Overview of Key Phytoplankton Toxins and Their Recent Occurrence in the North and Baltic Seas

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ABSTRACT: The frequency and intensity of harmful algal blooms (HABs) appear to be on the rise globally. There is also evidence of the geographic spreading of toxic strains of these algae. Consequently, methods had to be established and new ones are still needed for the evaluation of possible hazards caused by increased algal toxin production in the marine food chain. Different clinical effects of algae-related poisoning have attracted scientific attention; paralytic shellfish poisoning, diarrhetic shellfish poisoning, and amnesic shellfish poisoning are among the most common. Additionally, cyanobacteria (blue-green algae) in brackish waters often produce neurotoxic and hepatotoxic substances. Bioassays with mice or rats are common methods to determine algal and cyanobacterial toxins. However, biological tests are not really satisfactory because of their low sensitivity. In addition, there is growing public opposition to animal testing. Therefore, there has been increasing effort to determine algal toxins by chemical methods. Plankton samples from different European marine and brackish waters were taken during research cruises and analyzed on board directly. The ship routes covered marine areas in the northwest Atlantic, Orkney Islands, east coast of Scotland, and the North and Baltic seas. The first results on the occurrence and frequency of harmful algal species were obtained in 1997 and 1998. During the 2000 cruise an HPLC/MS coupling was established on board, and algal toxins were measured directly after extraction of the plankton samples. In contrast to earlier cruises, the sampling areas were changed in 2000 to focusing on coastal zones. The occurrence of toxic algae in these areas was compared to toxin formation during HABs in the open sea. It was found that the toxicity of the algal blooms depended on the prevailing local conditions. This observation was also confirmed by monitoring cyanobacterial blooms in the Baltic Sea. Optimal weather conditions, for example, during the summers of 1997 and 2003, favored blooms of cyanobacteria in all regions of the Baltic. The dominant species regarding the HABs in the Baltic was Nodularia spumigena. However, in addition to high concentrations of Nodularia spumigena in coastal zones, other blue-green algae are involved in bloom formation, with changes in plankton communities influencing both toxin profiles and toxicity. © 2005 Wiley Periodicals, Inc. Environ Toxicol 20: 1–17, 2005.

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HARMFUL ALGAL BLOOMS

Natural “contaminants” may accumulate via food chains. A typical example is the ingestion and accumulation of algal toxins in filter feeders such as mollusks. Because mollusks
do not distinguish between algae that have and algae that do not have toxins, a significant accumulation of toxins is plausible at any time.

The monitoring of toxins in seafood and the associated risks of human exposure require tight food-control measures. Consumption of seafood contaminated with algal toxins may yield serious diseases (Simon et al., 1997): damage to the nervous system [paralytic shellfish poisoning (PSP)] and to the intestinal system [diarrhetic shellfish poisoning (DSP)] as well as brain damage [e.g., loss of memory; amnesic shellfish poisoning (ASP)] were observed depending on the type of algal species involved in the bloom.

The global marketing of marine products poses a serious threat to consumers if proper safety-monitoring programs are not in place. Therefore, an important task is the accurate and cost-effective analysis of marine food samples for algal toxins according to national and international regulations including compliance measures.

Residue analysts who specialize in toxin determination in principle are confronted with the same problem as any other chemist dealing with complex biomatrices where high sensitivity is called for. The simultaneous realization of these demands is often accompanied by many difficulties. Although adequate detection methods have been established for several contaminants, only recently has the complexity and diverse specificity of algal toxins been recognized. The following physicochemical methods for routine determination of algal toxins were established during the last decade, and these methods were applied to control samples from different locations.

**CHEMICAL STRUCTURE AND ANALYSIS OF ALGAL AND CYANOBACTERIAL TOXINS**

Empirical observations on harmful algal blooms and their effects on toxicity of mussels were already scientifically manifest in 1937. It was found that toxic mussels were free of poison after being kept for 2 weeks in filtered salty water, that is, water without algae. Returned into salty water, the harmless mussels again became toxic within a short time (Sommer et al., 1937). At that time, some algae were described as being responsible for the production of toxins, and a biological test, the mouse bioassay, was developed to identify the level of toxicity produced by these algae in shellfish.

At present, the mouse bioassay is still the most important international detection method for determining the level of accumulated algal toxins, and all of the following analytical methods have to be reevaluated against this bioassay, using it as a yardstick measure.

However, such biological tests only reveal the total toxicity of a sample without detecting toxin specificity. The limited validity of the test, therefore, resulted in the need for physicochemical methods for the detection of individual algal toxins. Another important shortcoming of the mouse bioassay is the growing resistance of the public to routine animal experimentation in which test specimens may die.

**Paralytic Shellfish Poisoning Toxins**

Algal producers of paralytic shellfish poisoning (PSP) toxins are mainly the dinoflagellate species of the genus *Alexandrium* spp. These occur both along the Atlantic and the Pacific coastlines and may increase in population size, reaching great cell densities.

Toxins produced by *Alexandrium* were designated PSP toxins because of their consumption was observed to cause symptoms of poisoning in warm-blooded species similar to paralytic phenomena such as cramps, signs of paralysis, and blockage of respiration. PSP toxins are potential neurotoxins that specifically block the excitation current in nerve and muscle cells.

A change in the ratio of sodium and potassium ions causes an action potential in excitable membranes. This excitation opens the sodium channels and sodium ions stream into the cell. PSP toxins reversibly bind to the receptor on the outside of the cell membrane. This interrupts the flow of sodium ions, preventing the formation of an action potential, which interrupts the excitation current finally results in paralytic effects.

Consequently, the development of analytical methods for the early detection and quantification of toxins causing PSP was and still is an important task.

The mouse bioassay unambiguously gives evidence of the potential PSP toxicity of a tissue samples at higher toxin concentrations, and the time to death of laboratory animals is relatively short. However, this biological test reveals the total PSP toxicity of a sample only, which is expressed in mouse units or PSP per kilogram (MU/kg or PSP/kg). Specification of the PSP toxins is not possible with this bioassay. Variations in toxin profiles cannot be monitored, and determination of individual PSP toxins requires chemical isolation and structure identification.

In 1957 a PSP toxin was isolated from *Saxidomus giganteus* (clams) from Alaska, and in 1975 its chemical structure was classified as a so-called saxitoxin (STX). Later on, more PSP toxins were identified, all of which were related to *N*-1-hydroxy-saxitoxin (NEO) or saxitoxin (Schantz, 1986). Today it is generally accepted that three groups of PSP toxins can be distinguished: carbamate toxins, *N*-sulfocarbamoyl toxins, and decarbamoyl toxins (see Fig. 1). *N*-Sulfocarbamoyl toxins only exhibit low toxicity, whereas carbamate and decarbamoyl toxins show significantly higher toxicity. During the processing of PSP toxins in contaminated canned seafood, low-toxic *N*-sulfocarbamoyl toxins may be hydrolyzed to more toxic carbamate
or decarbamoyl toxins, resulting in a noticeable increase in total PSP toxicity.

In 1975 it was recommended that, in addition to the use of the mouse bioassay, a fluorometric method be used for the determination of PSP toxins in samples (Bates and Rapoport, 1975). PSP toxins, which exhibit neither UV absorption nor fluorescence, were oxidized in alkaline solution to fluorescent pyrimidino purines. After acidification the fluorescence intensity of the oxidation products can be measured in the solution. However, individual PSP toxins differ both in toxicity and fluorescence intensity after oxidation (Luckas, 1992). Therefore, it was suggested that chromatographic separation of the PSP toxins be performed prior to the determination of fluorescence (Jonas-Davies et al., 1984).

The importance of complete chromatographic separation of all PSP toxins that contribute to total PSP toxicity prior to quantification with a fluorescence detector was recently confirmed in an intercalibration exercise. The results clearly demonstrated that high-performance liquid chromatography (HPLC) methods for the determination of PSP toxins are superior to enzyme-linked immunosorbent assays (ELISA) especially developed for the determination of STX (van Egmont et al., 1994). In addition, the intercalibration exercise demonstrated that alkaline oxidation of PSP toxins has to be performed after the chromatographic separation of the underivatized PSP toxins (postcolumn derivatization).

On the other hand, varying results obtained with different chromatographic methods suggested that determination of PSP toxins always requires a careful evaluation of the results (Quilliam, 1995).

Independent of the determination method, PSP toxins have to be quantitatively extracted from the sample materials. The standard method uses 0.1 N HCl as the extraction solvent, as suggested by the Association of Official Analytical Chemists (AOAC). The sample and the extraction solvent are heated at 100°C for 5 min, thereby converting the N-sulfocarbamoyl toxins into their respective carbamate toxins. B1, B2, and C1–C4 toxins are no longer present in the extracts. These extracts are still suited for both the mouse bioassay and for other PSP determination methods (Hollingworth and Wekell, 1990).

The breakthrough in the use of HPLC methods for PSP determination took place when Sullivan and Wekell (1984) succeeded in separating underivatized PSP toxins by ion-pair chromatography with alkylsulfonic acids followed by postcolumn oxidation with periodic acid. The stationary phase consisted of polystyrene-divinylbenzene. A solvent gradient was used with a phosphate buffer containing n-hexane and n-heptane sulfonic acid as ion-pair formers. This method allowed good separation of the carbamate toxins. The only disadvantage of the method was that coelution of STX and dc-STX resulted in a false reading of total PSP toxicity because STX is twice as toxic as dc-STX. Oshima et al. (1989) described the entire process of HPLC separation of PSP toxins. In dependence on the polarity, three groups of PSP toxins were determined after isocratic elution in three HPLC systems. This method has the disadvantage of high expenditure, that is, three chromatographic runs are needed for the quantitation of all PSP toxins in samples.

It is generally recommended positive findings of PSP toxins be confirmed via mass spectrometry (Luckas, 2000a). However, phosphate and ion-pair formers in the eluents prevent efficient application of HPLC/MS coupling. Therefore, an HPLC method was developed for PSP determination that allows direct coupling of HPLC with mass spectrometry. The separation of the PSP toxins was obtained on ion exchange resins using an aqueous eluent, with ammonium acetate as the only additive (Jaime et al., 2001).

The more perfect is the determination of the concentrations of the respective PSP toxins, the more exact will be the calculation of total PSP toxicity. This follows from total PSP toxicity after HPLC separation being obtained by summing individual PSP toxicities. Therefore, unambiguous assignment of the peaks in HPLC chromatograms to specific PSP toxins is necessary to properly quantify these toxins. Then the total PSP toxicity value obtained from the HPLC chromatograms (STX equivalents/kg) will be in good agreement well with the results of the mouse bioassays. Although the mouse bioassay is still obligatory for the export of seafood, the HPLC methods for the determination of PSP toxins already have reduced the number of experiments using animal (Luckas, 2003). Recently, Gerds et al. (2002) described a fast and easy way to perform assay for the detection of PSP toxins in natural phytoplankton blooms by combining the initial fluorometric assay of Bates and Rapoport (1975) with the chemistry of Thielert et al. (1991). Because of its simplicity and speed, the FFA can be applied to gain a rough estimate of PSP toxins in water samples before an accurate quantitative and qualitative analysis by HPLC.

**Diarrhetic Shellfish Poisoning (DSP) Toxins**

Several groups of DSP toxins have been discovered since 1978 (Yasumoto and Oshima, 1978). Okadaic acid (OA) was isolated from the sponge *Halichondria okadai* (Tachibana and Scheuer, 1981), and later the dinophysistoxins (DTXs) were detected (Yasumoto and Murata, 1985). Okadaic acid and their derivatives constitute the so-called okadaic acid group (Fig. 2).

The first report about DSP in Europe was in 1979. Mussels were found to be contaminated with DSP toxins, and correlation of the toxicity with the presence of *Prorocentrum* spp. and *Dinophysis* spp. was evident. Okadaic acid (OA) and the dinophysistoxins (DTXs) were the dominant toxins (Kat, 1979, 1983; Dahl and Yndestad, 1985; Lassus and Bandouil, 1985; Edebo et al., 1988).

In the following years the chemical structures of new DSP toxins were elucidated, adding pectenotoxins (PTXs), yessotoxins (YTXs), and azaspirazines (AZAs) to the list of those considered DSP compounds (Murata and Sano, 1986; Murata and Kumagai, 1987; Satake et al., 1998). However, at present only yessotoxins and azaspirazines have been detected in algae and mussels from northern European waters (Daiguji et al., 1998; Ramstad et al., 2001; James et al., 2002, 2003).

Bioassays were the first methods developed for the determination of DSP toxins (Hamano et al., 1985). Later, immunoassays (Uda and Itoh, 1989), protein phosphatase assays (Tubaro et al., 1996; Klöpper et al., 2003), and HPLC methods based on precolumn derivatization with fluorescence markers (Luckas, 1994) supplemented the experiments with rats or mice.

If the goal of a study is to determine individual DSP toxins, a chromatographic separation must be performed prior to DSP determination. Pleasence et al. (1990) already reported on the application of atmospheric pressure ion source/electrospray ionization mass spectrometry (API/ESI-MS) to the determination of algal toxins. API/ESI-MS is now commonly applied to the determination of algal toxins after HPLC separation (Hummert, 2000). Because several companies offer cheap HPLC/MS systems equipped with an API/ESI interface, coupling HPLC with mass spectrometry currently is the most promising technique for the determination of DSP toxins in marine organisms (Goto et al., 2001).

**Amnesic Shellfish Poisoning (ASP) Toxins**

Quilliam and Wright (1989) reported on the great efforts in 1987 to elucidate the cause of the so-called Prince Edward Island disease. At that time more than 100 persons who had consumed mussels harvested on this eastern Canadian island were affected; three persons died, and the survivors suffered memory loss. Therefore, this type of intoxication was named amnesic shellfish poisoning (ASP).

During the isolation of the ASP toxin from mussels, several toxic and nontoxic mussel extracts were investigated using both biological and chemical methods. The respective HPLC chromatograms showed characteristic differences in comparison to nontoxic fractions. On the basis of the UV spectrum, the corresponding compound was identified as
the amino acid domoic acid (DA; Fig. 3), which affects the nervous system and mimics the biochemical action of glutamic acid. This explains why amnesia is the final effect of ASP (Addison and Stewart, 1989).

In the case of ASP toxin, it was surprising that the diatom *Nitzschia pungens* widely spread along the Canadian coast was the key producer of domoic acid (Subba Rao et al., 1988). *Nitzschia pungens* is a species also widely found in European waters. Therefore, mussels harvested in Europe also may be contaminated with ASP toxin.

Domoic acid became a subject for food quality control laboratories after the introduction of a preliminary residue limit of 20 mg DA/kg of mussel meat (Todd, 1983). HPLC separation of the underivatized domoic acid on an RP-C18 column followed by UV detection at 242 nm was suggested as the official monitoring method (Quilliam et al., 1995).

The method of mass spectrometric determination suggested by Lawrence et al. (1994) is another sensitive and selective way of analyzing domoic acid. This HPLC/ESI-MS was applied to the unambiguous detection of domoic acid in the presence of other substances. However, a thorough purification of sample extracts is also necessary for the determination of DA with HPLC/MS (Hatfield et al., 1994).

**Spirolides**

The term *spirolide* describes a group of biologically active compounds first isolated from the digestive glands of Canadian shellfish (Hu et al., 1995). Later, spirolides also were determined in plankton samples from Nova Scotia, Canada (Cembella, 1998).

With molecular weights in the range of 691–711 and a macrocyclic polyether structure, they belong to the family of polyether toxins containing, for example, the okadaic acid group, brevetoxins, pectenotoxins, and yessotoxins, too. A toxin pair with a difference in molecular weight of 2 Da related to one double bond is typical of spirolides-A/B, -C/D, and -E/F (Hu et al., 2001). In addition, two structure variations, both desmethyl derivatives, have been discovered (Hu et al., 1996). These new compounds were formed by demethylation of spirolide C and spirolide D, involving a location other than carbon 31 [Fig. 4(a,b)].

Spirolides are so-called fast-acting toxins that cause death with characteristic neurotoxic symptoms within several minutes when injected intraperitoneally (i.p.) into mice.

### Table: Spirolides

<table>
<thead>
<tr>
<th>Toxin</th>
<th>R₁</th>
<th>R₂</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirolide A</td>
<td>H</td>
<td>CH₃</td>
<td>Δ₂,₅³</td>
</tr>
<tr>
<td>13-desMethyl spirolide C</td>
<td>CH₃</td>
<td>H</td>
<td>Δ₂,₅³</td>
</tr>
<tr>
<td>Spirolide B</td>
<td>H</td>
<td>CH₃</td>
<td>693</td>
</tr>
<tr>
<td>desMethyl spirolide D</td>
<td>CH₃</td>
<td>H</td>
<td>693</td>
</tr>
<tr>
<td>Spirolide C</td>
<td>CH₃</td>
<td>CH₃</td>
<td>Δ₂,₅³</td>
</tr>
<tr>
<td>Spirolide D</td>
<td>CH₃</td>
<td>CH₃</td>
<td>707</td>
</tr>
</tbody>
</table>

**Fig. 4.** (a) Chemical structure of spirolides. (b) Structure of spirolides (Hu et al., 1996, 2001).
Although the mechanism of toxicity is still not fully understood, the cyclic iminium moiety is believed to be the active pharmacophore. This theory is supported by the observation that the keto-amine derivatives (spirolides E and F) are biologically inactive (Hu et al., 1996).

The origin of spirolides remained cryptic until recently, when their annual recurrence was related to the marine dinoflagellate *Alexandrium ostenfeldii* (Cembella et al., 2000). *Alexandrium ostenfeldii* is morphologically closely related to *A. tamarense* and *A. fundyense*, which are well known as important producers of paralytic shellfish poisoning (PSP) toxins. However, the strains of *A. ostenfeldii* isolated from Nova Scotia, Canada, which are responsible for the occurrence of spirolides in that region, do not produce PSP toxins (Cembella et al., 2001).

In Graves Shoal and Ship Harbour, two important areas of shellfish aquaculture in eastern Canada, annually recurring blooms of spirolide-producing algae have been monitored since 1996. Here, the toxin profiles observed at both locations revealed remarkable differences. In phytoplankton from Graves Shoal, spirolides B and D dominated, whereas spirolides A and C were minor components. In contrast, the profile of spirolides found at Ship Harbour was dominated by the demethylated derivative of spirolide C (Cembella et al., 1999). Although the number of spirolide-producing algal strains that had been successfully cultivated was still rather small, totally different profiles of spirolides were observed (Maclean et al., 2003).

**Cyanobacterial Toxins**

Cyanobacteria (blue-green algae) can, under given circumstances, also produce toxins (Carmichael, 1988). However, only a few blooms of toxic cyanobacteria from surface waters are known to have caused health risks to humans, although numerous cyanobacteria are able to produce both neurotoxins and hepatotoxins (Codd, 1995).

Several lethal poisonings of cattle, game, fish, and seabirds, as well as humans, have been documented. (Ressom et al., 1994). However, analytical methods for the specific determination of selected cyanobacterial toxins were developed only very recently (Chu, 2000). Therefore, it will be a task of future research to apply these powerful analytical methods to estimating toxicity during strong algal blooms that may occur, mainly in brackish water lagoons and in drinking water reservoirs or in coastal and inland aquaculture systems (Carmichael, 1993).

**Neurotoxins**

It was recognized in earlier times that the consumption of mussels from waters contaminated with cyanobacteria caused toxic effects similar to those cause by PSP toxins (Mundt, 1988). Later, it was discovered that the cyanobacteria *Aphanizomenon flos aquae* and *Anabaena circinalis* may produce PSP toxins (Mahmood et al., 1986; Onodera et al., 1996) and that the latter may produce the neurotoxins anatoxin-a and anatoxin-a (S) in addition to PSP toxins (Watanabe, 2000). Therefore, it was recommended that the methods be those specifically developed for the detection of the various neurotoxins produced by cyanobacteria separately (Codd, 1997).

**Hepatotoxins**

The cyanobacteria *Microcystis* and *Nodularia* are known to be found in brackish waters and have been identified as major components of blooms (Kononen and Sellner, 1995). Bioassays have been carried out with both these species. Because the symptoms that *Microcystis* and *Nodularia* induce are different than those that occur with neurotoxin exposure, it was demonstrated that these species produce hepatotoxins, not neurotoxins (Konst et al., 1961; Runnegar et al., 1981, 1988).

Isolation of the toxins called, depending on the origin, microcystins or nodularin and elucidation of their structures revealed these hepatotoxic substances to be cyclic peptides (Sivonen et al., 1989). Microcystins are composed of seven and five amino acids, respectively. Interestingly, both microcystins and nodularin always contain the 8-amino-acid 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienic acid, Adda (Rinehart et al., 1988).

**Microcystins**: Microcystins generally contain five amino acids—Adda, D-alanine, D-erythro-8-methyl-iso-aspartic acid, D-iso-glutamic acid, and N-methyl-dehydro-alanine—together with two varying amino acids, X and Y, in the cyclic peptide, with the general formula microcystin-XY (Fig. 5; Carmichael et al., 1988; Sivonen et al., 1992).

**Nodularin**: Blooms of *Nodularia spumigena* are a common phenomenon in Europe, particularly in the Baltic Sea. A hepatotoxic cyclic pentapeptide called nodularin was isolated from Baltic *Nodularia* strains (Sivonen et al., 1989). The hepatotoxic nodularin is composed of D-iso-
glutamic acid, N-methyl-2-amino-butene acid, α-erythro-β-methyl-iso-aspartic acid, l-arginine, and Adda (Fig. 6).

Antibodies against microcystins were developed that were used in immunoassays (Nagata et al., 1995). Unfortunately, these immunoassays proved to be nonspecific. Cross reactions between microcystin-RR, -LR, -YR, and nodularin were observed, and later on, mainly protein phosphatase assays were applied (Honkanen et al., 1995). Falsely-positive or -negative results only could be excluded by using a combination of an immunoassay with a protein phosphatase assay (An and Carmichael, 1994). Consequently, HPLC methods were developed for the selective determination of microcystins and nodularin, with special consideration for the most toxic structures.

HPLC determination of toxic peptides was performed with RP phases and UV detection or with a diode-array detector (DAD; Lawton et al., 1994). These HPLC methods often suffer from coelutions of toxins and matrix components that hinder the quantitative determination of hepatotoxic toxins. Therefore, the protocol for cleanup of toxin-containing extracts prior to HPLC separation has been improved (Hummert et al., 1999).

The unambiguous identification of hepatotoxic toxins is only possible by application of LC/MS (Poon et al., 1993). By application of this analytical technique, especially using tandem mass spectrometry (MS/MS), it is possible to determine the most abundant microcystins without problems. In addition, the applied chromatographic conditions allow the isolation and structure elucidation of substances suspected to be “new” microcystins (Hummert et al., 2001a, 2001b).

**SIMULTANEOUS DETERMINATION OF ALGAL AND CYANOBACTERIAL TOXINS**

The threat for human consumers from algal and cyanobacterial toxins requires powerful analytical methods. Such methods have been developed for most toxin groups. Because of the structural differences among toxins, analytical methods are typically based on HPLC separation using different stationary phases coupled to UV or fluorescence detection. By application of optical detection methods, pre- or postcolumn derivatization procedures are necessary for toxins without chromophoric groups, and cumbersome cleanup procedures prior to chromatographic separation are typically necessary to exclude interferences from matrix compounds.

These toxin-specific methods have their benefits and are in use at numerous analytical laboratories. However, there is a need for simultaneous detection methods, especially if large batches of samples have to be screened for toxins. Recently, liquid chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS) methods, which combine efficient separation power with generic detection of different toxin groups, have become available. By application of the highly selective mass detector, reliable results are obtained, and sample preparation protocols are usually simpler and less laborious (Dahlmann et al., 2003).

Ideally, risk assessments should provide information about these toxins in the phytoplankton under investigation. This information should become available immediately, for example, during research cruises or when complex phytoplankton communities from aquacultures have to be analyzed. Therefore, a universal solvent is needed for extraction of the different algal and cyanobacterial toxins, and highly selective and sensitive mass spectrometry is required in order to measure these toxins with one analytical method (Hummert et al., 2002).

**OCCURRENCE OF TOXINS IN PLANKTON FIELD SAMPLES FROM DIFFERENT LOCATIONS**

In 1997, 1998, 2000, and 2003 bulk plankton samples (net tows) from different European marine areas were taken and in part directly analyzed on board research vessels. During North Sea cruises in 1997, 1998, and 2000, transects sampled by the research vessel Heincke covered stations around the Orkney Islands, the east coast of Scotland, and a few stations from the central North Sea. The first results on the occurrence and frequency of harmful algal species were obtained in 1997 and 1998. In addition, in 1999 samples were taken along the Scottish coast during a 4-day transect, stored frozen, and analyzed later. In 2000 an HPLC/MS-coupling was established on board, and the algal toxins were measured directly after extraction of the plankton samples.

In the summer and autumn of 2000, water samples from Helgoland Roads (German Bight) as well as samples of mussel (Mytilus edulis) hepatopancreas were examined for the presence of okadaic acid with the enzymatic PP2A assay (Klöpper et al., 2003).

During the summers of 1997 and 2003 cyanobacterial blooms in the Baltic Sea were monitored by the research vessel Alexander von Humboldt.
PSP Toxins in Plankton Samples from Northern and Western European Coastal Zones

It was assumed that the blooms of the PSP producer *Alexandrium* spp. would show higher total toxicity in coastal zones than in offshore regions because of higher cell density. The first measurements, in 1997–1998, revealed the highest toxicity was in samples from coastal regions near the Orkney Islands, whereas PSP contamination in the inner part of the islands (Scapa Flow) was rather low (see Fig. 7).

The PSP profiles found in these marine areas were typically of *Alexandrium tamarense* (see Fig. 8). Nevertheless, temporal but also slightly geographical differences in toxin profiles could be observed. Shown in Figure 9 in multidimensional ordination (similarity matrix, euclidean distances of toxin compositional data) are 110 toxin profiles from plankton samples whose PSP content was greater than 100 ng L⁻¹ from different years and different sampling areas along the Scottish coast. In addition, the results of a long-term incubation experiment with a toxic *A. tamarense* strain isolated in 1997 southeast of the Orkneys are included for estimation of the influence of toxin profiles of aging dinoflagellates on MDS ordination. It is obvious that the samples from 1998 and 2000 form a distinct and dense cluster, which comprises sampling locations from the Orkneys to areas southeast of the Firth of Forth and from the coast to remote stations in the North Sea up to 100 nautical miles away. Five samples from May 1998 form a subcluster. Because other samples from this set, which were taken in a confined area during a 3-day sampling campaign, clearly fall in the main cluster, it remains unclear whether the ordination is a result of genetic differences in the population or of physiological adaptation. In contrast, all samples from 1999 form their own cluster, presumably indicating a genetically different population. The initial toxin profile of *A. tamarense* culture 57OK1A was related to the *in situ* profiles of plankton samples from 1998 and 2000 but changed remarkably during the aging process of the culture. Further

Fig. 7. PSP content of plankton samples off the Orkney Islands and the Scottish Coast in May 1997.

Fig. 8. PSP profile, typically for the strains of *Alexandrium* in the northwest Atlantic.
field studies are needed to differentiate genetic from physiological impacts on toxin profiles of *A. tamarense* blooms by combining molecular and chemical-analytical studies (Medlin et al., 1998).

**DSP Toxins in Plankton Samples from Northern and Western European Coastal Zones**

It is well known that algae communities in the North Sea produce high amounts of okadaic acid (Kat, 1979, 1983; Dahl and Yndestad, 1985; Lassus and Bandouil, 1985; Edebo et al., 1988), an observation confirmed by analyses of plankton samples from the North Sea for DSP content. During the cruise in May 2000 DSP determination was carried out directly on board by injecting undervatized sample extracts into the LC/MS coupling. The measurements revealed the presence of DTX-2 in addition to OA. DTX-1 was found only along the British coast as a minor component in addition to OA and DTX-2. Near the Orkney Islands DTX-2 was the dominant DSP toxin (Fig. 10).

In the German Bight (North Sea) around the island of Helgoland, two toxic *Dinophysis* species exhibited successive biomass maximums in the summer of 2000 (*D. norvegica* and *D. acuminata*). In contrast to findings in many other marine areas, the toxicity of *Mytilus edulis* (hepatopancreas) could be clearly attributed to the observed increase in the abundance of cells of both species (Klöpper, 2003).

Interestingly, elevated toxin levels in the hepatopancreas tissue of mussels were detected long before the dinoflagel-lates reached their peak intensities (Fig. 11). Chemical analytical measurements by LC/MS revealed the presence of OA and DTX1.

**Fig. 9.** MDS ordination plot (multidimensional scaling) of PSP toxin profiles (similarity matrix, euclidic distances) of plankton from the eastern Scottish coast sampled in May of 1998, 1999, and 2000 and of an *A. tamarense* 57OK1A culture, isolated in May 1997 southeast of the Orkneys.

**Fig. 10.** HPLC-MS chromatograms of plankton samples from different sampling sites. (a) DSP profile in plankton sample from the North Sea. (b) DSP profile in plankton sample from near the Orkney Islands.
ASP Toxins in Plankton Samples from Northern and Western European Coastal Zones

The presence of domoic acid also was reported in European waters (Lundholm et al., 1995; Dizer et al., 2001). Therefore, during the May 2000 cruise plankton samples also were analyzed for domoic acid directly on board by application of LC/MS. The greatest amounts of domoic acid were found in samples from the southeast Orkney Islands and along the Scottish coast (Fig. 12).

Spirolides in Plankton Samples from Northern and Western European Coastal Zones

To obtain data about spirolides in European waters, analytical tools for their identification and determination were established. An LC-MS method allowing the simultaneous determination of different algal toxins was developed (Hummert, 2003). This method was applied to bulk plankton samples (net tows) on board the research vessel Heincke during the research cruise to coastal areas on the east coast of Scotland in May 2000 to obtain data directly where toxins are produced. The results are shown in Figure 13.

Because the volume of the water sampled with the plankton net was not recorded in the present study, qualitative results indicating the presence or absence of spirolides were obtained. Hence, the high, medium, and low expression shown in Figure 13 are reflecting only the relative spirolide content of net tows. The exact quantification of the spirolide concentration in natural plankton samples will be a subject of future studies.

Because samples from the cruise were also examined microscopically (Utermöhl counts) and by fluorescence in situ hybridization using taxon-specific gene probes, it could be shown that spirolide-producing dinoflagellates (A. ostenfeldii) were present in the phytoplankton of the Scottish coast (John et al., 2003). From the qualitative results, it is obvious that a large area of the Scottish East Coast was affected by spirolides. Figure 14 shows the LC/MS chromatogram of an extract from sample S 161. The two signals with the highest intensity were observed at RT 7.7 and RT 9.6 min, whereby the SIM chromatograms showed characteristic spirolide-related masses.
The results show clearly that the occurrence of spirolides is not restricted to Canadian coastal waters, and therefore this toxin class is not only a North American problem. During May 2000 spirolides were detected in a European coastal zone 240 by 60 nautical miles in size, in which the total content of spirolides differed over a broad range. In addition, the profiles of toxins showed clear variation between regions. The spirolide composition at the sampling sites south of the Firth of Forth (S 130–S 179) appeared to be relatively constant, with all samples from this area obtained on 4 days between May 18 and May 21. One day later a different profile was observed on the latitude of Dundee at S 189 (north of the Firth of Forth), and the toxin composition here was similar to that obtained 2 weeks earlier at S 15 (approximately 60 km northeast of S 189). The estuary system of the Firth of Forth seems to form a borderline between two different spirolide-producing plankton communities or distinct spirolide-producing species.

These profile variations were obviously not time related because only 24 h passed between sampling at S 172 (south of the Firth of Forth) and S 189 (north of the Firth of Forth). Nevertheless, absolutely different profiles were observed. On the other hand, two stations north of the Firth of Forth (S189 and S15), sampled 2 weeks apart, displayed very similar spirolide profiles. It can generally be stated that the spirolide content in the sample material obtained north of Aberdeen was much lower than that in the rest of the area under investigation. However, a characteristic spirolide profile also could be determined for the area east of the Orkney Islands.

The causative organism in European waters also could be an *Alexandrium* species, probably also *A. ostenfeldii*. On the other hand, the different spirolide profiles observed on the Scottish East Coast may indicate different species or strains. Because of the sampling procedure, a large amount of water was sampled by the net. Organisms of higher trophic levels also were included in the samples. Hence, spirolides that

### TABLE I. Nodularin content of plankton samples from different areas of the Baltic Sea in July 2003

<table>
<thead>
<tr>
<th>Station</th>
<th>Sea Area</th>
<th>Date</th>
<th>Nodularin (µg. L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>Mecklenburg Bight</td>
<td>25.07.03</td>
<td>258</td>
</tr>
<tr>
<td>213</td>
<td>Bornholmsea (East)</td>
<td>25.07.03</td>
<td>228</td>
</tr>
<tr>
<td>245</td>
<td>Gotlandsea (West)</td>
<td>28.07.03</td>
<td>149</td>
</tr>
<tr>
<td>240</td>
<td>Gotlandsea (West)</td>
<td>28.07.03</td>
<td>804</td>
</tr>
<tr>
<td>286</td>
<td>Gotlandsea (East)</td>
<td>29.07.03</td>
<td>404</td>
</tr>
<tr>
<td>271</td>
<td>Gotlandsea (East)</td>
<td>29.07.03</td>
<td>166</td>
</tr>
<tr>
<td>259</td>
<td>Gotlandsea (South)</td>
<td>31.07.03</td>
<td>158</td>
</tr>
</tbody>
</table>
Fig. 15.
had already entered the food web by ingestion of microalgae and that had been metabolized also were analyzed. Unfortunately, little is known about the fate of spirolides in passage through the food web. Therefore, further studies are needed about the species affected and how spirolides are metabolized.

**Cyanobacteria Toxins in Plankton Communities from the Baltic**

During the summers of 1997 and 2003 extensive blooms of *Nodularia spumigena* were observed in the entire Baltic Sea area, also resulting in production of the hepatotoxic nodularin (see Table I).

Independent of sampling site, the toxin profile revealed no differences in all the plankton samples from the open sea. The peak of nodularin dominated the chromatograms obtained from extracts of the plankton samples [Fig. 15(a,b)]. However, the phytoplankton sample from the brackish water of the Fehmarn Island [Fig. 15(c)] turned out to be an exception. In these samples the microcystins, presumably produced by cyanobacterial species other than *N. spumigena*, occurred together with nodularin in the chromatograms. This observation underlines the importance of extending the monitoring of harmful algal blooms in coastal areas to all cyanobacterial toxins.

**MONITORING PROGRAMS AND REGULATIONS**

Because observed cases of human intoxication frequently occur after mussel consumption, governmental institutions as well as the fishery industry have established monitoring programs so that phycotoxin-contaminated seafood does not reach the consumer (Reguera et al., 1991). Since these monitoring programs were introduced, there has been no additional incidence in human beings, except when potential consumers or markets did not notice warnings or when the information was not forwarded to end users in time (van Egmond et al., 1993). The successful protection of consumers from algal toxin...
poisoning resulted from continuous actualization of the decrees and regulations of national authorities and international committees dealing with seafood trade. Furthermore, efforts were made to harmonize legal regulations and monitoring methods in order to provide a common basis for risk assessment (Luckas, 2000b; Commission of the European Communities, 2002).

REFERENCES


and sensitive assessment of okadaic acid contamination in mussels. Toxicon 34:743–752.


