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Design and Implementation of some Harmful Algal Monitoring Systems

By Dr. Per ANDERSEN

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FOREWORD

The IOC has over the past eight years given increasing attention to activities aimed at developing capacity in research and management of harmful marine microalgae. A comprehensive source of information and guidance on design and implementation of harmful algae monitoring, has been identified as a priority at a number of international workshops and conferences. In order to meet this need, the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB) decided to establish a Task Team specifically to prepare such a source of information. The compilation of information on existing monitoring practices have been carried out in cooperation with the ICES/IOC Working Group on the Dynamics of Harmful Algal Blooms, and has been made possible through the financial support of the Danish agency for development assistance, DANIDA.

The easy access to manuals, guides, and information documents is a important task of IOC and UNESCO and essential to facilitate knowledge exchange and transfer, the related capacity building, and for the establishment of systematic ocean and coastal area observations as envisaged in the Global Ocean Observing System. Detailed descriptions of methodologies in relation to harmful algal laboratory and field work can be found in IOC Manuals and Guides No. 33, "Manual on Harmful Marine Microalgae".

The United Nations Conference on Environment and Sustainable Development in 1992 (UNCED), generated Agenda 21 and the two Conventions on Climate Change and Biological Diversity, and fully recognized the need for scientifically based information and methods for management. This Report, together with other IOC activities, is also to be seen as a direct follow-up to UNCED, and implementation of Agenda 21.

The IOC is highly appreciative of the efforts of the ICES/IOC Working Group for assisting in compiling the data, and Dr. Per Andersen (Bio/consult, Denmark) as author of the present volume. The report was reviewed by Drs. Catherine Belin (IFREMER, France), Bernt Dybern (IMR, Sweden), Lars Edler (SMHI, Sweden), Sherwood Hall (FDA, USA), Jennifer Martins (DFO, Canada), Karl Tangen (OCEANOR, Norway), and Choo Poh Sze (FRI, Malaysia), members of the IPHAB Task Team on Design and Implementation of Harmful Algal Bloom Monitoring programmes.

The scientific opinions expressed in this work are those of the authors and are not necessarily those of UNESCO and its IOC. Equipment and materials have been cited as examples as some of those currently used, and their inclusion does not imply that they should be considered as preferable to others available at that time or developed since.

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1. INTRODUCTION AND BACKGROUND

Occurrence of harmful algal blooms have been known from antiquity. During recent years harmful algal blooms have become an increasing problem in coastal marine waters, killing invertebrates and wild stocks and cultured fish, due to either toxicity, physical irritation of gill tissue or producing oxygen deficiency, or making shellfish and fish toxic due to accumulation of algal toxins which can intoxicate human consumers as well as wild life.

The numbers of algal species, which can occur at high concentrations so as to discolor the water, or produce potent toxins, are approx. 300 and 75 respectively. That is, only a small fraction of the approx. 5000 existing marine species. The harmful species are distributed among all major taxonomic groups - diatoms, dinoflagellates, other flagellates and cyanobacteria (blue-green algae).

It is suggested that the observed increase in frequency as well as number of species identified as harmful algal species basically is the result of eutrophication of some coastal areas (Smayda, 1990).

Furthermore the intensified world wide traffic may be responsible for the spread of harmful species, as live cells or resting cysts, in the ballast water of ships, or with the transfer of shellfish, between regions and continents (Hallegraeff, 1993).

The continued increase of the world population calls for a similar increase in the production of food protein and carbohydrates to prevent starvation. The need for an increase in the food production calls for intensified exploitation of marine living resources, either through fishery on wild stocks or, more likely, through large scale culture of fish, shellfish and algae in coastal areas.

To be able to proceed with this necessary intensified exploitation of the coastal marine waters for food production, the extent of harmful algal blooms, in time and space, must be controlled by reducing eutrophication, and the effects of harmful algal blooms must be minimized through proper management of the environment and the resources based upon well focused HAB monitoring programmes,

The aim of the present manual is to serve as a knowledge platform for aquaculturists, fishermen and public officials for establishing or revising monitoring programmes for HABs.

1.1 DEFINITION OF HARMFUL ALGAL BLOOMS

Harmful algal blooms (HAB's) can be defined as events where the concentration of one or several harmful algae reach levels which can cause harm to other organisms in the sea e.g. by killing fish and shellfish, or cause accumulation of algal toxins in marine organisms which eventually harm other organisms who will eat the toxic species, e.g. accumulation of algal toxins in shellfish who become toxic to human consumers (Table 1).

Table 1, Definition of the different harmful effects of algal blooms, and examples of algae responsible for the harmful effects, adapted from Hallegraef (1993).

<p>1. Blooms of species which produce basically harmless water discolorations, with the result that the recreational value of the bloom area decreases due to low visibility of the water and eventually, under exceptionally weather conditions in sheltered bays, the blooms can grow so dense that they cause escape reactions and indiscriminate fish kills and kills of benthic invertebrates due to oxygen depletion.</p> <p>Examples of species Dinoflagellates: <i>Noctiluca scintillans</i>, <i>Ceratium</i> spp., <i>Prorocentrummicans</i>, <i>Heterocapsa triquetra</i> Diatoms: <i>Skeletonema costatum</i> Cyanobacteria: <i>Trichodesmiumerythraeum</i> Other flagellates: <i>Eutreptiella</i> spp., <i>Phaeocystis pouchetii</i>, <i>Emiliania huxleyi</i> Ciliates <i>Mesodinium rubrum</i></p>
<p>2 Blooms of species which produce potent toxins which accumulate in food chains and cause a variety of gastrointestinal and neurological illnesses in humans and other higher animals such as;</p> <p>Paralytic shellfish poisoning (PSP) Examples of species: <i>Alexandrium tamarense</i>, <i>Alexandrium fundyense</i>, <i>Gymnodinium catenatum</i>, <i>Pyrodinium bahamense</i> var. <i>compressum</i> Diarrhetic shellfish poisoning (DSP) Examples of species: <i>Dinophysis fortii</i>, <i>Dinophysis acuminata</i>, <i>Dinophysis acuta</i>, <i>Dinophysis norvegica</i> Amnesic shellfish poisoning (ASP) Examples of species: <i>Pseudo-nitzschia multiseriata</i>, <i>Pseudo-nitzschia pseudodelicatissima</i>, <i>Pseudo-nitzschia australis</i> Ciguatera fish poisoning (CFP) Example of species: <i>Gambierdiscus toxicus</i> Neurotoxic shellfish poisoning (NSP) Example of species: <i>Gymnodinium breve</i> (= <i>Ptychodiscus brevis</i>) Cyanobacterial toxin poisoning Examples of species: <i>Anabaena flos-aquae</i>, <i>Nodularia spumigena</i></p>
<p>3. Blooms of species which, in most cases are non-toxic to humans but harmful to fish and invertebrates (especially in intensive aquaculture systems) e.g. by intoxication, damaging or clogging of the gills or other means, Examples of species: <i>Alexandrium tamarense</i>, <i>Chaetoceros convolutus</i>, <i>Gyrodinium aureolum</i>, <i>Chrysochromulina polylepis</i>, <i>Prymnesium parvum</i>, <i>Heterosigma akashiwo</i>, <i>Chattonella antiqua</i>, <i>Aureococcus anophagefferens</i>, <i>Phaeocystis piscimortuis</i>, <i>Nodularia spumigena</i>.</p>
<p>4. Blooms of species which produces toxins which are toxic to humans and which are transported by air in aerosols from the bloom area to the coast. Examples of species: <i>Gymnodinium breve</i> (= <i>Ptychodiscus brevis</i>), <i>Phaeocystis piscimortuis</i> (?)</p>

There is no general rule to define harmful concentrations of cells in an algal bloom, the concentration in a HAB is species specific.

Some algae cause harm at low concentrations, with no discoloration in the water, e.g. *Alexandrium tumarense* where PSP toxins are detected in shellfish at concentrations below 10³ cells/L, whereas other algae cause harmful effects when they occur in higher concentrations, with discoloration of the water as a result, a "red tide", For example *Gyrodinium aureolum* kills fish and benthic animals at concentrations higher than 10⁷ cells/L.

1.2 THE ALGAL TOXINS AND INTOXICATIONS

Types of marine algal toxins involved in shellfish and fish poisoning when consumed by humans are mentioned in Tables 2 and 3. The chemical structure of some algal toxins are shown in Fig. 1, See Steidinger (1993), Premazzi & Volterra (1993) and Flemming et al. (1996) for further information on toxin chemistry, different types of poisonings and their causes.

Many species of fresh water cyanobacteria are toxic and can cause harm by killing fish and domestic animals as well as imposing a threat on drinking water resources as a result of bad smell and taste and potential toxicity of the water (Premazzi & Volterra (1993) and Falconer (1993)).

Table 2. Families of toxins involved in human food poisonings with the indication of syndromes, solubility of toxins and the target of the toxins, adapted from Anderson et al. (1993). (Numbers in brackets shows the numbers of toxins involved),

TOXIN FAMILY (number of toxins)	SYNDROME	VOLUBILITY	ACTION ON
Brevetoxin (10)	NSP (Neurotoxic Shellfish Poisoning)	Fat	Nerve, muscle, lung, brain
Ciguatoxin (multiple)	CFP (Ciguatera Fish Poisoning)	Fat	Nerve, muscle, heart, brain
Domoic Acid (11)	ASP (Amnesic Shellfish Poisoning)	Water	Brain
Okadaic Acid (3)	DSP (Diarrhetic Shellfish Poisoning)	Fat	Enzymes
Saxitoxin (18)	PSP (Paralytic Shellfish Poisoning)	Water	Nerve, brain

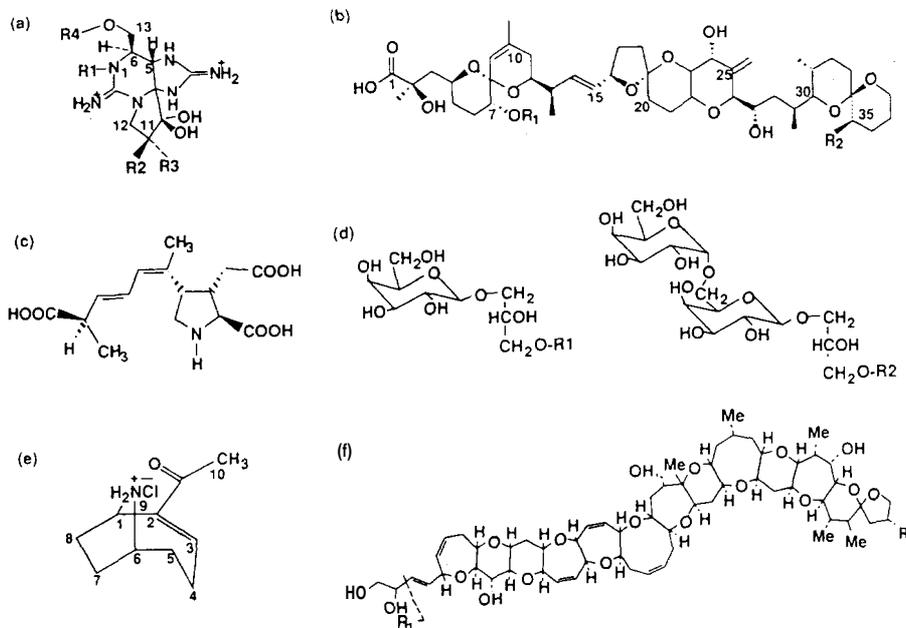


Figure 1. Examples of chemical structures of algal toxins a. Paralytic shellfish poisons from the dinoflagellates *Alexandrium* spp., *Gymnodinium catenatum* and *Pyrodinium bahamense*; $R_1 = H$ or OH , $R_2 = H$ or OSO_3^- , $R_3 = H$ or OSO_3^- , $R_4 = CONH_2$, $CONHSO_3^-$ or H . b. Diarrhetic shellfish poisons from the Dinoflagellates *Dinophysis* spp. and *Prorocentrum lima*; $R_1 = H$, $R_2 = H$, $R_3 = CH_3$. c. Domoic acid from the diatoms *Pseudo-nitzschia* spp.. d. Hemolysins from the dinoflagellates *Amphidinium carterae*, *Gyrodinium aureolum* and the prymnesiophyte *Chrysochromulina polylepis*; $R_1 = acyl(C_{18:4\omega 3})$, $R_2 = acyl(C_{18:4\omega 3})$ or $acyl(C_{18:5})$, $acyl(C_{20:5})$ or $acyl(C_{20:5})$. e. Anatoxin-a from the cyanobacteria *Anabaena flos-aquae*. f. Ciguatoxin from the dinoflagellate *Gambierdiscus toxicus*; $R_1 = CH_2 = CH-$; $R_2 = H$ (*Gambierdiscus*) or OH (moray eels), from Hallegraeff (1993).

Table 3 Various types symptoms of various types of fish and shellfish poisoning: Clinical symptoms, treatment and species implicated. Modified from Hallegraef (1993).

Paralytic Shellfish Poisoning (PSP)	Diarrhetic Shellfish Poisoning (DSP)	Amnesic Shellfish Poisoning (ASP)	Ciguatera
Causative organism: <i>Alexandrium catenella</i> , <i>Alexandrium minutum</i> , <i>Alexandrium tamarense</i> , <i>Alexandrium ostenfeldii</i> , <i>Gymnodinium catenatum</i> , <i>Pyrodinium bahamense</i>	<i>Dinophysis acuta</i> , <i>Dinophysis acuminata</i> , <i>Dinophysis fortii</i> , <i>Prorocentrum lima</i>	<i>Pseudo-nitzschia multiseriata</i> , <i>Pseudo-nitzschia pseudodelicatissima</i> , <i>Pseudo-nitzschia australis</i>	<i>Gambierdiscus toxicus</i> , <i>Ostreopsis siamensis</i>
Symptoms: Mild Case Within 30 min: tingling sensation or numbness around lips, gradually spreading to face and neck; prickly sensation in fingertips and toes; headache, dizziness, nausea, vomiting, diarrhoea	After 30 min to a few h (seldom more than 12 h): diarrhoea, nausea, vomiting, abdominal pain	After 3-5 h; nausea, vomiting, diarrhoea, abdominal cramps	Symptoms develop within 12-24 h of eating fish. Gastrointestinal symptoms, diarrhoea, abdominal pain, nausea, vomiting
Symptoms: Extreme Case Muscular paralysis; pronounced respiratory difficulty; choking sensation; death through respiratory paralysis may occur within 2-24 h of ingestion	Chronic exposure may promote tumour formation in the digestive system	Decreased reaction to deep pain, dizziness, hallucinations, confusion, short-term memory loss, seizures	Neurological symptoms, numbness and tingling of hands and feet, cold objects feel hot to touch, difficulty in balance, low heart rate and blood pressure, rashes. In extreme cases, death through respiratory failure
Treatment: Patient has stomach pumped and is given artificial respiration. No lasting effects of poisoning	Recovery after 3 d. irrespective of medical treatment	Gastric evacuation. No other effective treatment known	No antitoxin or specific treatment as available. Neurological symptoms may last of months and years. Calcium and mannitol may help relieve symptoms

1.3. HARMFUL ALGAL SPECIES

New species of harmful algae are continuously detected. A comprehensive list of species with indication of the kind of toxicity is compiled in Table 4. A few common toxic or potentially algae are shown in Fig. 2. Scenarios showing how algal toxins can accumulate in mussels and fish are shown in Fig. 3 and 4 respectively.

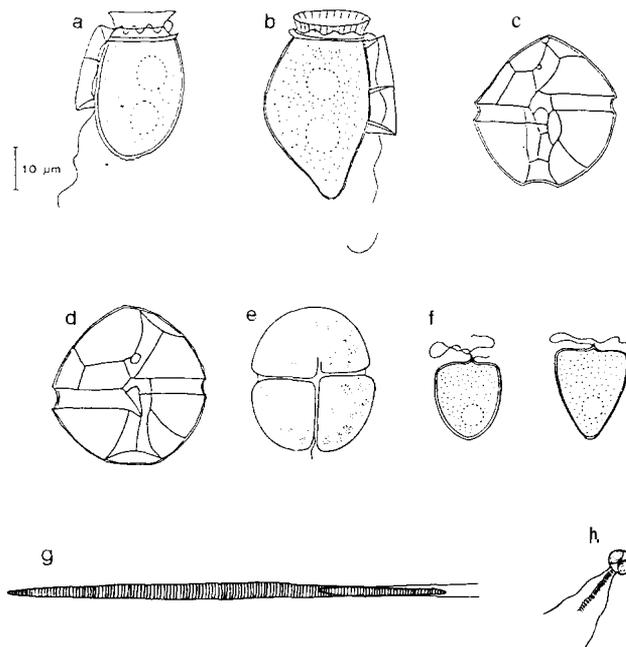


Figure 2. Examples of typical harmful algae: a. *Dinophysis acuminata*, b. *Dinophysis norvegica*, c. *Alexandrium tamarense*, d. *Alexandrium ostenfeldii*, e. *Gyrodinium aureolum*, f. *Prorocentrum minimum*, g. *Pseudo-nitzschia* spp. and h. *Chrysochromulina* spp.

Table 4, Harmful algal species with indication of the type of toxicity, known presence of toxin/toxins and references. The information is compiled from several different sources. Species names in parenthesis are synonyms (Steidinger (1983, 1993), Taylor (1984, 1985), Shumway (1990), ICES (1992), Premazzi & Volterra (1993))

Toxic algae	DSP	NSP	PSP	ASP	Ciguatera	Fish kills	Temporary ecosystem damage	Toxic substances	References
<i>Alexandrium acatenella</i>			X					X	Prakash & Taylor (1966)
<i>Alexandrium catenella</i>			X					X	Onoue et al. (1980, 1981a, b), Schantz et al. (1966)
<i>Alexandrium</i> cf. <i>cohorticula</i>			X					X	Tamiyavanichet al. (1985), Balech (1993)
<i>Alexandrium fundyense</i>			X			X		X	Franks & Anderson (1972)
<i>Alexandrium lusitanicum</i>			?					X	Silva (1979)
<i>Alexandrium minutum</i>			X					X	Oshima et al. (1989), Hansen et al. (1992), Belin (1993)
<i>Alexandrium monilatum</i>						X		X	Sievers (1969), Williams & Ingle (1972), Loeblich & Loeblich (1979)
<i>Alexandrium ostenfeldii</i>			X					X	Hansen et al. (1992), Balech & Tangen (1985)
<i>Alexandrium tamarense</i> (<i>A. excavatum</i>)			X			X		X	Schmidt & Loeblich (1979a, b), Franks & Anderson (1992), Tangen (pers. comm.)
<i>Amphidinium carterae</i>					?	?		X	Nakajima et al. (1981), Ikawa & Sasner (1975), Ikawa & Taylor (1973), Davin et al. (1988)
<i>Amphidinium klebsii</i>					?	?		X	Nakajima et al. (1981), McLaughlin & Provasoli (1957)
<i>Amphidinium rhynchocephalum</i>					X			X	McLaughlin & Provasoli (1957)
<i>Amphora coffaeiformis</i>				X					ICES (1992), Skov et al. (1995)
<i>Aureococcus anophagefferens</i>							X		Cosper et al. (1988)
<i>Chaetoceros</i> spp. - probably all species with long setae, e.g. <i>atlanticus</i> , <i>conconvicornis</i> , <i>convolutus</i> , <i>danicus</i> , <i>decipiens</i> and <i>eibenii</i> .						X		-	ICES (1992)

Table 4 (continued). Harmful algal species with indication of the type of toxicity, known presence of toxin/toxins and references. The information is compiled from several different sources. Species names in parenthesis are synonyms (Steidinger (1983, 1993), Taylor (1984, 1985), Shumway (1990), ICES (1992), Premazzi & Volterra (1993)).

Toxic algae	DSP	NSP	PSP	ASP	Ciguatera	Fish kills	Temporary ecosystem damage	Toxic substances	References
<i>Chrysochromulina</i> spp. (several species, e.g. <i>leadbeateri</i> , <i>polylepis</i> etc.)						X	X	X	Moestrup (1994)
<i>Cochlodinium polykrikoides</i>			?			?		X	Yuki & Yoshimatsu (1989)
<i>Cochlodinium</i> sp.			?			X		X	Yuki & Yoshimatsu (1989)
<i>Coolia monotis</i>					?	?		X	Yasumoto et al. (1987)
<i>Dictyocha speculum</i>						X ?		?	ICES (1992), Sournia (1991)
<i>Dinophysis acuminata</i>	X							X	Kat (1983), Yasumoto (1990), Belin (1993)
<i>Dinophysis acuta</i>	X							X	Yasumoto (1990)
<i>Dinophysis caudata</i>	X							X	Karunsagar et al. (1989)
<i>Dinophysis fortii</i>	X							X	Yasumoto (1990)
<i>Dinophysis mitra</i>	?							X	Yasumoto (1990)
<i>Dinophysis norvegica</i>	X							X	Yasumoto (1990)
<i>Dinophysis rotundata</i> (<i>Phalochroma rotundatum</i>)	X							X	Yasumoto (1990)
<i>Dinophysis sacculus</i>	X							?	Lassus & Berthome (1988), Alvito et al. (1990), Belin (1993)
<i>Dinophysis tripos</i>	?							X	Yasumoto (1990)
<i>Gambierdiscus toxicus</i>					X	?		X	Adachi & Fukuyo (1979)
<i>Lingulodinium polyedra</i> (<i>Gonyaulax polyedra</i>)			?					X	Schradie & Bliss (1926), Bruno et al. (1990)
<i>Gymnodinium breve</i> (<i>Ptychodiscus brevis</i>)		X				X		X	McFarren et al. (1965), Baden (1983)
<i>Gymnodinium catenatum</i>			X					X	Morey-Gaines (1982), Mee et al. (1986)

Table 4 (continued). Harmful algal species with indication of the type of toxicity, known presence of toxin/toxins and references. The information is compiled from several different sources. Species names in parenthesis are synonyms (Steidinger (1983, 1993), Taylor (1984, 1985), Shumway (1990), ICES (1992), Premazzi & Volterra (1993)).

Toxic algae	DSP	NSP	PSP	ASP	Ciguatera	Fish kills	Temporal ecosystem damage	Toxic substances	References
<i>Gymnodinium galatheanum</i>						X		X	Larsen & Moestrup (1989), Nielsen & Stromgren (1991)
<i>Gymnodinium mikimotoi</i>						X		X	Tangen (1977), Takayama & Matsuoka (1991), Hansen et al. (1992), Yasumoto et al. (1990)
<i>Gymnodinium sanguineum</i>						X		X	Woelke (1961), Nightingale (1936), Cardwell et al. (1979)
<i>Gymnodinium veneficum</i>						?		X	Abbot & Ballantine (1957)
<i>Gyrodinium aureolum</i> (<i>Gymnodinium mikimotoi</i> , <i>G. nagasakiense</i>)						X	X	X	Shumway (1990), Tangen (1978), Sournia (1991)
<i>Gyrodinium flavum</i>						X		?	Lackey & Clendenning (1963)
<i>Heterosigma akashiwo</i>						X		X	ICES (1992)
<i>Noctiluca scintillans</i>						X	X	X	ICES (1992)
<i>Nodularia spumigena</i>									Falconer (1993)
<i>Ostreopsis heptagona</i>					X			X	Norris et al. (1985)
<i>Ostreopsis lenticularis</i>					?			X	Tindall et al. (1990), Ballantine et al. (1988)
<i>Ostreopsis ovata</i>					?			X	Nakajima et al. (1981)
<i>Ostreopsis siamensis</i>					?			X	Nakajima et al. (1981)
<i>Peridinium polonicum</i>						X		X	Nakajima et al. (1981)
<i>Phaeocystis pouchetii</i>									Moestrup (1994)

Table 4 (continued). Harmful algal species with indication of the type of toxicity, known presence of toxin/toxins and references, The information is compiled from several different sources. Species names in parenthesis are synonyms (Steidinger (1983,1993), Taylor (1984, 1985), Shumway (1990), ICES (1992), Premazzi & Volterra (1993)).

Toxic algae	DSP	NSP	PSP	ASP	Ciguatera	Fish kills	Temporary ecosystem damage	Toxic substances	References
<i>Phisteria piscimorte</i>						X		X	Burkholder et al. (1995)
<i>Prorocentrum balticum</i>						X		?	Paredes (1962, 1968), Silva (1953, 1963), Pinto & Silva (1956)
<i>Prorocentrum concavum</i>					X	?		X	Fykuyo (1981), Nakajima et al. (1981), Yasumoto et al. (1987)
<i>Prorocentrum hoffmannianum</i>					X	?		X	Aikman et al. (1993), Tindall et al. (1984), Faust (1990)
<i>Prorocentrum lima</i>	X				?			X	Marr et al. (1992), Nakajima et al. (1981), Tindall et al. (1984)
<i>Prorocentrum mexicanum</i>					?	?		X	Nakajima et al. (1981), Tindall et al. (1984)
<i>Prorocentrum minimum</i>						X		X	Nakajima et al. (1981), Tindall et al. (1984)
<i>Prymnesium parvum</i>						X	X	X	Moestrup (1994)
<i>Pseudo-nitzschia australis</i>				X				X	Skov et al. (1995)
<i>Pseudo-nitzschia multiseries</i>				X				X	Skov et al. (1995)
<i>Pseudo-nitzschia pseudodelicatissima</i>				X				X	Skov et al. (1995), Martin et al. (1990)
<i>Pseudo-nitzschia seriata</i>				X				X	Skov et al. (1995)
<i>Pyrodinium bahamense</i> var. <i>compressum</i>			X			X		X	Maclean (1977), Harada et al. (1982)

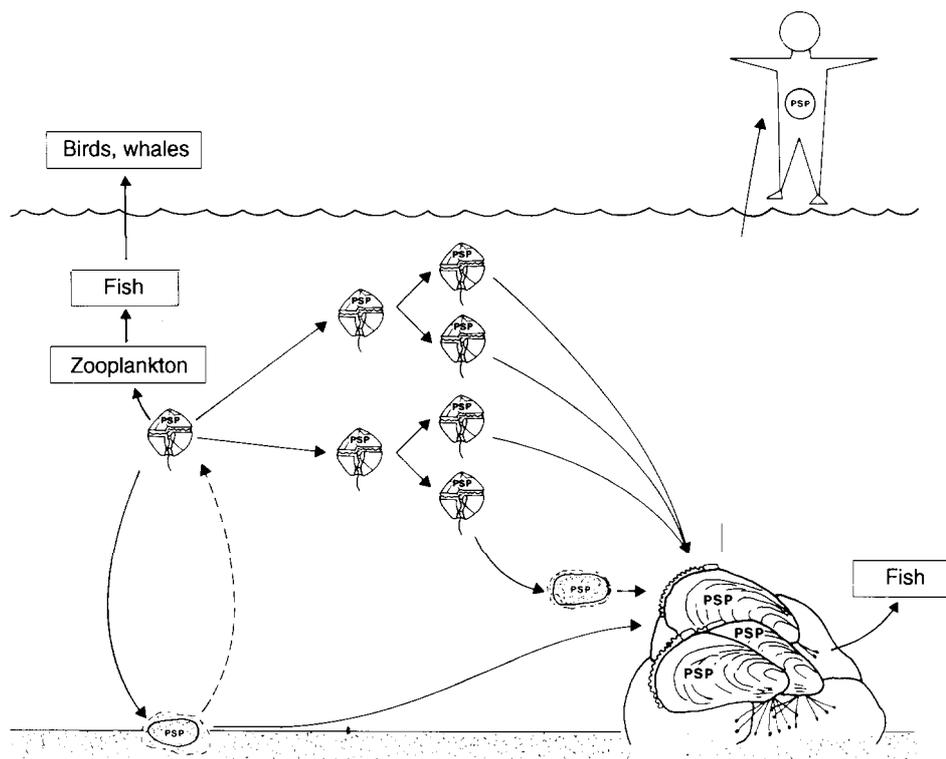


Figure 3. Scenario illustrating how algal toxins can accumulate in shellfish, zooplankton and fish when the toxic algae or their resting cysts are grazed and can intoxicate humans, birds and whales.

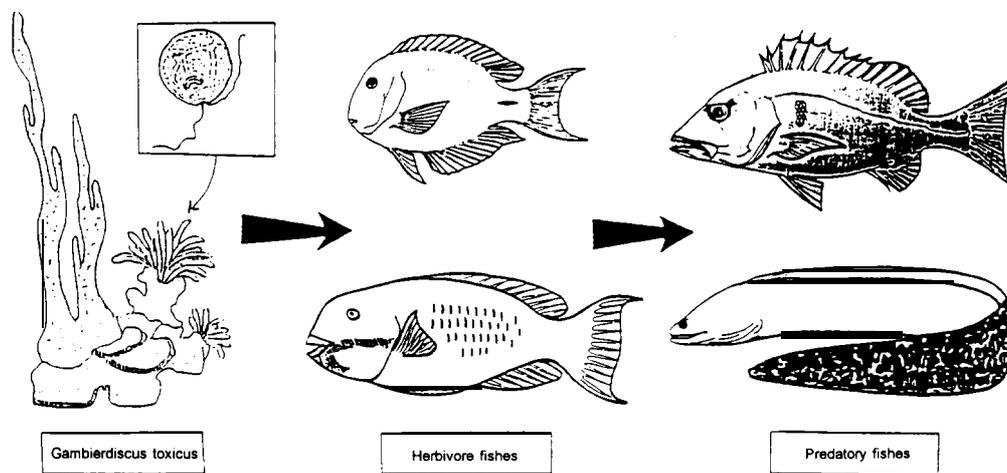


Figure 4. Scenario illustrating how algal toxins can accumulate in herbivorous and carnivorous fish on coral reefs, when the toxic algae are grazed by herbivorous fish (Legrand, 1991).

Useful taxonomic references: Bjergskov et al. (1990); Sournia et al. (1991); Premazzi & Volterra (1993); Hallegraeff et al. (1996); ICES Identification Leaflets for Plankton (Fiches d'Identification du Plankton) see Leaflet no. 182. Potential y Toxic Phytoplankton. 4. The diatom genus *Pseudo-nitzschia* (Diatomophyceae, Bacillariophyceae).

1.4 SHELLFISH POISONINGS, FISH POISONINGS AND FISH KILLS

PSP is the most widespread shellfish poisoning occurring all over the world, followed by DSP, (Fig. 5 and 6). The other poisonings, ASP and NSP, have more restricted geographical occurrences, (Fig. 7 and 8). CFP are only localized in tropical waters, (Fig. 10).

Fishkills of both cultured and wild fish, caused by either toxic algae or physical irritation of the gill tissue, are observed worldwide, (Fig 11).

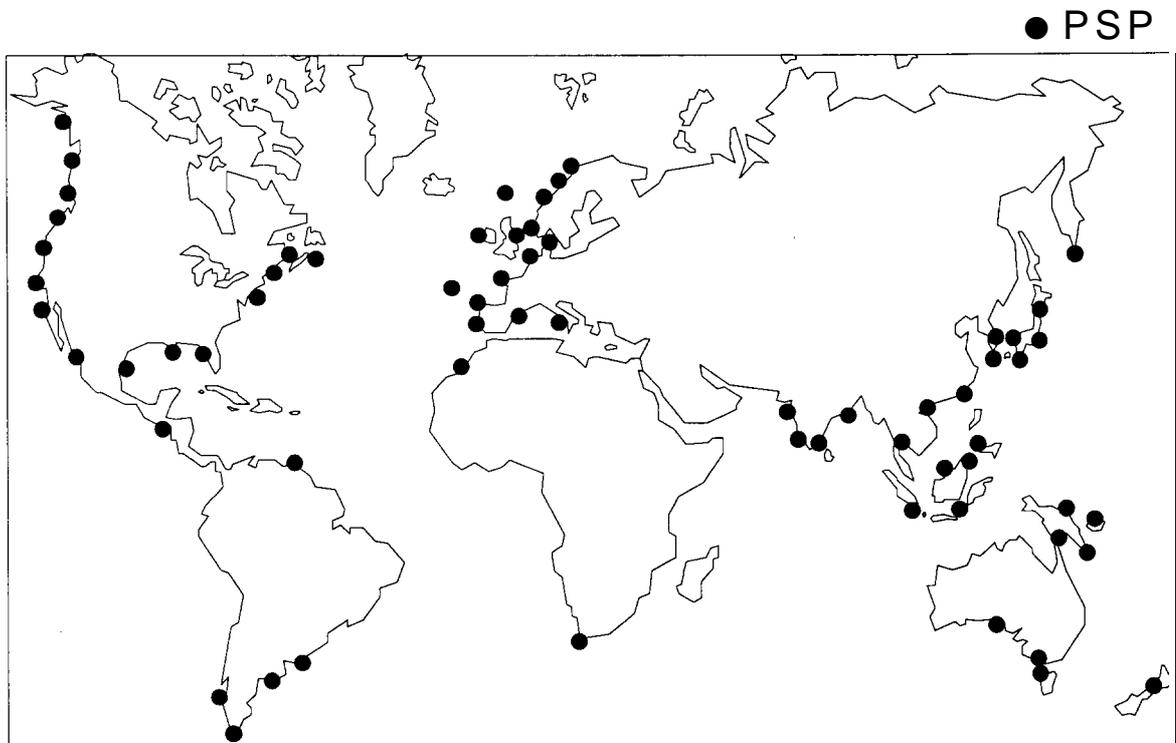


Figure 5. Global distribution of paralytic shellfish poisonings (PSP) and toxins (PST).

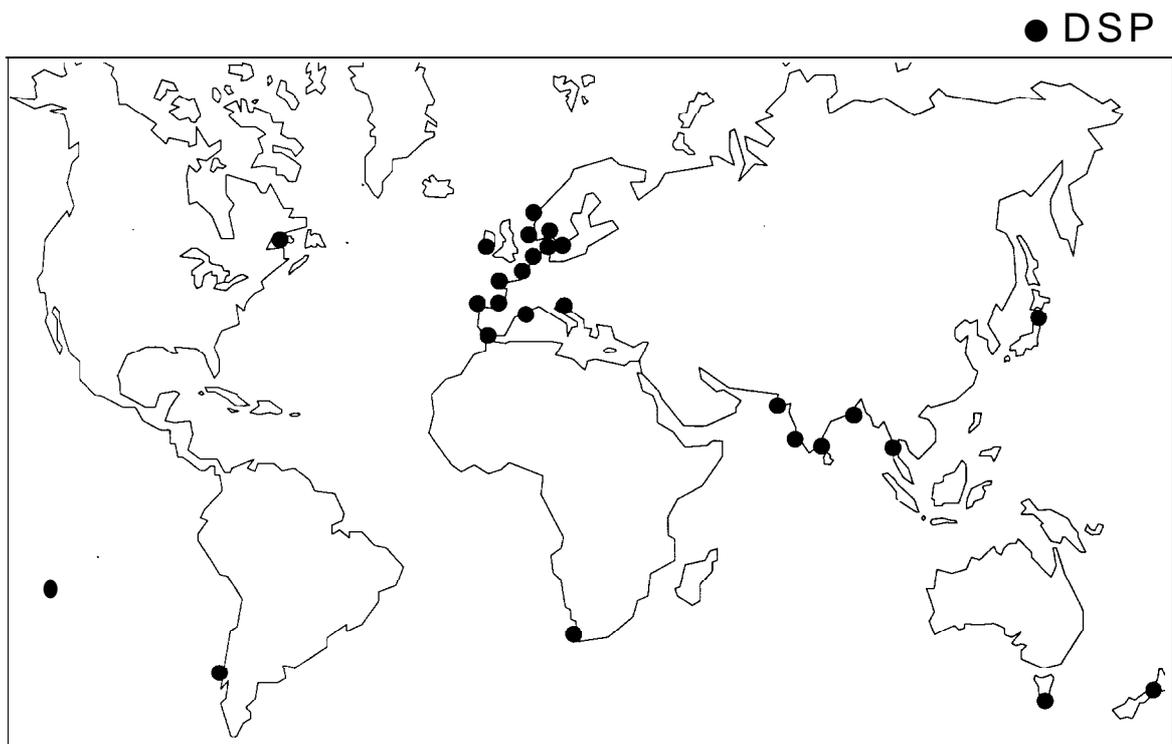


Figure 6. Global distribution of diarrhetic shellfish poisonings (DSP) and toxins (DST),



Figure 7. Global distribution of neurotoxic shellfish poisonings (NSP) and toxins (NST) and NSP-like shellfish toxins.



Figure 8. Global distribution of amnesic shellfish poisonings (ASP) and toxins (domoic acid).

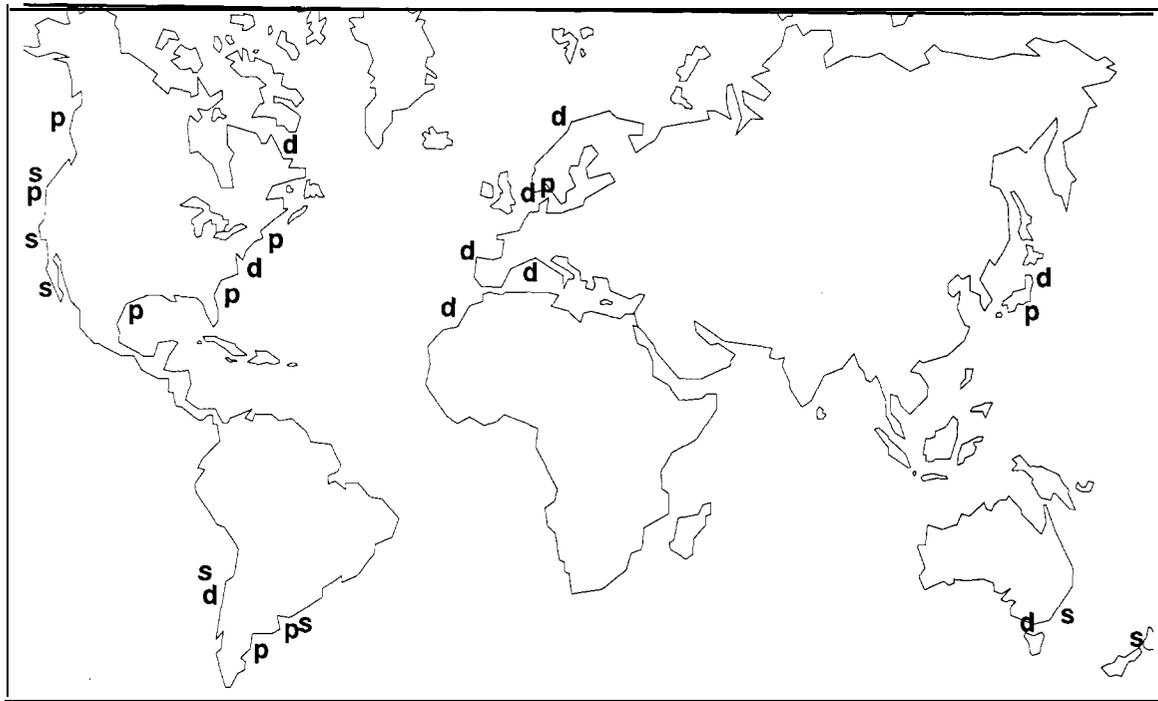


Figure 9. Global distribution of the *Pseudo-nitzschia* species potential causative of ASP. p: *Pseudo-nitzschia multiseries*; d: *Pseudo-nitzschia pseudodelicatissima* and s: *Pseudo-nitzschia australis* (= *Pseudo-nitzschia pseudoseriata*), from Hallegraeff (1993).

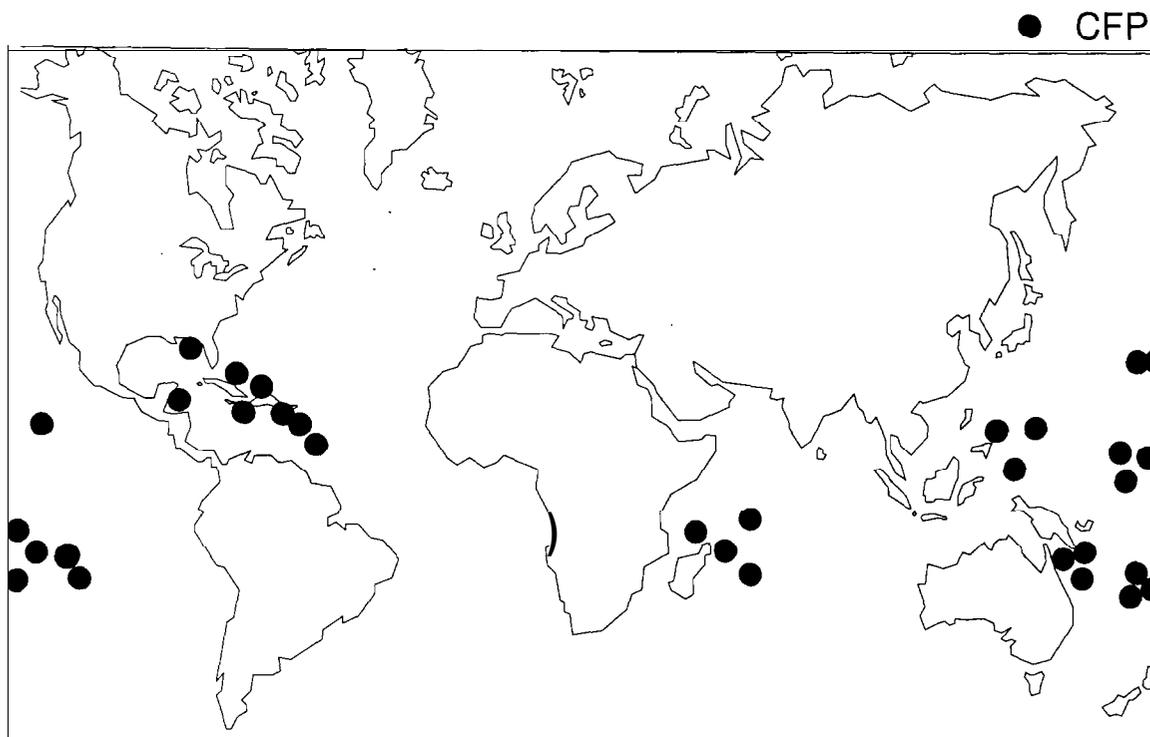


Figure 10, Global distribution of ciguatera fish poisoning (CFP), modified from Lassus (1988)

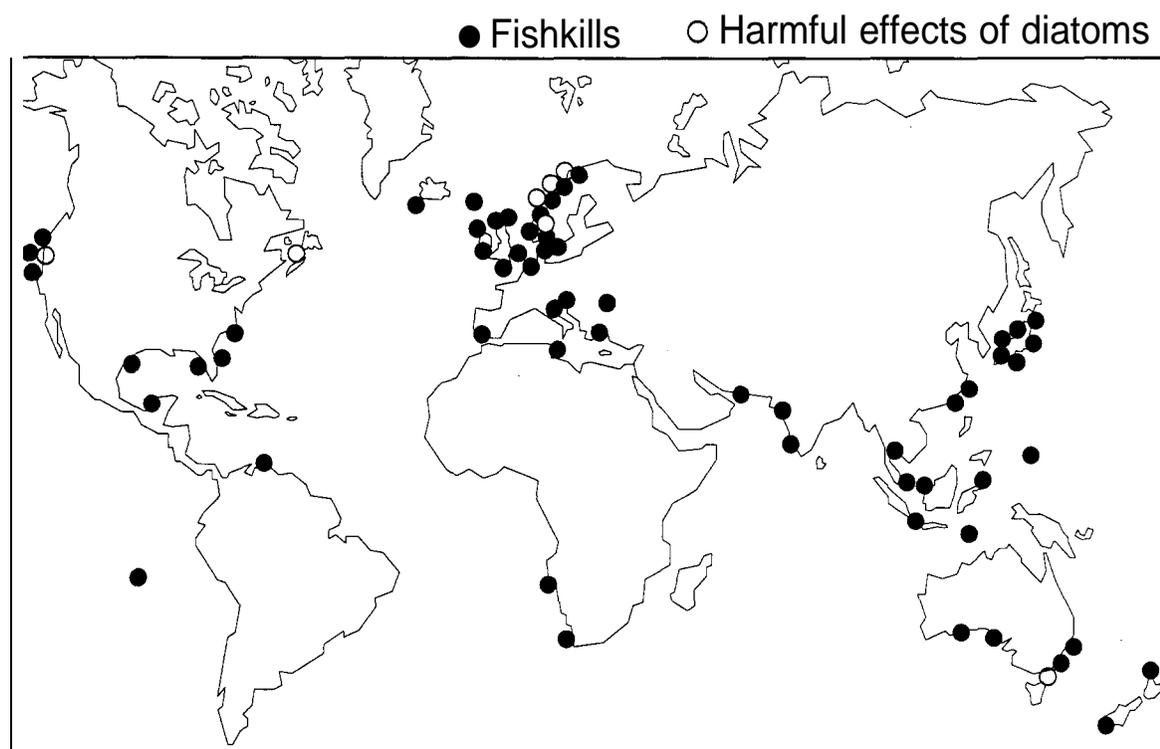


Figure 11. Global distribution of wild and cultured fish kills (various causes) (Granéli et al., 1989)

1.5 SHELLFISH AND FISH INVOLVED IN POISONINGS

The different algal toxins responsible for shellfish poisonings are known to accumulate in many species of shellfish, e.g. *Argopecten irradians*, *Cardium edule*, *Mya arenaria*, *Mytilus edulis*, *Pecten maxim us*, *Saxidomus gigantus* and *Spisuda solidae* (Shumway et al., 1990; Emsholm et al., 1995). Oysters also accumulate algal toxins but in most cases they tend to exhibit lower levels of toxicity than other species of mussels irrespective of the species of oyster or toxic algae. According to Shumway et al. (1990) accumulation of DSP-toxins in oysters is negligible while accumulation of PSP-toxins is not un-common, while Aune & Yndestad (1993) report that oysters accumulate DSP-toxins but at a lower level than other mussels. Shumway (1995) reported on the accumulation of phycotoxins in higher order consumers frequently harvested for human consumption.

More than 400 different species of fish are involved in CFP which are limited to tropical herbivore fish that feed on toxic dinoflagellates and detritus of coral reefs as well as reef carnivores that prey on the herbivores, e.g. grouper, sea bass, rock cod, snapper or sea perch, barracuda, emperor fish or porgies, spanish mackerel, jack, trevallie, king-fish or carang, wrasse, dog teeth tuna, moray eel, trigger fish, surgeon fish, parrot fish and mullet (Bagnis, 1993). Fish belonging to the same species and fished at the same time and locality maybe toxic or non-toxic. It is a common native habit to eat a small piece of fish and wait for several hours to determine if any signs of intoxication occur, before the fish is chosen for a meal. Measures to be taken to avoid CFP are summarized by Premazzi & Volterra (1993). The geographical distribution of some of the species are presented in Table 5, See Legrand (1991) and Premazzi & Volterra (1993) for further information.

Table 5. Fish involved in ciguatera fish poisoning (CFP) in different regions (adapted from Premazzi & Volterra, 1993).

Geographical region	Examples of toxic species
Caribbean	Several species of snapper, barracuda, amberjack, grouper and dolphin
Florida	Grouper, snapper, kingfish, amberjack, barracuda, jack mullet and dolphin
French Polynesia	Sphyreana, barracuda, grouper, snapper, wrosse, surgeon fish
Hawaii	Jack, amberjack, eel, flagtail fish, mullet, wrasse, goatfish, surgeon fish, groupers and parrot fish

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Hawaii	Jack, amberjack, eel, flagtail fish, mullet, wrasse, goatfish, surgeon fish, groupers and parrot fish
Australia	Different species of mackerel, giant dart, barracuda, coral trout, grouper, red snapper, yellow sweet-lip, yellow-tail kingfish, kingfish, trevally, lowly trevally, Maori wrasse, venus tusk fish, dart, southern fuselster, barramundi

1.6 CAUSES OF BLOOMS

Blooms of algae are natural phenomena which occur occasionally in upwelling areas and estuaries, where a combination of enrichment and physical conditions can result in high concentrations of algae, (Fig. 12).

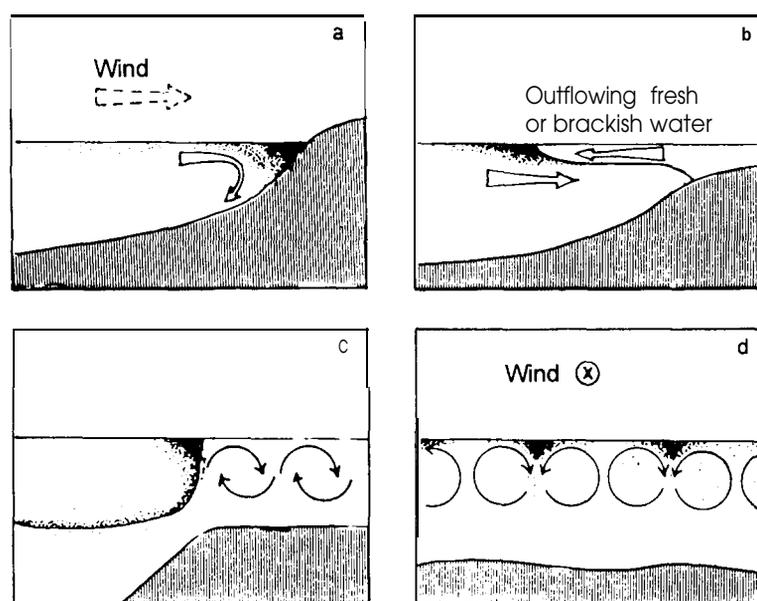


Figure 12, Scenarios illustrating how algae can accumulate due to physical and biological processes. a. Wind induced down-welling at the coast; b. Down-welling of coastal water caused by a freshwater plume; c. an algal bloom in a frontal area due to enhanced supply of nutrient from up-welling water or as a result of lifting a subsurface population to the surface; d. Local accumulation of algae at convergence lines due to wind induced Langmuir circulation, from Taylor (1987).

In situations where algae occur in low concentration in a region, physical conditions can lead to local blooms. That is, the bloom is not the result of local growth. Furthermore blooms can be transported from the area of origin into other areas by currents.

In other situations blooms occur as a result of anthropogenic causes, mainly eutrophication of the coastal areas and fjords with nitrogen and phosphorous, which can lead to increased local production of algae with an increased risk of algae blooms as a result. See Smayda (1990) for further discussion.

Local kills of benthic organisms due to oxygen deficiency or toxic algae may facilitate algal blooms, because grazing on the algae is suppressed.

1.7 DEPUTATION OF TOXINS

PSP: Once mussels and scallops have become highly toxic, toxins can be stored for several months or longer. During deputation of mussels and scallops two stages may be observed: a fast decrease in toxicity followed by a more slow decrease remaining above the acceptable limit of 80 $\mu\text{g}/100\text{ g}$. The toxins GTX-3 and GTX-8/epiGTX-8 are the dominant toxins in the early stages of the deputation phase, whereas GTX-2 is the predominant toxin during the slow deputation phase. At low levels of toxicity mussels and oysters might deplete within 2 weeks, and it is reported that toxicity can decrease 60% within 24 h (see Premazzi & Volterra, 1993).

Since PSP toxins are extremely resistant to destruction by ionizing radiation, this method can not be used on mussels for human consumption (see Premazzi & Volterra, 1993).

Commercial heating of mussels and clams above 100°C reduces toxicity, if the product is not acidic. It has been reported that the cannery processing, involving steaming under alkaline conditions followed by acid conditions in cans reduces the toxicity 80-90%. One of the reasons for the decrease in toxicity is that mussels produce large amounts of highly toxic juice during the steaming process, of which only a part is filled in the cans. Pre-cooking mussels with steam for 10 min. might reduce the toxin content in mussels by 90% (see Premazzi & Volterra, 1993).

Domestic steaming, boiling or pan-frying reduces the toxicity by approximately 30%. If the juice is discarded the toxicity can be further reduced. Pan-frying seems to be more effective in destroying the toxins even though the juice is not discarded, apparently because the temperatures during pan-frying are higher than during steaming or boiling (Premazzi & Volterra, 1993).

DSP: Depuration of mussels with moderate to high toxicity, in the absence of *Dinophysis* spp. occurs within 14 days at water temperatures 13- 14°C, and within a month at water temperatures below 9°C (Kat, 1987). Increasing the water temperature, as well as letting the mussels starve in experiments on the Swedish west coast, however failed to eliminate the toxicity for several months (Lindahl & Hageltorn, 1986).

Boiling of mussels for 163 min. was required to reduce the concentration of okadaic acid by 50% (Edebo et al., 1988).

ASP: Toxic shellfish can deplete in days if exposed to uncontaminated water (Grimmel et al., 1990), Boiling or steaming, even for prolonged periods, does not reduce toxicity of mussels.

2. MONITORING OF COASTAL WATERS IN RELATION TO HAB'S

To have an effective monitoring programme it is necessary to define precisely the local needs for information on a short or long time range.

It is necessary to have basic knowledge about the biological, chemical and physical conditions as well as temporal and geographic variation within the region of interest. The first priority information includes the occurrence in time and space of potentially toxic algae and historical evidence of their effects. Long time information on phytoplankton populations (toxic, harmful and others) may help to obtain a more comprehensive understanding of the dynamics of the phytoplankton and the function of the ecosystem and lead to a more efficient monitoring. If for instance long time series of phytoplankton populations exist it is possible to decide if the sudden appearance of a species is new to the area, or if an endemic species has suddenly become toxic.

Important supporting parameters include temperature, salinity, presence of surface water stratification, chlorophyll (phytoplankton biomass), and surface current circulation (transport of harmful algae). Knowledge of the temporal and geographic distribution of inorganic nutrients and their sources, as well as other phytoplankton growth factors, will also be important when planning and operating a monitoring programme.

2.1 IDENTIFICATION AND DEFINITION OF USER DEMANDS

The design of a HAB monitoring programme should start by defining the kind of information which is needed to protect the specific resource. This should be done in close cooperation between the users of the results of the monitoring and the authorities/institutions/companies involved in the monitoring and evaluation of monitoring results.

Mariculture: In the case of cultures of fish the user demand of a HAB monitoring programme would typically be an early warning that a HAB, of a certain species with indication of the kind of harmful effects could be expected, is under development. An early warning allows the fishfarmers to put specific contingency plans in action. Furthermore the HAB situation should be monitored until the risk of any harm has passed.

Ecosystem damage: In the case of HAB monitoring in relation to more general ecosystem damage, the user demand could also typically be an early warning that a HAB, of a certain species with indication of the kind of harmful effects could be expected to harm which components of the ecosystem, is under development. An early warning allows the appropriate environmental protection agency to monitor the components of interest and to put specific contingency plans in action to protect specific species. Also in this situation the HAB situation should be monitored until the risk of any harm has passed.

Fisheries: The user demands of a HAB monitoring programme in relation to fishery for mussels, other shellfish or fish would be a warning that a HAB of toxic algal species, of a certain species with indication of the kind of toxicity it introduces in seafood, is under development. Warning about the species composition of the HAB will indicate which toxins may be expected, and which shellfish should be considered for intensified monitoring, or allow for closure of fishing at the specific harvest sites, which might give the fishermen a chance to go fishing in other areas with none, or lower risk of algal toxins in the shellfish. The algal monitoring in combination with toxin analysis of the shellfish should prevent toxic seafood from entering the market and protect the consumers. The HAB situation should be monitored until the risk of any toxicity has passed.

Eutrophication/climate-changes: If user demand of the HAB monitoring programme is to follow the occurrence of HAB's as an indication of local and global eutrophication or effects of long-term changes/climate, the kinds of species of algal blooms identified as HAB's should be carefully defined (level of concentration, HAB species of interest). Monitoring should be long term (>10 years) and sampling should be carried out at fixed stations or in well defined areas to allow for statistical analysis of the data.

2.2 USE OF EXISTING REGIONAL/LOCAL ENVIRONMENTAL INFORMATION

In the design of a local HAB monitoring programme local, regional and global information on the

following should be consulted :

Phytoplankton, especially toxic species.

Evidence of earlier harmful occurrences.

Physical/chemical characteristics of the water masses and there seasonal variation as well interannual variation.

Meteorologic phenomena such as seasonal rain fall, periods with storms or special wind regimes (e.g. monsoon etc.).

Location of ecosystem components and economic resources vulnerable to damage from HABs (e.g. coral reefs, fish farms, shellfish sites).

2.3 MONITORING METHODS AND TECHNOLOGY

Monitoring HAB's involves a plan for sampling in time and space, that is definition of sampling areas (grids) or stations and sampling frequency, algal sampling, identification and quantification of algal species.

Additional monitoring information may be obtained from: moored buoys, continuously measuring hydrographic parameters (salinity, temperature, current speed and direction) as well as wave amplitude, light, light attenuation and "biological" parameters as oxygen, chlorophyll and observations contributed by fishers and others out in the environment.

Satellite images would give more global insight into distribution and movement of water masses (from surface temperature) and biomass of algae in the surface waters (from color and intensity of color).

2.3.1. Sampling of planktonic algae

A detailed manual on methodology in relation to HAB's is available in the IOC Manual on Harmful Marine Microplankton (1996). The most important methods in relation to monitoring are summarized here.

In general sampling of harmful algae should take place as close as possible to the resources to be protected, as well as at central stations representing the different water masses in the investigation area, see Franks (1996) for further information on strategies of station location.

In periods of higher risk of HAB's sampling should be carried out at least weekly, During development of a HAB, sampling should be intensified to daily.

Sampling for qualitative and quantitative microscope analysis should be carried out using a plankton net (mesh size 20 μm) and water bottle (e.g. Niskin, Nansen) or a sampling hose respectively.

Qualitative concentrated samples (net samples) are collected by drawing the plankton net vertically to cover the depth range of interest, (Fig. 15). In shallow water locations (depth <20 m) the net should be drawn, several times, from bottom to the surface of the water, until the water in the sample collector becomes unclear or coloured by the concentrated algae.

The quantitative samples (water/ bottle samples) are collected using a water bottle, sampling at different depths, to cover the depth range of interest. Depth intervals between sampling should be 2-5 m, dependent on local conditions. The samples from the different depths can be pooled together and counted as one sample representative of the whole water column.

An alternative to sampling using water bottle could be to use a hose for sampling the whole water

column (Fig, 16), as described e.g. by Lindahl (1986).

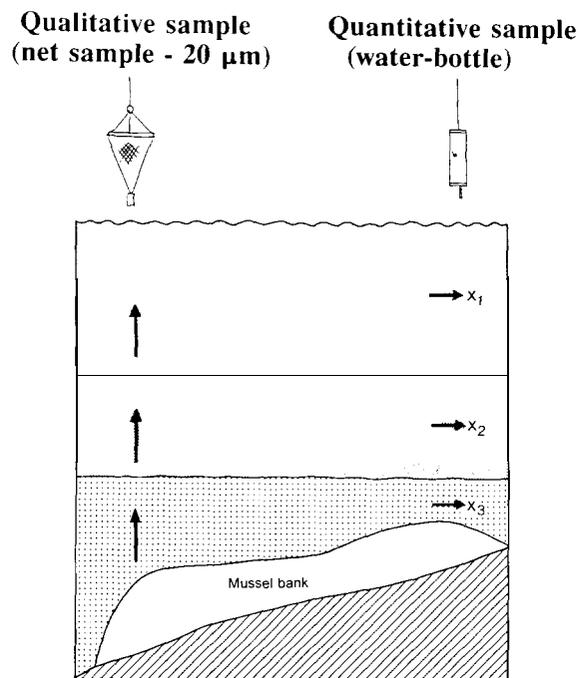


Figure 13. Collection of qualitative and quantitative algal samples for microscopical analysis of harmful algae using plankton net (mesh size 20 μm) and water bottle,

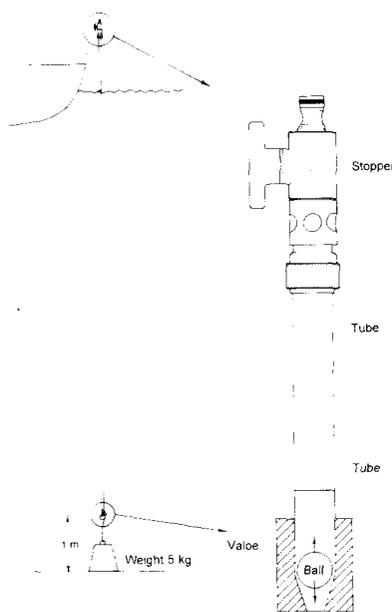


Figure 14. Collection of quantitative algal samples for microscopical analysis of harmful algae using a hose,

2.3.2. Sampling of benthic microalgae

Several methods have been used to quantify the benthic microalgae which causes ciguatera (Bagnis et al., 1980; Quod et al., 1995; McCafferey et al., 1992). According to Quod et al. (1995) benthic microalgae including the dinoflagellates responsible for ciguatera fish poisoning can be sampled for quantitative analysis by

the following procedure:

1. Collection of macro algae (20 g)
2. The macro algae collected are vigorously shaken in seawater
3. The seawater is sieved (mesh size 150 μm)
4. The dinoflagellates are counted in the fraction $<150 \mu\text{m}$

2.3.3. Fixation/preservation of algal samples

Immediately after the collection of the algal samples they must be preserved for later analysis in the laboratory. (Live samples can be very useful as a supplement to the fixed samples for taxonomic investigations).

Algal samples should be preserved using either neutral or acidic Lugol's, which produce good preparations for light microscopy, and which is low toxic to humans. If the brownish coloration of the algae, caused by Lugol's imposes a problem in the taxonomic investigations, the coloration can be removed by oxidizing the Lugol's using a few drops of a solution of sodium thiosulfate per ml (3 g $\text{Na}_2\text{S}_2\text{O}_3$ for 100 ml of water) of sample (Pomeroy, 1984).

Formaldehyde should be used with care, a fumehood is recommended, because of the toxicity to humans (potential carcinogenic), and its potential, eventually to develop allergic reactions in humans exposed to the fixative.

2.3.4. Qualitative analysis

Prior to the quantitative analysis of harmful algae a qualitative analysis of concentrated plankton, is a must to establish the species composition of the harmful algae as well as to identify the species which could be misinterpreted as harmful species during quantification.

Qualitative analysis can be performed using a normal compound microscope. The analysis can be facilitated if the microscope is equipped with facilities for phase contrast, interference or epifluorescence microscopy using specific fluorochromes as stains, e.g. Calco Flour White MR2, which is a specific stain for cellulose e.g. in the thecal plates of thecate dinoflagellates (Lawrence & Triemer, 1985).

2.3.5. Quantitative analysis

Compound microscope: In cases with high concentrations of harmful algae ($>10^4$ cells l^{-1}) counting using a compound microscope and a counting cell is simple and fast. If, on the other hand the cell concentration is low ($<10^2$ - 10^4 cells l^{-1}), the cells must be concentrated before counting, which can be a time consuming procedure, and counting using either inverted microscopy or epifluorescence microscopy is preferable.

Cells can be concentrated by a factor of x 10-100 by sedimentation in bottles, measure glass etc., or on filters. Note that cells can be lost during the concentration procedure. In most cases it is preferable that the algal samples are fixed/preserved before the concentration procedure is started.

Inverted microscope: Quantification of harmful algae using inverted microscopy, using sedimentation chambers, according to Utermöhl is very useful for counting of harmful algae in rather low concentrations ($<10^2$ - 10^4 cells l^{-1}) (Sournia, 1978). If concentrations are high the samples can be diluted using filtered sea water.

Settling of cells in the sedimentation chamber lasts from a few hours to several days depending upon the sample volume (the height of the chamber) and the linear dimension of cells to be counted. In general small cells have much longer sedimentation times than large cells. As a rule large cells ($L >10 \mu\text{m}$) must be allowed to settle for at least 12 hours before counting, while smaller cells must be allowed to settle for approx. 24 hours before counting.

NB: An inverted microscope is also excellent for e.g. qualitative examination of normal slide

preparations or counting cells, using suitable slide holders.

Epifluorescence microscopy: In general quantitative epifluorescence microscopy is based upon concentration and staining of cells on membrane filters, followed by quantification of cells. The methodology is very useful for counting of harmful algae in rather low concentrations ($< 10^2$ - 10^4 cells l^{-1}). If concentrations are high the samples can be diluted using filtered sea water. For thecate dinoflagellates the fluorochrome Calco Flour White MR2, which is a specific stain for cellulose is excellent (Andersen & Kristensen, 1995). For quantification of harmful algae in general other stains like e.g. Acridine Orange (Andersen & Sørensen, 1986), DAPI (Porter & Feig, 1980) can be very useful.

A great advantage of this methodology is that large volumes of sample (50-100 ml) can be prepared for quantification in a few minutes, and that specific stains e.g. Calco Flour white allows for counting of thecate dinoflagellates in low concentrations in situations where the overall cell concentration is very high.

NB: A compound microscope can be transformed into an epifluorescence microscope if it is equipped with a halogen lamp/ mercury burner and suitable filter sets for the stains used. See Table 6 for a summary of methods.

Table 6. A summary of useful methods for quantification of harmful algae

Methods for quantification of algae	Volume	Sensitivity	Preparation time
Compound microscope			
Sedgewick Rafter Cell (counting cell)	1 ml	1.000 cells/L	15 minutes
Palmer - Mahoney Cell (counting cell)	0.1 ml	10,000 cells/L	15 minutes
Drops on slide		5.000-10.000 cells/L	1 minute
Inverted microscope			
Utermohl (sedimentation chamber)	2 - 50 ml	20-500 cells/L	2-24 hours
Epifluorescence microscopy			
Counting on filters (fluorochrome: Calco Flour)	1-100 ml	10-1.000 cells/L	15 minutes

Other methods: In situations with monospecific blooms of harmful algae a coulter counter, or measuring of chlorophyll can be useful for quantification of cells/biomasses.

More sophisticated electronical methods like flow cytometry might be useful in the future for quantifying harmful algae in mixed natural plankton using e.g. the stain FITC and an immunofluorescence approach (Anderson, 1995).

2.3.6. Counting Statistics

At a 95 % level of confidence the relative limits of expected concentrations = $\pm (2 \times 100\%) / (n^{0.5}/n)$.

Table 7. The relationship between number of cells counted and the relative and actual limits of expectation.

Counts	Confidence limits +/- (%)
1	200
2	140
4	100
5	90
10	63
20	45
40	32
50	28
100	20
200	14
400	10
500	9
1000	6

Example 1:

Sample volume = 100 ml, counts = 50 *Dinophysis acuminata*

$$(50/100) \times 1000 = 500 \text{ cells l}^{-1}$$

$$\pm (14/100) \times 1000 = 140 \text{ cells l}^{-1}$$

$$\text{Concentration} = 500 \pm 140 \text{ } *Dinophysis acuminata* \text{l}^{-1}$$

2.3.7 Detection and quantification of algal toxins

Algal toxins are detected and quantified using one or a combination of several techniques:

- Biological methods
- Chemical analysis
- Immunological methods

Biological methods

The biological methods (bio tests or bioassays) are based upon extraction of the toxins followed by exposure of a living organism to the toxin e.g. either intraperitoneal injection into mice, feeding it to mice, rats or application on flies. The reaction of the animals to the exposure is followed in time and the toxicity is estimated from the time used to a certain reaction.

The mouse bioassay, with intraperitoneal injection of the extracted toxins, is the most widely used bioassay to detect algal toxins.

Concerning the mouse bioassay it should be noted:

that different methods of extraction may be applied:

acetone - a quick extraction, but it fails to eliminate fatty acids which may give false positive responses;
methanol/hexane/dichloromethane - more time consuming but more specific for DSP-toxins.

that different thresholds exist (24 hours, 5 hours . . .)

that the advantages of mouse bioassay are the low cost, the simple procedure and the fast response.

that the disadvantages of mouse bioassay are the use of living animals, that it is not very precise and not very specific.

Cytotoxicity methods

Although not yet routinely used, methods based on cytotoxicity could be alternative to biological test. They have the same advantages with quick response and the same precision, but without the inconvenience of using live animals for experiment (see e.g. the method based on the cytotoxicity of okadaic acid on KB cell cultures and examination of toxin induced changes in cell morphology (Amzil et al., 1992), or the hemolysis test, using rat blood cells, to detect toxicity of *Chrysochromulina* and *Gyrodinium* (Yasumoto et al., 1990).

Chemical methods

Most chemical methods for toxin analysis are based upon detection of the toxins themselves or derivatives, produced by chemical derivation, detected by high pressure liquid chromatography (HPLC) or autoanalyzer.

Analysis of PSP-toxins can be based upon alkaline oxidation of toxins to fluorescent derivatives using an autoanalyzer with a fluorescent detector (Bates & Rapoport, 1975), or alternatively, by using high pressure liquid chromatography (HPLC) where each toxic component can be detected, using oxidation of toxins to fluorescent products (Sullivan et al., 1985).

Analysis of DSP-toxins can be based upon HPLC detection of the fluorescent esters of OA and DTX-1 produced by esterification of the DSP-components with 9-anthryl diazomethane (ADAM) (Lee et al., 1987).

Domoic acid are based upon a HPLC technique using UV-absorbance (Subba Rao et al., 1988)

Contrary to biological tests which measure the total response of an organism to a set of toxins, chemical methods allow to discriminate the different toxins in most cases. However, the high cost of chemical methods prevent them to be used in a large scale.

Immunological methods

The immunological methods, e.g. ELISA (Enzyme Linked Immunosorbant Assay), RIA (radioimmuno assay), EIA (competitive enzyme immunoassay) or S-PIA (solid-phase immunobead assay), Premazzi & Volterra (1993), are based upon extraction of antibodies from e.g. rabbit serum, from rabbits exposed to the algal toxin (the functional antigen provoking the rabbit to produce the antibody). The antibodies are marked with either a radioactive or fluorescent label. The extracted algal toxins or homogenized meat of e.g. mussels are exposed to the marked antibodies followed by detection of the amount of radioactivity or fluorescence of the antiserum-antigen complex, which is a measure of the amount of toxin in the sample.

The use of the different methodologies to detect the different algal toxins are compiled in Table 8. For further, and more detailed information on the different methodology see Hallegraef et al. (1996); Premazzi & Volterra (1993), ICES (1992), Sullivan (1993) and IOC Manuals and Guides No. 31 (1995). For further information see e.g. Hallegraef et al. (1996).

Table 8, Biological, chemical and immunological methods used for the detection and quantification of algal toxins in monitoring programmes. Data compiled from Premazzi & Volterra

	PSP-toxins	DSP-toxins	ASP-toxins	NSP-toxins	CFP-toxins	Ichtyotoxins
Biological methods	Mouse bio-assay	Mouse or Rat bio-assay	Mouse bio-assay	Mouse bio-assay	Mouse, fly or mosquito bio-assay	Hemolysis-test (using blood cells)
Chemical methods	HPLC	HPLC	HPLC	HPLC		
Immunological methods		ELISA		ELISA	RIA EIA S-PIA, Ciguatex	

2.4 DESIGN ELEMENTS OF HAB MONITORING programmes

The design elements of HAB monitoring programmes reflects the structure and function of the programme, and depends upon the specific demands of the users of the programme as well as the overall rules and regulations imposed by the responsible national or regional authorities.

Monitoring programmes must be adapted to local conditions and circumstances, if possible based upon results from a general monitoring programme, Table 9, 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 tradition for administration.

The basic elements of a HAB monitoring programme are:

- Environmental observations including field plankton observations, fish kills and other animal behavior
- Sampling of plankton, shellfish or fish
- Evaluation 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

see Fig. 13.

Table 9. Monitoring goals of HAB monitoring and general monitoring of the water quality in marine waters,

Monitoring goals	
HAB monitoring:	Monitoring the occurrence of harmful algae to prevent algal toxins from reaching the human consumers and to minimize damage to living resources such as shellfish and fish, as well as economic loss,
General monitoring:	Establish basic knowledge about form and function of the ecosystem investigated
	Establish detailed knowledge about selected ecosystem processes to make it possible to understand and predict ecosystem response to eutrophication or exceptional physical and biological events.
	Establish patterns and trends for algal populations,

In practice the structure of the monitoring programme can be rather complicated depending upon how many institutions are involved in the in the separate analysis, procedures at each level in the network (Fig. 14a and 14b). Most HAB monitoring programmes rely on different official authorities for sampling of algae and e.g. shellfish for analysis. However, in the state of California sampling of algae and shellfish are carried out on fixed stations at weekly intervals by local companies, organizations etc. on a voluntary basic under the guidance of State of California, Health and Welfare Agency, Department of Health services, which are also responsible for analysis of the samples.

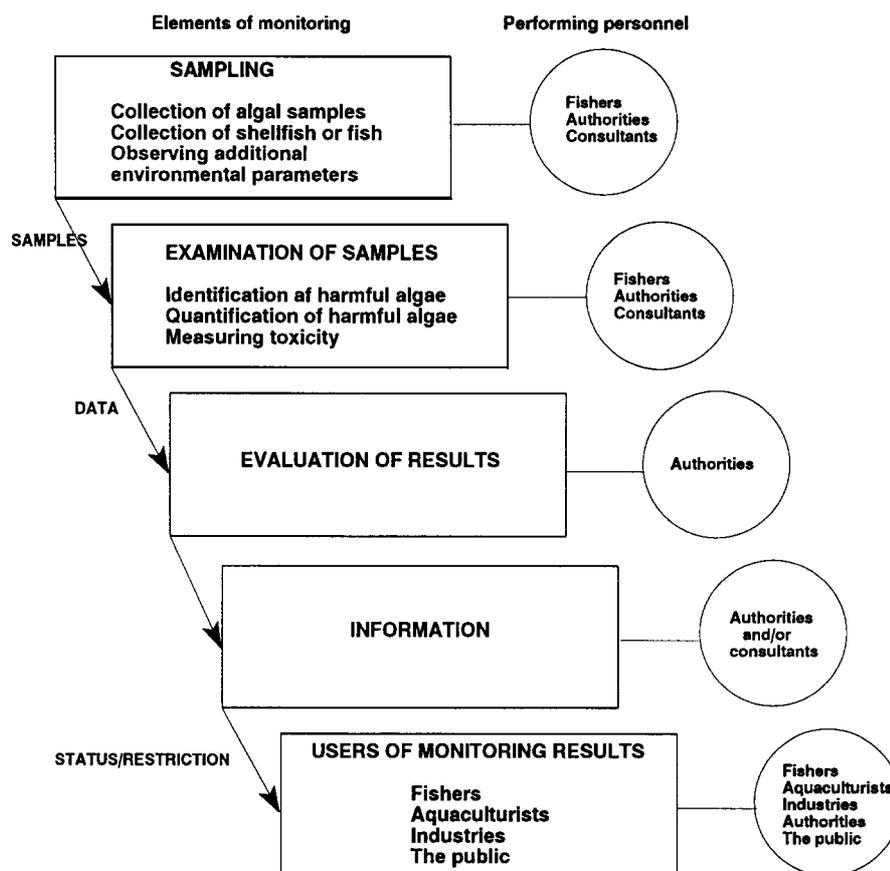


Figure 15. Theoretical basic monitoring network.

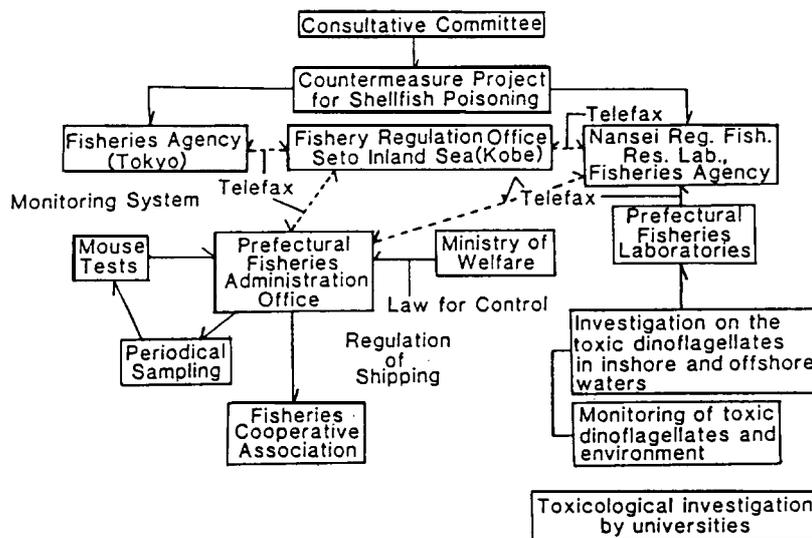


Figure 16a. Monitoring network used for shellfish poisoning monitoring and investigation systems in Seto Inland Sea, Japan (Okaichi, 1989).

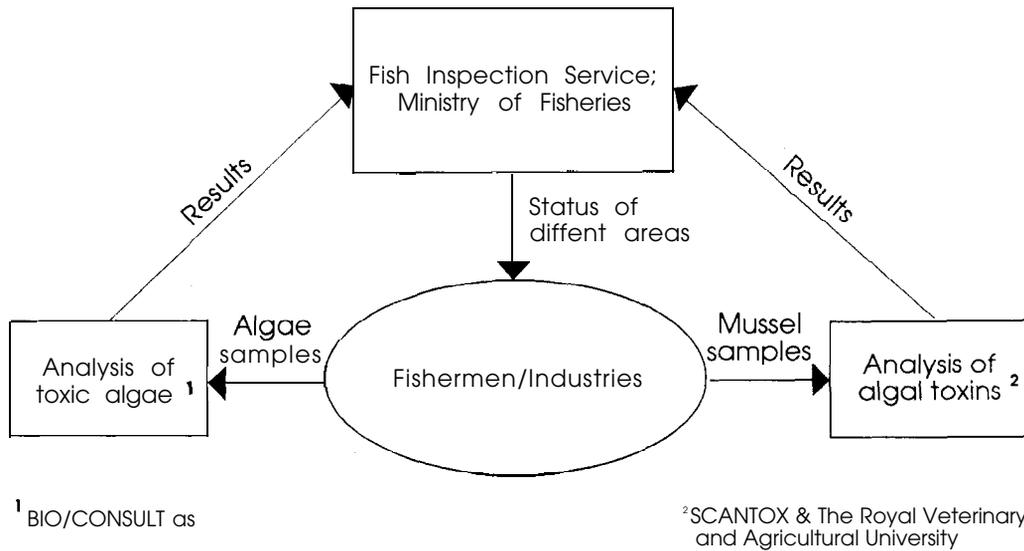


Figure 16b. Monitoring network used for shellfish poisoning monitoring in Danish coastal waters.

In general the structure of the' program must be kept as simple as possible to facilitate fast and uncomplicated flow of information between the individuals involved. It must be clear to all individuals involved in the programme who is responsible for which part of the programme. The operational structure of the programme should be well documented in the form of a short report distributed to all users of the programme, containing information on which institutions are involved (addresses, phone and fax numbers, E-mail addresses etc.), the responsible persons in the different institutions (addresses, phone and fax numbers, E-mail addresses etc.) and a clear description of which tasks of the programme for which each institution/person is responsible for.

Monitoring marine environmental conditions in relation to HAB's 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 harmful algal bloom is to be monitored.

Furthermore, depending upon the task of the monitoring, it can include a range of environmental parameters (Table 10).

Table 10. Examples of monitoring parameters in relation to monitoring of toxic algae.

Physical	Chemical	Biological
Temperature (vertical profile) Current speed and direction (vertical profile) Wind speed and direction Light attenuation/turbidity	Salinity (vertical profile) Oxygen content (vertical profile) Nutrients - Nitrogen Phosphorous - Silicate Chlorophyll (vertical profile)	Phytoplankton - Toxic species Mesozooplankton Protozooplankton Pelagic bacteria Fish Benthos Birds

Acquisition of data

Sampling: It must be clearly defined:

which kinds of samples should be collected and analyzed, as well as the methods used for sampling and the different kinds of analysis

which institution/who is responsible for collecting the samples
which institution/who is responsible for working up the samples

Results: It must be clearly defined how to present the monitoring data:
which forms must be used
which terms must be used
which units must be used

Quality control of analysis data: Before data are to be distributed through-out the monitoring system, the data should be properly checked by at least one person who did not perform the analysis. Raw analysis data should be kept in files for later investigation.

Evaluation of data

It must be clearly defined which institution/person is responsible for compiling/synthesis of the monitoring results, and how the results of the synthesis are presented to the users of the programme. E.g. the following kinds of restrictions can be imposed upon the shellfishery: fishery is allowed (= open); fishery is allowed under certain restrictions, and finally fishery is not allowed (= closed).

If mathematical models are used in the evaluation of monitoring data, e.g. to make predictions on the transport, physical concentration or dissipation of a bloom, or the temporal build up of a bloom due to in-situ growth of the bloom species, the models should be well established, that is defined and calibrated to be used in the specific monitoring programme. The following comment by Hallegraeff et al. 1996, on the use of predictive models should be kept in mind "The lessons learned from all the above efforts indicate that predictive models are likely to be site specific for the region for which they are developed. Moreover, ecological requirements of harmful algae vary from species to species and even among strains of the same species, and therefore can only be applied to other bloom situations with some approximations". Alternatively to the mathematical modelling, exploratory analysis, based upon the experience of the person in charge of evaluation of the situation, in most cases should be useful in the assessment of the risk of harm due to a HAB in time and space.

Forecasts

Based upon the monitoring results, forecasts which define risk-zones in time and space should be defined if possible. The temporal resolution of the forecast should be in the range of 1-7 days. The forecast should provide the users of the monitoring system with information to take proper action to prevent harmful effects on fish in fishfarms or harvesting of mussels in areas during periods with high risk of a harmful concentrations of algal toxins in the mussels, with the result that the mussels caught must be destroyed.

Reports

It must 'be clearly defined how to present results of the evaluation of the monitoring data and the forecast:
which forms, maps must be used
which terms must be used
which units must be used

Distribution of information to users

Results can be distributed instantly to the users of the monitoring system by telephone, telephone answering machine, fax, E-mail and Internet e.g. as The Baltic Sea Algaline in Internet World Wide Web: <http://www.fimr.fi> (Leppänen, pers. comm.) and with a time-lag of 1-several days using surface mail.

Contingency plans

To avoid or minimize the economic effects of a HAB, contingency plans should be prepared, and their reliability should be tested, to be ready if an area should be affected by a HAB.

2.5 DESIGN OF INFORMATION STRUCTURE AND CONTINGENCY PLANS

In general, contingency plans which aim to reduce acute problems in relation to HAB's should/must

include well defined and tested lines for information exchange to inform users of the monitoring system about the HAB situation. As mentioned earlier in this report results can be distributed instantly to the users of the monitoring system by telephone, telephone answering machine, fax, E-mail and Internet e.g. as The Baltic Sea Algaline in Internet World Wide Web: <http://www.fimr.fi> (Leppanen, pers. comm.) and with a time-lag of one to several days using surface mail.

In areas with risk of HAB's the public should be educated/informed about the risk of HAB's. The informational material should be adapted to local conditions, that is tradition and level of education using e.g. posters (Fig. 17), "cartoons" (Fig. 18), pamphlets and information folders (Fig. 19).

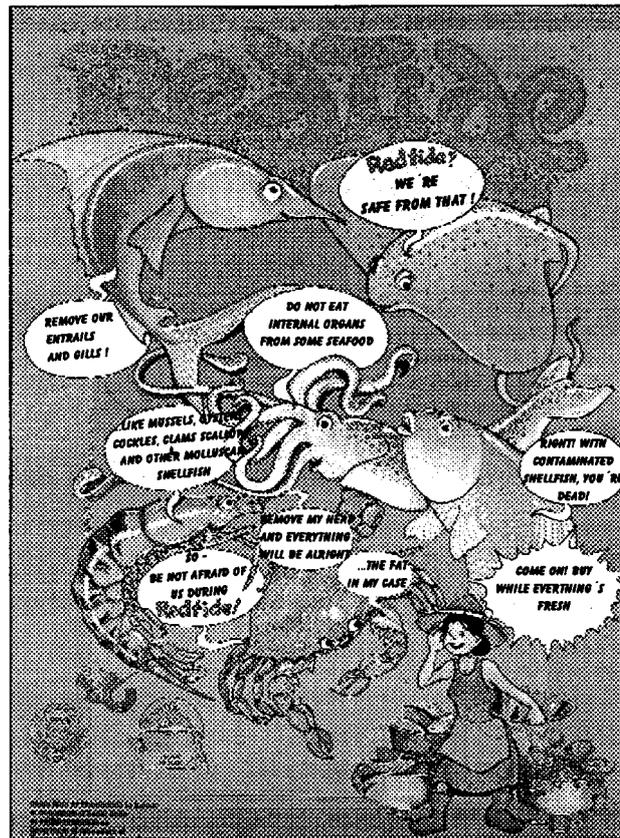


Figure 17. Philippine poster to inform the public about red tides (Bureau of Fisheries and Agriculture, translated by Dr. R. Choraless)

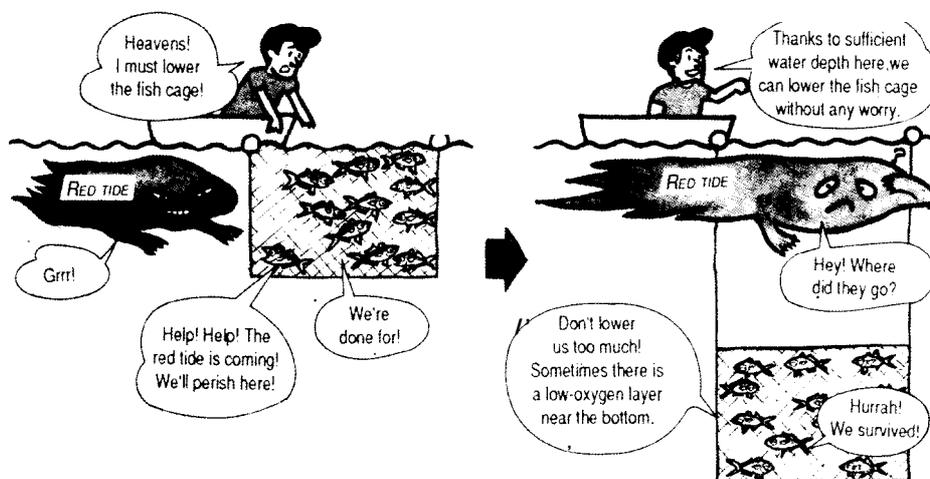
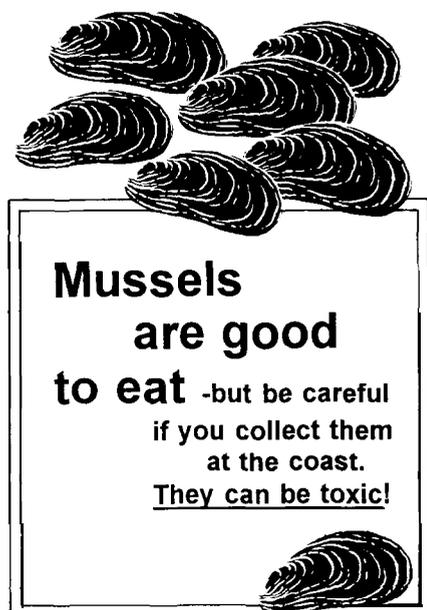
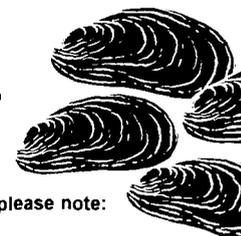


Figure 18. Information cartoon published by the Japanese Ministry of Agriculture and Fisheries to inform aquaculturists about red tides (text translated from Japanese).



Sometimes toxic algae appear among the algal food of mussels.



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 presence 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 immediately when you tick the shells.
Throw away all mussels which have not opened their shells during boiling

Figure 19. Front page of Danish information folder about shellfish and the risk of intoxication by algal toxins.
Translation from Danish

In case of a HAB with potential to harm the general public, instant global distribution of information through the public media (television, radio, newspapers) as well as local warning (posters in harbours, on beaches etc.) should be used.

Contingency plans should be based and currently updated upon access to current information about the HAB-situation. Furthermore, updates on the development of the HAB-situation (growth/spreading in time and space, toxicity) should be generated using hydrographic models to predict the physical spreading of a bloom in an area, and to define risk areas.

The specific contingency actions that can be initiated in relation to aquiculture or shellfish or fish poisonings are compiled in Tables 11 and 12.

Table 11. HAB contingency measures to avoid or minimize kill offs in aquaculture of e.g. fish, shrimp etc.

<p style="text-align: center;">AQUACULTURE: FISH FARMING</p> <p>Reduce or cease feeding to reduce the oxygen demand (stop feeding).</p> <p>Shielding pens using non-porous barriers (e.g. polyethylene sheets) to avoid contact with the HAB. (Additional oxygenation of the water inside the pens might be needed).</p> <p>Pumping of water containing no algae into shielded pens (e.g. by an air-lift pump).</p> <p>Relocation of farms into waters with less risk of intoxication of stocks e.g. by lowering the cultures into deeper waters, or transferring cultures to other localities.</p> <p>Harvest stocks before mortality occurs to minimize loss.</p>

Table 12. HAB contingency measures to prevent toxic shellfish or fish from reaching the human consumers and to ensure proper medical care for intoxicated persons.

<p style="text-align: center;">Shellfish or fish poisoning (PSP, DSP, ASP, NSP, CFP)</p> <p>To prevent or detect toxicshellfish</p> <p>Environmental monitoring network: system for rapid communication of observations field plankton observations observations on other indicators (animal behavior, water color etc.)</p> <p>Toxicity monitoring program</p> <p>Epidemiological surveillance network: Ensure that emergency room personel and other physicians can recognize seafood intoxications</p> <p>In response to the detection of toxicshellfish</p> <p>General warning of the public with clear recommendations on which species could be toxic and how to act in the case of intoxications (first aid, who to contact, phone numbers addresses etc.). Impose restrictions on fishing/harvestand processing (intensified monitoring/analysis, closing).</p> <p>Standby system for medical care to intoxicated persons (antidote, respirator etc).</p> <p>System for sample capture (particular clinical specimens from patients and samples of food consumed), analysis, and epidemiological followup</p>

3. EXISTING HAB MONITORING PROGRAMMES

Various HAB monitoring programmes exist on local, national and regional scales, related to historical events of shellfish poisoning epidemics, fish kills, or other effects of harmful algal blooms. When new monitoring programmes are planned, historical data should be taken into account.

3.1 EXISTING MONITORING PROGRAMMES

On-going HAB monitoring programmes have previously been presented, more or less detailed, in the literature and at meetings, USA (Hungerford & Wekell, 1993), Canada (Cerebella & Todd, 1993) Denmark (Emsholm et al., 1995), France (Belin & Berthome, 1991), Norway (Dahl, 1989), Japan (Fukuyo, 1992), Philippines (Corrales & Gomez, 1990). Furthermore reviews on HAB's and their monitoring have been published (Egmond et al., 1992; Shumway et al., 1996).

With the aim to provide an up-to-date overview of HAB monitoring programmes worldwide, a questionnaire was prepared as a collaboration between IOC and ICES, requesting the following information:

- Motivation
- Organization, planning and operation
- Funding
- Acquisition of data
- Evaluation and dissemination of data
- Management and use of HAB data
- Management regulations and guidelines
- Applied and basic research associated with monitoring
- Cost/benefit

In February 1995 the questionnaire was distributed (in print as well as on diskette) to IOC-action addresses world wide to be distributed to the institutions/persons actively taking part in monitoring HAB's, as well as to ICES-contact persons in all ICES countries. The completed Questionnaires were returned to IOC in Paris and distributed from there to Per Andersen who were responsible for handling and reporting the data.

Forty four questionnaires from different countries/regions were returned (Table 14). The returned questionnaires are kept at the IOC-secretariat in paper copies, on diskette as well as in a database, and can be acquired on request to IOC. The discussion on the on-going monitoring programmes in the present report is based upon questionnaires received not later than 15th of June 1995. Relevant individuals/institutions which either have not yet received or have not returned the questionnaire are urged to fill in the questionnaire and return it to IOC where it will be stored together with all other responses, and made available for further study on request to IOC. The questionnaire is presented in ANNEX I.

For some countries who had not responded to the questionnaire, information was compiled from other sources such as reports and other publications (Shumway et al., 1996) as well as personal communication.

Note: The data presented here may not be complete or fully correct in some cases, due to: 1) the fact that monitoring programmes are initiated, closed or changed currently and 2) problems with language, terminology and interpretation by the author of the returned questionnaires.

Results of the questionnaire

If data from the recent IUPAC questionnaire (Shumway et al., 1996) as well as data from Watson et al. (1989) or Hallegraef & McLean (1989) and the present IOC-ICES questionnaire are summarized, information on a total number of 76 countries and regions is available - of which 45 have on-going HAB monitoring programmes, (Table 13), and 31 countries and regions are reported to have no HAB monitoring at present (Table 13 and Fig. 20).

As expected the HAB monitoring programmes tend to fall into 2 major categories:

1. Focused routine programmes devoted to monitoring and management of HAB's in relation to shellfish harvesting and/or fish farming.

2. programmes run as integrated parts of the general environmental monitoring, with no specific focus on the detection of HAB's for management use.

In some countries/regions which at present have no HAB monitoring programmes, monitoring programmes are currently planned (e.g. China and Greenland).

Some countries/regions express their needs for, and wishes to develop HAB monitoring programmes and that guidelines for setting up such programmes are needed.

Table 13. Country status of HAB monitoring programmes according to the IOC-ICES survey as well as the IUPAC-questionnaire¹ (Shumway et al., 1996) Hallegraeff & McLean (1989)² or Watson et al. (1989)³.

Country/region	Have replied to the IOC-ICES questionnaire	Have HAB monitoring
Argentina	X	X
Australia ¹		X
Bahamas	X	
Bangla Desh	X	
Bolivia ¹		X
Brazil	X	
Brunei Darussalam ¹		X
Burkina Faso ¹		
Cameroun	X	closed since 1990
Canada (Maritime)	X	X
China	X	
Chile	X	X
Columbia (Pacific)	X	
Columbia (Carribbean)	X	
Denmark	X	X
Ecuador ¹		
Egypt ¹		
El Salvador ¹		
Ethiopia ¹		
Finland	X	X
France	X	X
Germany	X	X
Greece	X	X
Guatemala ¹		X
Guinée Bissau ¹		
Honduras ¹		X
Hungary ¹		
Hong Kong ¹		X
India ¹		
Indonesia ²		X
Iceland	X	
Ireland	X	X

Table 13. (cont.) Country status of HAB monitoring programmes according to the IOC-ICES survey as well as the IUPAC-questionnaire¹ (Shumway et al., 1996) Hallegraef & McLean (1989)² or Watson et al. (1989)³.

Country/region	Have replied to the IOC-ICES questionnaire	Have HAB monitoring
Italy	x	x
Japan ¹		
Jordan ¹		
Kenya ¹		
Kuwait	x	x
Lebanon	x	
Malawi ¹		
Malaysia ²		x
Mauritius	x	x
Mexico ¹		
Netherlands	X	X
New Zealand ¹		X
Nigeria	X	
Norway	X	X
Panama	X	
Peru ¹		
Philippines	X	X
Philippines (Bataan)	X	X
Portugal	X	X
Quatar ¹		
Romania ¹		
Singapore ¹		X
South Korea ²		X
Spain (Catalonia)	X	X
Spain (Galicia)	X	X
Spain (Valencia)	X	X
Sudan ¹		
Sweden (Baltic)	X	X
Sweden (Kattegat/Skagerak)	X	X
Switzerland ¹		
Syria ¹		
Thailand ¹		X
Tonga	X	

Table 13. (cont.) Country status of HAB monitoring programmes according to the IOC-ICES survey as well as the IUPAC-questionnaire¹ (Shumway et al., 1996), Hallegraef & McLean (1989)² or Watson et al. 1989³.

Country/region	Have replied to the IOC-ICES questionnaire	Have HAB monitoring
UK (England & Wales)	x	x
UK (Northern Ireland)	X	X
UK (Scotland)	x	x
Uruguay	x	
USA (California)	X	X
USA (Connecticut)	X	X
USA (Maine)	X	X
USA ("New York")	X	X
USA (Washington incl. Puget Sound)	X	X
Venezuela	X	X
Yemen ¹		

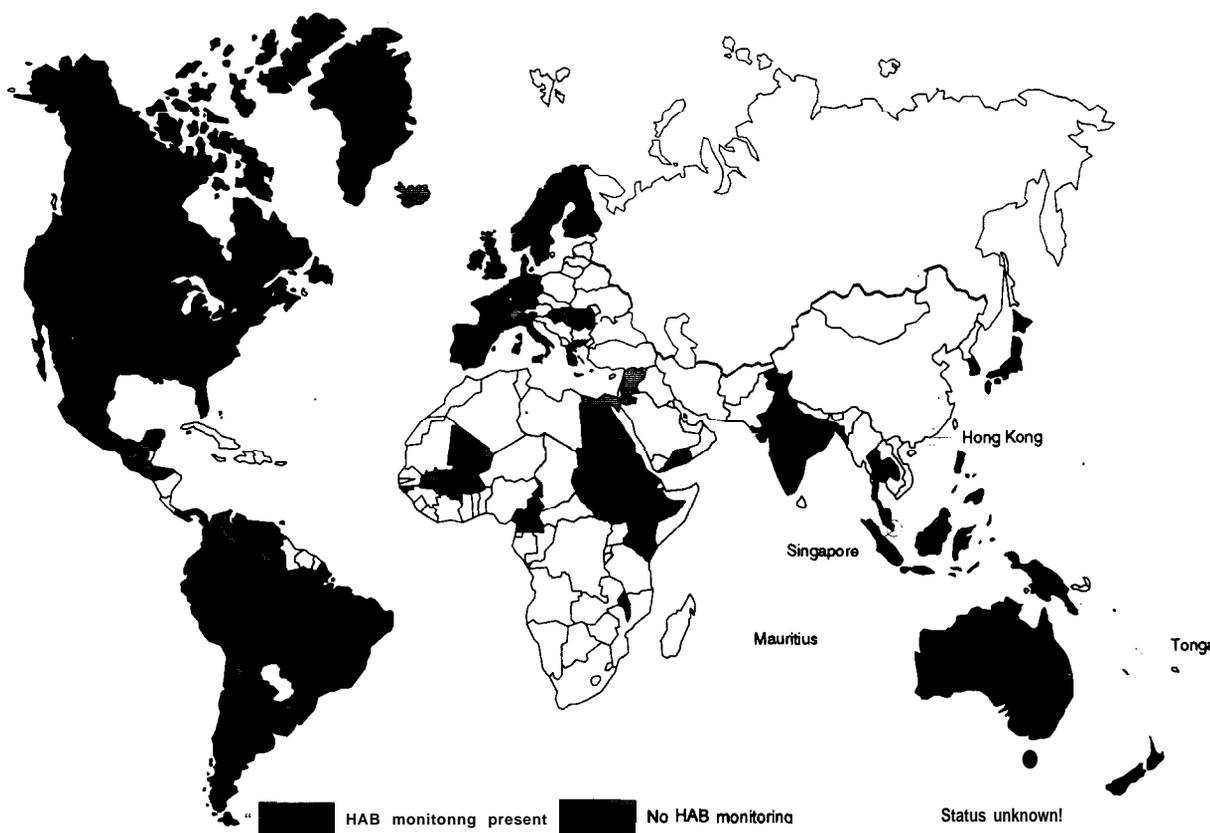


Figure 20. Country status of HAB monitoring programmes according to the IOC-ICES survey as well as the IUPAC-questionnaire (Shumway et al., 1996) Hallegraef & McLean (1989) or Watson et al, 1989.

National/regional HAB monitoring programmes

HAB monitoring programmes can either be national, or regional, covering relevant geographic regions.

National programmes can be broad, covering both monitoring for toxic algae and algal toxins in shellfish as well as fish, toxic algae and fishkills, e.g. France; or narrow, only covering monitoring for toxic algae and algal toxins in shellfish, e.g. regional programmes in Spain, or only covering toxic algae in relation to fishfarming, as in Chile.

In some countries several HAB monitoring programmes, with different purposes, are in operation. This is e.g. the case in the USA where different states can have different monitoring programmes running with different purposes (programmes concerning either shellfisheries or aquiculture or both), in Japan (different programmes concerning shellfish and aquiculture) and Denmark (separate programmes concerning shellfisheries, fishfarming and environmental quality).

Purpose/goal of HAB monitoring programme

The results of the IOC-ICES survey shows that most HAB monitoring programmes (70%) are initiated for management of molluscs (shellfish/mussels) either in culture or in wild stocks and fish culture (55%). Only Portugal has monitoring in relation to the culture of crustaceans,

In the case of natural ecosystems, about one third of the countries/regions indicated that HAB monitoring is also initiated for protection of natural ecosystems (Table 14).

Table 14. HAB Monitoring for protection of various resources.

Country	Fish culture	Fish wild stocks	Mollusc culture	Mollusc wild stocks	Crustacean culture	Public safety	Natural ecosystems
Canada (Maritime)	X	X	X	X		X	X
Chile	X						
Denmark	X		X	X		X	
Finland						X	X
France	x		x	x		x	x
Germany			x			x	
Greece						x	
Italy	x	x				x	
Ireland	x		x			x	
Kuwait	x	x				x	x
Mauritius		x				x	x
Netherlands			x	x		x	
Norway	x	x	x	x		x	x
Philippines			x			x	
Philippines (Bataan)		x		x		X	
Portugal	X		X	X	X	X	X
Spain (Balearic Islands)			X	X		X	
Spain (Galicia)	X		X	X		X	X
Spain (Catalonia)						X	
Spain (Valencia)			X			X	
Sweden (Baltic)		X				X	X
Sweden (Kattegat/Skagerak)	X	X	X	X		X	X
Thailand	X		X			X	
Uruguay				X		X	
USA (California)						X	
USA (State of New York)				X		X	X
USA (State of Washington)	X		X			X	
USA (Coast of Maine)			X	X		X	
USA (State of Connecticut)			X	X		X	
UK (Scotland)	X		X	X		X	
UK (England& Wales)			X	X		X	
UK (Northern Ireland)						X	
Venezuela		X	X	X		X	X

Organization/institution responsible for initiating and planning of HAB monitoring programmes

82% of the countries/regions answer that the HAB monitoring programme/programmes are initiated and planned by governmental authorities, only 4 countries, Canada (West Coast), Chile, Norway and Denmark (explain mussel fishery/fish farming-PA), have HAB monitoring initiated by private organizations (Table 15).

Table 15. The type of organization/institution responsible for the initiation and planning of the HAB monitoring.

Country	Governmental	Private	Combination
Canada (Maritime)	X	X	
Chile		X	
Denmark	X	X	X
Finland	X		
France	X		
Germany	X		
Greece	x		
Italy			x
Ireland	x		
Kuwait	x		
Mauritius	x		
Netherlands			X
Norway	X	X	X
Philippines	x		
Philippines (Bataan)	x		
Portugal	x		
Spain (Balearic Islands)	x		
Spain (Galicia)	X		
Spain (Catalonia)	X		
Spain (Valencia)			X
Sweden	X		
Thailand	x		
Uruguay	X		
USA (California)	X		
USA (State of New York)	X		
USA (State of Washington)			X
USA (Coast of Maine)	X		
USA (State of Connecticut)	X		
UK (Scotland)	x		
UK (England & Wales)	X		
UK (Northern Ireland)	X		
Venezuela	X		

Operation/ practical monitoring

In most cases (85%) the institution/organization which initiated the HAB monitoring programme is also responsible for carrying out the monitoring. In a few cases, Denmark, Finland, part of Spain and part of Sweden the practical monitoring is 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 are carried out by private consultancy laboratories and the data collected by the authorities, Table 16.

Table 16. The organization/institution carrying out the HAB monitoring.

Country	Same institution as responsible for initiating the programme	Several institutions in a network
Canada (Maritime)	x	
Chile	x	
Denmark		x
Finland		x
France	x	
Germany	x	
Greece	x	
Italy	x	
Ireland	x	
Kuwait	x	
Mauritius	x	
Netherlands	x	
Norway		x
Philippines	x	
Philippines (Bataan)	x	
Portugal	x	
Spain (Balearic Islands)		
Spain (Galicia)	x	
Spain (Catalonia)		x
Spain (Valencia)	x	
Sweden		x
Thailand	x	
Uruguay	x	
USA (California)	x	
USA (State of New York)	x	
USA (State of Washington)		x
USA (Coast of Maine)	x	
USA (State of Connecticut)	x	
UK (Scotland)	x	
UK (England& Wales)	x	
UK (Northern Ireland)	x	
Venezuela	x	

Funding source of the HAB monitoring

In most cases (91%) HAB monitoring is financed by governmental agencies. Exceptions from the rule are Denmark and Chile where the monitoring programmes are financed by the fisheries associations and Norway, Netherlands and part of USA and Canada where private users of monitoring data pay a part of the cost. In Finland, Norway and Portugal part of the HAB monitoring programmes are financed by the research institutions (Table 17).

Table 17. Funding sources of HAB monitoring programmes.

Country	Governmental authorities	Research foundations	Private users of monitoring data
Canada (Maritime)	x		x
Chile			x
Denmark			x
Finland	x	x	
France	x		
Germany	x		
Greece	x		
Italy	x		
Ireland	x		
Kuwait			
Mauritius	x		
Netherlands	x		x
Norway	x	x	x
Philippines	x		
Philippines (Bataan)	x		
Portugal	x	x	
Spain (Balearic Islands)	x		
Spain (Galicia)	x		
Spain (Catalonia)	x		
Spain (Valencia)	x		
Sweden	x		
Thailand	x		
Uruguay	x		
USA (California)	x		
USA (State of New York)	x		
USA (State of Washington)	x		x
USA (Coast of Maine)	x		
USA (State of Connecticut)	x		
UK (Scotland)	x		
UK (England& Wales)	x		
UK (Northern Ireland)	x		
Venezuela	x		

Identification and quantification of HAB species

In 64% of the countries/regions which have HAB monitoring, all species in the phytoplankton community are quantified along with the HAB species. In the rest of the countries/regions only HAB species are quantified. In 15% of the countries/regions only one or a few HAB species are quantified, where as in 36% of the countries/regions all potential HAB species are quantified. In most cases (87%) the algae are quantified as cell counts (cells/L). In 45% of the countries/regions the biomass of algae are quantified (Table 18).

Table 18. The type of identification and quantification of algae.

Country	Whole phytoplankton community	One or few HAB species	All potential HAB species	Cell Counts	Biomass
Canada (Maritime)				x	
Chile	x			x	
Denmark			x	x	
Finland	x			x	x
France	x		x	x	x
Germany	x			x	x
Greece	x			x	x
Italy	x			x	x
Ireland			x	x	
Kuwait	x			x	x
Mauritius	x	x		x	
Netherlands			x	x	
Norway	X		X	X	X
Philippines	x			x	
Philippines (Bataan)		x		x	
Portugal	x		x	x	x
Spain (Balearic Islands)	x			x	x
Spain (Galicia)	x			x	x
Spain (Catalonia)	x		x	x	x
Spain (Valencia)	x		x	x	
Sweden (Baltic)	X		X	X	X
Sweden (Kattegat/Skagerak)	X		X	X	X
Thailand	X			X	
Uruguay			x	x	
USA (California)	x			x	
USA (State of New York)		X		X	X
USA (State of Washington)	x			x	x
USA (Coast of Maine)					
USA (State of Connecticut)		x			
UK (Scotland)			x		
UK (England & Wales)			x	x	
UK (Northern Ireland)			x		
Venezuela		x		x	

Concentration limits of HAB species in relation to shellfishery

For a total of 27 HAB species or species groups, belonging to the genus *Alexandrium*, *Aureococcus*, *Dinophysis*, *Gonyaulax*, *Gymnodinium*, *Nodularia*, *Prorocentrum*, *Pseudo-nitzschia*, *Ptychodiscus* (= *Gymnodinium*) and *Pyrodinium* concentration limits exist in relation to shellfishery.

Concentration limits can be very variable between countries, and eventual restrictions implemented are in many cases not clear.

For all EU countries Council Directive No L 268 of 15 of July 1991, enforces the following in relation to HAB monitoring (extract):

“The control system must include:

1. Periodic monitoring of live bivalve mollusc relaying and production areas in order to:

(a) -----

(b) -----

(c) check the possible presence of toxin-producing plankton in production and relaying waters and biotoxins in live bivalve mollusks. ”

2. Sampling plans, as provided for in point 1, must in particular take account for:

(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, and

- toxicity test using the molluscs from the affected area which are most susceptible to contamination. ”

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, require analysis for toxins in shellfish or closing of areas for harvesting in several countries, whereas concentrations of *Alexandrium catanella* in Australia and part of Spain may reach $>10^4$ cells/L before closures are initiated (Table 19). In Norway a semi-quantitative measure based on net samples imposes analysis of toxins.

For the different species within the DSP-toxin producing genus *Dinophysis* concentrations from $<10^2$ - 10^3 cells/L 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) before action is taken (Table 19).

In the case of *Gymnodinium catenatum*, regulations are initiated at concentrations from presence to 2×10^3 cells/L (Table 19).

Concentration limits for the filamentous cyanobacteria (blue-green algae) *Nodularia spumigena* only exist in Denmark (10^5 colonies/L) (Table 19).

In the case of the genus *Prorocentrum*, concentration limits only exists for the species *Prorocentrum lima*, and they are within the range from detectable to 500 cells/L (Table 19).

For the ASP-toxin producing diatom genus *Pseudo-nitzschia* concentrations from 10^3 to 2×10^5 imposes regulations (Table 19). In most cases intensified monitoring involves HPLC analysis of shellfish extracts.

For *Gymnodinium breve* (= *Ptychodiscus brevis*) restriction are imposed in Florida at concentrations $>5 \times 10^3$ cells/L (Table 19).

In the case of *Pyrodinium bahamense* var. *compressum*, in the Philippines, closures occur at a concentration of 200 cells/L (Table 19).

The large differences in concentration levels which imposes restrictions, even between species from the same genus, are the result of variation in toxicity between species. The difference in concentration which imposes restrictions within a single species might be partly explained by geographical variability in the toxicity of the species, the environmental conditions (e. g. nutrient deficiency), or clones of one species, or simply reflect the tradition of regulation.

Table 19. Examples of concentrations of toxic algae which result in implementation of restrictions on shellfishery. Some countries are not cited in the table, since they do not use the algal concentration as a parameter for management decisions; but for many of the countries, the presence of toxic species induces intensified monitoring of toxins” Total of unidentified species,

Species/country-region	Cell concentration (cells/L)	Implemented actions
<i>Alexandrium catanella</i> Australia Spain-Valencia	> 4 x 10 ⁴ 2 x 10 ⁷ -5 x 10 ⁷	measure toxins?
<i>Alexandrium fundyense</i> Canada (Maritime)	presence	measure toxins
<i>Alexandrium lusitanicum</i> Portugal	5 x 10 ³	
<i>Alexandrium minutum</i> Spain-Balearic Islands	10 ³	intensified monitoring/closed
<i>Alexandrium ostenfeldii</i> Denmark UK-Northern Ireland UK-Scotland	500 presence presence	intensified monitoring/closed restrictions?? restrictions??
<i>Alexandrium tamarense</i> Denmark	500	Intensified monitoring/closed
<i>Alexandrium</i> sp. Denmark	500	intensified monitoring/closed
<i>Alexandrium</i> spp. Netherlands Norway Spain-Balearic Islands	10 ³ -10 ⁴ presence in net-hauls 10 ³	restrictions-alert/closed? restrictions/closed restrictions/closed??
<i>Aureococcus anophagefferens</i> USA-New York	2.5 x 10 ⁸	replanting of scallops
<i>Dinophysis acuminata</i> Denmark Portugal Spain-Balearic Islands Spain-Valencia Region	500 200 10 ³ 2 x 10 ⁷ -5 x 10 ⁷	intensified monitoring/closed restricted restrictions/closed?? measure toxins??
<i>Dinophysis acuta</i> Denmark Portugal	500 200	intensified monitoring/closed restricted
<i>Dinophysis norvegica</i> Denmark	10 ³	intensified monitoring/closed
<i>Dinophysis rotundata</i> Denmark	10 ³	intensified monitoring/closed
<i>Dinophysis sacculus</i> Spain-Valencia	2 x 10 ⁷ -5 x 10 ⁷	measure toxins?
<i>Dinophysis</i> 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??
Total <i>Dinophysis</i> spp. Denmark Italy Norway	1.2 x 10 ³ 10 ³ and DSP in mussels 500-1.2 x 10 ³	intensified monitoring/closed restrictions/closed- depending on species

Table 19. (cont.) Examples of concentrations of toxic algae which result in implementation of restrictions on shell fishery. Some countries are not cited in the table, since they do not use the algal concentration as a parameter for management decisions; but for many of the countries, the presence of toxic species induces intensified monitoring of toxins.

Species/country-region	Cell concentration (cells/L)	Implemented actions
<i>Gymnodinium catenatum</i> Portugal Spain-Andalusia UK-Northern Ireland	2 x 10 ³ > 500 presence	restrictions? restrictions??
<i>Gymnodinium sp.</i> Venezuela	no cons. limit	restricted
<i>Nodularia spumigena</i> Denmark	1 x 10 ⁵ -2 x 10 ⁵ colonies/L	intensified monitoring
<i>Prorocentrum lima</i> Denmark UK-Northern Ireland UK-Scotland Spain-Andalusia	500 presence presence	intensified monitoring/closed restrictions?? restrictions?? restrictions??
<i>Pseudo-nitzschia seriata</i> -group Denmark	2 x 10 ⁵	intensified monitoring/closed
<i>Pseudo-nitzschia delicatissima</i> -group Denmark	2 x 10 ⁵	intensified monitoring/closed
<i>Pseudo-nitzschia multiseriata</i> Canada (Maritime)	5 x 10 ⁴	monitor shellfish
<i>Pseudo-nitzschia pseudodelicatissima</i> Canada (Maritime)	1 x 10 ⁵	measures toxins
<i>Pseudo-nitzschia pungens</i> UK-Northern Ireland	> 10 ³	restrictions??
<i>Pseudo-nitzschia spp.</i> Netherlands	10 ⁴ -10 ⁵	restrictions-alert/closed?
<i>Gymnodinium breve</i> (= <i>Ptychodiscus brevis</i>) USA-Florida	> 5 x 10 ³	remain closed if toxins previously detected
<i>Pyrodinium bahamense var. compressum</i> Philippines-Bataan Philippines	200 cells l ⁻¹ ?	restrictions?? restrictions??

Detection of algal toxins and their effects

In 64% of the countries/regions with on-going HAB monitoring programmes toxins are quantified in molluscs. In Canada, Italy, Portugal, Spain-Galicia, USA-California and in Venezuela toxins are also quantified in fish (Table 20).

Fauna/fish mortality is monitored in 30% of the countries/regions including Chile, Denmark, France, Italy, Norway, Portugal, Spain (Galicia), Sweden, USA-California and Venezuela (Table 20).

Table 20. Parameters measured during monitoring,

Country	Toxins in molluscs	Toxins in fish	Fauna/fish mortality
Canada (Maritime)	X	X	X
Denmark	X		
Finland			
France	X		X
Germany	X		
Greece	X		
Italy	X	X	X
Ireland	X		
Kuwait			
Mauritius			
Netherlands	X		
Norway	X		X
Philippines	X		
Philippines (Bataan)	X		
Portugal	X	X	X
Spain (Balearic Islands)	X		
Spain (Galicia)	X	X	X
Spain (Catalonia)	X		
Spain (Valencia)	X		
Sweden (Baltic)	X		
Sweden (Kattegat/Skagerak)	X		X
Thailand			
Uruguay	X		
USA (California)	X	X	X
USA (State of New York)			
USA (State of Washington)	X		
USA (Coast of Maine)	X		
USA (State of Connecticut)	X		
UK (Scotland)	X		
UK (England & Wales)	X		
UK (Northern Ireland)	X		
Venezuela	X	X	X

Evaluation and communication of monitoring data

In 90% of the cases the evaluation and communication of monitoring results are carried out by the data collecting institutions/organizations, in the rest of the countries and regions various users are involved (Table 2 1).

Table 21. Institutions responsible for evaluation and communication of the monitoring data.

Country	Data collection institution	Various users
Canada (Maritime)	x	
Chile	x	
Denmark	x	
Finland	x	
France	x	
Germany	x	
Greece	x	
Italy	x	
Ireland	x	
Japan		
Kuwait	x	
Mauritius		
Netherlands	x	
Norway	x	
Philippines	x	
Philippines (Bataan)	x	
Portugal	x	
Spain (Balearic Islands)		x
Spain (Galicia)	x	x
Spain (Catalonia)		x
Spain (Valencia)	x	
Sweden (Baltic)	X	X
Sweden (Kattegat/Skagerak)	X	X
Thailand	X	
Uruguay	X	
USA (California)	X	
USA (State of New York)	x	x
USA (State of Washington)	X	
USA (Coast of Maine)	x	x
USA (State of Connecticut)	x	
UK (Scotland)	x	
UK (England& Wales)	x	
UK (Northern Ireland)	x	
Venezuela	x	

In 51 % of the cases raw data are disseminated. In 21 % of the cases forecasts on the situation is reported and in 88 % of the cases the data are summarized in reports.

In 85% of the cases the data are disseminated using paper/fax, in 33 % of the cases data are communicated using computers via e.g. Internet, E-mail. In France Geographical Information Systems (GIS) will be used to summarize the results (Table 22).

Table 22. Types of data released to various users and the method used for dissemination of data.

Country	Raw data	Forecasts	Summary report	Paper/fax	Internet, E-mail, GIS
Canada (Maritime)	x	x	x	x	x
Chile			x	x	
Denmark	x		x	x	
Finland		x	x	x	x
France	x		x	x	x
Germany			x	x	
Greece	x		x	x	x
Italy	x	x	x	x	
Ireland					
Kuwait	x				x
Mauritius					
Netherlands	x		x	x	
Norway	x	x	x	x	x
Philippines			x	x	
Philippines (Bataan)		x	x	x	
Portugal			x	x	
Spain (Balearic Islands)			x		x
Spain (Galicia)	x	x	x	x	x
Spain (Catalonia)	x	x	x	x	
Spain (Valencia)	x		x	x	
Sweden (Baltic)			X	X	
Sweden (Kattegat/Skagerak)	x	x	x	x	
Thailand			x	x	
Uruguay		x		x	
USA (California)			X	X	X
USA (State of New York)	X		X	X	X
USA (State of Washington)	X		X	X	X
USA (Coast of Maine)	X		X	X	
USA (State of Connecticut)			X		
UK (Scotland)	x		x	x	
UK (England& Wales)			x	x	
UK (Northern Ireland)	X		X	X	
Venezuela	x		x	x	

Institutions responsible for management actions

The HAB monitoring data is in many cases used by several authorities and private organizations. In 60% of the cases management based upon the HAB data are used by the governmental food control authorities. In 24% of the cases the data are used by the governmental pollution control authorities. In 61 % of the cases the data are used for management action by the public health authorities, and in 64% of the cases the data are used for management purposes by aquaculturists and fishermen (Table 23).

Table 23. Institutions responsible for the management actions related to public health and/or aquiculture,

Country	Food control authorities	Pollution authorities	Public health authorities	Aquaculturists/ fishermen
Canada (Maritime)	X			X
Chile				X
Denmark	X			
Finland		X	X	
France	X		X	
Germany	x		x	x
Greece		x		
Italy	x		x	x
Ireland			X	X
Kuwait		X	X	X
Mauritius				
Netherlands	X			X
Norway	X	X	X	X
Philippines	X		X	X
Philippines (Bataan)			X	X
Portugal	X		X	X
Spain (Balearic Islands)			X	X
Spain (Galicia)	X	X	X	X
Spain (Catalonia)	X		X	
Spain (Valencia)	X	X		
Sweden (Baltic)			X	
Sweden (Kattegat/Skagerak)	X			
Thailand		X		
Uruguay	X			
USA (California)	X		X	X
USA (State of New York)		X	X	X
USA (State of Washington)	X		X	X
USA (Coast of Maine)	X		X	X
USA (State of Connecticut)	X		X	X
UK (Scotland)	X		X	X
UK (England& Wales)	X			
UK (Northern Ireland)			X	
Venezuela	X		X	X

Monitoring parameters used for management of shellfisheries

In 30% of the countries/regions management actions can be initiated based upon the quantitative occurrence of HAB species. In 47% of the cases occurrence of algal toxins in mussels are used to initiate management actions, where as in 57% both parameters can be used together for management (Table 24).

In Denmark the mussel fishery can be closed based upon cell concentration of HAB species, whereas in most other countries the concentration of HAB species are used as guidance for initiating analysis of algal toxins in shellfish.

Table 24. Monitoring parameters used for management of harvest of shellfish.

Country	Occurrence/concentratio of HAB species	Occurrence of algal toxins in shellfish
Canada (Maritime)		X
Denmark	X	X
France		x
Germany	x	x
Greece		
Italy	x	x
Ireland	x	x
Kuwait		
Mauritius		
Netherlands	x	x
Norway	x	x
Philippines	X	X
Philippines (Bataan)	X	X
Portugal	X	X
Spain (Balearic Islands)	x	x
Spain (Galicia)	x	x
Spain (Catalonia)		x
Spain (Valencia)	x	x
Sweden (Kattegat/Skagerak)		
Thailand		
Uruguay		X
USA (California)	x	x
USA (State of New York)	x	
USA (State of Washington)		x
USA (Coast of Maine)		x
USA (State of Connecticut)		
UK (Scotland)		x
UK (England& Wales)	x	
UK (Northern Ireland)	x	x
Venezuela		x

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Methods for analysis and tolerance concentrations of algal toxins in shellfish

ASP-toxins are monitored in 26% of the countries/regions with a commercial shellfishery. HPLC analysis is used alone or in combination with the mouse bioassay (Table 25).

DSP-toxins are monitored in 45 % of countries/regions using bioassay, in some cases supplemented by chemical methods, most frequently HPLC. The mouse bioassay is used i 85% of the cases, whereas the rat bioassay is used in 14% (Netherlands and UK-Northern Ireland) (Table 26).

For all European Union (EU) countries the Council Directive No L 268, of 15 of July 1991, inforces 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).

PSP-toxins are monitored in 81 % of the countries/regions using mouse bioassay (AOAC 1995), except for Netherlands where HPLC analysis is used alone. In Denmark, Japan and UK-Scotland the bioassay is supplemented by HPLC analysis (Table 27).

The critical concentration limit of 80 $\mu\text{g}/100\text{ g}$ STX-equivalent to approx. 400MU/ 100 g is used in 89 % of the countries/regions for analysis of PSP-toxins. The concentration is the official critical concentration in all EU countries. In the Philippines and Norway the critical concentration limit is 40 $\mu\text{g}/100\text{ g}$ (200MU/100 g). In UK-Northern Ireland the critical concentration limit is 32 $\mu\text{g}/100\text{ g}$. In Ireland the critical concentration limits is presence-of PSP toxins measured by mouse bioassay (Table 28).

For Canada, products having PSP-toxin concentrations up to 160 $\mu\text{g}/100\text{ g}$ may be tamed.

Table 25. ASP tolerances and analysis methods,

Country	ASP	
	Tolerance Critical concentration limit	Method of analysis
Canada (Maritime)	2 mg/100 g	HPLC
Denmark	2 mg/100 g	HPLC
France		
Germany		
Greece		
Italy		Mouse bioassay
Ireland		
Kuwait		
Mauritius		
Netherlands	2 mg/100 g	HPLC
Norway		
Philippines		
Philippines		
Portugal		Mouse bioassay
Spain (Balearic Islands)		
Spain (Galicia)	2 mg/100 g	Mouse bioassay and HPLC
Spain (Catalonia)		
Spain (Valencia)		
Sweden (Kattegat/Skagerak)		
Thailand		
Uruguay		
USA (California)	2 mg/100 g (3 mg/100 g crab meat)	HLPC
USA (State of New York)		
USA (State of Washington)		
USA (Coast of Maine)		
USA (State of Connecticut)		
UK (Scotland)		
UK (England& Wales)		
UK (Northern Ireland)		
Venezuela		

Table 26. DSP tolerances and analysis methods .”data from Shumway et al. (1996).

Country	DSP	
	Critical concentration limit	Method of analysis
Canada (Maritime)	20 µg/100 g	Mouse bioassay, HPLC, ELISA
Denmark	Presence (2 out of 3 mice die within 24 h)	Mouse bioassay, HPLC
France	Presence (2 out of 3 mice die within 5 h)	Mouse bioassay
Germany		
Greece		
Italy	5 hours mouse test	Mouse Bioassay
Ireland	Positive bioassay	Mouse bioassay + LC-MS
Japan	5MU/100 g (= 20 µg/100 g)	Mouse bioassay
Korea	5MU/100 g (= 20 µg/100 g)	Mouse bioassay
Kuwait		
Mauritius		
Netherlands	0.2-0.4 µg/g digestive gland	Rat bioassay
Norway	5-7 MU/100 g (= 20-30 µg/100 g)	Mouse bioassay
Philippines		
Philippines		
Portugal	Presence (200 µg/100g)	Mouse bioassay
Spain (Balearic Islands)	Presence	Mouse bioassay
Spain (Galicia)	Presence	Mouse bioassay
Spain (Catalonia)	Presence	Mouse bioassay
Spain (Valencia)		
Sweden	0.4-0.6 µg/100 g	
Thailand		
Uruguay	Mortality in 24 h.	Mouse bioassay
USA (California)		
USA (State of New York)		
USA (State of Washington)		
USA (Coast of Maine)		
USA (State of Connecticut)		
UK (Scotland)		
UK (England& Wales)		
UK (Northern Ireland)	200 µg/100g	Rat bioassay
Venezuela		

Table 27. PSP tolerances and analysis methods. *data from Shumway et al. (1996). **Data from Bates et al. (1993). (1MU/100 g approx. = 8.18 STX-equivalents / 100 g (Premazzi & Volterra, 1993)).

Country	PSP	
	Critical concentration limit	Method of analysis
Australia*	80 µg/100 g	Mouse bioassay
Austria*	80 µg/100 g	Mouse bioassay
Canada (Maritime)	80 µg/100 g	Mouse bioassay
Denmark	80 µg/100 g	Mouse bioassay, HPLC
Finland	80 µg/100 g	
France	80 µg/100 g	Mouse bioassay
Germany	80 µg/100 g	
Greece	80 µg/100 g	
Guatemala*	400MU/100 g (approx. 30 µg/100 g)	Mouse bioassay
Hong Kong*	400MU/100 g (approx. 30 µg/100 g)	Mouse bioassay
Italy	80 µg/100 g	Mouse bioassay
Ireland	Positive bioassay	Mouse bioassay
Japan*	400MU/100 g (approx. 30 µg/100 g)	Mouse bioassay
Korea*	400MU/100 g (approx. 30 µg/100 g)	Mouse bioassay
Kuwait		
Mauritius		
Netherlands	80 µg/100 g	HPLC
New Zealand**	80 µg/100 g	Mouse bioassay
Norway	200MU/100 g (approx. 15 µg/100 g)	Mouse bioassay
Panama*	400MU/100 g (approx. 30 µg/100 g)	Mouse bioassay
Philippines	40 µg/100 g	Mouse bioassay
Portugal	80 µg/100 g	Mouse bioassay
Spain (Balearic Islands)	80 µg/100 g	Mouse bioassay
Spain (Galicia)	80 µg/100 g	Mouse bioassay
Spain (Catalonia)	80 µg/100 g	Mouse bioassay
Spain (Valencia)		
Singapore*	80 µg/100 g	Mouse bioassay
Sweden*	80 µg/100 g	Mouse bioassay
Thailand		

Table 27 (cont.). PSP tolerances and analysis methods. *data from Shumway et al. (1996). **Data from Bates et al. (1993). (1MU/100 g approx. = 8.18 STX-equivalents/ 100 g (Premazzi & Volterra, 1993)).

Country	PSP	
	Critical concentration limit	Method of analysis
Uruguay	80 µg/100 g	Mouse bioassay
USA (California)	80 µg/100 g	Mouse bioassay
USA (State of New York)	80 µg/100 g	Mouse bioassay
USA (State of Washington)	80 µg/100 g	Mouse bioassay
USA (Coast of Maine)	80 µg/100 g	Mouse bioassay
USA (State of Connecticut)	80 µg/100 g	Mouse bioassay
UK (Scotland)	80 µg/100 g	Mouse bioassay/HPLC
UK (England & Wales)	80 µg/100 g	
UK (Northern Ireland)	32 µg/100 g	Mouse bioassay
Venezuela	200-400 MU/100 g (approx. 15-30 µg/100 g)	Mouse bioassay

Management actions imposed upon shellfish fishery

If the HAB monitoring shows absence or low concentrations of HAB species and algal toxins in shellfish, the fishery is open, that is, allowed without restrictions in most countries and regions, (Table 28).

If presence or high concentrations of HAB species or algal toxins are detected, the shellfish harvest can be restricted, and the public warned, or the shellfish harvest is closed and the public warned.

Restrictions to shellfish harvest can imply more frequent sampling of algae and mussels for analysis of algal toxins, and that shellfish must be excluded from the market until the results of the analysis are available.

Table 28. Management actions imposed upon the shellfishery,

Country	Status of fishery - open	Status of fishery - restricted (public warned)	Status of fishery - closed (public warned)
Canada (Maritime)	X	X	X
Denmark	X		X
France	X	X	X
Germany	X		
Italy	X		X
Ireland	X	X	
Netherlands	X	X	X
Norway	X	X	X
Philippines	X	X	X
Portugal	X	X	X
Spain (Balearic Islands)	X	X	X
Spain (Galicia)	X		X
Spain (Catalonia)	X		X
Spain (Valencia)	X	X	
Sweden	X	X	X
Uruguay	X		X
USA (California)	X	X	.X
USA (State of New York)	X		
USA (State of Washington)	X		
USA (Coast of Maine)	X		X
USA (State of Connecticut)	X		
UK (Scotland)	X	X	X
UK (England& Wales)	X	X	X
UK (Northern Ireland)	X	X	X
Venezuela	X	X	

3.2 COST-BENEFIT ANALYSIS OF HAB MONITORING PROGRAMMES

Based upon the data provided through the present survey it is evident that monitoring of HAB's in relation to shellfisheries is much more expensive (1-5% of production value) than monitoring in relation to fish farming or the fisheries in e.g. Chile, Norway and Denmark (cost 0.02-0.05% of production value) (Table 29, Fig. 21). The reason for this difference is that monitoring in relation to the shell fisheries includes the expensive analysis of algal toxins in shellfish and in some cases also analysis of the occurrence of toxic algae, whereas monitoring of HAB's in relation to fish farming and fisheries only includes the analysis of the occurrence of toxic algae.

Table 29. Approx. annual production value (shellfish and fish) versus the approx. cost of the monitoring of HAB's in relation to the fisheries in US \$.

Country/region	Total cost (US\$)	Production value (US\$)	Total cost/Production value (%)
Canada (Maritime - shellfish)	135.000	10M	1.4
Chile (fishfarming)	20.000	400M	0.05
Denmark (shellfish)	500.000	46M	1.1
Denmark (fishfarming)	4.000	25-30M	0.02
France	800.000		
Norway	300.000	1.000M	0.03
Portugal	425.000	200M	2.1
Spain (Balearic Islands)	11.250	225.000	5.0
Spain (Catalonia)	200.000		
UK-Scotland	280.000	22M	1.2
Uruguay	35.000	3M	1.2
USA (Washington)	660.000	50M	0.1

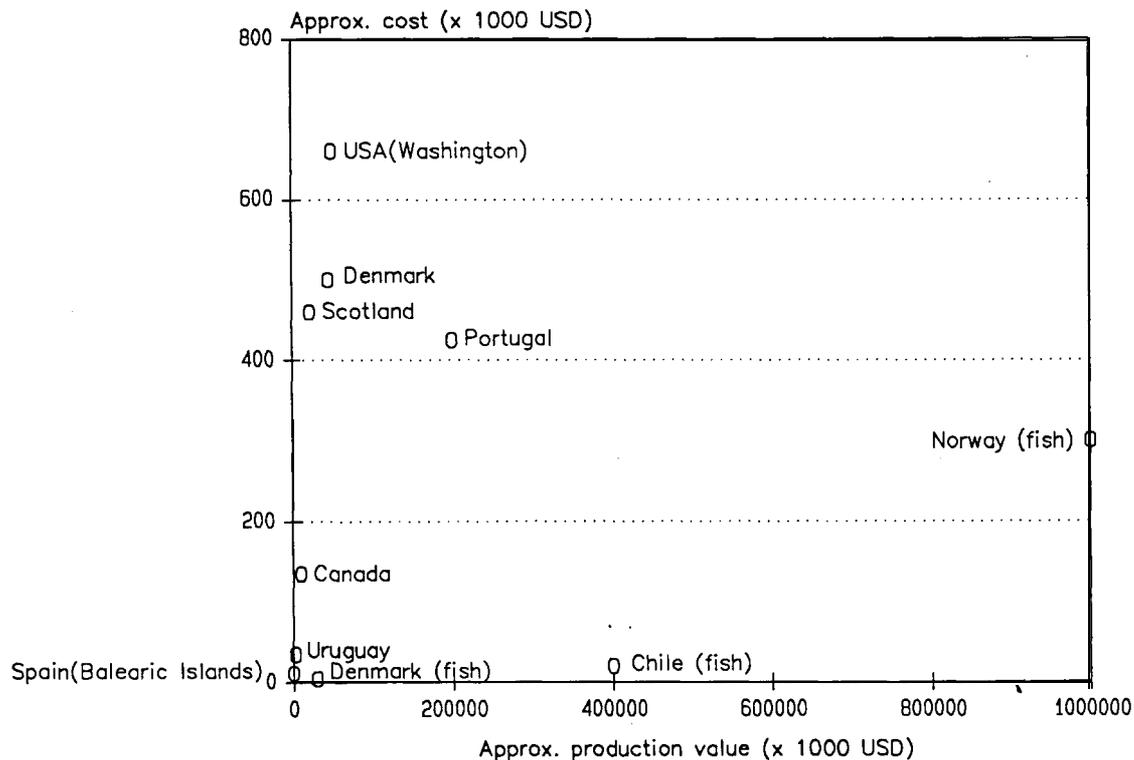


Figure 21. Approx. annual production value (shellfish and fish) versus the approx. cost of the monitoring of HAB's in relation to the fisheries in US\$.

3.3 MONITORING HARMFUL ALGAE IN RELATION TO SHELLFISHERY

In the following examples of monitoring systems in relation to shellfishery, either on wild populations or cultures will be presented.

3.3.1 Wild populations

Canada

Outbreaks of PSP as well as pressure to develop a canning industry for mussels, resulted in an extensive monitoring programme being initiated in eastern Canada in 1943, to study and measure all species of shellfish for paralytic shellfish poisoning. This was the first comprehensive bioassay programme established in the world and used the mouse bioassay, based upon the AOAC (1992). This programme is presently operated by regional laboratories of the federal Department of Fisheries and Oceans (DFO). Until 1983, bioassay analyses were carried out by the Foods Directorate of Health Canada (formerly the Department of National Health and Welfare) for DFO, but these are now done by regional DFO laboratories. Following the 1987/88 mussel incident, ASP analysis using high performance liquid chromatography (HPLC) also became a routine part of DFO's biotoxin monitoring programme. However, when a sickness and/or fatality is reported, Health Canada is notified and may participate in the investigation that follows

The primary purpose of the shellfish toxin monitoring programme is- public health protection, i.e. to provide a reasonable assurance that seafood from either wild stocks or aquaculture does not contain algal toxins in exceeding tolerance limits. As a secondary objective, the control program aims to enhance utilization of seafood resources for domestic and export markets by ensuring product safety.

Harmful algal bloom monitoring programmes are being conducted in various regions in Canada. For example, a study was initiated in eastern Canada in the southwestern Bay of Fundy in 1987 with three main objectives:

to act as an early warning to industries such as salmonid aquaculture and shellfish for HAB species that might occur

to establish patterns and trends in the occurrence of HAB's

to determine whether the aquaculture industry might have an effect on the natural environment.

The responsible HAB species for the PSP outbreaks in Atlantic Canada is *Alexandrium fundyense* and *Alexandrium tamarense*, whereas the responsible species at the pacific coast are *Alexandrium tamarense* and *Alexandrium catenella*.

Sampling and communication of results

Before 1988 shellfish harvesting areas were monitored according to their classification which reflected the frequency of PSP-toxicity in the areas (Table 30). Since 1988 sampling has increased spatially and temporally. A total of 15.342 samples are analyzed nationwide at 381 key stations (Table 32).

On the pacific coast approx. 70% of the coastline (approx. 20.000 km) is permanently closed to shellfish harvesting. The closures are due to lack of resources required for effective monitoring in the comparatively isolated locations, as well as to the chronically high levels of PSP toxicity in many areas.

The principal species from the Pacific coast, tested in the PSP monitoring include: blue mussel (*Mytilus edulis*), California mussel (*Mytilus californianus*), Alaskan butter clam (*Saxidomus giganteus*), Japanese little neck clams (*Tapes philippinarum*), native little neck clams (*Protothaca staminea*), geoduck clams (*Panopea abrupta*), Japanese oysters (*Crassostrea gigas*) and cockles (*Clinocardium nuttallii*). Those from the Atlantic coast include: blue mussel (*Mytilus edulis*), surf clams (*Spisula solissima*), soft-shelled clams (*Mya arenaria*), giant sea scallops (*Placopecten magellanicus*), European oyster (*Ostrea edulis*) and whelks (*Buccinum undatum*). Additional species such as razor clam, lobsters, Dungeness crabs and moon snails are also periodically included in the PSP

monitoring programme.

Methods for distributing monitoring data include mail, e-mail and fax. The public is alerted by posting signs on beaches advising persons to avoid harvesting shellfish during periods when shellfish are unsafe for consumption.

Table 30. PSP management program for the Bay of Fundy, Atlantic Canada (1945-1987), from Cerebella & Todd (1993).

Area classification	Sampling programme	Closure requirements
Key stations	Twice monthly (November-April) Weekly (rest of the year)	
Class I: Shellfish rarely if ever toxic	Monitored when class II areas are closed	Closed if a single sample > 80µg STX/100 g. Open to canners under permit when PSP levels are >80 and <160 µg STX/100 g Closed to canners if one sample >160 µg STX/100 g
Class H: Shellfish free from PSP for long periods	Weekly (June-October)	closed if a single sample >80 µg STX/100 g Open to tamers under permit when PSP levels are >80 and <160 µg STX/100 g Closed to canners if one sample >160 µg STX/100 g
Class III: Shellfish potentially toxic all year round	Weekly (June-October) weekly in any area open to harvest under permit for canning	Open for canning under permit; closed to canners if a single sample >160 µg STX/100 g Closed to canners if two consecutive samples at the same location >160 µg STX/100 g

Table 31. Key stations and shellfish samples analyzed for PSP, during monitoring in 1988, from Cerebella & Todd, 1993. The Gulf and Scotia-Fundy regions have been joined to form a new region called the "Maritime; region", The Quebec region is now called the "Laurentian Region".

Fisheries and oceans regions	Area	Key stations	No. of samples analyzed
Maritimes	North-east Nova Scotia	13	656
	North-east New Brunswick	16	807
	Prince Edward Island	43	3167
	South-east New Brunswick	16	776
	West Newfoundland	10	141
	South-west New Brunswick	23	2209
	East Nova Scotia	19	374
	South-west Nova Scotia	53	892
	Georges Bank	7	1685
	Total	112	10707
Laurentian	Magdalene Islands	12	327
	North shore, lower St. Lawrence estuary	44	1187
	Gaspé	28	528
	South shore, lower St. Lawrence estuary	6	277
	Total	90	2319
Newfoundland	South and east Newfoundland	40	480
Pacific	British Columbia	51	1836
Grand total		381	15.342

Methods

Toxic algae: An example of monitoring for harmful algal species is the Bay of Fundy programme where water samples are collected weekly from May through October, biweekly during November and May and monthly during December through april. Quantitative analyses are done by settling 50 ml and counting the algal species using an inverted microscope (Uthermöhl, 1958). Qualitative analyses are made using vertical and horizontal net hauls with 20 µm mesh nets.

Algal toxins: PSP has been monitored using mouse bioassay since the early 1940's in Canada (Fig. 22

and Table 32). Mice are observed for 4 h but may be kept overnight if they continue to exhibit abnormal behavior. HPLC analysis to confirm the presence of PSP toxins is performed in the following cases:

- if mouse death occurs after 15 min.
- if toxicity occurs at a key station where PSP toxicity is not expected to occur
- if epidemiological information implicates a sample in a case of illness

ASP was identified for the first time in the world in Atlantic Canada in the autumn of 1987. Initially, ASP-toxicity was detected using the mouse bioassay, but this method was soon replaced by the more sensitive HPLC method (Table 32).

Routine DSP monitoring has been going on at the Atlantic Coast (off Nova Scotia) in the Maritimes Region since 1990 using the mouse bioassay and HPLC (Yasumoto et al., 1984). If a positive response is observed from the mouse bioassay or high concentrations of cells in the production of DSP toxins are observed, samples may be further investigated using HPLC or ELISA methodologies (Fig. 23 and Table 32).

Table 32. Methods used for detection of algal toxins as well as the critical concentrations of toxins and the regulations imposed.

Toxin		Critical concentration limit	Regulation status
Type	Method		
PSP	mouse bioassay	80 µg STX/100 g	closed
DSP	mouse bioassay (HPLC/ELISA)	20 µg/100 g meat (not official !)	
ASP	HPLC	2 mg/100 g	closed

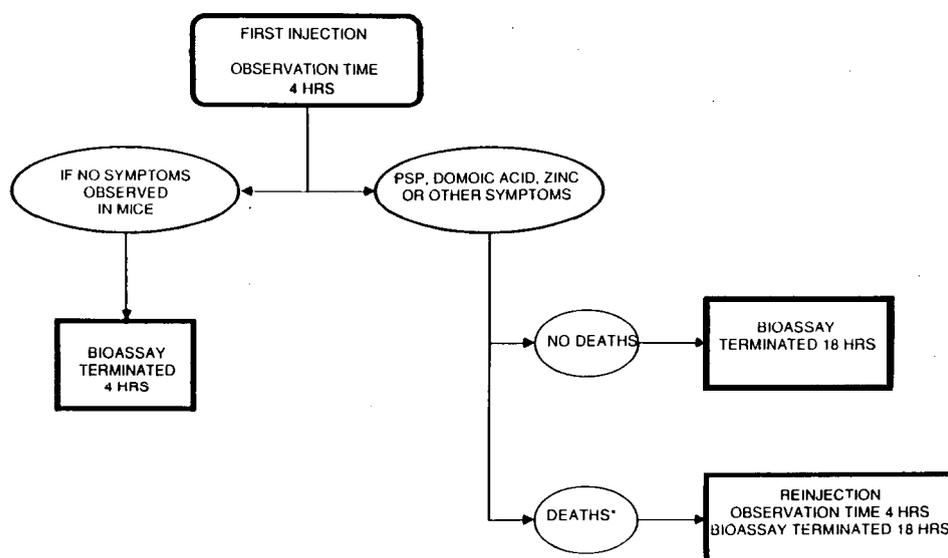


Figure 22. Flow chart of the modified AOAC (1989) mouse bioassay procedure implemented in Canada in response to domoic acid symptoms and other toxic artifacts.

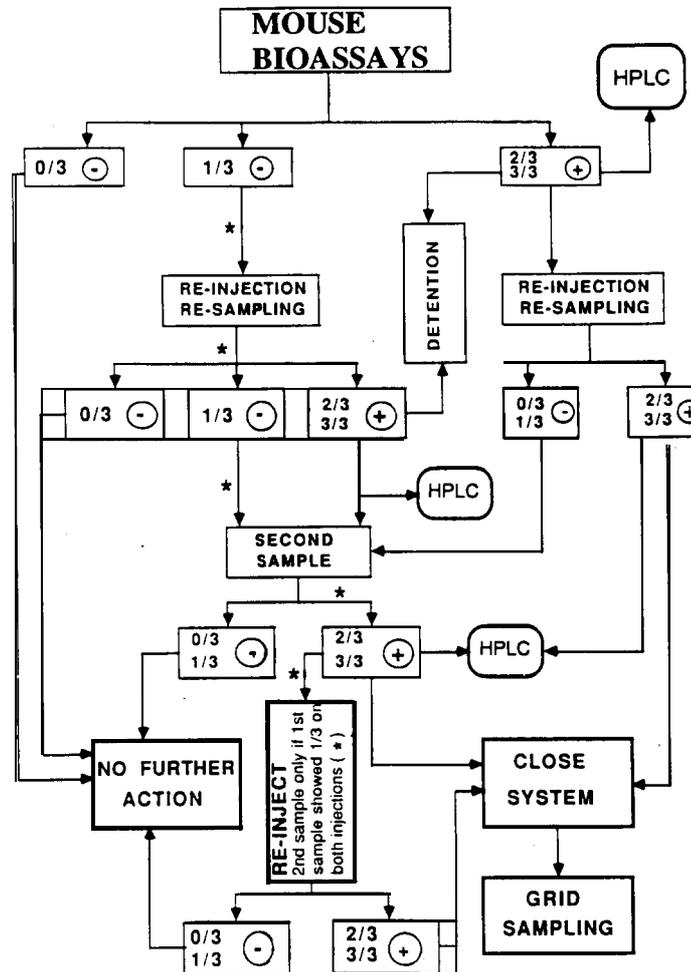


Figure 23. Early warning flow chart for the monitoring of marine molluscs for the presence of biotoxins, as well as strategic regulatory action regarding closure of contaminated areas, from Cerebella & Todd (1993).

Regulation and management strategies

The Inspection Branch of the Dept. of Fisheries and Oceans is responsible for opening and closing shellfish harvesting areas.

Concentrations of toxic algal species are used in the Maritimes Region to help determine when the frequency of monitoring of algal toxins in shellfish should be initiated (Table 33).

Table 33. Critical concentrations of toxic algae and the regulations imposed,

Algal species	critical concentration(cells/L)	Regulation
<i>Alexandriumfundyense</i>	presence in the water	measure toxins in shellfish
<i>Pseudo-nitzschiamultiseriis</i>	>50.000	measure toxins in shellfish
<i>Pseudo-nitzschiapseudodelicatissima</i>	>500.000	measure toxins in shellfish

Results

Toxins have been detected in shellfish from various regions in Canada. Examples of areas from eastern Canada are shown in Fig. 24.

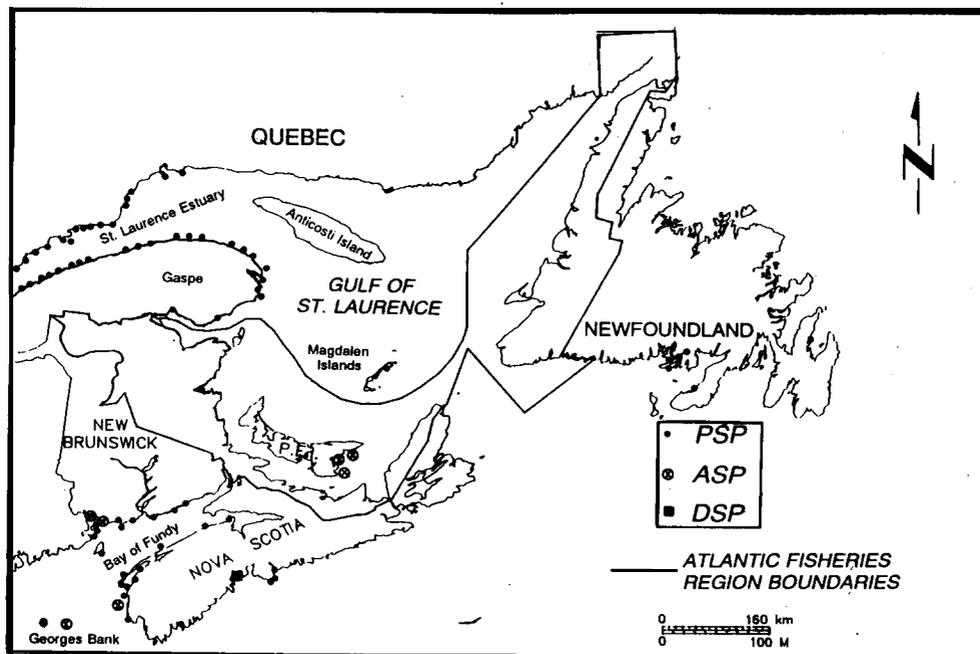


Figure 24. Map of Atlantic Canada with indication of different fisheries regions and sites where toxicity due to PSP, ASP and DSP toxins have been identified in marine shellfish

Research projects

Table 34. On-going HAB research projects in Canada.

Title of project	Institution	Funding institution
Patterns and trends of phytoplankton populations	Biological Station, St. Andrews, N. B. and Gulf Fisheries Center, Moncton, N. B.	Government of Canada
Examining 50 years of shellfish toxicity in relation to populations of <i>Alexandrium fundyense</i>	Biological Station, St. Andrews, N. B.	Government of Canada
Modelling blooms	Biological Station, St. Andrews, N. B.	Government of Canada
Uptake and deputation of toxins	St. Andrews, Halifax	Government of Canada

Cost

The cost of the local HAB monitoring programme for shellfish in Maritimes Region in eastern Canada is 135.000 US\$, in relation to a value of harvested shellfish of 10.000.000 US\$, that is approx. 1.4% (J. Martin, pers. com.).

Denmark

Since 1991 an intensive monitoring programme for detection of toxic algae and algae toxins in mussels in Danish coastal waters and fjords has been carried out. The primary goals of the programme are:

to prevent toxic mussels from reaching the consumer

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

The primary motivation for the Danish fishers and the mussel industry to initiate a monitoring programme was the occurrence of DSP-toxins in mussels for export in the previous years. These events lead to a dramatic decrease in the export of Danish mussels.

Sampling and communication of results

The Danish monitoring programme deals with the simultaneous occurrence of both a) toxic algae and b) algal-toxins in mussels at weekly intervals in the fishing areas.

The Danish coastal waters where mussels (primarily *Mytilus edulis*) are fished are divided into areas ; e.g. the Limfjord is divided into 22 areas (Fig. 25).

To start fishing for mussels in an area, qualitative and quantitative algae samples as well as samples of mussels are collected by the fishermen in the area, the week before, and sent to approved laboratories for analysis of the quantitative occurrence of toxic algae and algae toxins. If harvesting is started, each boat must collect plankton samples as well as mussel samples to be analyzed on the first fishing day every following week in an area.

The plankton and mussel samples are collected by the fishers, who have been instructed in how to sample and handle the samples at a training course taught by the consultants responsible for analysis of the samples. The qualitative, concentrated, algal sample is collected by the fishermen using a planktonnet (mesh size 20 µm). The quantitative algal sample is collected using a water sampler, and is a mixture of water sampled at the surface, in the middle of the water column as well as approx. 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 sending at the consultancy companies.

The results of the different analysis are distributed 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 Mussel fisheries (Fig. 26).

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.

The fishers and the industries can be continuously informed about the status of the different fishing areas at a telephone answering machine located at the Danish Fish Inspection Service.

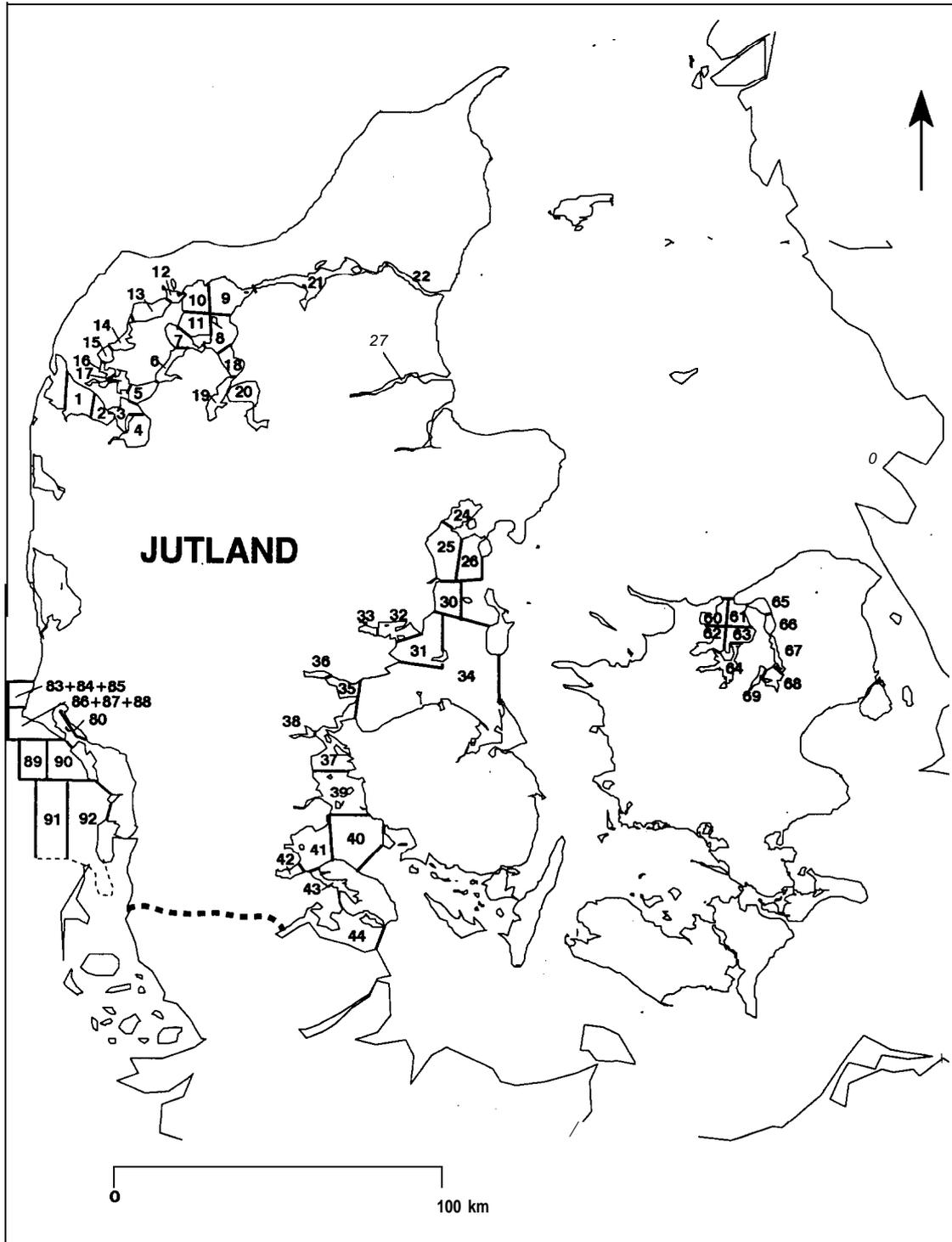


Figure 25. Map showing the different areas of the Danish coastal waters and fjords where monitoring of harmful algae and algal toxins in mussels in relation to the Danish mussel fisheries is carried out.

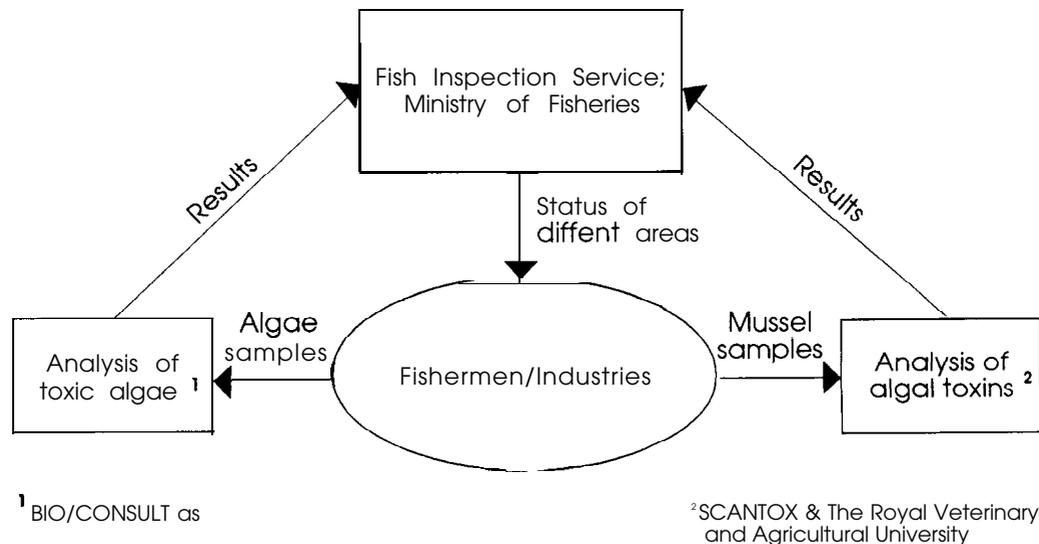


Figure 26. Flow of communication through the Danish monitoring programme for toxic algae and algal toxins in mussels.

Methods

Toxic algae: 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 modified from the procedure described by Lawrence & Triemer (1985).

The quantitative investigations of plankton samples (25-200 ml) are carried out using a combination of inverted microscopy, according to Uthermöhl (1958) see also Hallegraeff et al. (1996), and quantitative epifluorescence microscopy, using the fluorochrome Calco-fluor White, according to Andersen & Kristensen (1995). The toxic and potentially toxic algae registered in Danish waters are listed in Table 35.

The recommended concentration limits of the toxic and potentially toxic algae are shown in Table 36. The concentration limits were originally based upon information from the literature combined with educated guesses. The concentration limits are continuously evaluated and revised if necessary. During the years the monitoring has shown that even extreme concentrations ($>1 \times 10^6$ cells/L) 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 are that there is no fixed concentration 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 per L. This change was based upon the experience that accumulation of DSP-toxins has never been detected in mussels, even in situations with very high concentrations ($>10^3$ cells per L) of *Dinophysis norvegica*.

Table 35. Toxic and potentially toxic algae reported from Danish waters

DSP	PSP	Domoic acid	Other toxins
<i>Dinophysis acuminata</i> ¹	<i>Alexandrium tamarense</i>	<i>Pseudo-nitzschiaseriata</i> -group	<i>Prorocentrum balticum</i> ¹
<i>Dinophysis acuta</i> ¹	<i>Alexandrium ostenfeldii</i>	<i>Pseudo-nitzschia delicatissima</i> -group	<i>Prorocentrum minimum</i> ¹
<i>Dinophysis norvegica</i> ¹	<i>Alexandrium minutum</i>		<i>Prorocentrum micans</i> ¹
<i>Dinophysis</i> spp. ¹	<i>Gymnodinium catenatum</i> ²		<i>Nodularia spumigena</i>
<i>Dinophysis rotundata</i> ¹			<i>Aphanizomenon flos-aquae</i>
<i>Prorocentrum lima</i> ¹			<i>Microcystis aeruginosa</i>
			<i>Microcystis viridis</i>

¹Quantified using epifluorescence according to Andersen & Kristensen, 1995

²not observed in Danish waters but found in adjacent waters

Table 36. Recommended concentration limits of the toxic and potentially toxic algae in Danish waters.

Species	Closed/intensified monitoring (cells/L)
Dinoflagellates	
<i>Dinophysis acuminata</i>	500
<i>Dinophysis acuta</i>	500
<i>Dinophysis norvegica</i>	10 ³
<i>Dinophysis rotundata</i>	10 ³
Total <i>Dinophysis</i> spp.	1.2 x 10 ³
<i>Prorocentrum lima</i>	500
<i>Prorocentrum balticum</i>	
<i>Prorocentrum micans</i>	
<i>Prorocentrum minimum</i>	
<i>Alexandrium ostenfeldii</i>	500
<i>Alexandrium tamarense</i>	500
<i>Alexandrium</i> spp.	500
Diatoms	
<i>Pseudo-nitzschiaseriata</i> -group	2 x 10 ⁵
<i>Pseudo-nitzschia delicatissima</i> -group	5 x 10 ⁵
<i>Pseudo-nitzschia</i> spp.	2 x 10 ⁵
Cyanobacteria	
<i>Nodularia spumigena</i>	1 - 2 x 10 ⁵ (colonies)

Algal toxins: Mussels are examined for DSP by a modification of Yasumoto's mouse bioassay (Yasumoto et al., 1981). Through-out 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 & Fernández, 1993).

The methodologies used for monitoring of algal toxins are summarized in Table 37.

Table 37. Summary of guidelines for monitoring algal toxins in relation to the Danish mussel fisheries.

Algal toxin	Period	Method
DSP	All year round	Mouse bioassay -ether extraction (official method) -acetone extraction (used for <i>Mytilus edulis</i> under normal surveillance) Verification by HPLC
PSP	Minimum April-September	Mouse bioassay Verification by HPLC
Domoic acid	When blooms of <i>Pseudo-nitzschia</i> spp. occur	HPLC

The concentration limits of algal toxins in mussels follow the guidelines outlined by EU Council directive No. L268, of 15th of July 1991. That is, DSP toxins must not be detectable using the mouse bioassay, and the concentration of PSP toxins, detected by the mouse bioassay must be below 80 µg per 100 g. There are no official EU guidelines for ASP-toxins but the general accepted concentration limit of 2 mg per 100 g detected by HPLC is used in the Danish monitoring”.

Results

Every year since 1991, more than 2000 samples of plankton and mussels were collected annually. Of these more than 1000 samples were analyzed each year, During that period there have been several periods with closings or restrictions because of the occurrence of *Dinophysis acuminata*, *Dinophysis norvegica*, *Alexandrium tamarense*, *Alexandrium ostenfeldii*, *Pseudo-nitzschia seriata-group* and *Pseudo-nitzschia delicatissima-group*, and the first years also of *Prorocentrum micans*, *Prorocentrum minimum* and *Prorocentrum balticum*.

No cases of shellfish intoxication, due to consumption of Danish mussels have been reported since 1991. PSP has not been detected in shellfish since 1990 from Danish waters. Okadaic acid has been found every year in Danish mussels from 1991 to 1994.

Temporal and geographical distribution of the species *Dinophysis acuminata* is shown in Fig. 27.

The geographical distribution of DSP-toxins in mussels from 1991 to 1994 is summarized in Fig. 28, Detection of DSP is indicated when there was a positive mouse-bioassay (ether extraction) or if okadaic acid/DTX- 1 was detected by HPLC.

1991: DSP was detected in May in one sample of blue mussels (*Mytilus edulis*) at the east coast of Jutland. The area was already closed for fishing because of high concentrations of *Dinophysis acuminata* and *Dinophysis norvegica*.

1992: DSP was detected in August at the east coast of Jutland in blue mussels after a period with relatively low concentrations of *Dinophysis acuminata*. There might have been high concentrations of *Dinophysis acuminata* at certain depths. The maximum concentration was 2,6 µg OA/g hepatopancreas, and large amounts of already harvested mussels had to be destroyed. Since then, harvesting is usually restricted to the east coast of Jutland during most of the summer period at low concentrations of *Dinophysis*.

In 1992 DSP was also detected in low concentrations in surf clam (*Spisula solidae*) and in cockles (*Cardium edule*) on the west coast of Jutland when low concentrations of *Dinophysis* species were observed.

mussels had to be destroyed. Since then, harvesting is usually restricted to the east coast of Jutland during most of the summer period at low concentrations of *Dinophysis*.

In 1992 DSP was also detected in low concentrations in surf clam (*Spisula solidae*) and in cockles (*Cardium edule*) on the west coast of Jutland when low concentrations of *Dinophysis* species were observed.

Many areas were closed to harvesting because of large blooms of *Pseudo-nitzschia pseudodelicatissima* (up to 16.5×10^6 cells per L), but no domoic acid was detected.

1993: During a bloom of *Pseudo-nitzschia pseudodelicatissima* and the *Pseudo-nitzschia seriata*-group with maximum concentrations of 5.6×10^5 and 4.1×10^5 cells per L respectively, domoic acid was detected in small amounts (0,4-0,6 mg domoic acid/100 g mussel) in two mussel samples from the east coast of Jutland, but the result was not confirmed by another laboratory. The species *Pseudo-nitzschia seriata* has been found to be toxic in Danish waters.

1994: At the end of May and beginning of June 1994 there was a bloom with up to 1.2×10^4 cells per L of *Dinophysis acuminata* on the east coast of Jutland, but only one mussel sample had a trace of DSP. During the following months the concentrations of *Dinophysis acuminata* were much lower and the fishery was restricted. In August DSP accumulated in mussels (max. cone. $4,3 \mu\text{g}$ OA/g hepatopancreas) in two areas with maximum concentrations of *Dinophysis acuminata* of 1.2×10^3 cells per L and previously harvested mussels were destroyed because of risk of DSP contamination.

In 1994 DSP was also detected in surf clam (*Spisula solidae*) (max. cone. $1,4 \mu\text{g}$ OA/g hepatopancreas) and blue mussels (*Mytilus edulis*) (max. cone. $1,8 \mu\text{g}$ OA/g hepatopancreas) on the west coast of Jutland in July-August. After several weeks with very hot and calm weather the counts of *Dinophysis acuminata* suddenly rose to levels up to 4.2×10^4 cells per L, showing great variation within the area monitored. Trace of DSP was detected in the Isefjord in July.

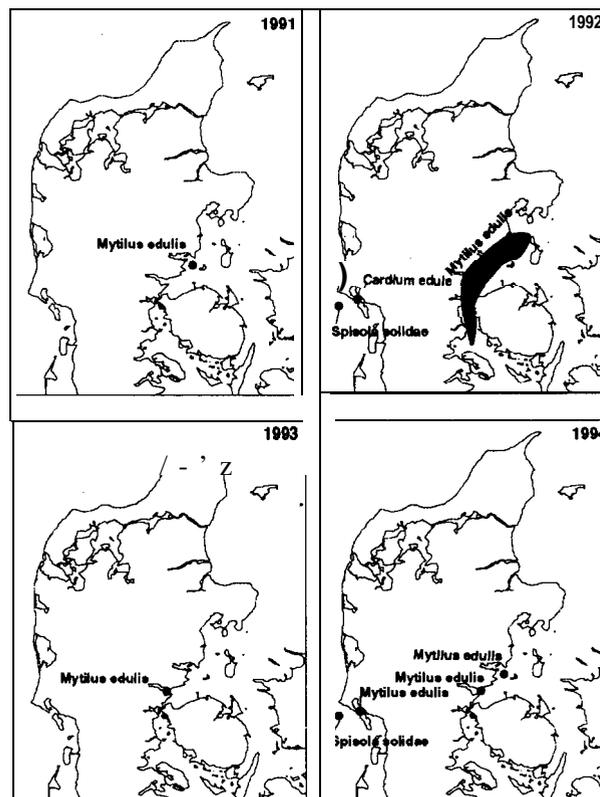


Figure 27.

Geographical distribution of DSP-toxins in mussels in Danish Waters during the period 1991-1994

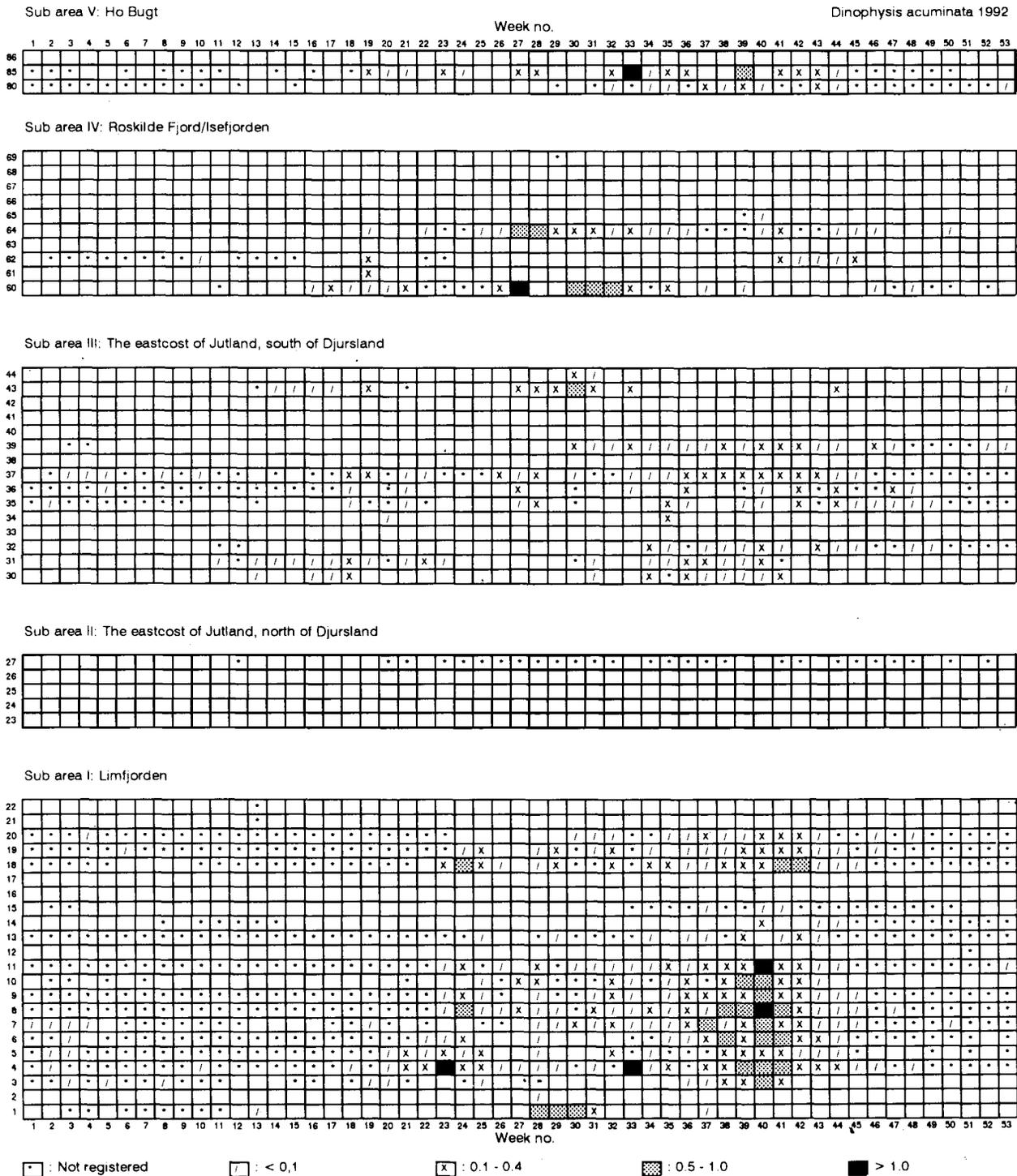


Figure 28. Concentration levels of *Dinophysis acuminata* in Danish coastal waters and fjords during 1992. The area numbers (1 -86) corresponds to the area numbers in Fig. 25.

Research

Table 38. On-going research projects in relation to the Danish HAB monitoring.

Title of project	Institution	Funding institution
Toxicity of <i>Dinophysis</i>	The Royal Veterinary and Agricultural University Bio/consult as	The Danish Ministry of Agriculture and Fisheries

Costs

The total cost of running the monitoring programme is covered by the mussel industry and fishers and was in 1992 approx. 2,5 mill, dkr. (approx. 400.000 US\$), which constitutes approx. 1% of the total export income of the mussel industry.

3.3.2 Mussel culture

France

HAB monitoring, including monitoring phytoplankton and phycotoxins in shellfish, is carried out by the French Phytoplankton Monitoring Network (REPHY). The monitoring program was initiated after the extensive development of *Dinophysis* which led to poisoning of shellfish consumers during the summers of 1983 and 1984 and has been revised. The institution responsible for REPHY is IFREMER (Institut Français pour la Recherche et l'Exploitation de la Mer).

REPHY is a national programme covering the whole French coast, although additional monitoring is performed by universities for research purposes.

REPHY has three complementary objectives : (i) acquisition of information on French coast phytoplankton populations, discolored waters and exceptional blooms, (ii) human health protection, through monitoring of species producing toxins which accumulate in shellfish, (iii) marine animal health protection, through monitoring of species toxic to fish and shellfish. Contingency planning/action plans to reduce acute problems is a part of the programme.

Concerned with human health protection, the REPHY monitoring strategy is primarily based upon detection of toxic species in water. That is the detection of toxic species in water samples determines the implementation of toxicity tests in shellfish. This strategy avoids a permanent monitoring of phycotoxins in shellfish. However, management actions and decisions are only based upon the toxicity level in shellfish, and not upon the concentration of toxic species.

In practice, an intensive monitoring is performed in periods and areas which, each year are known by experience to be affected by occurrences of toxic algal species.

The estimated annual production of cultured shellfish in France, by approx. 140 shellfish farmers, is 200.000-250.000 tons.

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

Sampling and communication of results

Twelve coastal laboratories distributed along the entire French coast perform sampling, observations, analysis and data acquisition from more than 100 sampling stations distributed along the entire length of the French coast (Fig. 30). The whole phytoplankton community is identified and counted on a few stations twice a month, simultaneously with measurements of physical-chemical parameters and chlorophyll. Other stations are sampled throughout the year as well,

measurements of physical-chemical parameters and chlorophyll. Other stations are sampled throughout the year as well, but only for identification of potentially harmful species. On the remaining stations, water and shellfish are sampled when toxic species are detected in the area.

All data from the respective coastal laboratories are directly entered and stored in a national database. The data can be consulted and extracted in real time (Fig. 30).

A new database using "client-server" architecture is currently under development. Data are stored at a national server into a database SYBASE, together with all other results from IFREMER monitoring programmes 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 softwares will soon allow statistical and cartographic consultation and treatments.

Results of shellfish toxicity are disseminated out 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...) and the local and regional media are informed. The public is informed the media and/or notice boards.

Methods

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 Uthermöhl (1958).

Algal toxins: Information about the methodology used for detection of algal toxins in mussels is compiled in Table 39.

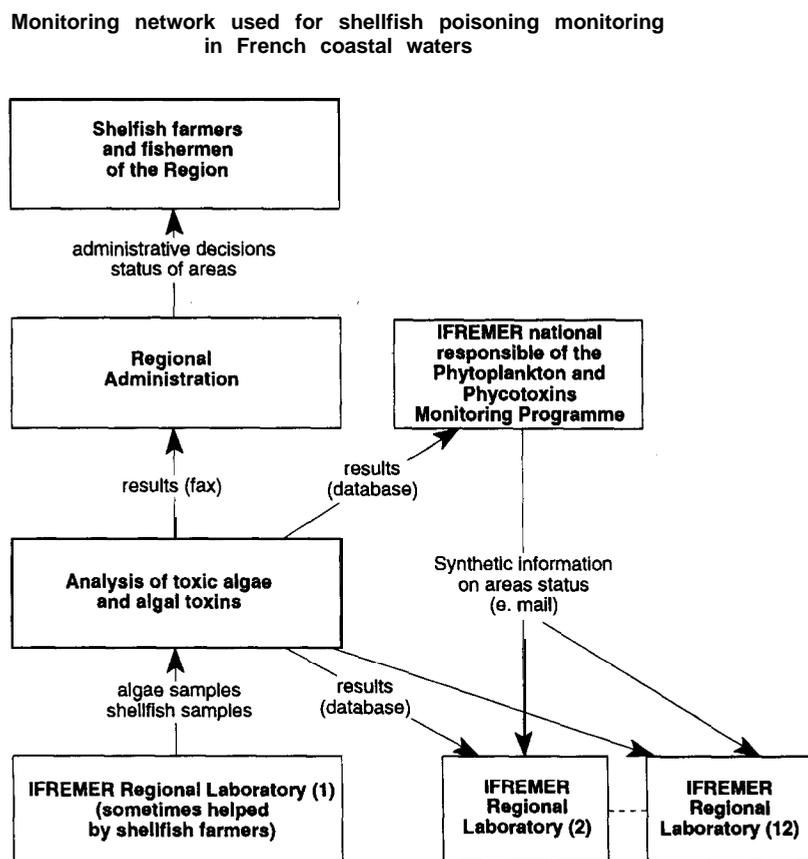


Figure 29. Monitoring network used for shellfish poisoning monitoring in French coastal waters.

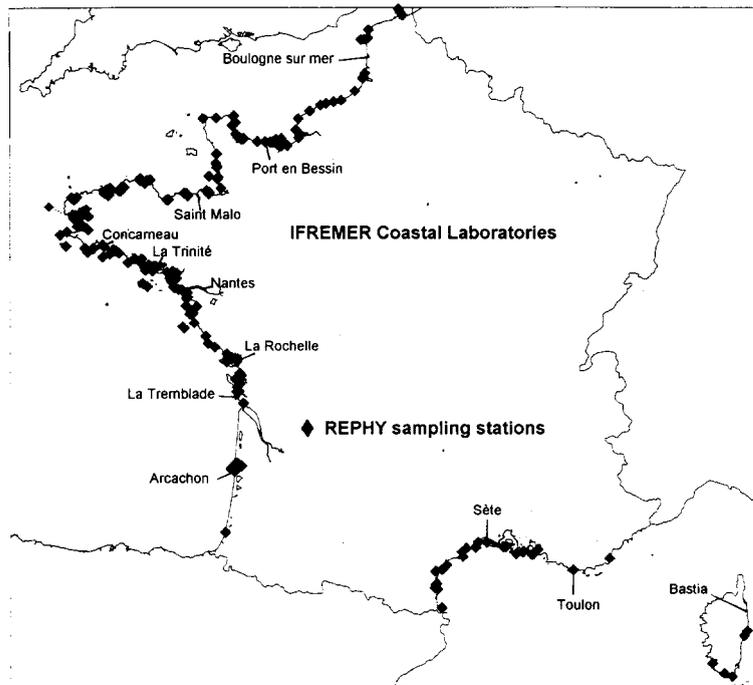


Figure 30. Geographic localization of sampling the REPHY monitoring and warning stations

Table 39. Methods used for detection of algal toxins as well as the critical concentrations of toxins and the resulting action.

Type	Toxin	Method	Critical concentration limit	Regulation status
DSP		mouse test	2 or 3 mice dead before 5 hours (equivalent of between 2 and 4 µg OA per g. of digestive gland)	closed
PSP		mouse test	80 µg equ. STX per 100g. of flesh	closed

Regulations

As mentioned earlier, the management actions and decisions are only based upon the toxicity level in shellfish, and not upon the observed concentrations of toxic algal species.

Results

In France most toxic episodes are DSP related, but some PSP episodes as well as fish kills caused by other HAB's are also observed, Fig. 31. Toxic and potential toxic algae registered in French waters are listed in table 40. 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 diarrhetic episodes, occurrence of toxic species from the genus *Dinophysis* were observed. Okadaic acid is the major DSP-toxin. The maximum observed concentrations of *Dinophysis* spp. observed along the French coasts from 1984 to 1990 are presented in Fig. 32. PSP-toxins were observed, only since 1988, in northern Brittany, and were always caused by the species *Alexandrium minutum*. The major PSP-toxins were GTX 2 and 3 and C 1 and C2 toxins. The maximal toxicity detected to date in mussels and oysters was 400 µg STX equiv./ 100 g meat. *Gymnodinium nagasakiense* caused scallop mortality and/or growth inhibition from 1976 to 1987 in Western Brittany. During the summer of 1995 very large blooms of this species lead to considerable marine animal kills (fish, shellfish, worms, urchins...) all 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 carterae* was also responsible for fish mortality in Western Brittany in 1994.

In late 1992 and 1993 stypic or 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.

Table 40. Toxic and potential toxic algae registered in French waters. "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

DSP	PSP	Other toxins
<i>Dinophysis cf. sacculus</i> <i>Dinophysis cf. acuminata</i> <i>Dinophysis cf. norvegica</i> <i>Dinophysis caudata</i> <i>Dinophysis tripos</i> <i>Dinophysis rotundata</i> (<i>Phalacrocoma rotundatum</i>) <i>Dinophysis</i> sp. <i>Prorocentrum lima</i>	<i>Alexandrium minutum</i>	<i>Gymnodinium cf. nagasakiense</i> = <i>G. cf. mikimotoi</i> = <i>Gyrodinium aureolum</i> <i>Gymnodinium</i> sp. <i>Prorocentrum minimum</i> (VSP) <i>Heterosigma carterae</i> = <i>H. akashiwo</i> <i>Dictyocha speculum</i> <i>Pseudo-nitzschia pseudodelicatissima</i> (ASP)

Research projects

Several interdisciplinary research projects are carried out in French coastal waters, see Table 41.

Table 41. Ongoing research projects in relation to the French HAB monitoring

Title of project	Institution	Funding institution
PNEAT (National Programme on Toxic Algal Blooms)	several universities laboratories, private laboratories, governmental agencies	ministries, governmental institutions
PNOC (National Programme on Coastal Oceanography)	several universities laboratories, private laboratories, governmental agencies	ministries, governmental institutions

costs

About 50 people are involved on a part time basis, that is equivalent to 16 full time persons a year. The total cost of running REPHY is 6.000.000 F (= 1.200.000 US\$). This estimated annual budget includes the personnel cost which is about 4.000.000 F (= 800.000 US\$).

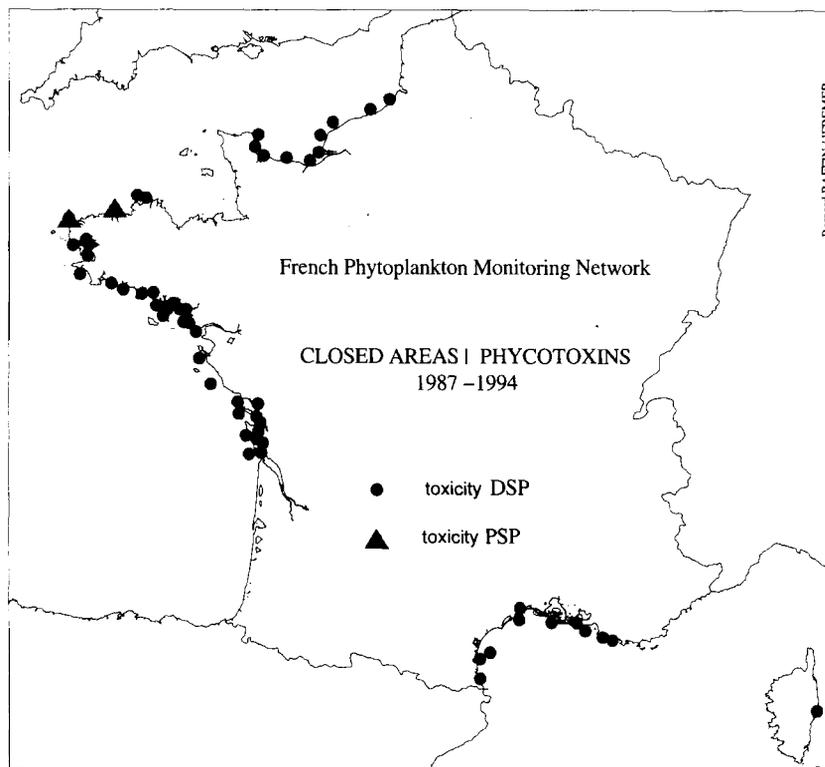


Figure 31. Areas closed due to occurrence of phycotoxins in shellfish during 1987-1994.

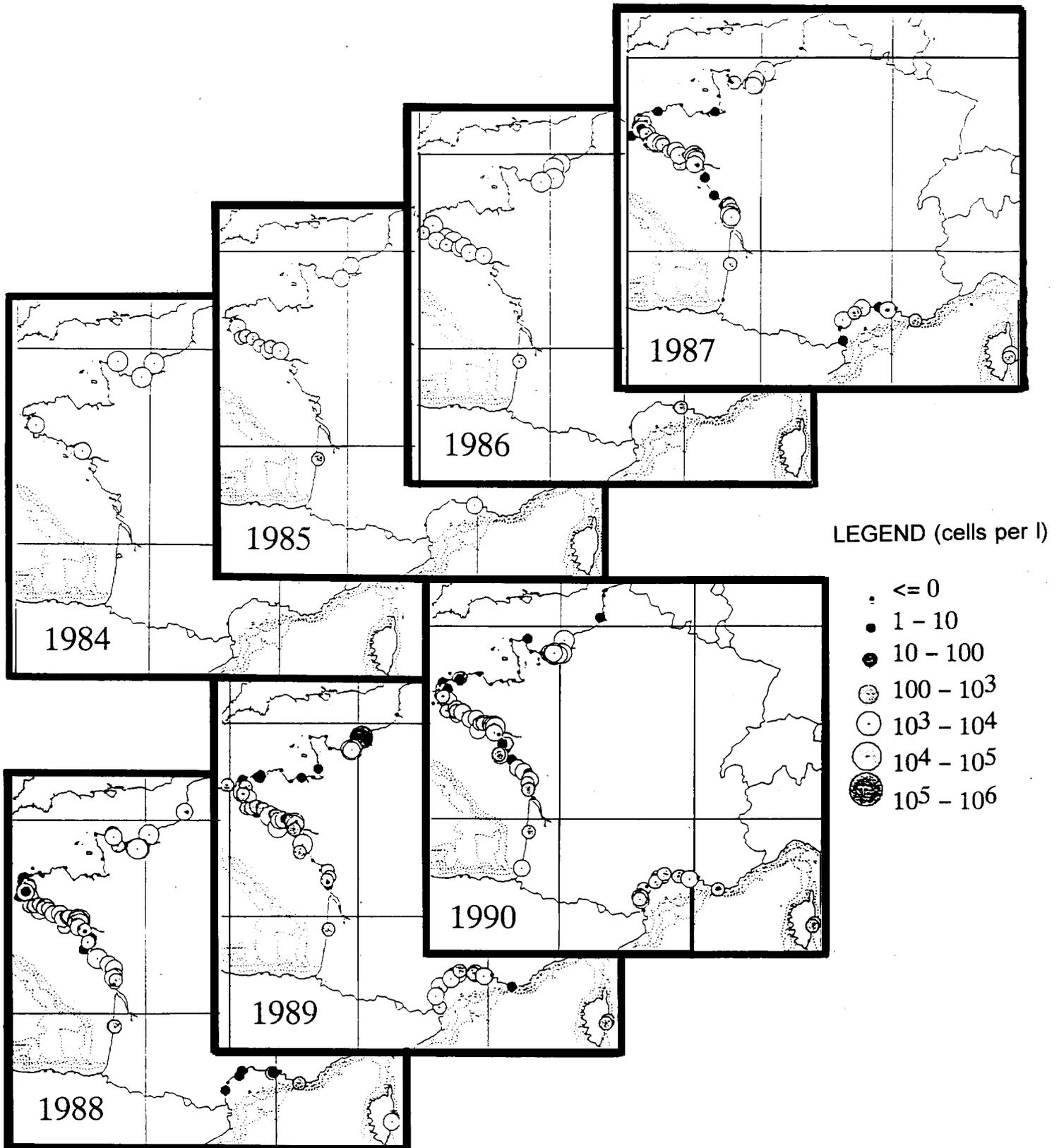


Figure 32. Maximum concentrations of *Dinophysis* spp. along the French coasts from 1984 to 1990, Belin & Berthome 1991.

Philippines

Following several cases of PSP in humans in 1983 due to consumption of the green mussel (*Perna viridis*) grown in government-initiated mussels cultures, as well as Asian moon scallop (*Amusium pleuronectes*), a national HAB programme in relation to mussel culture was initiated in 1984 by the Bureau of Fisheries and Aquatic Resources (BFAR) a governmental institution. Furthermore a local program is run by the Department of Science and Technology in one part of Manila Bay.

The PSP-toxins in the mussels were later found to be caused by the dinoflagellate *Pyrodinium bahamense* var. *compressum*.

The objective of the programme was to secure public safety by preventing contaminated seafood from reaching the human consumer.

The programme involves qualitative and quantitative investigations of the whole phytoplankton community including toxic species as well as the measurement of algal toxins (PSP-toxins) in mussels.

The results of the different analyses are stored on computer.

The Philippine HAB monitoring programme has recently been discussed by Corrales and Gomez (1990), Gonzales (1989) and Gonzales et al. (1989).

Sampling and communication of results

Plankton and shellfish samples are collected twice a month from areas with previously documented *Pyrodinium* blooms (Fig. 3 1), and at least weekly during blooms at several stations in the areas affected by blooms, by the Bureau of Fisheries and Aquatic Resources (BFAR).

During red tide blooms, aerial surveillance (using helicopters or light aircrafts of the Philippine Air Force and the Department of Agriculture (DA)), are carried out to determine the extent and movement of the blooms. Based upon the information gathered from these investigations, the residents in the affected areas are alerted. Furthermore, during blooms, stomach content of shellfish from shellfish farms, public markets and fish landing sites are examined periodically. Shellfish samples for analysis are chucked in the field, frozen and are transported for analysis to the Bureau of Food and Drugs (BFAD).

Physical, chemical and biological parameters such as water temperature, salinity, pH, dissolved oxygen, inorganic phosphorous and the quantitative occurrence of *Pyrodinium* cysts are determined once a month, during neap tide, to minimize the tidal effect on the sampling of algae. Furthermore meteorological parameters such as the amount of rainfall, wind speed and direction are available from the Philippine Atmospheric, Geophysical and Astronomical Services Administration.

The results are distributed from the BFAD to the DA and DOH by traditional methods like mail and fax.

Methods

Toxic algae: Algal samples are preserved either in Lugol's or in a 10% solution of formalin. Quantification of toxic algae (*Pyrodinium bahamense* var. *compressum*) are carried out at the central laboratory, on 1 ml samples, using a compound microscope and a Sedgewick-Rafter counting chamber,

Algal toxins: The PSP-toxins are measured using the mouse bioassay (AOAC, 1995).

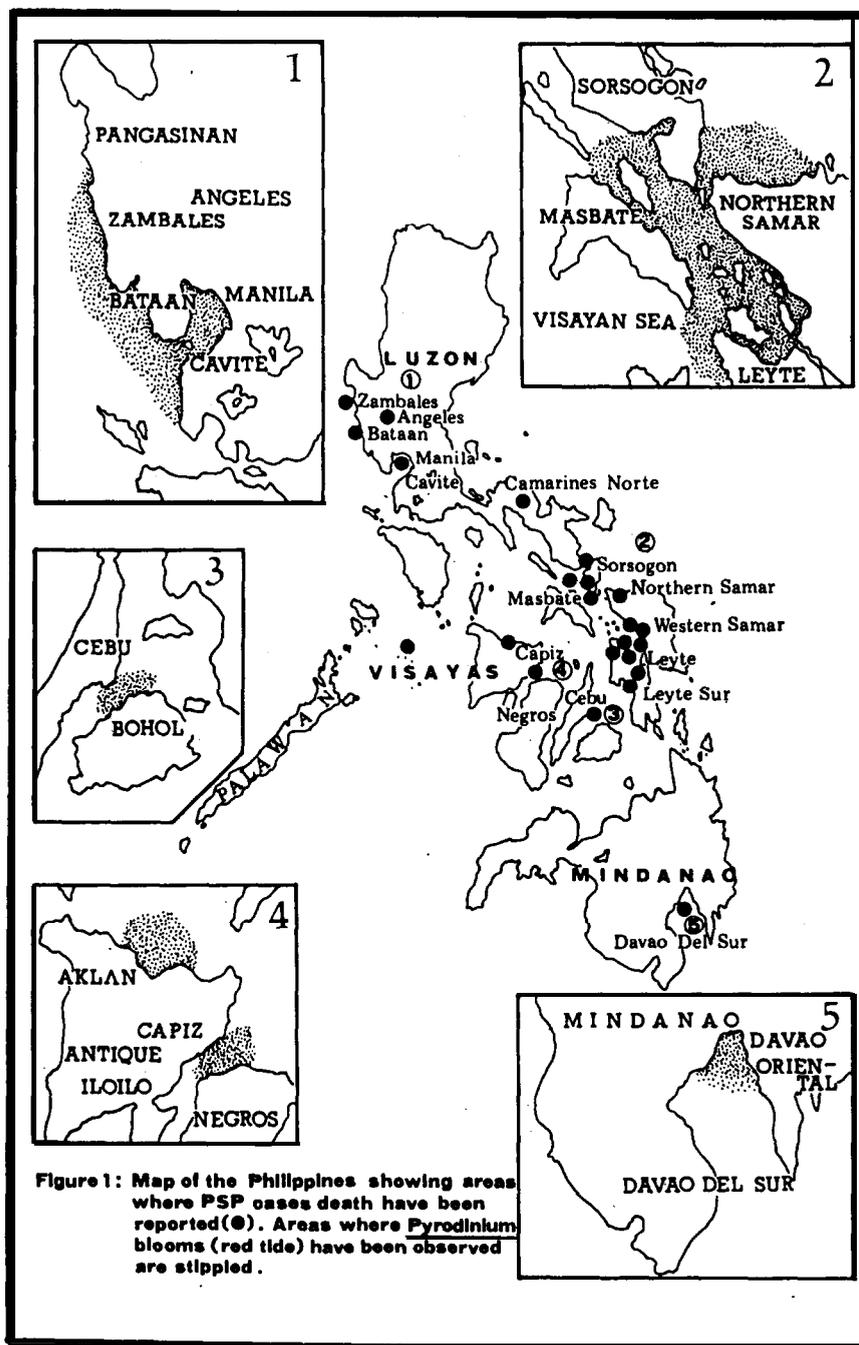


Figure 33. Map of the Philippines showing areas with fatal PSP cases and areas where blooms of *Pyrodinium* (red tide) have been observed (Corrales and Gomez, 1990),

Regulations & management

The regulation of the harvest of mussels is based upon the toxicity of the mussels, where as the quantitative occurrence of toxic algae is used to follow the temporal and geographical coverage of the blooms.

The concentration limit of PSP-toxin is 40 $\mu\text{g}/100\text{ g}$ shellfish meat. If the concentration of PSP-toxins exceeds this limit the DA imposes a temporary ban on the harvesting, marketing and transport of all kinds of marine shellfish from the affected areas (Table 42 and Fig. 32).

Table 42. Methods used for detection of algal toxins as well as critical concentrations of toxins and regulations imposed

Type	Toxin Method	Critical concentration limit	Regulation status
PSP	mouse test	40 µg SIX/loo g	closed

A “new” management scheme for HAB management is proposed in Corrales & Gomez (1990), with the following important features (Fig. 33):

PSP detection at the municipal or regional level by the local action personnel, instead of at a national level by the authorities in Manila.

Increased frequency of plankton sampling (weekly sampling during non-bloom periods and daily during blooms).

Active involvement of the local authorities and responsible citizens in a local red tide committee for public information and implementation of bans.

Tests to confirm toxicity to be done at the Bureau of Food and Drug in Manila

Creation of a National Red Tide Committee to act as an advisory or regulatory body.

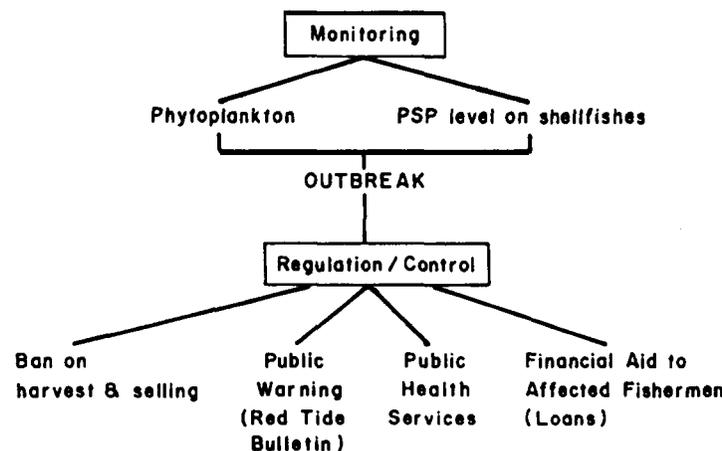


Figure 34. Diagram of the present red tide management scheme in the Philippines (Corrales & Gomez, 1990),

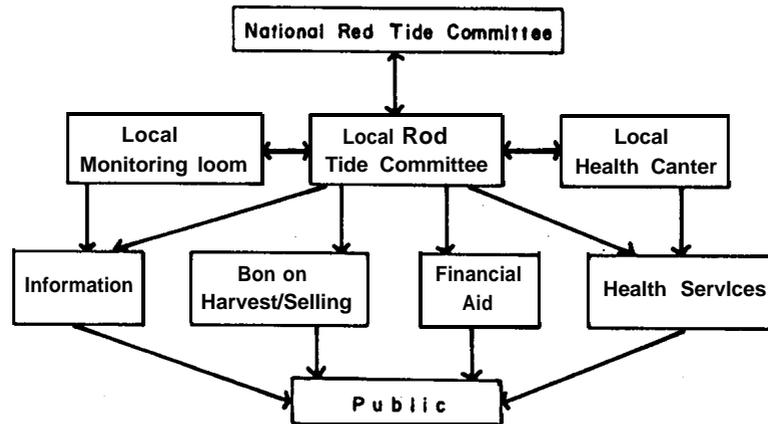


Figure 35, Diagram of the proposed "new" red tide management scheme for the Philippines (Corrales & Gomez, 1990),

The National Red Tide Committee should also be responsible for, when and how red tide announcements will be made to ensure public safety, and ensure public confidence in the system. Public health services for PSP victims are provided by local centers and doctors dispatched from the "Manila office of the Department of Health. The public is informed through print (newspapers) and broadcast, whenever the toxicity of shellfish exceeds the regulatory limit of $40 \mu\text{g} / 100 \text{ g}$. During such situations, the public is advised not to eat any kind of shellfish from the affected areas, and at the same time is informed that fish and other invertebrates caught from red tide areas are safe for human consumption.

Public meetings and seminars are organized, especially in the fishing villages, to inform the fishermen and the public properly about the current red tide situation. In addition, village criers are sent out to issue warnings to the villagers that toxic red tides are present in their area and that they should avoid eating shellfish.

To help fishermen financially in periods with closure of shellfish harvesting the authorities provide, soft loans from a Natural Livelihood Support Fund (NLSF).

Results

During the period from 1983-1989 several *Pyrodinium bahamense* var. *compressum* red tides have been documented in Philippine waters (Table 43). In total, more than 1,000 persons were PSP-intoxicated in the period and 34 fatal cases were reported (Table 45).

Table 43. Summary of the dates and places of *Pyrodinium red tides* in the Philippines (Corrales & Gomez, 1990)

Dates	Places
1983, June	Samar Sea, Maqueda Bay and vicinity
1983, July	Balete Bay, Davao
1987, June	Samar Sea, Maqueda Bay and Zambales area
1988, July-september	Samar/Leyte area
1988, August-september	Bataan area
1988, September-october	Manila Bay area
1988, December	Negros Island
1989, February	Cebu Island

Table 44. Philippine records of PSP cases and deaths from 1983-89 (Corrales & Gomez, 1990)

Province	Cases	Deaths	Period
Bataan	44	1	1988, Aug.-Sept.
Zambales	?	?	1987, June
Manila	14	2	1988, Sept.
Cavite	8	1	1988, Sept.
Sorsogon	?	?	1988, Sept.
Samar/Leyte	211	6	1983, June
	691	14	1987, June
	22	0	1988, July-sept.
Capiz	3	1	1988, December
Negros	109	4	1988, December
Cebu	24	5	1989, February
Davao	1	0	1983, August
Total	1.127	34	

Research projects

The monitoring and management functions are supplemented by research in collaboration with the universities, at present under a project, funded by the Bureau of Fisheries and Aquatic Resources (BFAR), with the title "Study on some climatological and hydrological parameters and their effects on the distribution and abundance of *Pyrodinium bahamense* var. *compressum* in Manila Bay and Masinloc Bay, Zambales, Philippines".

Cost

The HAB monitoring programme involves 9 persons and has a total annual budget of approx. 60.000 US\$. It is funded by governmental authorities.

3.4 MONITORING HARMFUL ALGAE IN RELATION TO THE FISHERIES

In the following, examples of monitoring programmes in relation to fisheries, either wild stocks or cultures are presented.

3.4.1 Wild populations

French Polynesia

No HAB monitoring is going on in French Polynesia, but a mosquito bioassay (Hall & Shimizu, 1985) is used to check large samples of dangerous species of fish for the presence of ciguatoxin, and HPLC methodology is currently under development. The veterinary limit for ciguatoxin in fish for human consumption is 0,06 µg/g. The following nine fish species are not allowed in the market, *Gymnothorax javanicus*, *Plectropomus leopardus*, *Epinophelus fuscoquattatus*, *Lutjanis bohar*, *Lutjanus monostignus*, *Lutjanus nivulatus*, *Sphyaena barracuda*, *Ctenochaetus striatus* and *Balistoides vireescens*, see Egmond et al. (1992).

In the state of Hawaii the sale of fish known to be toxic is prohibited. Consumer complaints are investigated, and if necessary, legal action is taken. The industry has maintained a self-imposed policy of not selling Amberjacks over 9 kg in weight. In Puerto Rico sale of Barracuda and Amberjack is prohibited (Hungerford & Wekell, 1993).

3.4.2 Fish culture

Norway

The Norwegian HAB monitoring programme in relation to the fish farming industry was initiated by the Norwegian Association of Fishfarmers in 1987. The history of harmful algae in Norway is compiled in Table 45. The reason for the initiation of the HAB monitoring was extensive mortalities of salmon in fish farms situated in coastal waters and fjords with great economical losses, caused by from several blooms of the fish toxic dinoflagellate *Gyrodinium aureolum*. The first bloom was observed in 1976. During the last few years several species of *Chrysochromulina* and *Prymnesium* as well as different diatoms have also been implicated in fish kills.

Table 45. The history of harmful algae in Norway.

Year	Episode
1870	First human intoxication (DSP) (Sognefjord)
1901	First PSP epidemic in Norway (two deaths) (Oslo - 60 °N)
1966	<i>Gyrodinium aureolum</i> - first bloom in Europe
1968	PSP epidemic (<i>Alexandrium excavatum</i>) in mid Norway (63.5 °N)
1971	DSP epidemic (<i>Dinophysis</i>) in south Norway (Oslofjord - 59.5 °N)
1976	First mortality in fish farms due to <i>Gyrodinium aureolum</i>
1979	First bloom (in Scandinavia) of <i>Prorocentrum minimum</i>
1981	Massive wild fish mortality due to <i>Gyrodinium aureolum</i>
1982	First fish mortality in north Norway due to <i>Gyrodinium aureolum</i>
1984	Documented toxicity in <i>Gymnodinium galatheanum</i>
	First bloom (in Scandinavia) of <i>Alexandrium (Goniodoma) pseudogoniaulax</i> (Gonidomin toxins ?)
1986	First detection (in Europe) of DTX-1 as dominant DSP toxin
1987	Detection of undefined toxins (not PSP or DSP) in mussels Detection of DSP in mid Norway (Trondheimsfjord - 63.5 °N)
1988	<i>Chrysochromulina polylepis</i> causing massive mortality in fish farms and marine ecosystems
1989	<i>Skeletonema costatum</i> associated with fish kills First mortality in fish farms due to <i>Prymnesium parvum</i>
	Detection of DSP in north Norway (Dønna - 66 °N)
1991	<i>Phaeocystis pouchetii</i> associated with fish kills Mortality in fish farms due to <i>Chrysochromulina leadbeateri</i>
1992	Fish mortality caused by <i>Alexandrium excavatum</i> Detection of PSP in northernmost Norway (Tromsø - 70 °N)
1993	Detection of PSP in the winter period (January)

The monitoring programme covers most of the coastal waters and fjords of Norway (Fig. 36). Information in relation to the occurrence of HAB's is gathered from fish farmers, mussel farmers and the State Food Control Authority as well as from moored buoys - the SEAWATCH-buoys, which currently measure a whole 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).

All data in relation to fish farming is collected by the private consultancy company OCEANOR, which is responsible for the advice to fish farmers if a HAB should occur.

The monitoring programme was revised in 1989, 1991 and 1992.

Other parts of the Norwegian HAB monitoring programme include monitoring in relation to mussel farming and environmental quality in the coastal waters.

Sampling and communication of results

Algae are sampled weekly by a number of fish farmers, mussel farmers and the State Food Control Authority. Fish farmers collect additional samples and if algal concentrations are high, an evaluation is made based upon secchi depth and water color. Guidelines for secchi depth determination are available for the fish farmers. A total of 80 stations are currently sampled.

A list of harmful algae and algal toxins harmful to fish are presented in Tables 47 and 48.

Table 46. Examples of harmful algae in relation to Norwegian fish farming and mussel fishery.

Dinoflagellates	Diatoms	Other algae
<i>Alexandrium excavatum</i>	<i>Chaetoceros borealis</i>	<i>Chrysochromulina leadbeateri</i>
<i>Alexandrium minutum</i>	<i>Chaetoceros concavicornis</i>	<i>Chrysochromulina polylepis</i>
<i>Alexandrium ostenfeldii</i>	<i>Chaetoceros convolutus</i>	<i>Chrysochromulina spp.</i>
<i>Alexandrium pseudogoniaulax</i>	<i>Chaetoceros spp.</i>	<i>Phaeocystis pouchetii</i>
<i>Amphidinium carterae</i>	<i>Leptocylindrus minimus</i>	<i>Prymnesium parvum</i>
<i>Dinophysis acuminata</i>	<i>Pseudo-nitzschia pseudodelicatissima</i>	<i>Prymnesium patelliferum</i>
<i>Dinophysis acuta</i>	<i>Pseudo-nitzschia multiseriis</i>	<i>Dictyocha speculum</i>
<i>Dinophysis norvegica</i>	<i>Pseudo-nitzschia delicatissima</i>	<i>Dictyocha fibula</i>
<i>Dinophysis rotundata</i>	<i>Rhizosolenia spp.</i>	<i>Heterosigma akashiwo</i>
<i>Dinophysis spp.</i>	<i>Skeletonema costatum</i>	
<i>Gymnodinium galatheanum</i>		<i>Nodularia spumigena</i>
<i>Gyrodinium aureolum</i>		
<i>Prorocentrum lima</i>		
<i>Prorocentrum minimum</i>		

Table 47. Types of algal toxins which can be harmful to fish in Norwegian waters

ICHTHYOTOXINS	
Prymnesin PSP Polyethers Glycolipids Hemolysins	Present in planktonic algae and may accumulate in filter feeders

The occurrence of medusae, which have been involved in fish kills are also monitored.

Other environmental parameters which can have a negative impact on fish farms, like extreme weather conditions (wind speed, wave height, temperature) as well as information concerning oil spills and other chemical pollution events are monitored as well.

The role of the fish farmers in the monitoring programme is very important. The fish farmers routinely measure and report on secchi depth, color of the water and water temperature. All results are collected at OCEANOR.

The fish farmers are urged not to hesitate to contact OCEANOR if the fish behave abnormally or if death occurs, but are also advised to routinely consult the veterinarian, because problems can be caused by eg. infections (Table 48).

Data are communicated by fax, phone and Internet.

The updated information is evaluated by a marine biologist and an oceanographer at OCEANOR at a routine surveillance meeting at the beginning of each day (Fig. 37). 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.

Table 48. OCEANOR guidelines to fish farmers (translated from Norwegian).

Monitoring 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 behaviour or death, which can not 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	
08.00-16.0073 52 xx xx	
16.00-08.00	Cellular phone: xx xx xx xx Beeper: xx xx xx xx

LOCAL OBSERVERS REPORTING DAILY TO OCEANOR FOOD AND DRUG STATIONS ALGAE AND TOXIN SAMPLES (WEEKLY)

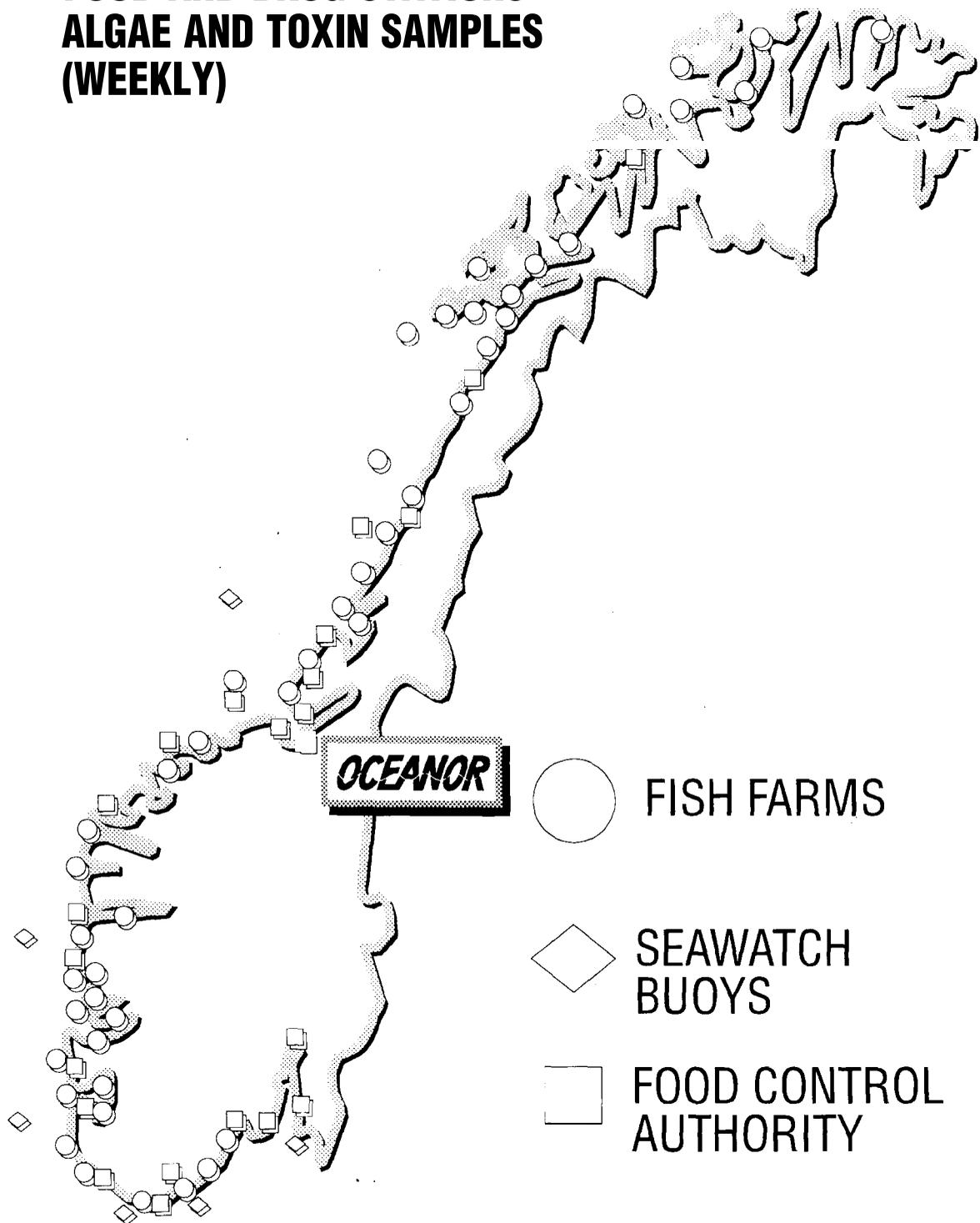


Figure 36. Sources of information in the Norwegian HAB monitoring programme in relation to fish farming, that is location of fish farmers SEAWATCH buoys and stations monitored by the Statens Naeringsmiddeltilsyn (OCEANOR).

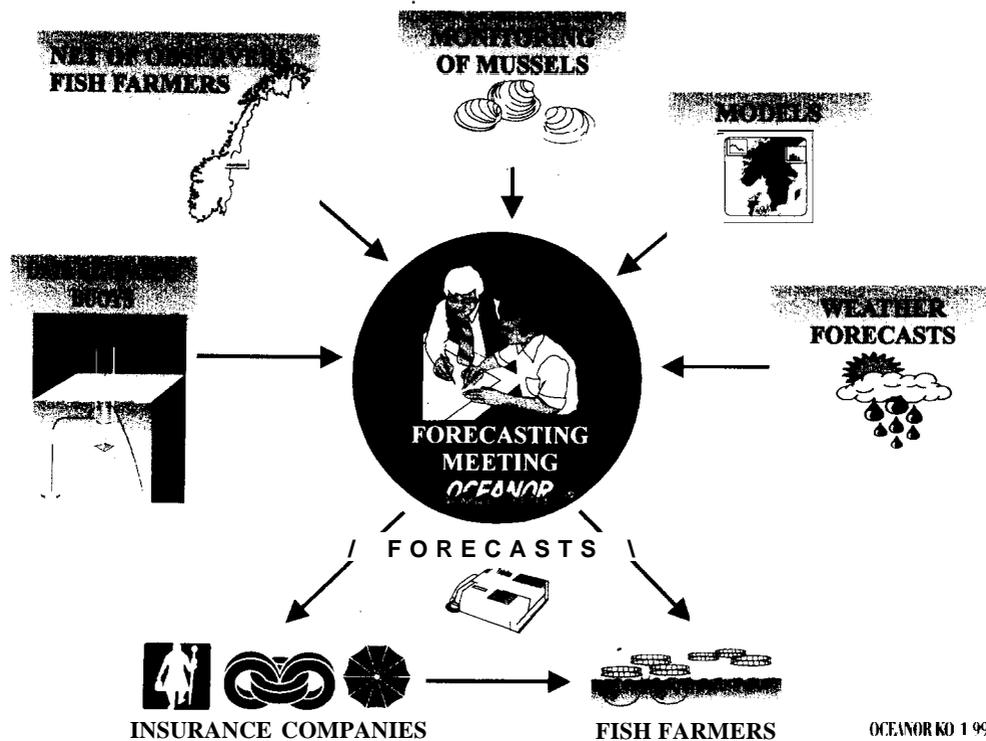


Figure 37. Scenario showing how information about eventual HAB situations is collected, evaluated and communicated to fish farmers and insurance companies in Norway,

Methods

Phytoplankton samples are collected by the observers/fish farmers at the farm site with a water sampler, usually at 0.5 m and 3(4) m depth. From each depth the sample is split in two: one unpreserved sample for live analysis and one preserved with formaldehyde. Standard samples are collected in 25 ml sterile plastic containers protected by transport containers (Nunc) and then sent by mail to OCEANOR for analysis. Since the time aspect is considered to be extremely important, 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. Samples from the observers are collected weekly on a routine basis or additionally when the water becomes turbid and the color changes.

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. In addition to the water samples, net samples are also collected as vertical hauls (0-15 m depth) with 20 μm mesh nets and then preserved with formaldehyde.

Water samples are examined in Palmer-Mahoney 0.1 ml counting chambers, or as a simplified routine in a Pasteur-pipette-two-drop sample on a standard glass slide, which equals 0.1 ml. This procedure is considered to be satisfactory for monitoring related to fish farming, since problems are observed only when concentrations of potentially harmful algae are fairly high (e.g. above 0.5 mill cells/L). A few diatoms which may be harmful also at low concentrations (*Chaetoceros convolutus/concavicornis*) are monitored primarily in the net haul samples. When these species are detected, the colonies are counted from the water samples, either after settling in 2 ml chambers or on membrane filters after filtration of 20-25 ml.

Regulations & management

It is considered very important that the fish farmers themselves can take immediate action if a HAB 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 measures are used:

Current generating propeller, which can dilute or spread a local bloom. Please note that the propeller can increase problems during blooms of medusae, because the medusae are divided into many small pieces which harm the fish.

moving the culture pens into waters with less risk of contact with HAB's

stopping feeding the fish

preparing the fish for harvest

transporting the most valuable fish to another location with no risk of contact with HAB's.

Fish farmers have arranged with insurance companies that the (free of charge) can consult OCEANOR for evaluation of HAB situations and advice on what to do to minimize losses.

The monitoring results are evaluated at OCEANOR using computer models to simulate currents and make forecasts of the spread of blooms (Fig. 38).

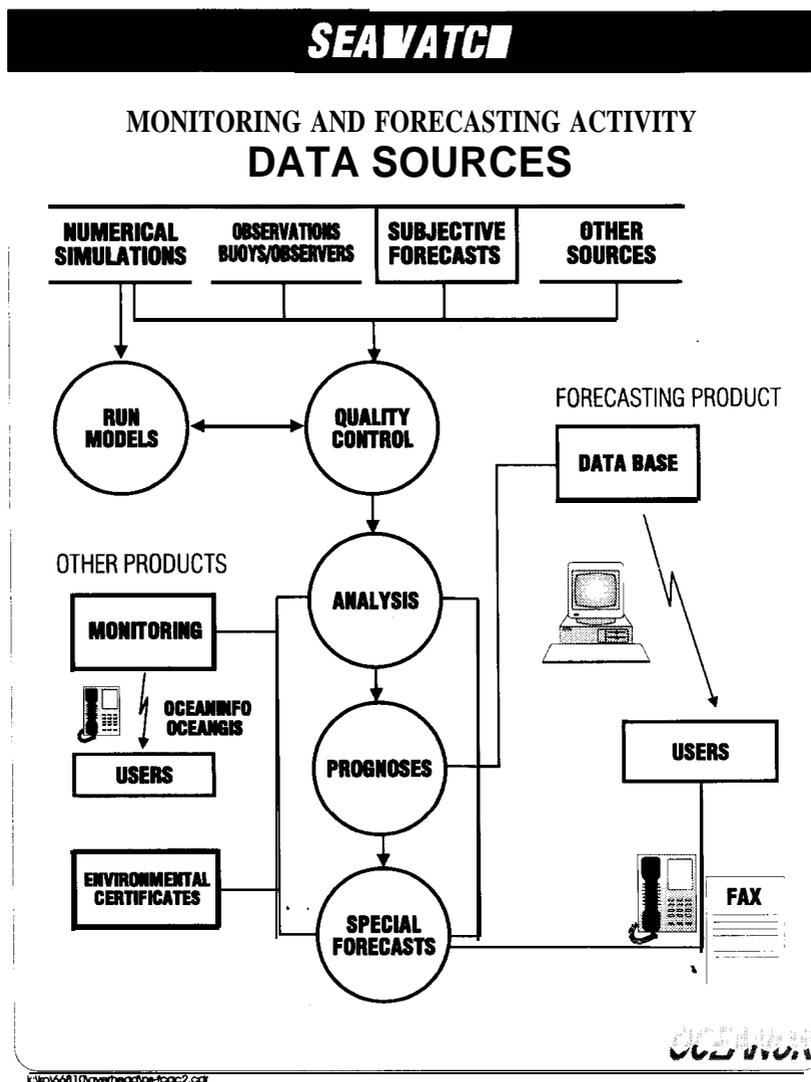


Figure 38. Overview of the elements included in the forecasting activities at OCEANOR.

Results

The occurrence of harmful algae which have caused fish kills in Norwegian waters are shown in table 49. Blooms of *Gyrodinium aureolum* in Norwegian waters are shown in Fig 39.

Table 49. HAB's in Norway after 1985 in which major mitigation actions to reduce losses of fish were in action."

Year	Causative species	Season	Region of coast
1985	<i>Gyrodinium aureolum</i>	Autumn	South and west coasts
1988	<i>Chrysochromulina polylepis</i>	Early summer	South and south west coasts
	<i>Gyrodinium aureolum</i>	Autumn	South and south west coasts
1989	<i>Prymnesium parvum</i> **	Summer	Inner fjords, west coast
1990	<i>Prymnesium parvum</i>	Summer	Inner fjords, west coast
	<i>Gyrodinium aureolum</i>	Autumn	South coast
1991	<i>Chrysochromulina leadbeateri</i>	Early summer	North
	<i>Phaeocystis pouchetii</i>	Early spring	West coast
1992	<i>Alexandrium excavatum</i>	Early summer	West coast
	<i>Prymnesium parvum</i>	Summer	Inner fjords, west coast
	<i>Gyrodinium aureolum</i>	Autumn	South west coast
1993	<i>Prymnesium parvum</i>	Spring	Inner fjords, west coast
1994	Diatoms (e.g. <i>Skeletonema costatum</i>)	Spring	Fjords, west coast
	<i>Prymnesium parvum</i>	Summer	Inner fjords, west coast
1995	<i>Prymnesium parvum</i>	Summer	Inner fjords, west coast

*A large number of smaller incidents (fish kills < 10 tonnes) as well as fish kills due to mass occurrences of jellyfish are not mentioned.

***Prymnesium parvum* + *Prymnesium patelliferum*

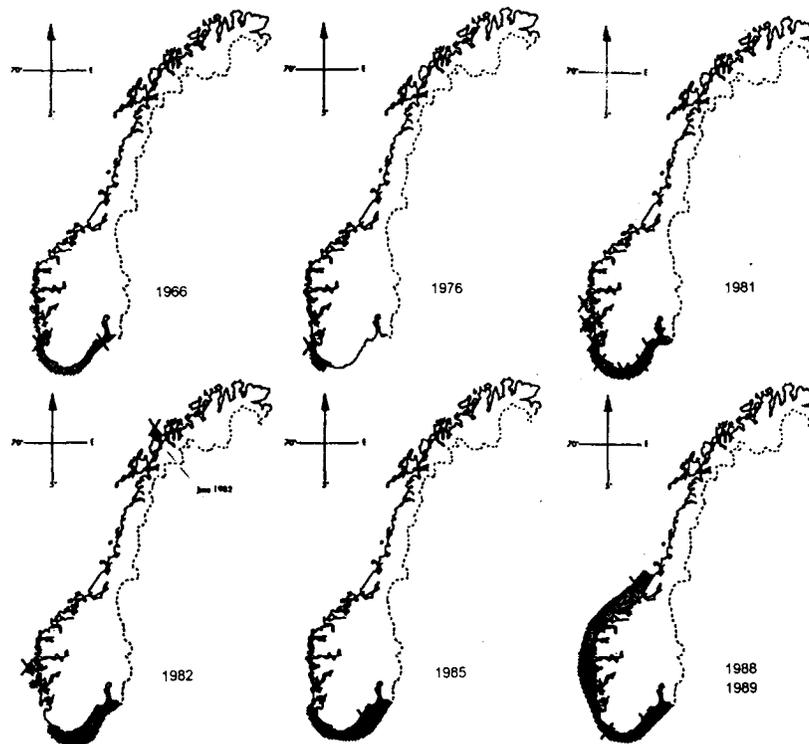


Figure 39. Map showing the occurrence of *Gyrodinium aureolum* in Norwegian waters.

Research projects

At present 3 research projects in relation to are going on (Table 50).

Table 50. Ongoing research projects in relation to the Norwegian HAB monitoring,

Title of project	Institution	Funding institution
SEAWATCH	OCEANOR	Norwegian Research Council & State Pollution Control Authority
Toxicology	Norwegian College of Vet. Medicine	Norwegian Research Council
Photobiology	University of Trondheim	Norwegian Research Council

cost

The monitoring programme is financed by the fish farmers together with insurance companies and the State Food Control Authority. The total cost of the monitoring is 300.000 US\$ per year. The production value of salmon from fish farms is 1.000 mill. US\$ per year. It is estimated that the value of the annual average loss of fish due to HAB's is worth 3 mill. US\$ per year, and the estimated reduction in economical loss due to HAB monitoring is 2 mill. US\$ per year.

Japan

Algae causing harmful blooms in Japan are divided into two groups according to their harmful effects. That is algae which cause mass mortalities of marine organisms and toxic species which can result in accumulation of toxins in shellfish and cause human intoxication. Only monitoring of algae causing mass mortalities among marine organism such as fish and invertebrates will be dealt with here.

During the 1970's the frequency of HAB's increased in the coastal waters of Japan due to eutrophication. This was especially the case in the Seto Inland Sea which is an important area for aquiculture of fish (Fig. 40).

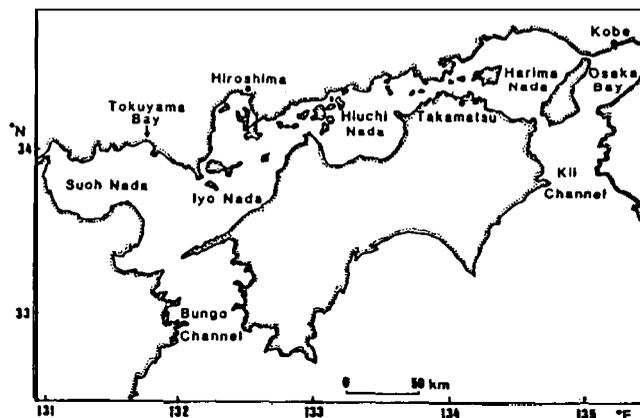


Figure 40. The Seto Inland Sea (Okaichi, 1989)

The increased frequency of HAB's also increased the economical loss, due to fish mortality of caged fish. The fish kills were due to intoxication or oxygen deficiency.

The Seto Inland Sea Environmental Law was implemented in 1973, following a massive bloom of *Chatonella antiqua* in 1972, in which the fishermen lost 14 million yellowtail, worth 71 billion yen (0.5 billion US\$), to counteract the increasing frequency of HAB's. A result of the law was a decrease in the eutrophication of the Inland Sea (with the

nutrients phosphorous and nitrogen) which led to a decrease in the frequency of HAB's (Fig. 41).

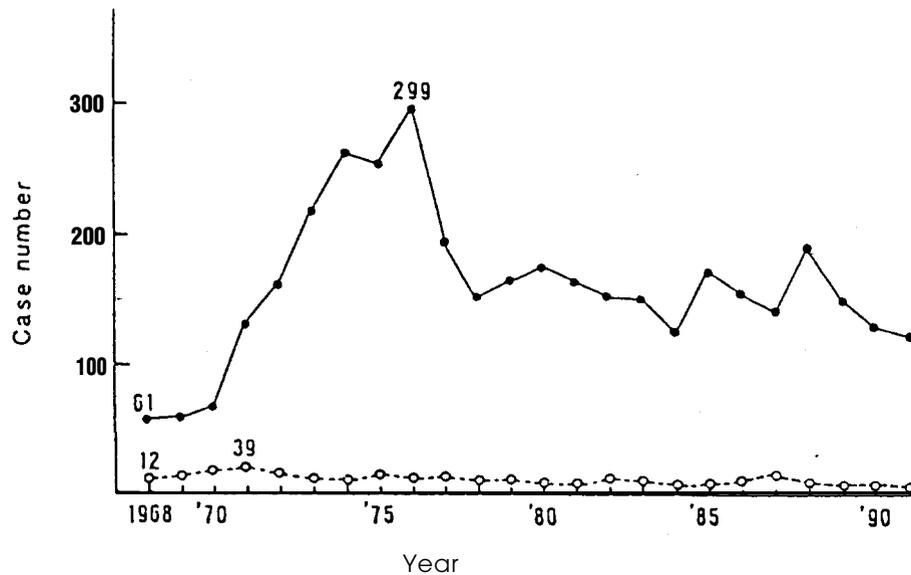
The frequency of red tides of HAB species is still approx. 10 per year. The economical loss caused by HAB's between 1972 and 1991 in the Seto Inland Sea was 165 million US\$.

The monitoring programme involves regular qualitative and quantitative investigations of the whole phytoplankton community including toxic species at fixed stations.

The HAB monitoring programme has recently been reviewed by Fukuyo (1992) and Okaichi (1989).

Sampling and communication of results

Plankton samples are collected regularly from fixed stations by the universities. During red tide blooms, observed by fishers, information is collected at the Seto Inland Sea Fisheries Coordination Office, which distribute all necessary information to the Fisheries Agency, other national institutions and prefectural authorities concerned. Aerial surveillance, using light aircrafts is carried out to determine the extent and movement of blooms. The dissemination of information by fax is completed within one hour (Fig. 42).



Case number of red tides in Seto Inland Sea,
.: red tides
O: red tides with fish kills

Figure 41. Number of red tides in the Seto Inland Sea (Fukuyo, 1992).

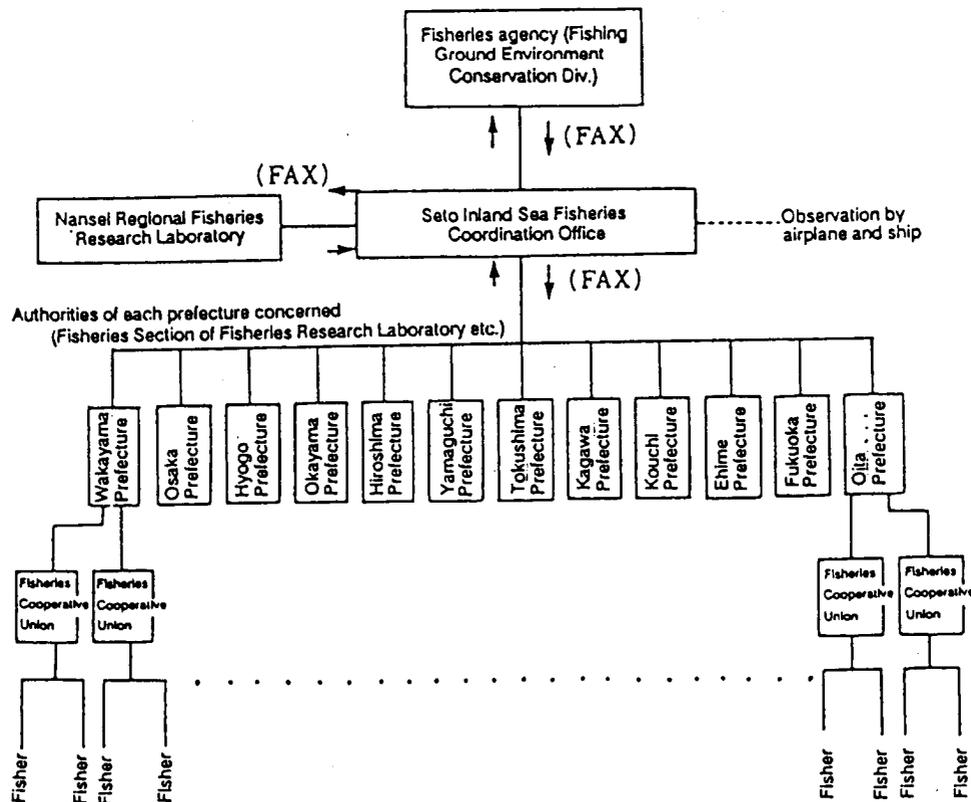


Figure 42. Information system on red tides in The Seto Inland Sea (Fukuyo, 1992).

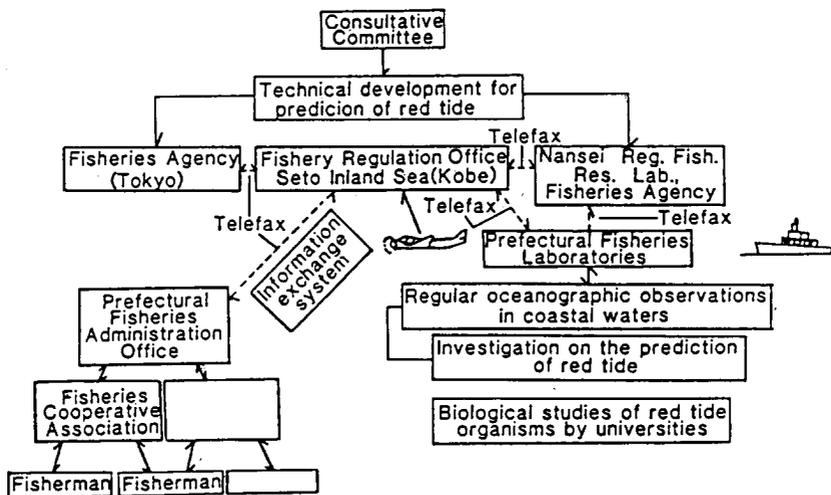


Figure 43. Information exchange and red tide investigations in The Seto Inland Sea (Okaichi, 1989).

Regulations & management

To counteract HAB's a counterplan involving direct and indirect measures is imposed. The direct counter actions aims to eliminate the HAB species using chemicals, destruction using ultrasonication or collection of the concentrated algae at the sea surface, whereas the indirect measures work on a longer time scale imposing regulations to lower eutrophication, improving operation at the aquaculture sites to improve water and sediment quality or involving transfer of pens to other areas (Fig. 44).

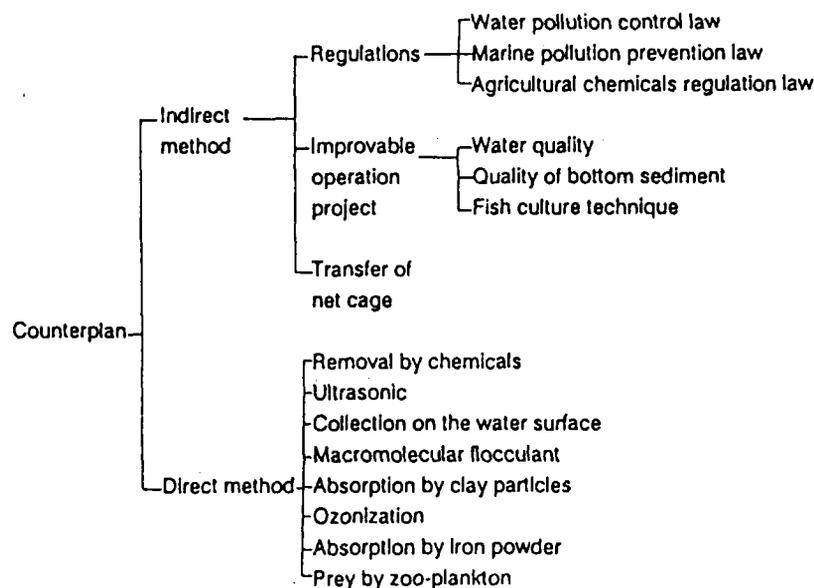


Figure 44. Mitigation actions against HAB (Fukuyo, 1992).

Results

Several dinoflagellates and raphidophyceae are known to cause mass mortality of marine organisms in Japanese coastal waters (Table 51).

Table 51. HAB species known to cause mass mortalities in Japanese coastal waters (Fukuyo, 1992)

HAB species causing mass mortality	
Dinoflagellates: <i>Cochlodinium polykrikoides</i> <i>Gymnodinium mikimotoi</i>	Raphidophyceae: <i>Chatonella antiqua</i> <i>Chatonella marina</i> <i>Fibrocapsa japonica</i> <i>Heterosigma akachiwo</i>

Red tides in the Seto Inland Sea and Kyusyu district from 1987 to 1991, with indication of species and eventual fish kills, are shown in Table 52a and 52b.

Table 52a. Red tides in the Seto Inland Sea during the period 1987-1991. (Numbers in brackets are red tides with fish mortality. Economic loss is expressed in units of million yen (Fukuyo, 1992)

Causative organisms	1987		1988		1989		1990		1991	
	No. of cases	Economic loss								
<i>Skeletonema costatum</i>	11 (1)		14		19 (11)		11 (1)	2	14	
<i>Prorocentrum</i> spp	13 (1)		12		16		8 (1)		10	
<i>Noctiluca scintillans</i>	12		23		15		34		19 (1)	28
<i>Gymnodinium</i> spp.	20 (4)	22	32 (9)	6	13 (5)	4	5 (1)		19 (3)	1 501
<i>Chattonella</i> spp	18 (10)	2 400	0		6 81)	486	1		0	
<i>Heterosigma</i> spp.	20 (1)		24		22		27 (19)		28 (1)	
<i>Mesodinium</i> spp.	12		8		23		6 (2)		8	
Others	24		67 (1)		43		41 (1)		26	
Total	141 (17)	2 422	180 (10)	9	157 (7)	490	133 (7)	2	124 (5)	1 529

Table 52b. Red tides in the Kyusyu district during the period 1987-1991. (Numbers in brackets are red tides with fish mortality. Economic loss is expressed in units of million yen (Fukuyo, 1992)

Causative organisms	1987		1988		1989		1990		1991	
	No. of cases	Economic loss								
<i>Chaetoceros</i> spp.	9		6 (3)		5		6		5 (3)	
<i>Skeletonema costatum</i>	10		13 (5)		6 (3)		7	2	13 (3)	
<i>Prorocentrum</i> spp.	17		9 (1)		12 (1)		10		15	
<i>Noctiluca scintillans</i>	5		9		7		6		7	
<i>Cochlodinium</i> spp.	1 (1)		1		3		3 (3)	51	8 (2)	48
<i>Gymnodinium</i> spp.	10 (4)	59	15 (2)	5	10 (2)	256	12 (1)		17 (5)	173
<i>Chattonella</i> spp	1		7 (6)	41	6 (3)	63	9 (5)	1 579	0	
<i>Heterosigma</i> spp.	6 (1)		11 (3)	3	10 (1)		10 (2)		7 (2)	3
<i>Mesodinium</i> spp.	14		13		17		9		20	
Others	29		19 (6)		17 (6)		18 (1)	74	42 (12)	1
Total	102 (6)	61	103 826)	49	93 (16)	319	90 (12)	1 709	132 (27)	225

Research projects

A whole range of research projects have been going on in Japan in relation to red tides and HAB problems (Fukuyo, 1992). See Table 54, for a few examples.

Table 53. Examples of recent research projects in relation to the Japanese HAB monitoring (Fukuyo, 1992)

Title of project	Institution	Funding institution
Technical development on the ecological control to noxious red tide		Ministry of Agriculture, Forestry and Fisheries
Technical development for the prediction of the occurrence of red tide		Ministry of Agriculture, Forestry and Fisheries

3.5 MONITORING HARMFUL ALGAE IN RELATION TO RECREATIONAL USE OF COASTAL WATERS

In the following, examples of monitoring programmes in relation to recreational use of coastal waters are presented.

Denmark

In Denmark the counties are responsible for monitoring water quality in the coastal areas. Each county has several monitoring stations on which a whole range of parameters are sampled at least once every month including phytoplankton. If a bloom situation occurs the sampling programme can be expanded to involve more stations and more frequent sampling, such as during a bloom of *Chrysochromulina polylepis*. In addition, if a localized bloom situation occurs and is identified by the public, the public must contact the local county at a special environmental emergency 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 cyanobacteria *Nodularia spumigena* warnings are put up on local posters on beaches, and the local newspapers/radiostations/television stations are notified and advised to inform the public.

Italy

In the Adriatic coastal waters increasing algal problems have occurred during recent years due to eutrophication with nutrient from the Po River (Fig. 43). The major problems are an uncontrolled growth of planktonic algae causing enormous build up of biomass (Fig. 44), which is a problem in relation to the recreational use of coastal water and which eventually causes a. oxygen deficiency when mineralized, killing benthic organisms and b. formation of enormous masses of mucilage (gel), assumed to be produced by material produced by the large algal blooms, covering up to 10.000 km² of the coastal area in 1988 and 1989.

Blooms in regions with hundreds of millions of cells/L leading to deeply coloured 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 be chronic. The diatoms and dinoflagellates responsible for building up the large biomasses are listed in Table 54.

Table 54. Diatoms and dinoflagellates responsible for HAB's on the upper Adriatic coast (Marchetti, 1992),

Diatoms	Dinoflagellates
<i>Asterionella japonica</i>	<i>Gonyaulax polyedra</i>
<i>Chaetoceros lacinosus</i>	<i>Gymnodinium</i> sp.
<i>Nitzschia longissima</i>	<i>Protoperidinium pellucidum</i>
<i>Skeletonema costatum</i>	<i>Prorocentrum micans</i>
<i>Thalassiosira</i> sp.	<i>Scrippsiella trochoidea</i>

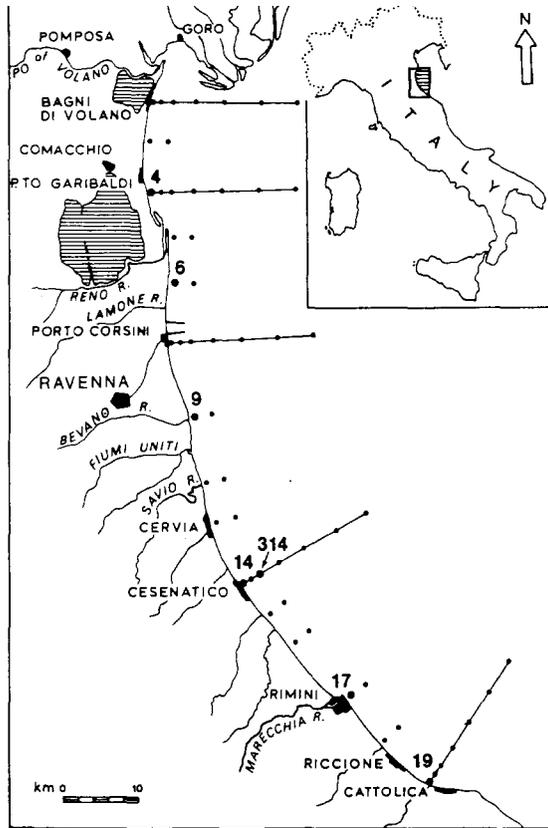


Figure 45, The coastal area of the upper Adriatic coast of the Emilia-Romagna region, affected by the eutrophication from the Po River, showing a monitoring grid (Vollenwieder et al., 1992).

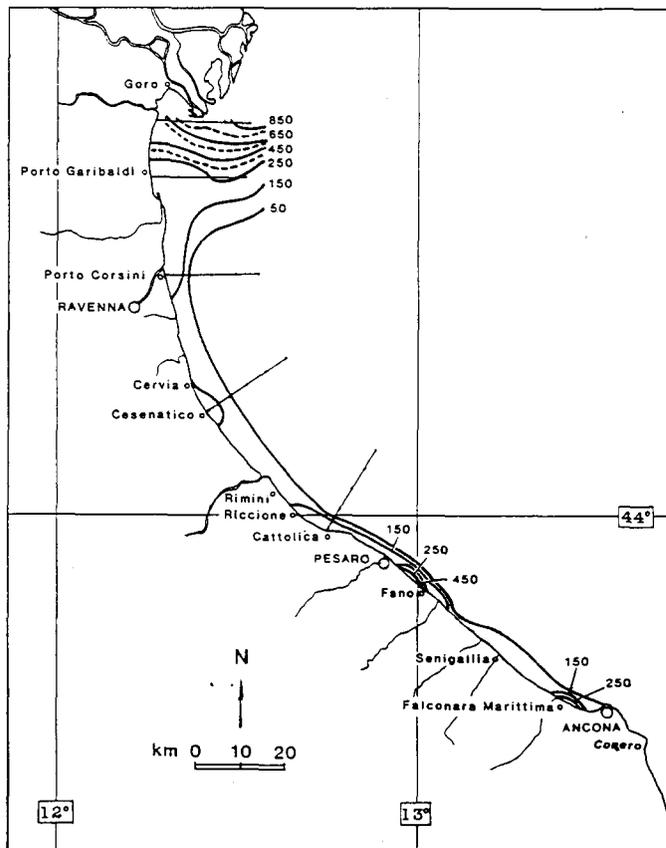


Figure 46. Concentrations of chlorophyll from the coastal area of the upper Adriatic coast of the Emilia-Romagna region, affected by the eutrophication from the Po River, in December 1984 (Marchetti, 1992).

A pilot HAB monitoring programme conducted in the area in relation to recreational use of the coastal water involved sampling at 4 stations within 300 m from the coast in the period June - October 1989. Samples were analyzed for nutrients and qualitative and quantitative determination of phytoplankton using the Utermöhl method (Bonalberti et al., 1992).

The aim of the pilot monitoring programme was to assess the feasibility and reliability of "simplified" coastal monitoring, run by the local public authorities.

From 1989, a revised monitoring programme, conducted by Centro Ricerche Marine, Cesenatico, has been going on in the area monitoring biotoxins, nutrients and problems involved with mucilage (gel).

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ANNEX

Questionnaire

on

Design of Harmful Algal Bloom (HAB) Monitoring Systems

IOC-FAO Intergovernmental Panel on Harmful Algal Blooms
ICES/IOC Working Group on Harmful Algal Bloom Dynamics

Responding institution

Name:

Address:

No. of employees:

Year of foundation:

Field of activity:

Questions related to the institution's involvement in HAB monitoring

Do you represent a national programme ? Yes/No

Geographical area covered by the monitoring:

Are there other HAB monitoring programmes in the same geographical area or in the neighbour area? If yes, specify responsible institution(s):

HAB monitoring was started in which year:

HAB monitoring was revised in which year(s):

Is the programme also monitoring other environmental conditions (e.g. oilspills, acute pollution, extreme sea states) ? If yes, specify

Is contingency planning/action plans to reduce acute problems a part of the programme ? Yes/No

On the next pages we ask you to answer questions on

**MOTIVATION, ORGANIZATION, FUNDING, EVALUATION AND
DISSEMINATION OF DATA, MANAGEMENT, ACQUISITION OF DATA,
ASSOCIATED RESEARCH PROJECTS, COST/BENEFIT ISSUES**

A. MOTIVATION

What is the main motivation for your HAB monitoring programme?

(Mark/encircle actual elements below)

- 1 Protection of resources (indicate species) from mortality or production loss
 - 1.1 Fish culture
 - 1.2 Mollusc culture
 - 1.3 Wild mollusc stocks
 - 1.4 Crustacean culture
 - 1.5 Other cultured organisms
 - 1.6 Wild fish stocks
 - 1.7 Natural ecosystems
- 2 Prevention of contamination of seafood/human intoxication
 - 2.1 Public safety
 - 2.2 Quality control of products
 - 2.3 Recreational/tourist aspects
- 3 Management of eutrophication/pollution
- 4 Scientific aspects
 - 4.1 Basic science
 - 4.2 Applied research
- 5 The HAB programme is a subordinate part of other monitoring

Comments and additional information. Historical background.

B. ORGANIZATION, PLANNING, OPERATION

Describe how your HAB monitoring at present is organized and operated.

(Mark/encircle actual elements)

- 1 What type of organization/institution is responsible for the initiation and planning of the monitoring:
 - 1.1 Governmental
 - 1.2 Private
 - 1.3 Combination
- 2 Which operational institution is carrying out the monitoring:
 - 2.1 Same as 1)
 - 2.2 Principal institution different from 1)
 - 2.3 Several institutions in a network
- 3 Indicate number of persons involved _____
- 4 In cases where there is more than one monitoring programme in your country:
 - 4.1 Data are exchanged without restrictions
 - 4.2 Some data have a restricted distribution

Comments and additional information

C. FUNDING

Funding the HAB activities. Economic support and budgets.

(Mark/encircle actual elements)

- 1 What type of funding source is supporting the monitoring and associated' activities:
 - 1.1 Governmental authorities
 - 1.2 Research foundations
 - 1.3 Private sponsors (e.g. insurance companies, aquiculture association)
 - 1.4 Private users of monitoring data
- 2 Indicate approximate size of annual budget (US\$ or local currency) _____

Comments and additional information

D. ACQUISITION OF DATA

Data collection. Field and lab. Type of data. Data management.

(Mark/encircle actual elements)

- 1 Field data/measurements/observations available
 - 1.1 From regular ship/boat cruises
 - 1.2 From nearshore/shorebased observers
- 2 Biological parameters
 - 2.1 Algal species identification
 - 2.1.1 Whole phytoplankton community
 - 2.1.2 Only harmful algae
 - 2.1.2.1 One or a few species
 - 2.1.2.2 All potentially harmful species
 - 2.2 Cell counts/concentrations
 - 2.3 Biomass (chlorophyll etc.)
 - 2.4 Fauna (fish etc.) mortality
 - 2.5 Toxins/toxicity (PSP, DSP, ASP, NSP, other)
 - 2.5.1 In molluscs
 - 2.5.2 In fish or plankton
 - 2.6 Other biological parameters (specify)_____
- 3 Hydrography/chemistry
 - 3.1 Salinity, temperature, oxygen
 - 3.2 Nutrients “
 - 3.3 Other (specify)_____
- 4 Remote sensing data available
 - 4.1 Satellites
 - 4.2 Automatic buoys
- 5 Numerical models run on routine basis or in associated research
 - 5.1 Biological models (primary production, algal growth, etc.)
 - 5.2 Physical models (circulation/currents, spreading/dilution, etc.)
- 6 Data management
 - 6.1 Data are computer stored, only internal access
 - 6.2 Access to data from external network
 - 6.3 Data stored in paper archive

Comments and additional information

E. EVALUATION AND DISSEMINATION OF DATA

Evaluation and distribution of the data for different purposes and users.

(Mark/encircle)

- 1 Who is evaluating and communicating the data
 - 1.1 Same as operational (data collecting) HAB institution
 - 1.2 Various users evaluate data received from operational institution
- 2 Type of products and data released to various users
 - 2.1 Data sets (raw data) (e.g. documentation of algal analyses)
 - 2.2 Forecasts (weekly or shorter intervals)
 - 2.3 Summary reports (weekly/monthly/quarterly/bi-annual/annual)
 - 2.4 Special (tailored) data support for research projects
 - 2.5 Other products (specify) _____
- 3 Dissemination/communication of data and other information
 - 3.1 Traditional methods (e.g. paper/telefax)
 - 3.2 Computer based communication (e.g. Internet, E-mail, GIS)

Comments and additional information

F. MANAGEMENT AND USE OF HAB PRODUCTS/DATA

Management and decisions based on monitoring data.

(Mark/encircle actual institutions)

- 1 Management actions related to public health and aquiculture
 - 1.1 "Food control authorities "
 - 1.2 Pollution authorities
 - 1.3 Public health authorities
 - 1.4 Aquiculture managers/Shellfish growers/Fish farmers
 - 1.5 Insurance/finance managers
 - 1.6 Aquiculture associations
- 2 Users at research institutions
 - 2.1 Basic science/research projects (Universities, etc.)
 - 2.2 Applied research (Fisheries Res. Inst., etc.)
- 3 Nature protection associations
- 4 Media (newspapers/radio/TV)

Comments and additional information

G. MANAGEMENT REGULATIONS AND GUIDELINES

Which actions and guidelines are followed in the management of molluscs
(Mark actual elements below)

- 1 Management actions related to molluscs are primarily based upon
 - 1.1 Occurrence/concentration of specific toxic algae
 - 1.2 Occurrence of algal toxins in molluscs
 - 1.3 A combination of presence of algae and algal toxins
 - 1.4 Other criteria (specify) _____

Comments

- 2 Which guidelines are used when regulations are based on the occurrence of algae

Algal species	Critical concentration limit (cells/L)	Regulation status (free - restricted - closed)

- 3 Which guidelines are used when regulations are based on toxin content

Type	Toxin Method	Critical concentration limit	Regulation status (free - restricted -closed)

- 4 Types of regulation status defined by the management
 - 4.1 Harvesting is free/marketing allowed, no warning to the public
 - 4.2 Harvesting and marketing restricted, public informed/warned
 - 4.3 Harvesting and marketing closed, public alarmed
 - 4.4 Other regulations (specify) _____

Comments

H. APPLIED AND BASIC RESEARCH ASSOCIATED WITH THE MONITORING

If there are research projects associated with the HAB monitoring, please specify below or on a separate list.

Title of project	Institution	Funding institution

1. COST/BENEFIT

Attempt to assess funding vs. benefits; aquiculture, public interests, research

Approximate annual costs (local currency or US\$) to operate the HAB programme related to sectors:

Total costs	Aquiculture & wild stocks	Research	Public authorities	Other sectors

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ANNEX Page VIII

Approximate annual costs related to monitoring and management activities. Estimated potential value/production/loss of aquiculture organisms which may be exposed to HABs in the geographical area covered by the monitoring:

	Shellfish	Fish	Other
Sampling, including ship time			
Laboratory analyses			
Evaluation, management, data communication			
Estimated annual production value realized			
Losses due to HAB induced mortality (annual average, last 5 years)			
Production loss due to HAB/toxin contamination			
Reduced losses due to the HAB monitoring			

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