a system would prove useful in communication among the participants of the monitoring/management program, as well as with the public.

- General public education information on HAB issues should be prepared and distributed using the Internet, information folders, posters, broadcasts etc. In contrast, specific "alert" information material should be prepared in advance to ensure proper coordinated communication with the public in the case of a serious HAB event.

7.2 Finfish Mariculture and Monitoring

- Routine monitoring of phytoplankton for fish mariculture facilities is conducted in many countries with large-scale marine fish farms. It is used to help predict the onset of harmful algal blooms and to manage mitigation during blooms.

- Some countries have elaborate and coordinated phytoplankton monitoring for fish farms, that is commensurate with the risks and losses due to fish kills (e.g., Norway). Other areas such as British Columbia have monitoring responsibility resting with individual farms or companies. This is because farms are often hydrographically remote from each other and located on an immensely large coastline, which reduces the chance of area-wide harmful algae effects. Chile is the only country with an industry-wide monitoring program fully paid for by the mariculturists.

- Case histories of monitoring worldwide show that government is sometimes involved in phytoplankton monitoring at fish farms during the initial phases of an industry. When industry consolidates into large companies with many sites, the mariculturists or their organizations typically take over the responsibility.

- Monitoring by mariculturists follows typical strategies such as heightened observations during known periods of risk, net tows for general examination of algae species, and cell counts from vertical profiles during HAB events. Consultants may conduct some of this work, but often mariculturists do so after training with local university, government experts or from fellow mariculturists.

- In periods of heightened bloom risk, visual surveys by fixed wing aircraft, with some ground truthing surveys, have proven to be an effective tool for early warning and bloom tracking. If conducted as discussed in this report, they can also quickly help determine if "refuge areas" from blooms exist for towing cages. This may also be critically important in designing relocation of fish culture zones, should that occur in the future.

- Observation of abnormal fish behavior is typically one of the first early warnings noted by fish mariculturists at the onset of a harmful algal occurrence or environmental hypoxia. In many cases and with most toxic HABs, it may be too late to avoid fish loss once such behavior is noted, hence the need for some type of early warning system.

- Certain species of cultured fish may be used as sensitive bioindicators for the rest of a mariculture fish population subjected to environmental hypoxia. *Chysophrys major* (red seabream) and *Rabdosarga sarba* (gold-line seabream) are two such species. There is anecdotal evidence that some species are

more sensitive to toxic HABs than others (e.g., rainbow trout versus salmon), which should be researched.

7.3 Finfish Mariculture: Mitigation of Fish Kills

- There is no single system of mitigation for harmful algae at fish mariculture facilities. Selection of method must be tailored to local hydrographic conditions (e.g., depth, current velocity, mixing properties), the nature (e.g., vertical distribution, mode of fish kill action) of the harmful algae likely to occur and the needs and behavior of the cultured fish.

- Site selection involving the deployment of net pens in areas less subject to fish killing blooms is a suitable means for fish mariculturists to avoid adverse effects of HABs. In practice, siting is usually done by a trial and error basis and from lessons learned from other sites in a region, but it can be accomplished through site evaluation prior to net-pen placement.

- Moving cages to refuge areas with fewer algae is a preferred method of mitigation by some companies and fish farmers in several countries. The trend towards use of very large cages in some countries has increased risks of this method, but many small cages may be suitable for movement, if refuge areas can be identified and potential interference with navigation issues addressed.

- Another widely used method is to displace algae from within the net pens. Typically, this is done by pumping deep water into the cages by airlifts or by using "open" aeration diffusers set well below the pens that will lift deep water with the rising air bubbles. This method is cost effective if the target HAB species are usually surface oriented.

- Use of perimeter skirts of reinforced polypropylene fabric may be highly effective to prevent harmful microalgae from killing cultured fish in pens. For environmental hypoxia producing blooms or upwelling of low DO water, a perimeter skirt would be ideal to retain water that could be aerated and effectively stripped of ammonia from fish excretion. It may also be used to retain clay treatment for flocculation treatment.

- Fish may be killed very quickly by HABs, so that no mitigation method would necessarily be effective if mariculturists wait until the last moment to react. One late-reaction method would be to deploy perimeter skirts, which can be done very fast if they are stored in a rolled up manner around the outside of the cages. The retained water could be treated with small amounts of appropriate flocculant, such as certain types of clay that were stored on site, ready to deploy. The environmental impact of this on the sea bottom is currently being assessed, but culture areas are already highly impacted for the most part and better ones have non-depositional sea bottoms of sand or coarser material that would prevent any long-term impact if the clay was used sparingly.

- A cost effective method to aerate smaller pens is through the use of inexpensive and easily maintained venturi-style, aspiration nozzles that transfer air from the surface to the pumped water, mixing it in specially designed chambers. This technology is more suited to small-scale systems as large-scale venturi systems are not commercially available and may have depth limitations.

- Cessation of fish feeding or handling and pre-emptive harvesting are practiced by many fish farmers worldwide when dealing with fish-killing algae. This will not entirely mitigate the problem, but helps by reducing stress and oxygen consumption by the fish, which may have damaged gill surfaces.

- Many other candidate methods have been suggested for mitigating blooms, but some are environmentally unacceptable (e.g., most oxidizing agents, copper sulphate, etc.), some are too expensive (e.g., ozone, oxygen injection), simply not effective (e.g., swirl separators) or unproven in practical use (e.g., drugs).

- Alternative fish mariculture systems are available but some may not be economically or technically feasible at present. Onshore farms are expensive to build and operate and demand low elevation, deepwater shoreline areas often used for other purposes. Bag culture (i.e., pens made of impervious, flexible material supplied by water pumps) are also relatively expensive and do not perform as a means to control wastes. They may be suitable for certain high value fish culture operations. Offshore pens may also be practical, if the scale of production could be increased and siting considers nutrient sensitivity issues. These pens are generally not placed in true "offshore" areas but are placed in exposed coastal areas. Some are well suited to sinking below the surface. Bag culture and onshore tanks farms do not solve localized waste discharge problems, as claimed by some proponents, as there are no inexpensive means to remove the dissolved and solid wastes that are ladened with salt, which prevents direct land application as fertilizer.

7.4 Fish Mortality and Toxic Blooms

- Many fish toxins cause death of mariculture fish by damaging or disrupting the gills, which leads to blood hypoxia and fish death. This process occurs quickly in most cases and toxins are apparently not transferred to the body of the fish or the edible portions. There are exceptions, so generalization is dangerous. Some fish toxins damage other parts of the fish too, mainly the liver, gut and heart, but neurotoxins may cause paralysis and death by blood hypoxia.

- The toxic actions on fish associated with several HAB species including *Gymnodinium spp.* and *Heterosigma akashiwo* are not well described. The available evidence, however, suggests that toxins or killing agents are not taken up and retained by dead fish for these species of toxic algae. Some low levels of toxins have been detected in fish killed by *G. breve* in Florida. The problem with many *Gymnodinium* spp. involves their delicate structure, so the cells rupture easily when pumped through the fish gill chamber or when mariculturists attempt to filter or pump the water with the alga present. Fish death causes from *Heterosigma* blooms remain an enigma, and multiple, possibly overlapping mechanisms have been described.

7.5 Effects of Harmful Algae on Shellfish

- Losses, mass mortalities and reduced production, of shellfish attributed to the direct (toxicity) and/or indirect effects (e.g. hypoxia) of harmful algae have been documented worldwide, especially for wild and cultured bivalves (Shumway 1990) and for shrimp in aquaculture ponds. It is important to emphasize that HABs that have no known human health effects (e.g. *Aureococcus anophagefferens*, *Heterocapsa circularsiquama*, *Prorocentrum minimum*, *Gyrodiniuym aureolum* and *Chrysochromulina polylepis*), can, however, have severe adverse effects on bivalve mollusc populations.

- Other algal species produce toxins that are detrimental to both humans and shellfish, e.g. *Alexandrium* spp. and *Pfiesteria* spp. and yet a third category affects humans but is generally not known to impair shellfish (e.g. *Pseudo-nitzschia* and *Dinophysis* spp.), although compromised motor (and thus escape) response has been observed in the scallop species, *Chlamys hastata*, contaminated with DA (Whyte et al. 2001) and stress responses were reported in DA-contaminated oysters (Jones et al. 1995).

7.6 Biotoxins

- Toxin monitoring programs worldwide are mainly designed to monitor shellfish in growing waters, although lot testing of wild and cultured shellfish is conducted in some cases. This reduces consumer risk and economic losses and hostility generated by product embargo at the market.

- In order to facilitate international trade, there is increasing demand that countries meet uniform requirements worldwide with respect to biotoxins and other seafood safety issues.

- Tagging of market product is required in many parts of the world to track the origin of local or imported contaminated product.

- Action limits for most algal toxins in shellfish and finfish are well established on a worldwide basis.

- Action limits for concentrations of HAB species have been established only in a few countries/regions. Many countries use detection/presence of HAB species or their concentrations as a trigger to initiate or increase sampling of fish and shellfish toxins. Local experience is needed to adapt the monitoring and management procedures to local needs.

- Established shellfish toxin monitoring programs have used their long-term historical data towards developing some predictive capabilities on the spatio-temporal occurrence and toxin levels of contaminated shellfish within a year, but are still unable to predict whether a toxic outbreak is likely to occur in a given year. Knowledge of local physical oceanography has proved useful in interpreting spatio-temporal patterns in the occurrence of shellfish toxicity.

- Effective communication between regulatory authorities and scientists involved in biotoxin monitoring and research, across institutional and political boundaries (most notably between Canada and the USA), is important in responding to HAB crises.

- A crisis situation is usually the impetus for development and/or expansion of a biotoxin monitoring program. The absence of toxic outbreaks for a few consecutive years often leads to funding cutbacks and downsizing of the program. However, red tides are a recurrent phenomenon, and a sustained monitoring effort is essential to protect public health and fisheries resources.

- Three elements of the monitoring program are essential to ensure efficient response to HAB outbreaks: a) a detailed, clear outline of agency roles and responsibilities (e.g. via a memorandum of understanding and regular meetings); b) a well defined set of procedures (action plans) to respond to different degrees of danger (e.g. routine, watch, or alert modes, similar to categories used in severe storm warnings); c) supportive legislation and authority for implementation and enforcement of the program.

- Multiple openings and closures of harvest areas and larger-scale closures than necessary to protect public health discredit the monitoring program, and result in lack of public compliance of closures or warnings.

- Shellfish species vary greatly in their capacity for toxin accumulation and retention. Indicator or sentinel species are often used in monitoring programs worldwide, because they provide early warning of toxic events and allow evaluation of historical/regional toxicity trends. Bivalve species

characterized by high toxin uptake and detoxification rates, which attain high toxicity maxima, and occur in relative abundance over a wide distribution range, are ideally suited as indicator species. Use of different sentinel species may be required for different biotoxins.

- A high degree of variability in toxin levels among individuals (with coefficients of variation, CV, of up to 98-99%) has been documented within regional bivalve mollusc populations. The magnitude of variation and the reasons for its occurrence need to be considered in designing regional sampling and monitoring programs.

- Effective shellfish monitoring can greatly reduce public health risk. However, human illnesses and fatalities due to biotoxins still occur in countries, which conduct intensive monitoring. They are mostly caused by disregard of warning notices posted in contaminated areas by recreational harvesters. This emphasizes the need to direct efforts towards public education, as well as monitoring.

- Public advisories to identify most susceptible groups to specific toxins within a population (e.g. individuals with poor renal function for domoic acid) may contribute to reduce public health risk.

- Dissemination of information to the consumer and seafood industry can reduce the economic impact of the "halo effect" as well as reduce public health risk. Food products (species or tissues) that are unaffected by toxins, or show a low risk of contamination should be specifically identified. Consumers should be informed of effective means of handling potentially contaminated food product, and of unfounded practices ingrained in public folklore. For example, household cooking or boiling of shellfish does not inactivate PSP toxins, since these toxins are heat stable. Inclusion of whole crabs, which have DSP contaminated viscera in soups is not a recommended practice, because cooking, can release these water-soluble toxins. Public education is also required to encourage compliance with posted warnings and advisories.

- Harvest closures due to contamination by PSP, ASP, AZP and DSP toxins do not affect swimming and other related recreational activities in these waters.

- Monitoring of both shellfish and phytoplankton provides the best strategy for prediction and understanding of toxic outbreaks, if resources are available. Testing of edible product is mandated by law and essential to protect consumers. Suspension-feeding bivalves also provide time- and space integrated information on toxic phytoplankton present in the water column. Routine phytoplankton monitoring is still restricted to measurement of cell densities rather than toxin concentrations. Even if water column phycotoxin concentration is measured, the same toxin concentration may lead to large variation in the toxin levels attained by different shellfish species. The advent of new automated technologies is likely to expand the capabilities for phytoplankton monitoring in the future.

- Toxic outbreaks do not preclude harvesting of fish and shellfish in areas affected by HABs if an effective surveillance program is in place. Selection of candidate shellfish species, which accumulate low toxin levels and detoxify rapidly, can mitigate the economic impacts of HABs.

- Mouse bioassays remain the internationally accepted method for determination of some of the major toxin groups. Improved standardization of routinely used mouse bioassay (e.g. the AOAC mouse bioassay for PSP toxins) protocols is recommended to improve reproducibility of results. Alternative toxin analysis methods, which show great promise, have been developed and are undergoing initial steps towards AOAC certification. For example, a new immunoassay allows non-invasive detection of ciguatoxins in individual fish.

- There is a growing demand for rapid, simple, inexpensive toxin screening methods (e.g. immunoassays and receptor-based assays) to reduce or avoid the need for live animal assays. Samples identified as positive (exceeding the safety level) by these methods can then be tested by mouse bioassay or analyzed by more sophisticated quantitative methods in a two-tiered biotoxin monitoring program.

- The long-term effects on human health of repeated or chronic exposure to many recently discovered toxins, including domoic acid, are unknown. Additional epidemiological studies and laboratory research are needed in this area.

- An effective therapy exists for ciguatera fish poisoning (CFP) victims, but it must be applied soon after consumption of contaminated fish. This is clearly a method that should be communicated to the general public and the medical community.

- Periodic synthesis and analysis of fish and shellfish toxicity data generated by the monitoring program (e.g. via technical reports) is essential to determine long-term toxicity patterns, develop predictive relationships and allow re-evaluation of monitoring practices.

7.7 Early Warning and Prediction

- Early warning and prediction of algal blooms requires observations that characterize algal distributions in relation to environmental factors over temporal and spatial scales appropriate for describing bloom dynamics. For many HABs and areas, mariculturists and agencies have learned about general risk timing and weather conditions that may lead to blooms. Exact prediction of timing and severity of HAB events is not probable in the near future.

- Simple, relatively inexpensive fluorometers may be used to describe distributions of chlorophyll *in situ*. They are useful for vertical profilers, moorings, and underway systems on ferries. Spectral absorption/attenuation meters and spectral fluorometers provide much more information, but effective interpretation requires considerable knowledge. Passive sensors for measuring ocean color can describe temporal and spatial variability of phytoplankton. A simple string of subsurface sensors can detect subsurface layers of phytoplankton.

- Because harmful algae can reside in subsurface layers and can be transported to and between coastal regions in these layers, it is important to determine the distributions of phytoplankton, relative to density, in vertical profiles. Monitoring programs should use profiling instrument packages to measure chlorophyll fluorescence, in addition to conductivity, temperature, depth, oxygen, and light attenuation or turbidity.

- Systems could be installed on ferries to continuously record chlorophyll, temperature, and salinity. Some blooms would be detected during transects, and long-term records would describe changes associated with eutrophication and climate variability. Analysis of nutrients and chlorophyll could be added.

- Moorings with an array of optical sensors can effectively describe variability of phytoplankton. Additional instruments can describe hydrographic processes and provide other measures of phytoplankton. Fouling of flow-through sensors can be retarded, and data can be telemetered to shore. A small device is available to alert the user if the moored instrument is stolen or breaks loose. It can be tracked through GPS and satellite communication.

- A new moored system keeps the instruments on the bottom: periodically the package rises to the surface, records data; then it descends, driven by wave motions. The risk of theft or damage would be reduced. Temperature, salinity, fluorescence and optical properties can be profiled.

- Inexpensive, autonomous observation systems can be installed on fish farms, to measure multiple parameters at several depths. Security should be good, but proximity of the structures to the sensors compromises some measurements.

- The SEAWATCH™ system is the only complete operational marine monitoring and information system available on the open market. Systems are installed in several countries, and planned for others. The parent company, OCEANOR, has unchallenged expertise in systems integration for marine monitoring. SEAWATCH can benefit a broad range of users. Costs are very difficult to estimate because each system is tailor-made: for 10 instrumented buoys, the initial investment would be about US $3 million - $4 million, plus $500,000 per year for spare parts and consumables. Costs for personnel, information systems and third party damage would be additional. SEAWATCH buoys are equipped to detect signals from phytoplankton, but more sensitive instruments for detecting algae and other constituents of the water are available on the open market. OCEANOR is receptive to 3rd party products and might incorporate these instruments into a bloom-detection system. Important selling points for SEAWATCH are that it is the only integrated system available on the open market, and that the information benefits many users beyond those principally concerned with algal blooms.

- Aircraft surveys of ocean color can be extremely effective in mapping the distributions of blooms. Imaging radiometers can do this quantitatively when conditions are good, but they require a big investment of money and trained personnel. An observer with video backup could map many blooms effectively. Movements of blooms could be predicted by entering the observed distributions into models of water movements.

- Satellite remote sensing is excellent for synoptic assessment of temperature and ocean color. Some coastal processes can be resolved, but there are major problems with clouds. For ocean color, patterns of phytoplankton in coastal waters can be difficult to distinguish because spatial resolution is too low, and interference from runoff obscures the signal from phytoplankton. However, several new ocean color satellites, some with improved capabilities, will be launched and research on interpreting imagery is progressing rapidly. Detection of algal blooms from remote sensing and prediction of movement from numerical models is being attempted.

- Operational forecasting of algal bloom dynamics seems not to be possible at this time, even though retrospective analyses have been quite successful at describing important factors that influence the distributions and persistence of algal blooms. Simulation models can be effective in revealing which factors dominate in the control of algal bloom dynamics, but only rarely can the models be parameterized using species-specific physiological data from the environment to be modeled. Continued research is extremely important to understanding and modeling algal dynamics. However, it seems that the best predictions, for some time to come, will be made by people who have worked on the problem for a very long time, and have accumulated a great deal of information on which to base assessments and forecasts. The lesson to date is that if there is continuity in efforts to observe, describe and explain the dynamics of algal blooms, predictive capabilities will develop and improve.

7.8 Control and Mitigation of Algal Blooms

- One approach to bloom impact reduction involves general water quality improvements through reductions in nutrient loading that create excessive N and P or imbalances between them and silica. Policies of this type have been linked to decreases in red tide incidence in some parts of the world, but the policies take years to produce detectable results, and are not guaranteed to work. There are many factors that govern the extent and virulence of red tides/HABs, only one of which is eutrophication due to pollution.

- There are documented cases of unintentional introductions and transfers of non-indigenous species, including red tide organisms, through ballast water discharges and mariculture activities in coastal and marine waters. Long-term management of red tide/HAB problems may need to consider restrictions on ballast water discharges, as well as the manner in which live fish and shellfish are transported and dispersed. Such policies are important for fish and shellfish disease prevention too and many economies lack even attempts at such controls.

- One aspect of impact prevention relies on accurate predictions of the timing or transport pathway of red tide outbreaks. There are, however, no predictive models of population development, transport, and toxin accumulation for any of the major red tide species worldwide. This is an area where sufficient promise exists to justify an effort to develop predictive models for red tides. Expectations must be kept reasonable, however, as the uncertainties in these models are significant and will continue to be that way for many years to come.

- Another approach to impact prevention would be early warning based on remote sensing of HABs. Satellite remote sensing has long been considered a tool with great potential for detecting and tracking red tide populations, but this technology has not yet fully lived up to this promise. Remote sensing from aircraft can provide useful information with less reliance on clear weather conditions, but the expenses can be significant, and development efforts are again needed to determine the types of sensors and the oceanographic features that should be monitored in this fashion.

- It is logical that HAB mitigation and control would be a prime research topic in many countries, given the scale of the impacts and the increasing trends in incidence. There are, however, only a few active research programs in the area of bloom mitigation and control, and therefore most strategies are not sufficiently well tested to permit rapid implementation. Targeted research is needed on the most promising technologies.

- Many chemicals have been proposed for use as HAB mitigation agents. In general, these claims are unsupported by scientific studies that document effectiveness on blooms and overall environmental impact. Although chemicals and integrated pest management are used extensively in agriculture and insect control on land, application of this technology to the ocean is new and controversial. Environmental concerns are significant in this regard, as it seems unlikely that a chemical compound can be identified that specifically targets only HAB species and leaves other algae and marine animals unaffected. Each candidate chemical will require extensive testing for lethality, specificity, and general safety, and each must surmount significant regulatory hurdles. Although direct chemical control of HABs is not a strategy of choice given other potentially more benign alternatives (e.g. clays), the success of this approach in terrestrial systems suggests that it should not be completely ruled out for further exploration.

- One material that shows considerable potential for HAB mitigation or control is clay. This natural, non-polluting material is abundant in many riverine systems and the ocean and may have limited

environmental impacts on its own if used wisely. If clay treatments are to be used to control HABs, however, a number of issues must be resolved. First, a local source of inexpensive clay would have to be identified, since transportation costs would quickly render this method uneconomical for large-scale control programs. Smaller quantities of highly effective clays or flocculants might be imported for small-scale control, such as at a fish farm site. In addition, studies of the environmental effects of the clay and the sedimented bloom biomass are needed, especially in the context of shallow, poorly flushed fish mariculture sites. Clay flocculation techniques should be evaluated for both large-scale bloom control, as has been practiced in Korea, and small-scale mitigation at the scale of an individual fish culture site or even fish culture cage. Both are already being done, and the latter will likely be used more broadly than the former in the near future.

TABLE 7.1. Summary of HAB monitoring and management technologies.

Approach[1]	Countries Using Technique[2]	Comments
HAB Monitoring and Management Program	More Than 45	Most countries with significant shellfish or fish production or consumption have implemented programs of this type.
Phytoplankton Monitoring - Light Microscopy	Canada, US, Norway, Chile, New Zealand, Japan, Denmark, France, Portugal, Spain, Italy	Phytoplankton monitoring has proven to be a useful and cost-effective method in regions with multiple species with multiple toxins and impacts.
- Molecular probes	New Zealand	This technology is useful in research but has not been fully validated for regulatory purposes, other than in New Zealand.
Toxin Monitoring in Shellfish Mouse bioassay HPLC (ASP)	Most Canada, U.S., Denmark, Spain, Netherlands, New Zealand, Japan, many more	Widely used and certified as regulatory method.
Receptor based assays	None	Several are being certified, but these are generally not approved for regulatory purposes.
Antibody kits	None	Several being certified, not approved for regulatory purposes.

[1] Only in support of monitoring of HABs for regulatory purposes.
[2] Not meant to be a comprehensive listing but rather to illustrate major country involvement

Toxin Monitoring in Fish Ciguatera - Mouse bioassay - Antibody kits	None None	Typically only used to verify clinical cases. Needs to be tested against fish from different regions
Swimming Beach Monitoring and Closures	Hong Kong	History of public acceptance of closures during red tides suggests continuation of policies, but less frequent closures might be warranted
Remote Sensing for Bloom Detection and Tracking Moored sensors (including SEAWATCH) - Satellite sensors Aircraft-mounted sensors - Aircraft Visual Observations - Underway ferry monitoring	Norway None None US, Canada, Japan Finland, others	High expense/low specificity for HABs; generally not used in routine monitoring Still a research tool. Expensive, provides necessary resolution for certain bloom types. Excellent method for bloom mapping and tracking Can provide regular sampling along fixed transects, can provide high temporal and spatial coverage; requires extensive analysis of samples
Public Education and communication Campaign	Many countries	Public education can minimize over-reaction to both harmless and harmful blooms. This is important for public health issues (fish and shellfish consumption) and for recreation (beach closures)
Bloom modelling for prediction	None	Remains an active research topic, but RT/HAB modelling and prediction is not yet possible; conceptual models useful in mariculture, numerical models used in research.

Broad Scale Bloom Control		
- Chemicals	None	Unacceptable impacts
- Biological agents	None	Used effectively on land, but not developed for aquatic use yet.
- Flocculant (clay)	Korea, Japan	Effective for some sppp. and potentially environmentally benign; needs more research before field application
Impact Prevention: Shellfish		
- processing	Spain	Applicable for shellfish canning industry.
- depuration	None	Common for bacterial decontamination, but not for biotoxins.
- ozonation	None	Not effective in reducing toxins in shellfish.
Mariculture Bloom Mitigation - Aeration	US, BC, Canada, Chile, Denmark, Hong Kong, Norway, Japan, many others	Widely used to displace surface blooms or to offset hypoxia, but not effective to combat toxic effects or the HAB cells. May be much more effective when combined with perimeter skirts
- Oxygenation	BC, Canada	Expensive, may be effective for blooms of noxious species.
Mariculture Bloom Mitigation (continued) - Airlift Pumping	BC, Canada, Norway, US, elsewhere	Used to displace surface blooms
- Moving Pens	BC, Canada, Chile, Norway, New Zealand, Singapore, elsewhere	May be very effective, but requires coordination and knowledge of refuge areas
- Perimeter Skirts	BC, Canada, Chile, Norway, Singapore, U.S.	Widely used. Must be deployed in advance of HAB event.
- Ozone	None	Effective, but too expensive and fish are highly sensitive to ozone so residue must be removed
- Site Selection	Varies	Works in some cases, but in practice rarely used in new areas.
- Offshore Pens	Ireland, New Brunswick, Faroe Is., Canary Is., Washington State, Philippines, Cyprus, Gulf of Mexico, New Hampshire, Hawaii, China	Some designs allow rapid submersion to avoid storm waves or surface-oriented HABs, but this is a developing technology in general.

- Filter Systems	None (for net-pen mariculture)	Mostly not effective and too expensive; useful for hatchery systems
- Best Management Practices	All, to varies degree	Useful in reducing fish stress during blooms
- Sinking of Pens below Surface	Some areas using offshore pens, often done for other reasons (e.g., aesthetics)	Used to avoid surface blooms; requires special equipment and relatively deep sites.
- Pre-emptive Harvest	BC, Canada, Norway, Denmark, US, elsewhere	Requires adequate warning and probably not suitable for live fish sales.
- Deep Nets	BC, Canada, US, Chile, Singapore	Not suitable for blooms mixed into water column.
Clay Flocculation	Korea, Japan, China	Potentially very useful for *in situ* mitigation within or near mariculture cages, needs to be evaluated with respect to logistics, environmental impacts.

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ANNEX 1

Health Certificate required for import of live molluscs into EU member nations

ANNEX I

HEALTH CERTIFICATE (MODEL)

No ....

pertaining to live: __ *bivalve molluscs* (^{1}).
__ echinoderms (^{1}).
__ tunicates (^{1}).
__ marine gastropods (^{1})
intended for direct human consumption in the European Community.

Dispatching country: ....
Competent authority (^{2}): ....
Inspection service (^{2}): ....

I. Identification of the products
__ Species (scientific name): ....
__ Nature of packing: ....
__ Number of packages: ....
__ Net weight: ....
__ Necessary storage and transport temperature: ....
__ Reference number of the analysis report (if necessary): ....

II. Source of the products
__ Approved production area: ....
__ Name and official number of the approved establishment: ....
....

III. Destination of the products
Products are to be sent
from: ....
(place of dispatch)
to: ....
(countries and place of destination)
by the following means of transport (^{3}): ....
Name and address of the consignor: ....
....
Name of consignee and address of the place of destination: ....
....

(^{1}) Delete where not applicable.
(^{2}) Name and address.
(^{3}) Registration number of lorries, railway wagons or container, flight number or name of the ship.

IV. Health certificate

I, the undersigned official inspector, certify that the live products described above:

1. were collected, if necessary re-laid over a period at least two months, and transported under conditions at least equivalent to those laid down in Chapters I, II and III of the Annex to Directive 91/492/EEC;
2. were handled, and if necessary purified in acordance with the hygiene rules laid down in Chapter IV of the Annex to Directive 91/492/EEC;
3. were inspected in accordance with the requirements laid down in Chapter VI of the Annex to Directive 91/492/EEC;
4. were packaged, stored and transp;orted in accordance with the requirements laid down in Chapters VII, VIII and IX of the Annex to Directive 91/492/EEC:
5. bear a health mark in accordance with providsions laid down in Chapter X of the Annex to Directive 91/492/EEC;
6. were analyzed and are in conformity with the requirements laid down in Chapter V of the Annex to Directive 91/492/EEC and therefore are fit for direct human consumption.

I declare that I am aware of the provisions of Directive 91/492/EEC laying down the health conditions for the production and the placing on the market of live bivalve molluscs.

Done at: .... on ....
              (place)   (date)

....
(signature of the official inspector) ([1])

Official seal ([1])

....
(name, title and designation of the signatory in capitals)

([1]) The colour of the seal and of the signature must be different from that of the other printing on the certificate.

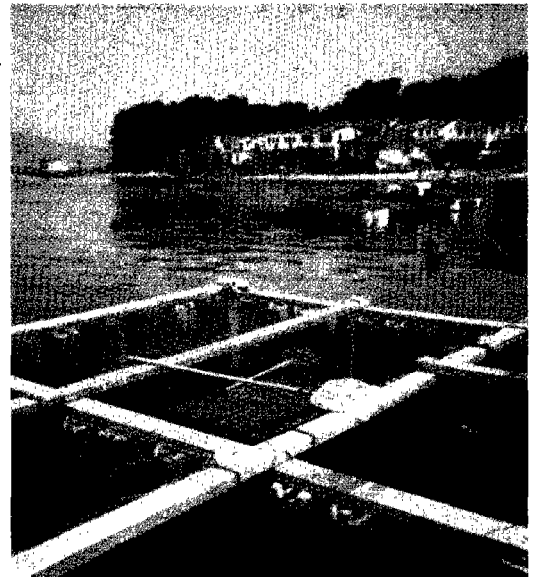## Export Health Certificate

STATE OF _____

ADDRESS _____

STATEMENT OF LICENSURE AND CERTIFICATION

Exported By: Certificate # _____   Consigned To:

License (*Check one*):   Shellstock Shipper ____   Shucker-Packer ____

Shipped Via:	Port of Embarkation:	Port of Debarkation:
Identifying Marks:	Total # of Containers:	Total Marked Weight:

Product:	Class, Type, Style:	Count:	Lot Weight:	Labels/Brand:

The above-named exporter hereby certifies through its authorized agent that this product was harvested from the following harvest area or areas:

Agent's Signature _____   Date: _____

The _____ State Department of Health routinely inspects shellfish operations and shellfish harvest areas to determine their compliance with state shellfish sanitation laws and the requirements of the National Shellfish Sanitation Program. The above named exporter is currently licensed and certified by the Department as indicated above. The above named harvest area is currently certified by the Department of Health as approved for harvest.

By: _____   Date: _____
(Appropriate state official/title)

Back cover photographs:

Top: Small scale net-pen system used for marine fish culture in NE Hong Kong waters near the Crooked Islands. Photo by J.E. Rensel.

Bottom: Pilot-scale operation of a series of bag system pens in Tasmania, Australia for rearing Atlantic salmon. Photo courtesy of Aquatas Tasmania.

Globally, the problem of harmful algal blooms has expanded considerably over the last several decades. Nearly every coastal country is now affected, often by multiple toxic or harmful algal species that threaten wild and farmed fisheries, coastal ecosystems, and recreational activities. The task of managing these diverse resources is a challenging one, made all the more difficult by the diversity of the potential impacts. This report provides a broad review of the many programs, technologies, and policies used worldwide in the monitoring and management of harmful algal blooms in coastal waters.