Algal Monitoring and Forecasting

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Recent international conferences on harmful algae have demonstrated the rapidly increasing interest in planktonic algae as key component in aquatic ecosystems and risk factor in aquaculture and coastal seafood harvest. The scientific challenges include description of newly detected species and toxins and the effects they may have on other organisms, and a number of projects are related to the development of new and better methods and measuring devices. Certain algal species pose athreat to public health due to their production of potent toxins causing contamination of bivalves and other seafood, and several species may cause serious economic losses in fish and shrimp farming by intoxications or other harmful effects causing acute mortality or retarded growth. Algal blooms ("red tides") are also associated with discharge of nutrients from land and are the primary effect of eutrophication on a local or regional scale. The historical record in Norway (Tangen & Dahl 1993) gives an indication of the expansion of the problem in a restricted geographical area, with respect to geographical spreading, occurrence of new species and toxins, fish mortality and human intoxications. The Norwegian example may be a good example of what may happen in the course of aquaculture development in new regions.

The need for algal monitoring systems is motivated by the needs of the basic responsibilities of public food control, health, and environment authorities and from private and public economic bodies, including fish farms, banks, and insurance companies. The design of a monitoring programme in a given geographical region should be based on a thorough evaluation of the needs of these institutions and interests. Basic scientific projects are associated with some algal monitoring programmes, although the monitoring itself may stand alone in most cases.

The main purpose of algal monitoring is to provide data to document the occurrence and most often the concentrations of certain species or the whole algal community. The other main elements are evaluation of the data, dissemination of information based on the evaluation, e.g. warning of the risk of human intoxication, and

additionally, in combination with other information forecasts may be worked out and distributed.

The documentation of the occurrence of algae on the species level still has to be based on sampling and use of microscopy. A new generation of marine data buoys (SEAWATCH) designed for real-time monitoring and forecasting of marine biological, physical and chemical variables have been in use since 1988 (Volent and Tangen 1989). The buoy gives a detailed documentation of the dynamics and duration of algal blooms and In the SEAWATCH Europe programme, which also included a network of observers and sampling stations, the combined data sets, a large number of algal blooms representing species from several algal classes (dinoflagellates, diatoms, prymnesiophytes, chrysophyles) could bedetected and documented (see Appendix). The data were used for the surveillance of algal toxins in mussels (Aune & al. 1994) and for operational forecasting of harmful algae for the Norwegian fish farming industry, and additionally extracts of the data have been used in the evaluation of the eutrophication status in parts of the North Sea region.

Enclosed:
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Harmful Phytoplankton in Norwegian Waters - An Overview

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ABSTRACT

Harmful algal blooms are part of the natural history in Norwegian coastal waters and date back at least a century. The recorded number of potentially harmful algae is increasing and includes about 30 different species representing several algae classes, including dinoflagellates, prymnesiophytes, diatoms, and various flagellates. Acute massive mortality in farmed and wild fish stocks has been associated with blooms of Gyrodiniwn aureole, Alexatidriiimexcavation, Chrysoclirointilitia polylepis, Chrysochromulina leadbeateri, and Prymnesium parvum. The estimated losses amount to at least 5000 metric tonnes. Shellfish toxicity, mainly documented in mussels, includes the detection of PSP related to the occurrence of Alexandrium species and DSP associated with various Dinophysis species. Serious PSP epidemics, in one case with two fatalities, date back to 1901. A large number of DSP epidemics have been recorded during the last 25 years. Shellfish toxicity (PSP and DSP) is recorded in north Norway during the last years, indicating a northward geographical expansion of the actual algae populations, and there is also a documented extended seasonal occurrence of PSP in mussels.

INTRODUCTION

Algal blooms occur regularly in Norwegian coastal and offshore waters and harmful blooms are so common that they may be considered as part of the natural history of these waters. The first documented case that may be related to toxic algae, human intoxication after consumption of mussels, extends back to 1870. Since then numerous incidents due to harmful phyloplankton have been recorded, including epidemics of human shellfish poisoning [1,2], widespread mortality in farmed and wild fish stocks [3,4,5], and serious damage of natural ecosystems by algae blooms has aroused great public attention [6]. Table 1 summarizes the history of the potentially harmful phytoplankton in Norway. in a chronological list of events. The table dates the first observations of certain species, toxins, and fish kills, and shows the documented geographical spreading or introduction to new districts.

The status in 1993 is serious economic effects on the fish farming industry and a retarded development in the mussels farming. The secondary effects include far-reaching political impacts on the international regulations and conventions related to the pollution of the North Sea with associated consequences on national budgets.

Table 1. The history of harmful algae In Norway.

1870	First human intoxication (DSP) (Sognefjord)
1901	First PSP epidemic in Norway (two deaths) (Oslo - 60°N)
1966	*Gyrodinium aureolum* - first bloom (in Europe)
1968	PSP epidemic *(Alexandrium excavatum)* in mid Norway (63,5 °N)
1971	DSP epidemic *(Dinophysis)* in south Norway (Oslofjord, 59.5 °N)
1976	First mortality in fish farms due to *Gyrodinium aureolum*
1979	First bloom (in Scandinavia) of *Prorocentrum minimum*
1981	Massive wild fish mortality due to *Gyrodinium aureolum*
1982	First fish mortality in north Norway due to *Gyrodinium aureolum*
1984	Documented toxicity in *Gymnodinium galatheanum* First bloom (in Scandinavia) of *Alexandrium (Goniodoma) pseudogoniaulax* (goniodomin toxins ?)
1986	First detection (in Europe) of DTX-1 as dominant DSP profile
1987	Detection of **undefined toxins** (not PSP or DSP) in mussels Detection of **DSP** in mid Norway (Trondheimsfjord, 63.5 °N)
1988	*Chrysochromulina polylepis* causing massive mortality in fish farms and marine ecosystems
1989	*Skeletonema costatum* associated with fish kills First mortality in fish farms due to *Prymnesium parvum* Detection of DSP in north Norway (Donna, 66 °N)
1991	*Phaeocystis pouchetii* associated with fish kills Mortality in fish farms due to *Chrysochromulina leadbeateri*
1992	Fish mortality caused by *Alexandrium excavatum* Detection of PSP in northernmost Norway (Tromso, 70 °N)
1993	Detection of PSP in the winter period (January)

RECORDS OF POTENTIALLY HARMFUL SPECIES

The species composition of the phytoplankton in Norwegian waters is fairly well known after more than a century of taxonomic studies and field surveys. A large number of classical species and new species from various algae groups were originally described from these waters and the seasonal and geographical distribution of the majority of species is in general known. After the first record of *Gyrodinitun aureolum* in Norwegian waters in 1966 [7] this species has become one of the most common bloom forming *dinoflagellates* in European waters [2]. During the last 15 years several harmless and potentially harmful species (e.g. *Prorocentrum minimum, Chrysochromulina polylepis, Chrysochromulina leadbeateri, Prymnesium parvum*) have made their appearance, first usually occurring in bloom proportions, and later apparently becoming natural members of the phytoplanklon, sometimes in repeated blooms. It is probable that some of the "new" species have long unattended history in these waters. The number of algae

associated with various adverse effects is now reaching about 30 species representing several taxonomic classes (Table 2). *Alexandrium excavatum* is the morphotype earlier described [8,9] to be different from the type of the former *Gonyaulax tamarensis* Lebour by possessing an excavated sulcus.

Table 2. Potentially harmful algae in Norway.

Dinoflagellates	
Alexandrium excavatum	*Dinophysis norvegica*
Alexandrium minutum	*Dinophysis rotundata*
Alexandrium ostenfeldii	*Dinophysis spp.*
Alexandrium pseudogoniaulax	*Gymnodinium galatheanum*
Amphidinium carterae	*Gyrodinium aureolum*
Dinophysis acuminata	*Prorocentrum lima*
Dinophysis acuta	*Prorocentrum minimum*
Prymneslophytes	
Chrysochromulina leadbeateri	*Phaeocystis pouchetii*
Chrysochromulina polylepis	*Prymnesium parvum*
Chrysochromulina spp.	*Prymnesium patelliferum*
Diatoms	
Chaetoceros borealis	*Pseudonitzschia pseudodelicatissima*
Chaetoceros concavicornis	*Pseudonitzschia pungens f. multiseries*
Chaetoceros convolutus	*Pseudonitzschia delicatissima*
Chaetoceros spp.	*Skeletonema costatum*
Other species	
Dictyocha speculum	*Heterosigma akashiwo*
Dicycha fibula	*Nodularia spumigena*

FISH MORTALITY

Fish that are kept in net pens are exposed to the ambient phytoplankton without being able to escape and the effects of harmful algae are easily observed. Documented mortality in farmed fish has been associated with species from the taxonomic classes *Dinophyceae*, *Pryninesiophyceae/Haptophyceae* and *Bacillariophyceae*. The dinoflagellates *Gyrodinium aureolum* (several blooms after 1966; [3,4] and *Alexandrium excavation* (one bloom, 1992 [10] and the prymnesiophytes *Prymnesium parvum*/ *patelliferum* (blooms every year after 1989), *Chrysochromulina polylepis* (one bloom, 1988 [11] and *Chrysochromulina leadbeateri* (onebloom, 1991 [5] have been associated with massive mortality. Except *Chr. leadbeateri* and *P. parvum* these species also have caused mortality in wild fish stocks and natural biota, mainly benthic organisms. Minor cases of mortality in fish farms have occurred during various blooms of the

prymnesiophytes *Phaeocystis pouchetii*, the diatom *Skeletonema costatum*, and mixed blooms of flagellates and diatoms [12]. *Chaetoceros spp*. and some flagellates (*Dictyocha speculum*, *Heterosigma akashiwo*), which have caused serious fish mortality in other waters, are frequently observed in Norwegian waters but are so far not documented to he associated with fish kills;

The fish kills in net pens are summarized in Table 3. The economic impacts in Norwegian fish farming have been on loss of dead fish, reduced productivity after reduced feeding and food uptake, lower rating of fish product quality, and market mechanisms resulting in lower first-hand prize of fresh fish during some of the major blooms. There is probably a considerable additional loss due to secondary effects on the health status of the fish, as seen after several blooms of *Gyrodinium aureolum* and the major blooms of *Chrysochromulina* and *Prymnesium*, which have been followed by outbreaks of lethal fish diseases (e.g. furunculosis, infectious salmon anemia, coldwater vibriosis).

Table 3. Mortality of salmon and rainbow trout in Norwegian fish farms due to algae.

Causative organism	Estimated loss	Season
Gyrodinium aureolum	2000 tonnes	Autumn (Summer)
Prymnesium parvum/patelliferum	1300 tonnes	Summer
Chrysochromulina polylepis	800 tonnes	Early summer
Chrysochromulina leadbeateri	620 tonnes	Early summer
Alexandrium excavatum	120 tonnes	Early summer
Other organisms	300 tonnes	Spring - summer

Some of the largest blooms of *Gyrodinium aureolum* (e.g. 1976, 1981) and the *Chrysochromulina polylepis* bloom in 1988 caused serious damage also to natural ecosystems [3,6,13,14] including wild finfish and invertebrates. Fishermen have reported that living catches of cod, eel, sprat, saithe, and lobsters that were kept temporarily in cages and net pens had an extraordinarily high mortality in localities where the water was discoloured by *Gyrodinium aureolum* during the 1976 and 1981 blooms. In addition to these cases of mortality, which were caused by exposure of the toxic algae or ichthyotoxins contained in the surrounding water, intoxication of finfish through the food

chain was reported by Tangen & al. [10] during the 1992 bloom of *Alexandrium excavatum*. In this case plaice and other species of flatfish were poisoned and killed by paralyzing shellfish poison (PSP) through the consumption of PSP contaminated sand-dwelling bivalves, whereas sprat were killed by PSP contained in their diet (copepods). It has not been possible to confirm that a significant reduction in the stock of eider ducks in Mid Norway during the last few years [15] is due to PSP contaminated mussels.

SHELLFISH TOXICITY

Shellfish toxicity in Norway, mainly documented in mussels, includes detection of PSP toxins related to the presence of *Alexandrium excavatum* and *Alexandrium ostenfeldii*, and detection of DSP toxins associated with the occurrence of various *Dinophysis* species (mainly *D. acuta*, *D. acuminata*, *D. Norvegica*, *D. rotundata*). Additionally, there is a number of cases of undefined "mouse toxicity" observed in various bioassays, where the corresponding toxins have not been identified. Several massive blooms of *Pseudonitzschia* species have occurred without the detection so far of the ASP toxin Domoic acid.

Serious PSP epidemics, in one case with two fatalities, date back to 1901 [16] and have occurred repeatedly thereafter [1], with the last documented case in 1992 [17]. PSP is shown to reach high concentrations in mussels (> 5000 MU/100 g mussel meat) when *Alexandrium excavatum* has occurred in very low concentrations (4000 cells/L) in the seawater [18]. In 1992 PSP values reached 96492 MU/100 g with 110000 cells/L as the highest concentration of *Alexandrium excavatum* detected at the same locality (Trondheim, 63.5^{o}N, 10^{o}E), indicating that this species in Norwegian waters is extremely toxic [10]. *Alexandrium ostenfeldii* and *Alexandrium minutum* are frequently observed in low concentrations but have so far not been shown to be the main source of PSP in mussels in Norway.

The observations of *Alexandrium excavatum* and PSP in mussels indicate that there has been a spreading northward during recent years. Repeated records of PSP in mussels in the Trondheim area during the last 30 years have been followed by several records further north during the last ten years, e.g. in the Helgeland district (66^{o}N) in l989[10], and in l992 PSP was detected for the first time in the Tromso area (70^{o}N)

associated with a minor bloom of *Alexandrium excavatum* [12]. There is also a clear change in seasonal occurrence. Before 1981 the records of PSP were restricted to the early summer (May-June) [1], then in the spring (April) [19], and during the last few years human poisoning due to PSP has occurred in the autumn (September-October) [12] and traces of PSP were detected in the winter (January-February) in 1993 [12]. In the Trondheims fjord, which has been monitored extensively during the last 10 years, there is a clear trend of an increasing presence of *Alexandrium excavatum* through the autumn-winter season and this species is now observed in most net hauls throughout the year.

Epidemics of DSP intoxication after consumption of mussels have been recorded repeatedly during the last 25 years [2,18], with *Dinophysis spp*. as the causative organisms[2]. Lee & al. [20] investigated the DSP toxin profiles in Norway and for the first time in Europe detected multiple toxin profiles, with either DTX-1 or OA as the major toxin and with a minor component of yessotoxin. Although DSP producing species (*Dinophysis spp*., *Prorocentrum lima*) are common along the entire Norwegian coast, DSP has not been detected in mussels from northernmost Norway. However, the monitoring of DSP during the last years give a clear indication of a northward spreading, from the west coast (60^oN) before 1986 to Mid Norway (64^oN) in 1987 and north Norway (66^oN) in 1989.

The first diatom documented to produce Domoic acid (DA-ASP), *Pseudonitzschiapungensf. mulliseries*, was originally described from the Oslofjord, and other potentially ASP species (*Pseudonitzschia delicatissima* and *P. pseudodelicatissima*), occur in bloom proportions in Norwegian coastal waters. So far DA is not detected in mussels collected from such blooms in Norway. In several cases toxicity has been demonstrated by the mouse biossay without the presence of known toxins (PSP, DSP, ASP) in detectable concentrations. The nature of this mouse toxicity is so far not clarified.

FREQUENCY AND GEOGRAPHICAL EXPANSION

As mentioned above, several species which are "new" in Norwegian coastal waters may seems to have expanded their geographical distribution after the initial observation, although for some species this may he a wrong interpretation, taking into

account the increased awareness and observation frequency. However, for several species there are good reasons to assume a geographical spreading.

After 1968 when incidents related to harmful algae were restricted to the coast south of 63^{o}N [3] such incidents have been observed along the whole coast from Sweden in the south (57-58^{o}N) to near Russia in the north (71^{o}N). The documented northward spreading of detection of PSP and DSP in mussels and an expansion of the season, resulting in the detection of PSP on a year-round basis, has coincided with a period of mild winters in the area and comparatively high seawater temperatures. The effect of the climatolocigal factors on the changes in geographical and seasonal occurrence on the Norwegian coast of important harmful species has so far not been thoroughly analyzed.

In a summary of dinoflagellate blooms in Norwegian waters Tangen [3] referred 21 cases from 1969 to 1978 and only 19 cases prior to 1969 in spite of a fairly large number of investigations along the Norwegian coast from the turn of the century. In the Oslofjord massive blooms of dinoflagellates have also appeared after 1978. Both in the Oslofjord and in a few other Norwegian localities, as well as in larger adjacent regions, like the southern part of the north Sea and the Baltic, there is good reason to claim that there has been a general increase in intensity and frequency of algal blooms due to eutrophication. The documented reduced annual number of phytoplankton blooms in the Seto Inland Sea, Japan, is assumed to be associated with reduced eutrophication [21], which may also explain the generally lower phytoplankton biomass in the Oslofjord during the last few years after reduced anthropogenic inputs of nutrients [22]. However, some of the major blooms of *Gyrodinium aureolum* and the blooms of *Chrysochromulina polylepis* and *Chrysochromulina leadbeateri* have occurred in water masses which are not expected to be overloaded with nutrients from anthropogenic sources.

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Algal Monitoring, A Useful Tool in Early Warning of Shelirish Toxicity

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ABSTRACT

In 1992 a new surveillance system for marine algal toxins was established in Norway. This system constituted three elements; algal monitoring, traditional mouse bioassays, and a combination of chemical analysis and invitro methods. The occurrence of the following potential toxin-producing algae was monitor at 17 stations along the Norwegian coast; Alexandrium spp., Dinophysis spp., Pseudonitzschia spp, Gyrodinium aurelum, Chrsochromulina spp. and Prymnesium spp., The purpose was to advice people on the risk associated with eating wild mussels. The occurrence of algae was estimated from their presence in water samples and/or net haul samples. Advice against consumption of toxic mussels was based on presence of the different algae above levels based on previous experience. After closing of an area, it was only opened again after negative results in the mouse bioassay. Chemical analyses and in vitro assays were used to furter clucidate the toxin profiles during episodes of toxic algae. According to the results from the surveillance program reliable information on toxicity of mussels was obtained from the monitoring of potential toxic alga (There were some exceptions concerning DSP, however. Dinophysis norvegica was present at concentration above what was considered a risky level for a two weeks period without causing toxins in mussels, and mor seriously, on a few occasions significant toxin concentrations were recorded in mussels by the mouse test without in advance warning of the potential toxic algae mentioned above. Psetidonitzschipseudodelicatissima was observed at very high concentrations on several occasions, but these episodes were not associated wit, toxins.

INTRODUCTION

There is an urgent need for internationally accepted procedures concerning monitoring of algal toxin in seafood. Such a system should include both sampling procedures, assay methods, and not least important unified limits of acceptance. Today, regulation concerning the very toxic paralytic shellfish poisons (PSP) i in operation in 21 countries, while regulation for the diarrheagenic toxins (DSP) is established in 12 countric (van Egmond et. al., 1991). In recent years, several countries are applying combinations of algal screening an, toxicity testing of infected seafood in their

monitoring programs. It is very important to verify whether such systems can reliably replace the previous system which mainly were based on animal testing of extracts from samples of seafood from selected areas. Hopefully, a combination of algal monitoring and chemical immunotoxicological method / *in vitro* tests can replace testing on live animals in the future. Before this ca be accepted, the reliability of such combinations needs 'to be verified. In this report, experience from the first year of a new surveillance system for marine algal toxins in Norway is discussed. The surveillance continued in 1993.

METHODS

Sampling stations: The 17 sampling stations for algae and mussels were chosen from a combination of known areas of mussel sampling and geographic spreading. They cover most of the country from the Swedish border in the south to Tromso in the north (see map. Fig.1).

Algal monitoring: The choice of algae monitored was based on previous experience associated with toxicity towards people via consumption of mussels or toxicity towards fish. Samples were collected twice per month in the spring until April 15. During summer until September 1 weekly samples were taken. Later in the fall bimonthly samples were collected.

The occurrence of potential toxic algae was estimated from the presence in two sets of samples, one water sample collected from the upper 0-3 m by tube, and another collected by a net, mesh size 20 μm, from the same surface layer by a combination of vertical and horizontal hauls. The presence of algae in water samples was given as cells/L. The presence in net samples was given according to a relative scale; ND-not detected, D-detected, and 1,2 and 3 which indicated that the algae of concern constituted 1 - 10, 10 - 50 or > 50% of the sample, respectively.

Monitoring of algal toxins in mussels: Blue mussels (*Mytilus edulis*) were placed in the upper 0-3 m of the sea, in nets anchored to buoys at the algal sampling sites. At two routine stations (Flodevigen and Vega, number 5 and 15, respectively), samples were collected and analyzed by means of the mouse bioassay every other week. Al the other stations, mussels were only analyzed 2-3 weeks after termination of episodes with

increased concentrations of toxin producing algae. The mouse bioassay applied for DPS is a slight modification of the Japanese method, as described previously (Stabell et al., 1991). For PSP, the AOAC method (1984) was used. Chemical methods for PSP (Lawrence at al., 1991[a]), and domoic acid (Lawrence et al., 1991[b]) were applied infrequently in order to verify results with the mouse bioassay. Furthermore, an in vitro method using freshly prepared *hepatocyles* (Aune, 1989) was used to further study specific samples at occasions where results from algal monitoring and the mouse bioassay showed diverging results.

Warning system: The public warning against consumption of mussels which might be contaminated with algal toxins was primarily based on levels of toxin producing algae. At the onset of the program, the following levels of warning were selected: *Alexandritim* spp.,"detected" in net haul sample; *Dinophysis* spp., level "1" in net haul sample; *Pseudonitzschia pseudodelicatissima*, 10^6 cells/L in seawater (hygienic evaluation); *Chrysochromulina* spp., *Gyrodinum aureolum* and *Prymnesium parvum*, >500 000 cells/L in seawater (hygienic evaluation). During the program, based on increased experience, the levels were adjusted upwards for both *Alexandrium* and *Dinophysis* to level "1"and "2", respectively, while previous levels were used as levels for the recommendation: "eat mussels with care". The closed areas were not opened for consumption before negative results in the mouse bioassay were obtained.

RESULTS

A comparison of the results obtained with two different ways of quantification of *Dinophysis* spp., net haul and water samples, are given in Fig. 2 (data from Flødevigen, station 5). Even though the amplitudes are higher with the water samples, the results from the two methods are quite similar. Due to the low number of *Dinophysis* cells per liter seawater, results from net haul samples were routinely used concerning DSP warning. For the algae having ichthyotoxins, like *Chrysochromulina* and *Gyrodinium*, and for *Pseudonitzschia*, presence of high numbers of cells per liter during episodes made counting of cells in water samples the method of preference.

In Fig.3 records of the dominating toxin producing algae at Flødevigen are plotted against the mouse toxicity of mussel extracts from the same station. During the 1992

summer season, no episode of high mussel toxicity was recorded al Flødevigen even during the about two weeks period around week 3 1, when concentrations of *Dinophysis norvegica* were above 2000 cells/L (Fig.2). The low toxicity, however, corresponded well with the concentrations of *Dinophysis* in the net haul samples. The mouse toxicity recorded corresponds quite well with the variations in *Dinophysis,* in contrast to the presence of *Chrysochromulina* and *Gyrodinium* which are also extracted in the DSP sample. These data were confirmed with the hepatocyte test which differentiates between DSP toxins and the ichthyotoxins (unpublished data). Results from the other routine station for mussel analyses, Vega, are shown in Fig. 4 None of the algal species recorded appeared at concentrations expected to yield high mussel toxicity. However, the correlation between concentrations of *Dinophysis* and mouse lethality was not convincing. Furthermore, the mice died with abnormal symptoms involving cramps from the DSP extracts on occasions of the highest toxicity. A study of the mussels Indicate a possible contribution to the mouse toxicity from algae not included in the routine monitoring (*Amphidinium cf. certerac* and *Prorecentrum lima*) growing on the shells of the blue mussels or in the mussel cultures.

From the other stations, the concentrations of *Dinophysis* in net haul samples usually varied between "detected" and level "1-2". Mouse bioassays of mussels from these stations taken two-three weeks after termination of elevated algal concentrations usually showed no toxicity. On a few occasions, however, high mouse toxicity was recorded even during periods with levels of toxin producing algae below warning levels. Especially at station 13 (Molde) on the south-west coast, high mouse toxicity (levels 3-4) in mussels persisted from August to the end of the programme in November, even though none of the known toxin producing algae appeared at elevated levels.

During 1992 only one episode of toxic levels was recorded for PSP. This incidence took place in the Trondheim fior where a high number of *Alexandrium* cells appeared in the spring. The episode was associated with very high toxicity in mussels, as can be seen from Fig. 5. The mouse lethality was at a high level of about 100 000 mouse units per 100 g mussel meat in the beginning of May as determined both by the chemical and the mouse bioassay, levelling off in a time-dependant manner after termination of the algal bloom, reaching the level of acceptance in the end of June, 6 weeks after the

disappearance of *Alexandrium*. Results from the water samples and the net haul corresponded well. The correspondence between algal monitoring for *Alexandrium* and mouse lethality from the routine stations was very good; levels of the algae varied between "not detected" and "detected", and on no occasion did the mouse lethality exceed the lower warning level corresponding to 200 mouse units per 100 g mussels meat (data not shown). At one station the *Alexandrium* concentration in the net haul samples varied between "detected" and level "1", and the corresponding mouse lethality raised to about 300 mouse units per 100 g mussel meat.

Concerning ASP, no episode of toxic mussels was registered even during episodes with elevated levels of *Pseodonitzschia psetudodelicatissima* lasting for two months with a peak level of 5 million cells per liter (Kragerø, data not shown).

DISCUSSION

The purpose of the new surveillance program was to establish a warning system for consumers of mussels toward the different algal toxins known to appear in Norwegian waters, based primarily on algal screening. In addition, possible appearance of the "new" ASP toxin from species of *Pseudonitzschia* at toxic levels in mussels was studied for the first time in our country.

Quantification of marine algae was performed by two methods; counting of number of cells in water samples or counting and giving relative number of cells from net haul samples. The former method is the method of preference for algae which appear in high numbers (and which are non-toxic at low concentrations, *Chrysochromulina, Gyrodinium, Prymnesium, Pseudonitzschia*), while relative quantification by the net haul method is more convenient for those species appearing and being toxic at low concentrations (*Alexandrium* and *Dinophysis*). In Figs. 2 and 5 the results obtained with the two algal monitoring methods are given for *Dinophysis* and *Alexandrium*, respectively. They confirm that the correlation between the two methods was satisfactory for the purpose of our surveillance program. Consequently, results from net haul sampling was used as basis for warning against both DSP (*Dinophysis*) and PSP (*Alexandrium*). Quantification of the so-called inchthyotoxin producing algae

(*Chrysochromulina, Gyrodinium, Pryimnesium*) and *Psetidonitzschia* was mainly based on cell counting from water samples.

One of the most important aspects of the new surveillance system was to reduce as much as possible the use of live animals (mice) in toxicity testing due to ethical considerations. Consequently, the surveillance was based on algal monitoring as the indicator for closing area by means of warning to the public. The mouse bioassay was only used for certifying the absence of toxins in mussels before opening the areas again, and this was achieved by performing mouse tests of mussel extracts 2-3 weeks after termination of episodes of toxin producing algae above predetermined limits. This means loss of information about the association between variations in algal concentrations and toxicity in mussels. To counteract this lack of information to some extent, two stations were selected for routine mouse tests of mussels every other week. In the south, the station of selection was Flødevigen, the location of a marine research station, in the county of Aust-Agder. The station in the north was Vega, in an area of shellfarming, in the country of Nordland.

Contrary to previous years, no episodes of toxic mussels appeared at Flødevigen during the 1992 season. These results corresponded well with the results from the algal monitoring except for the two weeks period with high concentrations of *D. norvegica*, a species which previously has occurred in a toxic mode in the area (Lee et. al., 1989). The toxicity toward mice varied between "not detected" and level "1", the latter corresponding to 5-7 mouse units or about 25-30 μg of okadaic acid per 100 g mussel meat. This is the limit of acceptance for mussels in Norway. The corresponding level of *Dinophysis* in net haul samples varied between "not detected" and level "1", with one episode at level "2". This further support that at least *D. norvegica*, which was dominating among the *Dinophysis* spp., may occur at rather high concentrations without causing toxic mussels. In this connection our results together with previous findings, support that different species or even strains of *Dinophysis* may vary considerably in their toxic potential. The levels of the inchthyotoxin never reached levels associated with toxicity towards fish, and obviously did not influence the toxicity of mussels. At the other routine station, Vega, none of the toxin-producing algae reached levels expected to render mussels toxic. Never the less, several episodes of high mouse toxicity were registered upon intraperitoneal

injection of DSP extracts. Before death, the mice sometimes showed unusual symptoms like cramps. Algal observations indicates a possible role of other toxic species growing on the shells of the mussels. Tests with freshly prepared hepatocytes verified presence of toxins not belonging to the DSP complex.

From most of the other stations in the surveillance program, the concentration of *Dinophysis* varied between "detected" and level "1-2". Mouse bioassays performed when levels were below I usually were negative. However, on a few occasions, high mortality against mice was found even during periods of low levels of *Dinophysis* (and other known toxin producing algae). During these episodes, the mice often displayed abnormal DSP symptoms with cramps. Even though faulty algal sampling cannot be excluded as an explanation for the discrepancy between results from algal monitoring and toxicity, presence of algae so far not expected to produce toxins in Norwegian waters cannot be excluded. The phenomenon with cramps associated with DSP assays has also been recorded in previous years, and it is studied in more detail in the 1993 programme. The successful use of algal monitoring as the basic method for public warning against toxic algae requires solution of this problem-, one cannot accept false negative results if the toxins found in the mouse bioassay represent a health threat to consumers.

During 1992 only one serious episode of PSP infected mussels was recorded. The algal monitoring functioned according to expectations, and consumers were warned about the danger of eating mussels from ft affected area. Furthermore, experience from the programme indicated that the warning level concerning P.SP could be changed to level "1" from net haul samples, instead of "detected" which was the level of warning at the onset of the program. No episode of amnesic shellfish poisoning (ASP) was registered during 1992, even though levels of *Pseudonitzschiapseudodelicatissima* reached several million cells/L for weeks at station 4 (Kragero) on the south coast. Lack of ASP toxins in mussels was verified with both chemical methods and a mouse bioassay which was consistent with other observations from the same bloom episode (Lundholm and Skov, 1993). This indicated that the dominating strain of *Pseudonitzschia* appearing in Norwegian waters is non-toxic, at least for the time being. Possible formation of ASP toxin in the future will be checked regularly.

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APPENDIX

INTRODUCTION

A new generation of marine data buoys designed for real-time monitoring and forecasting of marine biological, physical and chemical variables together with meteorological information have been in use since 1988 (Volent and Tangen 1989).

Combined data from SEAWATCH buoys (LBA, 02-saturation, temp and salinity) and observer network, i.e. 38 stations situated from the Swedish border (SW-coast) to N-Norway were measurements of secchi depths (turbidity and water color), surface sea temperature and salinity, general weather conditions, fish behavior and appetite, unusual biological events (e.g. red tides, yellyfish invation), water samples for enumeration and identification of phytoplankton.

RESULTS

Major toxic blooms:

A large number of potentially harmful algal blooms were recorded by the SEAWATCH Europe buoy system in 1993. All potential toxic species were detected in Norway, except *Glenodinium foliaceum* (dinoflagellate), and *Nodularia spumigena* (Cyanobacteria).

1. April - June :

Two blooms of *Pseudoniftschia pseudodelicatissima*

- April - June (Bloom 1): 0.5-5 mill/L in mid-Norway after the regular spring bloom.
- August - Nov: Max conc. early Sept. in the More district (2.8 mill/L) + late Sept. in Trondheimsfjord (2.5 mill/L).
- Both blooms lasted about 1.5-2 months.
- From spring to fall: Bloom conc. Obs. In samples along the coast (incl. Fjords) from the Swedish west coast to Tromso.
- No ASP toxin (domoic acid) detected along the Norwegian coast (large number of toxin analyses performed) by the Norwegian College for Veterinary Medicine

2. *Phaeocystis cf. globosa* in Dutch waters, May 1993:

- Indication of eutrophication in Southern North Sea.
- Netherlands II buoy: End of April (2mill/L)-12 May (>40mill/L)
- Phaeo-blooms usually extend to the German Bight and Danish west coast.

3. Extensive bloom of *Finiliania huxleyi* May-Sept-93:

- Bloom along Norway coast and offshore waters
- Last half of April: Started in Osterfjorden/Sorfjorden, NE of Bergen (>1 mill/L), obs. below the brackish layer.
- May-early June.- 1-5 mill/L from Oslofjord to Mid-Norway.
- Middle of June: Bloom spread to Haltenbanken, 200 km off Mid-Norway.
- End of June: >1 mill/L obs. from the Oslofjord to the Polar circle.
- July: Bloom started to decline at the south and west coast, but continued northwards to Vesteralen (69N, 20 July)
- August: Bloom reached Tromso area (70N, 10 August)
- September: Undocumented obs. of milky waters in the Barents Sea (fishermen)
- Upwelling situations in the southwest and west coast during early summer mixed nutrient rich deep water into the surface water above the pycnocline

4. *Skeletonema costatum*:

- The dominating species during the spring bloom and later local blooms from the Oslofjord to Finnmark (71N), sometimes associated with reduced appetite.
- Skeletonema bloom in Skagerrak in May.
- *Rhizosolenia* spp. were recorded frequently but did not induce mucus production in the gill tissues (danger of suffocation in caged fish).

5. June-July : *Prymnesium parvum* and *P. Patelliferum* bloom originated (as usual In Hylsfjorden) :

- Site: Hyls-and Sandsfjorden in Ryfylke (Rogaland).
- July: Bloom started in beginning of July with max. cell count of 1.7mill/L (end of July).

- August: Surface water from these two fjords flushed out into the Boknafjord causing fish kills in fish farms (5 tons of salmon).

6. Aug - Sept, *Gyrodinium aureolum*

- *G. aureolum* detected in the Skagerrak area, but did not reach bloom proportions in l993.
- *Gymnodinium galatheanum* was obs. together with *G. aureolum* in Aug-Sept and sporadically from the Oslofjord to Mid-Norway.

7. September: *Ceratium furca*

- Obs. in bloom proportions at the west coast of Norway during Sep.
- Increased in outer Oslofjord in Aug and early Sept.
- also reported at the Swedish west coast and Norwegian south coast (Flodevigen).
- Typical annual occurrence of *C. furca* in North Eur. waters.
- Up to 30 mill cells per liter.
- The bloom of *C. furca* on the west coast seemed to be associated with the advection of the Norwegian Coastal Current water masses from the Skagerrak during a large outflow observed in the middle of September.

8. *Dinophysis* spp. blooms autumn-winter 1993 (DSP)

- Discolored water obs. (patchiness, brownish/red, > 0.1 mill/L obs., i.e. bloom proportions) recorded from Skagerrak to Stad (62N)
- North of Stad *Ceratium furca* dominated, on the Norw Skagerrak coast *Ceratium lineatum* occurred in rel. high cell numbers while *C. furca* bloom was still increasing on the west coast.
- *D. niorvegica* (most abundant) with D. acuta (2nd) were abundant during the *Ceratium furca* bloom on the west coast in Aug-Sept.
- *D. rotundata* were abundant in some branches of Trondheimsfjord during summer + scattered obs along the coast up to 69N.
- May-fall: DSP detected in very high concentrations in mussels of south and west Norway-associated with the three *Dinophysis* species.

9. *Alexandrium* (PSP)

An *excavatum* (most common, reg. blooms not recorded in 1993, scattered obs. through the year, especially Mid-Norway. *A. minutum*, *A. ostenfeldii*: scattered obs.

- Highest PSP - value recorded from the Molde area (1622 MU/100g) in early May.
- Rel.high [PSP] detected in other localities in Mid-Norway (900 MU/100g).
- PSP was associated with moderate number of *Alexandrium excavatum.*
- During summer *A. excavatum* spread northwards (Tromso) with only traces of PSP north of 67N.

10. *Prorocentrum minimum*

- Fairly abundant in the outer Oslofjord area (Singlefjorden) in summer-fall.

11. *Chrysochromulina polylepis and C. leadbeateri*

Both species obs. during spring-summer.

- *C. polylepis* in mixed flagellate community associated with some mortality in a salmon farm north of Bergen (Oster and Sorfjorden, April-May), in addition to *C. polylepis*, cells of *Heterosigma akashiwo* and *Gymnodinium galatheanum* were identified (EM microscopy-Marine Botany, Oslo; toxin detection - Defence Research Establishment of Norway). 50 tons, mainly rainbow trout were killed.
- *C. polylepis* were also found sporadically in Mid-Norway and Skagerrak coast during summer.
- *C. leadbeateri* occurred in trace amounts in the Vestfjorden area (June, massive fish kills in 1991).
- *Chrysochromuina spp.*: March - April, fish kills in March in Masfjorden (rainbow trout and salmon) the cells accumulated under the brackish layer. Cells found: *Chrysochromulina* species (0.5 mill/L) and *Emiliania huxleyi* (5 mill/L).

12. Fall 1993, the *Siliconagellate Dictyocha speculum*:

- Abundant in net hauls from Mid-North Norway.