

Diarrhetic shellfish toxicity in relation to the abundance of *Dinophysis* spp. in the German Bight near Helgoland

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ABSTRACT: Diarrhetic shellfish toxicity is caused by the accumulation of okadaic acid and its derivatives, which are produced by particular species of *Dinophysis* Ehrenberg 1839 and *Prorocentrum* Ehrenberg 1833 (Dinoflagellata). In the German Bight (North Sea) around the island of Helgoland, 4 toxic *Dinophysis* species occur, of which 2 exhibited successive biomass maxima in summer 2000 (*D. norvegica* Claparède et Lachmann 1859 with cell concentrations of max. 400 cell l⁻¹ and *D. acuminata* Claparède et Lachmann 1859 with max. cell concentrations of over 4000 cells l⁻¹). In contrast to findings in other marine areas, toxicity of *Mytilus edulis* Linné 1758 could be clearly attributed to the observed increases in cell abundances of both species. In several *Mytilus* samples toxin concentrations (max. 460 ng diarrhetic shellfish poisoning [DSP] toxins g⁻¹ hepatopancreas) were in a range which is considered dangerous for human consumption. While mussel toxicity coincided with concentration increases of both *Dinophysis* species, toxicity of the particulate substance in the water (max. 26 ng l⁻¹) could be detected only during cell concentration maxima of *D. acuminata*. Mussel toxicity lasted 3 wk after the end of the *D. acuminata* bloom, and this is attributed to extended toxicity of decaying *Dinophysis* cells and detritus. Elevated summer temperatures and low silicate concentrations evidently supported the development of high concentrations of *D. acuminata*.

KEY WORDS: Toxic dinoflagellates · Diarrhetic shellfish poisoning · DSP · Harmful algal blooms · HAB · Phytoplankton

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INTRODUCTION

In the German Bight of the North Sea, around the island of Helgoland, dinoflagellates of the genus *Dinophysis* Ehrenberg regularly occur during summer. Long-term observations from the Helgoland Reede time-series indicate that highest cell concentrations arise in summer or late summer (Hickel et al. 1997, E. Hagmeier unpubl.). In various European coastal regions (e.g. Norway, Denmark and Sweden), *Dinophysis* spp. are suspected of being responsible for diarrhetic shellfish poisoning (DSP) (Séchet et al. 1990, Anderson et al. 1996, Lindahl & Anderson 1996). DSP results from a widely distributed type of contamination

of edible bivalves like mussels, which feed by filtering particles from seawater. These particles, which accumulate in the digestive glands and stomach (hepatopancreas), may potentially contain toxic phytoplankton cells like *Dinophysis* spp. In cases of high cell abundances of these toxic species, the consumption of only 1 mussel may be enough to cause toxic effects in humans. Although the first detailed report of DSP dates from 1978 in Japanese waters (Yasumoto et al. 1978), gastrointestinal poisoning of humans after consumption of mussels had been observed about 20 yr earlier in northern Europe (Krogh et al. 1985).

The toxins which cause DSP are the polyether molecules okadaic acid (OA), its derivatives dinophysistoxin-

1, -2 and -3 (DTX), 7-acetyl okadaic acid, okadaic ethyl ester, as well as yessotoxin and pectenotoxins (Yasumoto et al. 1989). The gastrointestinal symptoms of DSP, which causes extreme liquid loss, begin within 30 min to a few hours after consumption of toxic shellfish and consist of abdominal pain and cramps, incapacitating diarrhea, nausea, vomiting and chills (Tubaro et al. 1996). The ailment clears up within 3 d with or without medical treatment (Hallegraeff 1993). Fatal incidents based on DSP have not been recorded, but the DSP toxins may have the potential to induce tumors in the gastrointestinal tract (Suganuma et al. 1988).

Findings about correlations between *Dinophysis* cell concentrations and DSP toxicity of mussels have been controversial. For example, investigations in French coastal waters revealed no clear relationship between shellfish toxicity and *Dinophysis* spp. occurrence (Lassus et al. 1991). Similar observations were made in a Norwegian bay: no apparent relation between *Dinophysis* spp. concentrations and mussel toxicity was observed (Dahl & Johannessen 2001). On the other hand, in a Norwegian fjord DSP-toxin concentrations in blue mussels followed changes in *Dinophysis* spp. cell densities (Séchet et al. 1990).

We carried out our investigations in spring and summer 2000 in order to search for a relationship between *Dinophysis* spp. cell densities and mussel toxicity in the German Bight near the island of Helgoland.

MATERIALS AND METHODS

We investigated which *Dinophysis* species were potentially involved in causing DSP effects by monitoring their cell concentrations. Furthermore, we measured DSP toxins in mussels and in the particulate substance of the water column.

Investigation area and sampling. The investigations were carried out near the island of Helgoland in the German Bight (North Sea) from April to October 2000. Phytoplankton samples (1 l) were taken daily by means of sampling bottles from the surface parallel to the sampling of the Helgoland Reede time-series at the position 'Kabeltonne', which is located between the island and the dune (54° 11.3' N, 7° 54' E; Fig. 1; Hickel et al. 1997). An aliquot of 100 ml seawater was fixed with a hexamin buffered 20% formaldehyde solution (final concentration 0.5%). *Dinophysis* spp. cells and other potentially harmful phytoplankton species were identified and counted according to the method described by Utermöhl (1958). The

inverse microscope (Zeiss Axiovert 135) was equipped with light, phase contrast and differential interference contrast objectives and with a video imaging system. Additionally, samples were taken by net (20 µm mesh size) for qualitative analysis and documentation. Samples were stored in 200 ml bottles with a higher concentration of fixative (2% final concentration).

To determine the DSP-toxin content of the particulate substance in the water, 3 samples of a larger amount of seawater (up to 15 l) were taken by sampling bottle from the surface, parallel to the phytoplankton sample. Up to 5 l of water were vacuum filtered on GF/C filters (47 mm diameter, nominal pore size 1.2 µm), and were stored at -20°C until measurement.

Specimens of *Mytilus edulis* were collected at least once a week in an intertidal rocky area at the north-eastern part of the island. The number of mussels varied from 5 to 16, depending on their size. In the laboratory the mussels were steamed for 2 min and the hepatopancreas (stomach and digestive glands) was separated and analyzed immediately or stored in 3 aliquots of at least 1 g wet weight at -80°C until analysis. Due to the varying size of the collected mussels, 1 to 5 specimens were required per analysis.

Salinity and temperature data were obtained from the Helgoland Reede time-series (Hickel et al. 1997).

Determination of DSP toxins. Toxins from particulate substance and mussels were extracted according to Tubaro et al. (1996), with some modifications. The thawed GF/C filters with the particulate substance

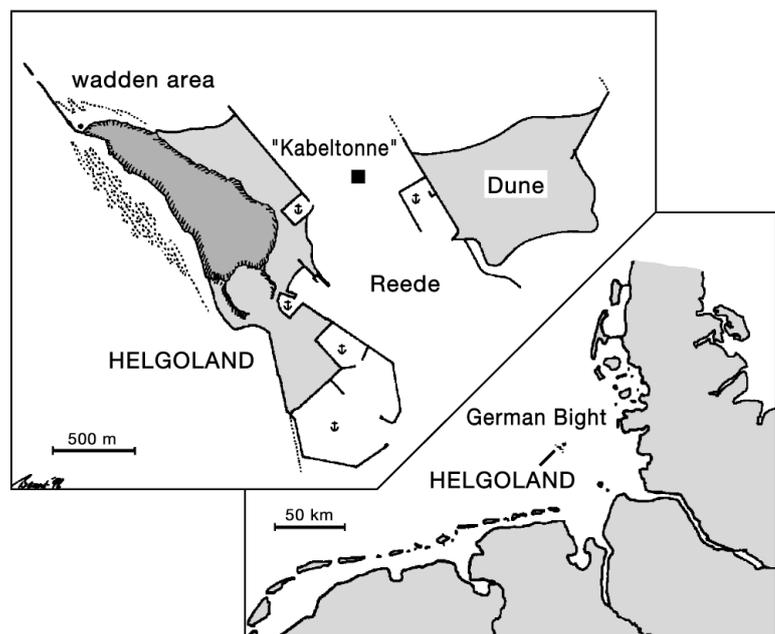


Fig. 1. Location of Helgoland in the German Bight (North Sea), showing the sampling sites 'Kabeltonne' (Helgoland Reede time-series) and the intertidal rocky area

samples were homogenized in 1 ml liquid (50% methanol, 50% Milli-Q) by ultrasound (1 min, 60 W). Subsequently, the liquid was taken up by a syringe and centrifuged (10 min, $16\,000 \times g$). The supernatant was evaporated and the dry matter was once more extracted in 100 μ l methanol. If not immediately used, the liquid samples were stored at -20°C .

Samples of *Mytilus edulis* hepatopancreas were homogenized for 1 min by Ultra-Thurrax in liquid (50% methanol, 50% Milli-Q). After centrifugation (10 min, $1993 \times g$) and addition of 10 ml Milli-Q, to lower the methanol portion, the supernatant was liquid-extracted twice with an equal quantity of ethyl acetate (10 ml). A formation of a third phase, which seemed to be a fatty emulsion, was observed in several samples. Sodium chloride (3 g) was added to support the division of the phases. The pooled organic phases were dried on 3 g sodium sulfate. After removing the liquid with a vacuum concentrator, the dry matter was dissolved in 100 μ l methanol. The samples were stored at -20°C until measurement.

The DSP-toxin contents of the particulate substance and hepatopancreas of *Mytilus edulis* were assessed with a protein phosphatase Type 2A (PP2A) inhibition assay, described by Tubaro et al. (1996), applied with a few modifications. In this assay, DSP toxins are measured as OA equivalents. The amount of enzyme added (0.01 or 0.02 U of protein phosphatase Type 2A, isolated from human blood cells, Biomol) differed because of the varying activity of the charges used. For the analyses, multiplate wells were used. Each well contained 20 μ l enzyme substrate (Sigma 104[®] P-nitrophenyl-phosphate, P-NPP, 0.282 M), 1 μ l sample, and 1 or 2 μ l PP2A-dilution. It was filled with buffer (40 mM Tris/HCl, 34 mM MgCl_2 , 4 mM EDTA, 4 mM DL-dithioereithol, pH 8.4) to a volume of 200 μ l. Measurements were carried out in a spectrophotometer (Anthos luminometer lucy 1 16800) at 405 nm wavelength. In order to inhibit the enzymatic activity before starting the measurement, the multiplate well was stored on ice. Nine OA concentrations, with 2 parallel batches in each set-up, ranging from 0 to 5 $\mu\text{g ml}^{-1}$ diluted in methanol, were prepared from a stock solution (5 $\mu\text{g ml}^{-1}$, from *Prorocentrum concavum* isolates, Sigma). One concentration series was measured on each plate to obtain a calibration curve.

RESULTS

Dinophysis spp. near the island of Helgoland

Four *Dinophysis* species were observed off Helgoland during summer 2000. The highest cell concentrations of *Dinophysis* spp. were recorded from mid-

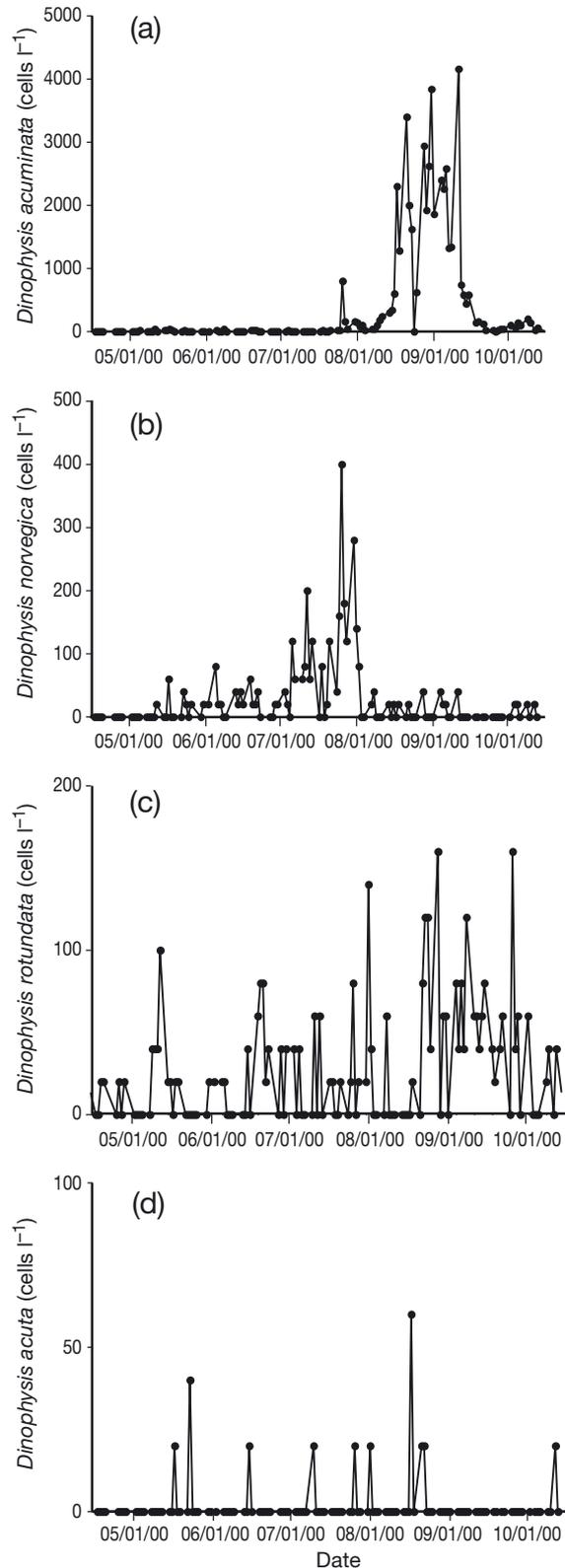


Fig. 2. *Dinophysis* spp. Concentrations measured during the investigation period (April 15 to October 15, 2000): (a) *D. acuminata*; (b) *D. norvegica*; (c) *D. rotundata*; and (d) *D. acuta*. Dates given as mm/dd/yy

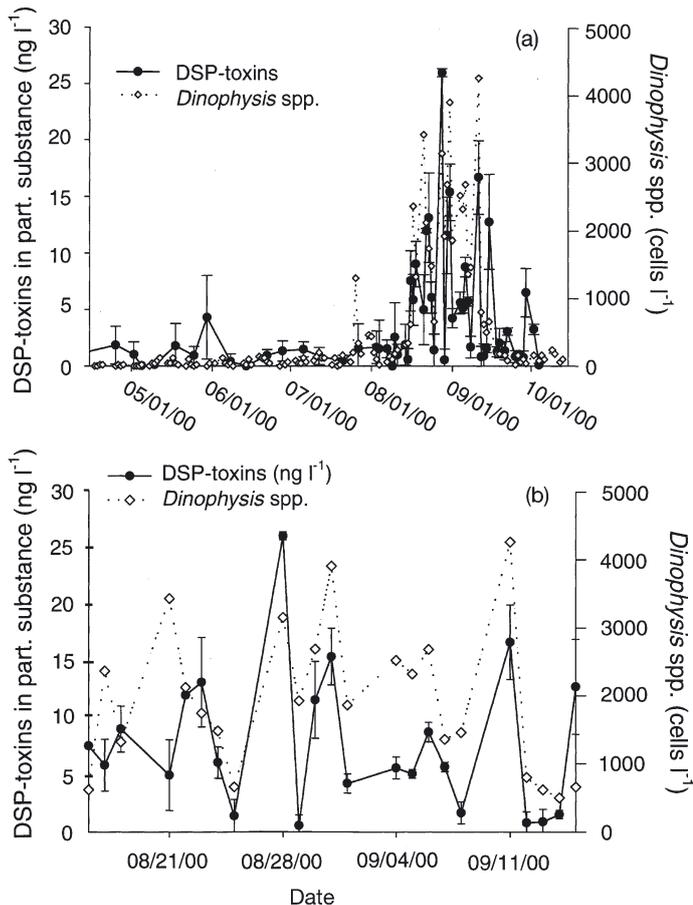


Fig. 3. Concentrations of diarrhetic shellfish poisoning (DSP) toxins in the particulate substance of the water column. Also indicated are concentrations of *Dinophysis* spp.: (a) during entire investigation period (mid-April until mid-October 2000); (b) from mid-August until mid-September. Dates given as mm/dd/yy

August to mid-September. The most abundant species was *D. acuminata* Claparède et Lachmann 1859, with a maximum of 4260 cells l⁻¹ in early September (Fig. 2a). Cell concentrations of *D. norvegica* Claparède et Lachmann 1859, which was the second most abundant *Dinophysis* species, increased during July to concentrations of 400 cells l⁻¹, about a tenth of maximum *D. acuminata* cell abundances (Fig. 2b). *D. rotundata* Claparède et Lachmann 1859 and *D. acuta* Ehrenberg 1841 (in order of detected cell maxima) played a minor role, and concentrations >160 cells l⁻¹ were never recorded (Fig. 2c,d).

DSP-toxin concentrations in particulate substance of the water column and in mussel hepatopancreas

The increase in DSP toxins in the particulate substance of the water column coincided with the peak

of *Dinophysis* spp. between mid-August and mid-September (mainly caused by *Dinophysis acuminata*, Fig. 3a). During this period, toxin concentrations increased ca. 5-fold and more from previous values of ca. 2 ng l⁻¹. Meanwhile, *Dinophysis* cell concentrations varied between 1000 and 4000 cells l⁻¹.

During the interval of highest *Dinophysis* cell concentrations (mid-August to mid-September) the slope of the linear regression of cell concentrations against particulate substance toxin concentrations indicates an average toxin content of 3.3 pg cell⁻¹ (DSP toxins measured as OA equivalents; Fig. 4). Interestingly, the toxin content in the water column stayed rather high until the beginning of October (Fig. 3a). At the beginning of this period, the oscillations of cell concentrations and DSP-toxin concentrations in the particulate substance took opposite courses. On days with higher concentrations of *Dinophysis* spp., less particulate substance toxicity was measured and vice versa. After approximately 1 wk, the situation changed and the development of cell and DSP toxin concentrations ran parallel (Fig. 3b).

The first noticeable rise in DSP-toxin content in mussel hepatopancreas tissue (HP) was measured on 14 June. Concentrations ranged between 140 and 190 ng g⁻¹ HP until the third week of August (Fig. 5). The described increase in *Dinophysis norvegica* took place in the middle of this period, during July. From the 4th week of August the toxicity of the mussels increased to concentrations over 400 ng g⁻¹ HP. The concentrations of OA and its derivatives in the HP fluctuated from then on, yet remained at a high level for 6 wk until the beginning of October (Fig. 5). High densities of *D. acuminata* were detected only until mid-September. During the period of very high HP DSP-toxin contents, some samples contained concentrations

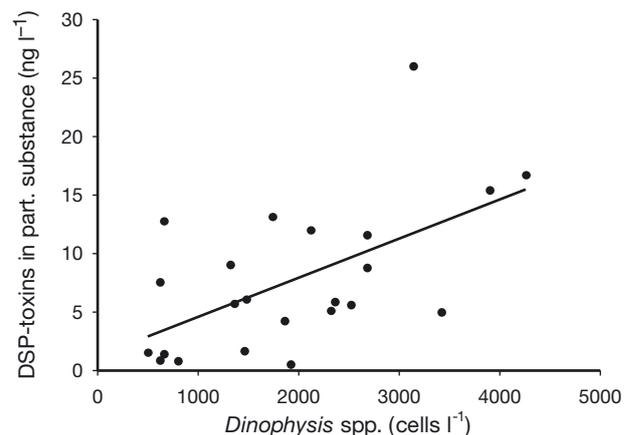


Fig. 4. *Dinophysis* spp. Linear regression of cell concentrations against particulate substance toxin concentrations during the period from mid-August until mid-September ($y = 0.0033x + 1.3224$; $r^2 = 0.33$; $p < 0.005$). Dates given as mm/dd/yy

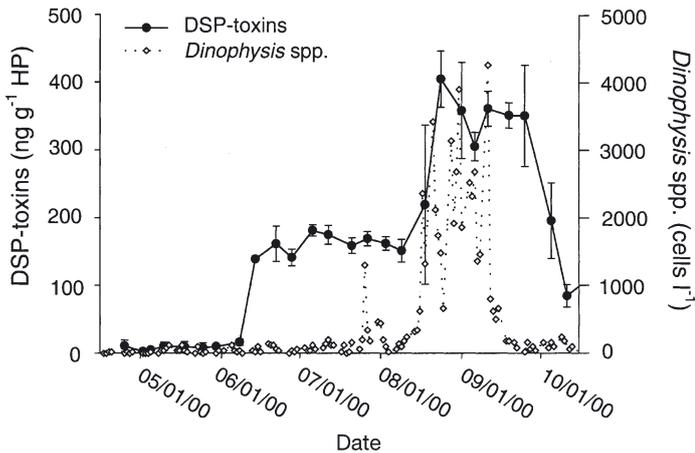


Fig. 5. Concentrations of okadaic acid and its derivatives (DSP toxins) in mussel hepatopancreas tissue (HP) during the investigation period (mid-April until mid-October 2000). Indicated as well are concentrations of *Dinophysis* spp. Dates given as mm/dd/yy

which were even above the highest OA concentration of the calibration curves (5 ng OA in 1 µl calibration liquid).

In the first week of October, mussel toxicity started to decrease, and in the second week values were lower than 100 ng g⁻¹ HP (Fig. 5).

The protein phosphatase 2A inhibition assay is an indirect method to determine DSP toxicity. Because it measures inhibition of phosphatase activity, the possibility exists that interference by phosphatase inhibitors other than the DSP toxins could occur, which may share the same binding site on the PP2A molecule (Takai et al. 1995). Substances known as inhibitors are microcystin-LR (isolated from fresh and brackish water cyanobacteria), calyculin A (marine sponge; Bagu et al. 1996) and tautomycin (soil bacteria; Serres et al. 2000). We can exclude those substances as the responsible inhibitors of the PP2A used in our assays, because these organisms do not occur in the German Bight of the North Sea. During our investigations some samples were measured by high performance liquid chromatography (HPLC). These measurements corroborated our results. During the increase in *Dinophysis* cell concentrations and in particulate substance and mussel HP toxicity (measured by PP2A inhibition assay), an increase in DSP toxins was measured as well by HPLC (B. Luckas pers. comm.).

Temperature and salinity

Salinities fluctuated from about 32 to 34 from mid-April until the end of July. During August salinities dropped to below 32 and started to increase again by

the end of the month, until the end of the investigation period. The highest temperatures (max. daily value 17.8°C) were also recorded in August. Temperatures started to decrease during the second week of September (Fig. 6).

During our investigation period from mid-April to mid-October, the lowest salinities coincided with the warmest period. The interval of highest cell densities of *Dinophysis acuminata* occurred during this period.

Nutrients

Nitrate concentrations were higher than 30 µmol l⁻¹ at the beginning of the investigation period and decreased to less than 10 µmol l⁻¹ at the end of May. In July the concentrations dropped further to less than 5 µmol l⁻¹. The lowest concentrations of nitrate were measured in August and September; towards the end of September the concentrations increased again (Fig. 7a).

Ammonium concentrations ranged from 0 to >3 µmol l⁻¹, with strong oscillations. Highest concentrations were measured in the period from the end of July until mid-August (Fig. 7a).

Similar to nitrate, the silicate concentrations were highest (>8 µmol l⁻¹) in April. They decreased until exhaustion in the first half of June but recovered during summer (with another maximum of >8 µmol l⁻¹ in the first half of July). A second drop in concentrations occurred in the second half of August, with a subsequent recovery (Fig. 7b).

In the third week of April, daily phosphate concentrations fluctuated between 0.7 and 1.1 µmol l⁻¹. In May, concentrations oscillated between 0 and 0.8 µmol l⁻¹. Afterwards, until the end of June, phos-

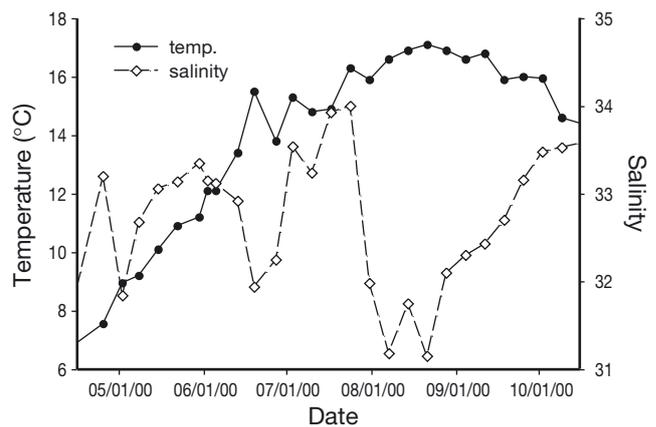


Fig. 6. Weekly medians of temperature and salinity at the sampling site 'Kabeltonne' during the investigation period (April 15 to October 15, 2000). Dates given as mm/dd/yy

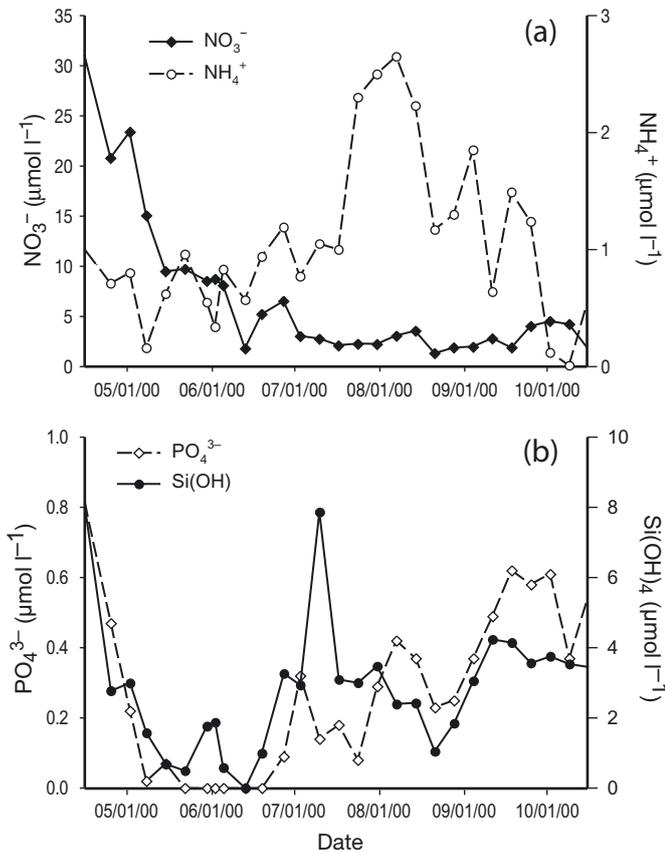


Fig. 7. Weekly median concentrations of inorganic nutrients at the sampling site 'Kabeltonne' during the investigation period (April 15 to October 15, 2000). (a) Concentrations of nitrate and ammonium; (b) concentrations of phosphate and silicate. Dates given as mm/dd/yy

phate was generally not detectable. Concentrations recovered towards July, with strong oscillations (0.2 to 1.1 μmol l⁻¹) but an overall tendency to increase (Fig. 7b).

DISCUSSION

Dinophysis abundance and DSP toxicity near Helgoland

Our investigations demonstrated, for the first time, the presence of DSP toxins in the German Bight near Helgoland. Two *Dinophysis* species, *D. acuminata* and *D. norvegica*, appeared to be mainly responsible for DSP toxins in the particulate substance of the water column and hepatopancreas of mussels *Mytilus edulis*.

The first conspicuous increase in OA and its derivatives in mussels was detected in mid-June 2000, when the total concentration of *Dinophysis* was around

100 cells l⁻¹. In this period, during which medium to high HP toxin concentrations were found, the only observed increase in concentrations of *D. norvegica* (beginning of July until beginning of August), as well as the first increase in concentrations of *D. acuminata* (end of July until mid-August) occurred. Both species exhibited relatively low concentrations, resulting in an average *Dinophysis* spp. concentration of ca. 200 cells l⁻¹. In contrast to the distinct increase in toxicity of mussel HP, the samples of the particulate substance of the water column did not reveal any comparable changes in DSP-toxin concentrations. Taking into consideration the DSP-toxin concentration of ca. 3.3 pg cell⁻¹ which we estimated for summer, concentrations in the water column would have increased by less than 1 ng l⁻¹, a signal not clearly distinguishable assuming average background concentrations of 1.3 ng l⁻¹ and its variability (Fig. 3). The increase in toxins in the HP can be related to the feeding behavior of mussels, which accumulate particles like phytoplankton cells, and hence their toxins, in their digestive tract. One mussel filters ca. 30 l of seawater d⁻¹ (Clausen & Riisgard 1996) (taking into consideration the tidally induced dry periods in the intertidal rocky area), resulting in an uptake of 6000 cells d⁻¹ at a cell concentration of 200 cells l⁻¹, provided that the filtration rates are not affected by *Dinophysis*. Consequently, each mussel took up 19.8 ng DSP-toxin d⁻¹ during that period. Taking into account the average weight of HP per mussel (0.54 g), the resulting concentration would have been 37 ng DSP toxin g⁻¹ HP. The concentrations measured during this period were ca. 5 times higher. Hence, it appears that the mussels indeed accumulated the toxins in their digestive tract. This estimation, furthermore, is conservative because it is implying a 100% absorption efficiency. However, there is evidence that *Mytilus* do not absorb the entire organic material they filter (Hawkins et al. 1998, Cranford & Hill 1999).

The above estimations were made assuming a direct relationship between the *Dinophysis* concentrations and HP toxin concentrations measured. The distance between the 2 sites is a little less than 1.5 km (Fig. 1), which implies that there may be some uncertainty regarding the *Dinophysis* concentrations present at the mussel sampling site itself. However, we do not expect the *Dinophysis* concentrations to be very different in the intertidal rocky area because, due to the tidal currents which change their direction nearly twice a day, close interactions between both sites is guaranteed. For logistical reasons, sampling of *Dinophysis* and the particulate substance always had to be carried out at the same time of day. In order to compensate for variability in *Dinophysis* and particulate substance concentrations due to variable tidal currents, we carried

out high frequency sampling (daily). In summary, we conclude that the measured concentrations of both parameters are adequately representative of the concentrations at the mussel sampling site.

Effects of toxic algae on mussels

Results of investigations on clearance rates of mussels exposed to toxic phytoplankton species vary. The presence of high densities of DSP-producing microalgae might reduce the clearance rates of *Mytilus edulis* as described by Pilet et al. (1995). Bauder et al. (2001) measured similar clearance rates when scallops *Argopecten irradians* Lamarck 1819 were exposed to a toxic microalgae diet, in contrast to exposure to non-toxic phytoplankton, but a lower absorption efficiency of organic matter was observed. Sidari et al. (1998) described a feeding preference of blue mussels *Mytilus galloprovincialis* Lamarck 1819 for *Dinophysis* spp., without detecting a correlation between the cell amounts in the digestive glands and the DSP-toxin content. The apparent insensibility of *Mytilus* spp. to DSP-toxin-producing species appears to have its origin in the apparently harmless effects on the animals themselves. *M. galloprovincialis* exposed to low cell densities of dinoflagellates *Heterocapsa circularis-quama* Horiguchi 1995, which induce mussel mortality by direct cytotoxicity, show significantly reduced clearance rates (Matsuyama et al. 1997).

Delayed detoxification of mussels

We detected high DSP-toxin levels in the mussel HP up to ca. 3 wk after the period of highest *Dinophysis* cell concentrations (until the first week of October). The evidently long residence time of DSP toxins in mussels has important implications for the handling of mussels for human consumption. In an area where *Dinophysis* spp. were apparently DSP-toxin producers, Dahl et al. (1995) lowered mussels to a water depth where concentrations of algae were presumed to be low. They concluded that this technique was not suitable for depuration from toxins because the mussels lost weight and quality due to starvation. A different approach was the transplantation of mussels from a 'toxic' environment to a 'less toxic' environment. An experiment at the Swedish coast showed an average decrease of 12 μg OA 100 g^{-1} mussel meat per day (Haamer et al. 1990b). Depending on the dimensions of a *Dinophysis* spp. bloom, the possibility of moving a mussel farm could effectively protect against or decontaminate mussels from toxification.

DSP toxins in *Dinophysis*

The relatively weak but significant correlation between *Dinophysis* concentrations and toxin concentrations in the water column during the *D. acuminata* bloom (mid-August until mid-September) might have been caused by variable toxin content in the cells during the bloom process. The dynamics of DSP-toxin production are poorly understood, especially the reasons for the broad range of toxin content per cell and the ratios of OA and its derivatives (Johannson et al. 1996). Ramstad et al. (2001) found that *Dinophysis* peaks and the concentrations of OA and its derivatives (DTX) in the water column do not always correlate. Provided that *Dinophysis* spp. are the only producers of DSP toxins in the investigation area, the occurrence of DSP toxins in the particulate substance of the water column until the end of September, 2 wk after cell concentrations of *Dinophysis* had decreased to prebloom levels, is remarkable. This phenomenon might have been caused by a possible retention of the toxins in dead *Dinophysis* cells and detritus after the disappearance of *Dinophysis*. On the other hand, it might be a result of the position of the sampling area and the sampling method, as will be discussed below. An extended presence of DSP toxins in the detritus fraction and decaying *Dinophysis* would imply a preceding toxin accumulation in mussels by the uptake of particles.

If we apply the decontamination rate measured by Haamer et al. (1990b), the most contaminated mussels we encountered during our investigations (around 400 ng g^{-1} HP) would have been detoxified in ca. half a day. The extended mussel toxicity present 3 wk after the end of the bloom might have been due to the survival of DSP toxins in detritus and decaying *Dinophysis*. However, in our case it might also have resulted from slower detoxification rates than those measured by Haamer et al. (1990b). It is known, for example, that *Mytilus* needs several weeks to detoxify paralytic shellfish toxins (Bricelj & Shumway 1998).

Public health concerns

The decontamination rate found in this study shows the need to continue monitoring toxin contents of commercial mussels well after *Dinophysis* concentrations have decreased to background levels. During the *Dinophysis acuminata* bloom, many mussel HP samples contained DSP-toxin concentrations close to, and some even above, the legal threshold of 450 ng g^{-1} HP (Bundesministerium für Gesundheit 2000). The respective EU directive (EU 1991) prohibits pro-

duction or marketing of mussels when test results are positive for DSP toxins in the edible parts. Compared to maximum *Dinophysis* spp. concentrations of the last 10 yr, the maximum cell density during our investigation period was relatively low, since abundances exceeded 10 000 cells l⁻¹ several times, and in one year reached 100 000 cells l⁻¹ (E. Hagmeier unpubl.). Regarding the observed mussel toxicity in our investigation, the public health risk of DSP is quite obvious. There is evidence that even lower concentrations of *Dinophysis* spp. can cause higher mussel toxicity than that found in our investigation (Sidari et al. 1998). In comparison to other toxic algae, bloom concentrations of *Dinophysis* spp. are in general relatively low. Yet Dahl et al. (1996) described one case of a mass occurrence of *D. norvegica* (23×10^6 cells l⁻¹), *D. acuta* (1.3×10^6 cells l⁻¹) and *D. acuminata* (1.3×10^6 cells l⁻¹) in combination with high cell concentrations of a heterotrophic dinoflagellate (*Polykrikos schwartzii* Bütschli, 11×10^6 cells l⁻¹) which caused a non typical coloration of seawater for *Dinophysis* spp. blooms.

Why does *Dinophysis* regularly bloom in summer?

Research on harmful algal blooms is focussed on the possible prediction of toxic algal events in order to avoid or reduce health and economic damage. Monitoring of physical and chemical parameters can be a tool for this. A decrease in salinities preceded the bloom (from the beginning of August, Figs. 2a & 6). These findings are in contrast to Haamer et al. (1990a), who found that coastal water with lower salinities appears to counteract the OA contamination of mussels by producing poor conditions for cellular OA synthesis. During the period of decreased salinities, seasonal temperatures were highest, which might be a prerequisite for *Dinophysis* blooms in that region. Lassus et al. (1991) concluded from their investigations at the French coast that *Dinophysis* spp. development occurs in relatively warm and low-salinity surface waters. Several authors mentioned a stratified water body as an additional condition (Lassus et al. 1991, Delmas et al. 1992). We assume that stratification occurred around the island of Helgoland from the beginning of August until mid-September because of calm seas and relatively high surface water temperatures (Fig. 6). We do not have evidence for this, as we did not measure vertical profiles in the water column. However, it is known that summer stratification regularly occurs in the German Bight (Dethlefsen & Westernhagen 1983, Budéus 1989). On the other hand, our particular sampling position 'Kabeltonne' of the Helgoland Reede time-series is situated in a relatively narrow channel

between the main island and the 'Helgoland Dune' (Fig. 1). Due to tidally induced currents, the body of water is mixed, even when the surrounding water is stratified.

Very contradictory information exists in the literature about the importance of an inorganic nutrient supply for the development of *Dinophysis* spp. blooms. Delmas et al. (1992) did not detect a relationship between cell concentrations of *Dinophysis* spp. and the availability of dissolved inorganic nutrients. Another study indicated that increasing nitrate concentrations might stimulate the development of high cell densities (Lassus et al. 1991). Our results provided evidence for a potentially indirect effect of decreased silicate concentrations on the occurrence of *Dinophysis* spp. Low concentrations of silicate, such as we observed in late spring and in summer, inhibit coastal diatom development (Nelson & Treguer 1992 and references therein, Kristiansen et al. 2000), which might, in combination with high temperatures, create favorable conditions for dinoflagellates including *Dinophysis* species. Similar observations were made in Swedish coastal waters (Haamer 1995). Another possible influence favoring the development of *Dinophysis* might be the presence of relatively high concentrations of ammonia in summer (Fig. 7a). The effects of inorganic nutrients on toxin concentrations were observed by Johansson et al. (1996), and he suggested that there might be a possible increase in *Dinophysis* toxin production under nitrogen-sufficient and phosphorus-deficient conditions. The difficulties in collecting biological data on *Dinophysis* are largely caused by the fact that species of this genus cannot be cultivated in the laboratory (Lassus et al. 1991, Delmas et al. 1992). To date, field observations are the only source for understanding *Dinophysis* biology. Our results showed not only that there is a clear connection between increased summer cell concentrations and mussel toxicity, but also that even slightly elevated concentrations can have a toxic effect. The persistence of particulate matter toxicity in the water column and HP toxicity of mussels which we detected well after the end of the *Dinophysis* bloom gives reason for concern that with the end of a *Dinophysis* bloom the threat of toxicity has not yet passed.

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LITERATURE CITED

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