

Pectenotoxin-2 and dinophysistoxin-1 in suspended and sedimenting organic matter in the Baltic Sea

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Abstract

Dinophysis acuminata, *D. norvegica*, and *D. rotundata* occur regularly in the Baltic Sea summer plankton. They are known to produce diarrhetic shellfish poisoning (DSP) toxins in coastal areas worldwide, but so far, evidence from the Baltic Sea is scarce, and the fate and transfer of their toxins in the ecosystem is poorly known. Occurrence of *Dinophysis* and DSP toxins was studied on the southwest coast of Finland in late July–September 2004 by sampling the water column down to the thermocline. DSP toxin profiles were analyzed using high-performance liquid chromatography–mass spectrometry from the thermocline sample from material collected with a sediment trap. Maximum abundances of *D. acuminata* (7,280 cells L⁻¹) and *D. rotundata* (880 cells L⁻¹) were above the thermocline, but *D. norvegica* (maximum 200 cells L⁻¹) was most abundant in the thermocline region. Pectenotoxin-2 (PTX-2) was found during the entire study period. Cellular PTX-2 content in *Dinophysis* varied between 1.6 and 19.9 pg PTX-2 cell⁻¹. Dinophysistoxin-1 (DTX-1) was found in samples after mid-August in concentrations ranging from 0.2 to 149 pg DTX-1 cell⁻¹. PTX-2 and DTX-1 were found in all sediment trap samples. The estimated sedimentation rate of PTX-2 was 0 to 15.4 ng m⁻² d⁻¹ and DTX-1 0 to 190 ng m⁻² d⁻¹, corresponding ~0.01% of PTX-2 and 1% of DTX-1 of the integrated water column DSP pool during a 6-week period. Sedimenting organic matter did not contain intact *Dinophysis* cells, but copepod fecal pellets found in the sediment traps indicated that fecal pellets are the major pathway of DSP toxins to the bottom. The major fraction of the PTX-2 and DTX-1 was either decomposed in the water column or transferred to higher trophic levels in the planktonic food chain.

Harmful algal blooms are widespread phenomena in coastal areas of the oceans. Among harmful algal bloom events, diarrhetic shellfish poisoning (DSP) syndrome in humans is caused mainly by representatives of the genus *Dinophysis* (e.g., Yasumoto et al. 1985; Subba Rao et al.

1993) after ingestion of shellfish containing *Dinophysis* toxins.

According to their chemical structure, the DSP toxins can be divided into (1) okadaic acid (OA) and its derivatives dinophysistoxins (DTX) (Murakami et al. 1982; Murata et al. 1982; Yasumoto et al. 1985), (2) pectenotoxins (PTX) (Yasumoto et al. 1985; Draisci et al. 1996, and references therein), and (3) yessotoxin (Murata et al. 1987). These toxins have been shown to cause acute gastrointestinal illness in humans, in addition to which they are cytotoxic, hepatotoxic, and tumor-promoting agents (Yasumoto et al. 1985; Van Egmont et al. 1993).

Dinophysis acuminata Claparède et Lachmann 1859, *Dinophysis norvegica* Claparède et Lachmann 1859, and *Dinophysis rotundata* Claparède et Lachmann 1859 are common members of the summer plankton community in the entire Baltic Sea (Hällfors 2004). *Dinophysis acuta* Ehrenberg 1839 only occurs in the southern parts of the Baltic where salinities are higher. In the northern Baltic,

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Dinophysis densities are the highest in late July–August, and especially *D. norvegica* is known to form dense populations at the thermocline region and contribute to the subsurface chlorophyll *a* (Chl *a*) maximum (e.g., Carpenter et al. 1995). In late summer and early autumn, when *Dinophysis* are abundant, *D. acuminata* can contribute to the total phytoplankton biomass by 20–95% on Finnish coastal areas (Finnish Environment Institute database unpubl. data).

Because there is no commercial cultivation of bivalves in the Baltic Sea, with the exception of the southwestern parts (Emsholm et al. 1996), the DSP toxins produced by Baltic *Dinophysis* have not caused any obvious intoxication episodes, and hence gained only little attention. However, it is not likely that the Baltic representatives of *Dinophysis* genus make an exception with respect to their toxicity. In fact, in the Gulf of Finland, Pimiä et al. (1997) have found OA in bottom-dwelling blue mussels (*Mytilus edulis*), co-occurring with reasonably high densities of *D. norvegica* and *D. acuminata* in the water column, and Sipilä et al. (2000) have detected OA in the liver tissue of common flounder (*Platichthys flesus*). Goto et al. (2000) found pectenotoxin-2 (PTX-2) and its seco acid, and after hydrolysis also dinophysistoxin-1 (DTX-1), from *D. norvegica* cells collected in the Baltic proper. Furthermore, Klöpffer et al. (2003) observed concurrent DSP toxicity in blue mussels (*Mytilus edulis*), suspended particulates, and the abundances of *D. norvegica* and *D. acuminata* in the southern Baltic Sea. They measured cellular content of DSP toxins as 3.3 pg cell⁻¹ OA equivalents, but did not differentiate between the toxins.

It is not well known to what extent the DSP toxins are transferred toward higher trophic levels (zooplankton and fish) by the planktonic food chain, or whether they sink to the bottom. In order to answer these questions, we have investigated the occurrence and possible downward flux of the DSP toxins. Two major hypotheses were tested: (1) Baltic *Dinophysis* contain DSP toxins, as do their oceanic counterparts, and (2) sedimentation is a pathway for DSP toxins from the pelagic ecosystem to the benthos. To test them, we have measured the toxin concentrations in suspended particulate matter and in sedimenting organic matter, and linked the measurements to microscopic analyses of occurrence of *Dinophysis* species.

Material and methods

Sampling—Water samples were taken in the Gulf of Finland open-sea area (Fig. 1), where salinity varies between 6 and 7. Because *Dinophysis* spp. in the area are considered late summer species, the sampling was started at the end of July. Water samples were taken on 6 occasions: on 27 July, 02 August, 05 August, 18 August, 01 September, and 15 September 2004.

A vertical series of water samples was taken for the analysis of phytoplankton community and measurement of Chl *a* concentration. Two samples were taken from the surface water (0 m and 10 m), several samples around the thermocline, and one or two samples below the thermocline. The thermocline was first determined with a conduc-

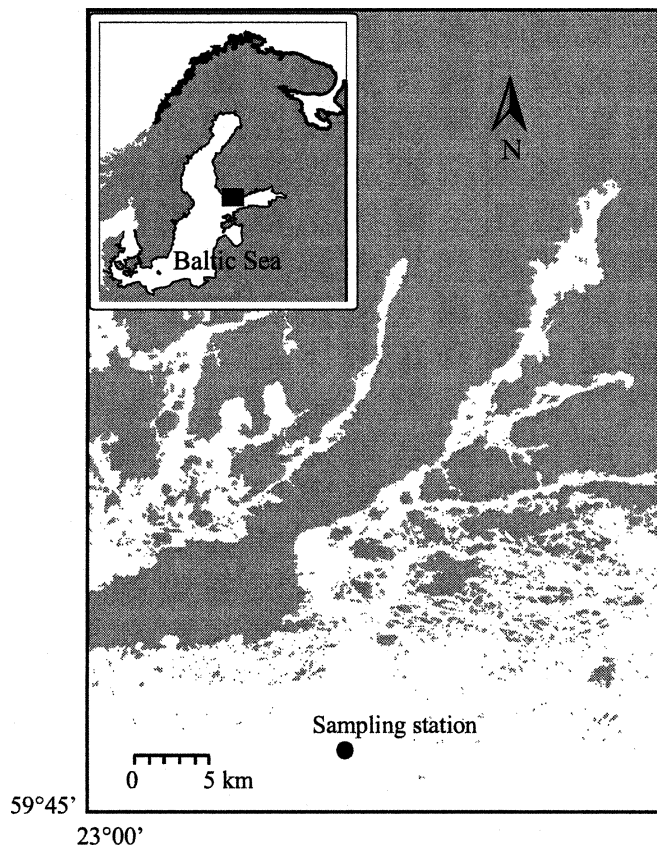


Fig. 1. Study area at the entrance to the Gulf of Finland, northern Baltic Sea.

tivity–temperature–density probe cast. On each sampling occasion, a 160-L water sample was collected for DSP toxin analysis from one or two depths (see Table 1) from the lower part of the thermocline with a hose and a peristaltic pump. The sample was first filtered through a 150- μ m mesh net in order to avoid zooplankton and larger aggregates, after which it was concentrated to 1–2-liter volume by inverse filtration through 20 μ m and 25 μ m mesh nets. A 200-mL subsample for microscopical analyses was fixed with acidic Lugol solution and stored at +4°C. From the concentrates, two or three replicate samples for DSP toxin analyses were prepared by filtering the concentrate on GF/A filters and frozen immediately (–24°C), corresponding to, on average, 3.2×10^5 (range 2.2×10^2 to 9.7×10^5) *Dinophysis* cells on the filter.

To measure sedimentation, an automatic sediment trap (Technicap, model PPS 4/3, height 1.2 m, collecting area 0.05 m²) was installed at a 40-m depth, close to the vertical water sampling site (59°47.383'N; 23°19.669'E; depth 50 m). The trap was programmed to collect sedimenting material from the beginning of August to the beginning of October. Each sample (volume 250 mL) was a result of collected sediment material for a 3-d period. Before the deployment of the trap, the sampling bottles were filled with 2% formaldehyde and brine (salinity 11) in order to prevent decomposition and diffusion of material from the sampling bottle, respectively, during the sampling period (Knap et al. 1996). In the laboratory, a 150-mL subsample

Table 1. Sampling date (in 2004), depth from where the sample for the DSP toxins was taken, abundance of *Dinophysis* in the concentrated sample, and percentage of *D. acuminata*, *D. norvegica*, and *D. rotundata* in the sample, concentrations of PTX-2 and DTX-1 in the suspended matter, and PTX-2 and DTX-1 concentrations calculated per cell (all *Dinophysis* species included).

Date	Sampling depth (m)	Cells L ⁻¹ in the concentrate	% <i>D. acuminata</i> <i>D. norvegica</i> <i>D. rotundata</i>	PTX-2 (ng L ⁻¹) CV%	DTX-1 (ng L ⁻¹) CV%	PTX-2 (pg cell ⁻¹)	DTX-1 (pg cell ⁻¹)
27 Jul	23	5.8×10 ⁴	59/39/2	2.5(23%)	0	6.7	0
02 Aug	25	1.7×10 ⁴	59/38/3	0.2(26%)	0	1.7	0
05 Aug	34	1.3×10 ⁵	76/0/24	1.3(64%)	0	1.6	0
	25	4.0×10 ⁴	72/0/28				
18 Aug	27	4.5×10 ⁴	97/0/3	5.5(7%)	0.05(35%)	19.9	0.2
01 Sep	23	3.6×10 ²	74/0/26	0.07(15%)	0.3(38%)	19.9	125
15 Sep	22	5.5×10 ²	97/0/3	0.1(9%)	1.0(69%)	19.2	149

from each sampling bottle was filtered onto GF/A glass fiber filter and stored frozen (−24°C) until the analysis of DSP toxins. For visual examination of the sedimented material, formaldehyde (37%) was added to another subsample, which was stored at +4°C until microscopy.

Analyses—The Lugol-fixed water samples, as well as the trap samples, were examined with inverted microscope by using the Utermöhl method (Utermöhl 1958). For Chl *a* analysis, duplicate 100-mL subsamples were filtered onto GF/F glass fiber filters and stored frozen (−18°C) until analysis. The filters were extracted with 5 or 10 mL ethanol for 24 h at room temperature in darkness, and analyzed fluorometrically (Shimadzu RFPC 5001; calibrated with pure Chl *a*; Sigma Aldrich).

DSP toxin profiles were analyzed from both the suspended samples and the sedimented material using high-performance liquid chromatography–mass spectrometry methodology (Goto et al. 2001, Quilliam 2003). Material on the GF/A filters was extracted with 80 : 20 (vol/vol) methanol–water solution. The DSP toxins were detected by LC/MS MS (Single Quadrupole LC/MS mass spectrometer API 165EX, Applied Biosystems). Because of low DSP toxin content in the trap samples, three successive samples (i.e., 9 d of sampling) were pooled for final DSP toxin analyses.

Calculation of sedimentation—Sedimentation rates (ng m⁻² d⁻¹) for PTX-2 and DTX-1 were calculated according to the Joint Global Ocean Flux Study methods, by dividing the measured toxin concentrations with the number of deployment days and the trap area (Knap et al. 1996). A rough overall budget of DSP toxin sedimentation was calculated for the period between 03 August and 19 September because it covered the sedimentation measurement parallel with most sampling occasions (03, 05, and 18 August, and 01 and 05 September). The measured PTX-2 and DTX-1 concentrations (ng L⁻¹) in the suspended sample from the thermocline region were calculated per m⁻², assuming that the concentration was constant throughout the water column above the sediment trap (0 to 40 m). The m⁻² values on each sampling occasion (ng m⁻²) were then integrated over the time period between 03 August and 05 September. Sedimentation rates (ng m⁻² d⁻¹) of the toxins were summed over a period that covered

the sampling occasions (August 3 to September 19, altogether 6 weeks), and divided by the integrated toxin concentration in the water column. This quotient (×100) was used as the percentage of the suspended PTX-2 and DTX-1 toxins (% 6 week⁻¹).

Because the target of our study was to test in general the occurrence of *Dinophysis* toxins in the Baltic Sea, and their possible transfer from the planktonic to benthic ecosystem, our data structure does not allow rigorous conclusions on the seasonal development and vertical positioning of the *Dinophysis* populations by statistical analyses.

Results

Distribution of *Dinophysis* species—The thermocline in the end of July was located at ~20-m depth, and the surface water temperature varied between 17°C and 18°C (Fig. 2). In the beginning of August, the water column was mixed as a result of strong winds, and the thermocline descended down to 35 to 40 m, to rise again to 20-m depth in September. In September, the water temperature decreased gradually to 10°C. Secchi disk transparency during the study was on average 6 m, which corresponds to a trophogenic layer of approximately 12 m.

Chl *a* was high at the surface layer in the beginning of the study (July–05 August), ranging between 13.8 and 16.9 µg L⁻¹ (Fig. 2), decreasing to 2.3 to 5.9 µg L⁻¹ in September along with decreasing water temperature and irradiance. The Chl *a* decreased rapidly with depth (Fig. 2), except on 02 August, when a subsurface chlorophyll maximum (6.2 µg Chl *a* L⁻¹) was observed at 26-m depth. This thin layer consisted mainly of the dinoflagellates *Dinophysis acuminata* and *Heterocapsa triquetra*.

Dinophysis acuminata was the most abundant member of the *Dinophysis* genus during our study period, with the cells being distributed throughout the water column, and the highest densities usually at the mixed surface layer (0 m, 10 m) (Fig. 2). The maximum abundance of *D. acuminata* (7,200 cells L⁻¹) was observed on 18 August (Fig. 2). On 02 August, the cell numbers of *D. acuminata* were also high at 22- and 26-m depths (4,500 and 5,400 cells L⁻¹, respectively), where the high Chl *a* concentrations were measured as well (14.9 and 12.4 µg Chl *a* L⁻¹, respectively).

D. norvegica was mainly found at the thermocline region (Fig. 2), with cell numbers ranging between 40 and

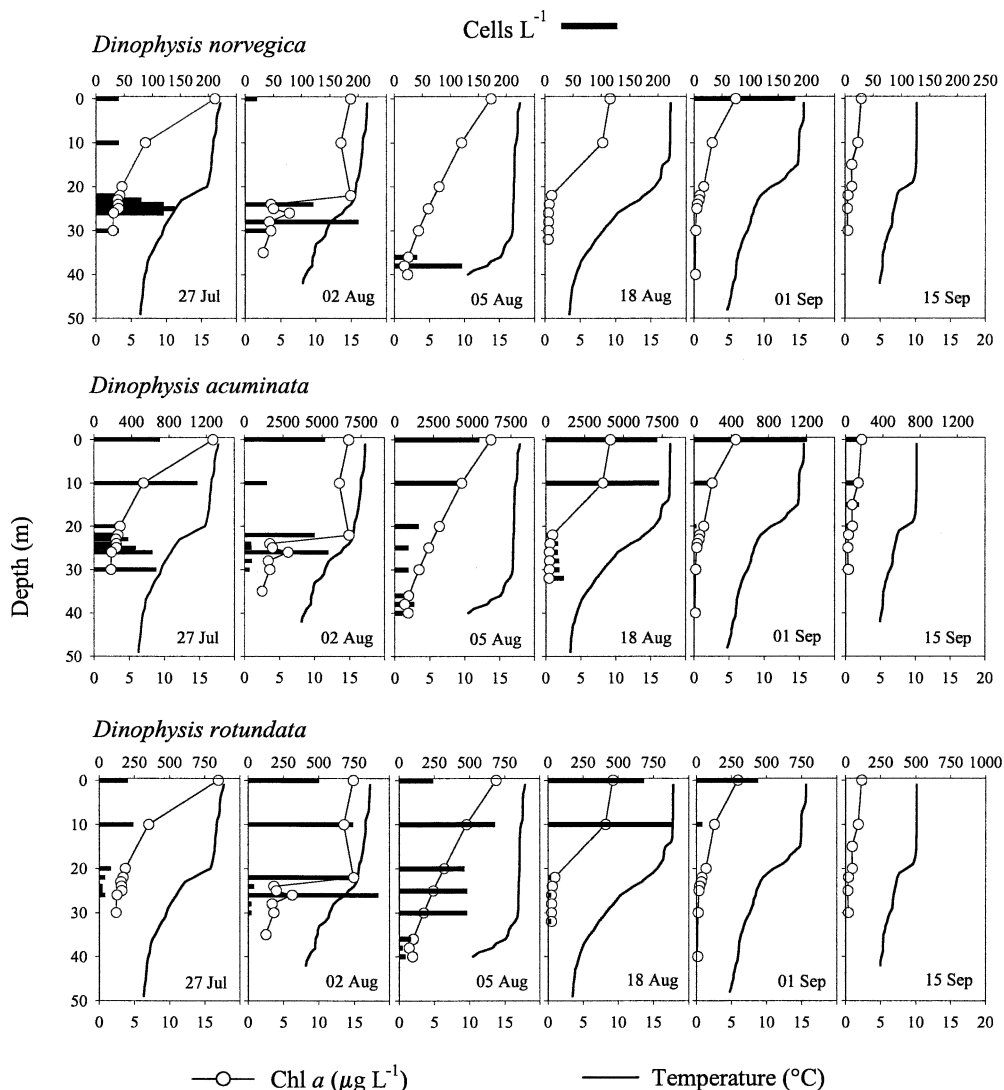


Fig. 2. Distribution of *Dinophysis acuminata*, *Dinophysis norvegica*, and *Dinophysis rotundata*, in relation to temperature and Chl a. Note different scales in the top x-axis.

200 cells L⁻¹ (27 July–05 August). After the beginning of August, the cells were not found any longer at this layer, except at the surface on 01 September, with abundance reaching 180 cells L⁻¹.

D. rotundata was relatively evenly distributed throughout the water column, with cell numbers varying between 20 and 920 cells L⁻¹ (Fig. 2). The high abundances were usually at the mixed surface layer, but the maximum was found at the depth of the thin subsurface Chl a layer at 26 m on 02 August (Fig. 2). Like *D. norvegica*, *D. rotundata* was not found in September.

In the concentrated water sample taken for the toxin analyses, *D. acuminata* was the most abundant species of *Dinophysis* at all sampling occasions, with a share of 59–97% of total *Dinophysis* cell numbers. *D. norvegica* was present only on the two first sampling occasions, while *D. rotundata* contributed to the assemblage in all samples with 2–28%.

DSP toxins in suspended matter—PTX-2 and DTX-1 were found in the concentrated samples from the thermo-

cline region. PTX-2 was measured throughout the study, whereas DTX-1 could be found during the last 3 sampling occasions. PTX-2 concentration in the suspended matter varied between 0.07 and 5.5 ng L⁻¹ (Table 1). The highest concentration was measured on 18 August, when *D. acuminata* was clearly the dominating *Dinophysis* species in the concentrated water sample (97%), whereas in September the PTX-2 concentration in the suspended matter noticeably decreased. The cellular PTX-2 concentrations (calculated on the sum of the 3 *Dinophysis* species) of PTX-2 varied from 1.7 to 19.9 pg PTX-2 cell⁻¹, with lower values in late July and beginning of August, and higher values when the *Dinophysis* cell numbers decreased (Table 1).

DTX-1 was detected only on the last three sampling occasions with concentrations increasing from 0.05 to 1.0 ng L⁻¹ toward mid-September (Table 1). The cellular DTX-1 concentrations also increased from low value in August (0.2 pg DTX-1 cell⁻¹) to 149 pg cell⁻¹ in September, because of the decrease in *Dinophysis* abundance with

Table 2. DSP toxins (PTX-2 and DTX-1) in the sedimenting organic matter and their calculated sedimentation rates.

Period	Toxin concentration per 9-d sample		Sedimentation rate	
	PTX-2 (ng sample ⁻¹)	DTX-1 (ng sample ⁻¹)	PTX-2 (ng m ⁻² d ⁻¹)	DTX-1 (ng m ⁻² d ⁻¹)
05–14 Aug	0	0	0	0
14–23 Aug	6.9	83	15.4	184
23–31 Aug	1.1	76	2.7	190
01–10 Sep	0.3	36	0.6	79
10–19 Sep	0.5	36	1.2	79
19–28 Sep	0.9	51	2.0	114
28 Sep–07 Oct	0.7	58	1.6	129

the concurrent increase in DTX-1 concentration in the suspended matter.

DSP toxins in the sedimenting matter—During the first collecting period (05–14 August), no DSP toxins were found in the trap material. Later on, the PTX-2 concentration in the sedimenting organic matter varied between 0.3 and 6.9 ng sample⁻¹ (Table 2; sample = 9-d collecting period), and the DTX-1 up to two orders of magnitude higher ranging from 35.5 to 82.8 ng sample⁻¹ (Table 2). The highest DSP toxin values in the sedimenting material were measured for the second collecting period (14 to 23 August). The sedimentation rates of PTX-2 and DTX-1 were 0.6–15.4 and 79–190 ng m⁻² d⁻¹, respectively (Table 2). Calculated PTX-2 sedimentation rates for the period between 03 August and 19 September, accounted for 0.01% of the toxin suspended in the water column; the corresponding value for DTX-1 was 1%.

Discussion

Distribution of Dinophysis—Temporal and vertical distribution of *Dinophysis* spp. in the water column varied within species. Although *D. acuminata* and *D. rotundata* were abundant in the mixed surface layer above the thermocline (Fig. 2), *D. norvegica* was mainly found at the thermocline region (except on 01 September; Fig. 2), as has been shown previously by, e.g., Carpenter et al. (1995). However, *D. acuminata* contributed also to the subsurface chlorophyll maximum observed on 02 August (cf. Fig. 2). *Dinophysis* populations have often shown uneven vertical distribution, e.g., in the Thermaikos Gulf, Mediterranean (Koukaras and Nikolaidis 2004), with vertical peaks positioned in or just above the pycnocline.

Published records on the temporal variation of *Dinophysis* in the study area are scarce. Kononen and Niemi (1986) have shown that there is large interannual variation in the occurrence of both *D. acuminata* and *D. norvegica* on the basis of long-term records, but no more detailed studies have been carried out since then. In our study, all *Dinophysis* spp. cell numbers declined toward September with decreasing day length and water temperature. Especially *D. norvegica* disappeared from the thermocline region after the beginning of August, possibly because of

insufficient irradiation at that depth (Gisselson et al. 2002). The peak of *D. norvegica* at the surface sample on 01 September remains unexplained.

DSP toxins—Observations of DSP toxin profiles show that the toxin content of *Dinophysis* varies between locations and times (e.g., Cembella 1989; Sato et al. 1996; MacKenzie et al. 2005). The most regularly measured DSP toxin in *Dinophysis* is OA and its derivatives DTX-1 and DTX-2 (e.g., Cembella 1989; Subba Rao et al. 1993; Morono et al. 2003). In this study, no OA was found in the samples, which corresponds to the finding of Goto et al. (2000), who did not find OA in their Baltic *Dinophysis norvegica* cells. Why was OA found in the bottom fauna of the Baltic Sea (Pimiä et al. 1997; Sipiä et al. 2000), but not in *Dinophysis* suspended in the water column? It is probable that OA, which is found in sterified form in *D. norvegica* and *D. acuminata* in the Baltic Sea, is transformed to OA when subjected to low pH in the guts of the animals (T. Yasumoto pers. comm.).

The first record of PTX-2 related to seafood poisoning in Europe is from Italian coastal waters connected to *Dinophysis fortii* (Draisci et al. 1996). PTX-2 has also previously been identified in *D. fortii* in Japan (Lee et al. 1989; Suzuki et al. 1996) and the Portuguese coast (Vale and Sampayo 2002), in *D. acuta* in Europe and New Zealand (e.g., Suzuki et al. 2003; Puente et al. 2004), and in *D. norvegica* on Swedish and Norwegian coasts (Goto et al. 2000; Miles et al. 2004). PTX-2 records in *D. acuminata* are from coasts of Norway and New Zealand (Miles et al. 2004; MacKenzie et al. 2005). This is the first record of PTX-2 connected to *Dinophysis acuminata* in the Baltic Sea.

In our study, PTX-2 was present in all suspended samples, although there was variability in the concentrations. The cellular concentrations (1.6 to 19.9 pg cell⁻¹) fit with the values measured from *D. acuminata* in New Zealand (MacKenzie et al. 2005) and are at the lower end of those reported for other *Dinophysis* species (Table 3). In addition, Karlsson et al. (2000) found in *D. norvegica* in the Trondheim fjord, Norway, only traces of PTX-2 but its seco acid in high concentrations (25 pg cell⁻¹). Our concentrations calculated per cell have included also counts of *D. rotundata*. Because there is evidence from Norwegian coast (Miles et al. 2004), that *D. rotundata* contains PTX-2 in equal concentrations to *D. norvegica* (mean 0.7 and 0.9 fmol cell⁻¹, respectively) we had no reason to exclude it from our calculations. Nevertheless, if *D. rotundata* were left out, the PTX-2 concentrations would have been in the range of 1.7 to 30.5 pg cell⁻¹.

DTX-1 is a derivative of OA. It was first identified in *Dinophysis fortii* as the main causative agent of DSP in Japan (Murata et al. 1982). Since then, it has been found in *D. fortii* cells in both Japan and Europe (Lee et al. 1989; Sato et al. 1996; Suzuki et al. 1996), *D. acuta* in New Zealand, Japan, and Europe (Lee et al. 1989; Johansson et al. 1996; MacKenzie et al. 2005), in *D. caudata* and *D. miles* in the Philippines (Marasigan et al. 2001), and in *D. acuminata* in New Zealand (Lee et al. 1989; MacKenzie et al. 2005). There are no published records of DTX-1 in Baltic *Dinophysis* species.

Table 3. Comparison of pectenotoxin-2 (PTX-2) and dinophysistoxin-1 (DTX-1) cellular concentrations measured in coastal areas.

Species	PTX-2 (pg cell ⁻¹)	DTX-1 (pg cell ⁻¹)	Habitat	Reference
<i>Dinophysis</i> sp.	1.6–19.9	0.2–149	Baltic Sea	This study
<i>D. fortii</i>	42.5	13–191	Japan	Lee et al. (1989)
<i>D. fortii</i>		252	Japan	Suzuki et al. (1996)
<i>D. fortii</i>		200	Japan	Suzuki and Mitsuya (2001)
<i>D. acuta</i>	82		New Zealand	MacKenzie et al. (2002)
<i>D. acuta</i>	20–80		New Zealand	Suzuki et al. (2004)
<i>D. acuta</i>	32.5–107.5	0.1	New Zealand	MacKenzie et al. (2005)
<i>D. acuta</i>		4.2	Europe	Lee et al. (1989)
<i>D. norvegica</i>	13.3		Southern Baltic	Goto et al. (2000)
<i>D. acuminata</i>	2.4–25.8	0.1–2.4	New Zealand	MacKenzie et al. (2005)
<i>D. norvegica</i>		2.5–14.0	Europe	Lee et al. (1989)
<i>D. norvegica</i>	13.3		Southern Baltic	Goto et al. (2000)
<i>D. caudata</i>		7.2–53.9	Philippines	Marasigan et al. (2001)
<i>D. miles</i>		3.1–10.7	Philippines	Marasigan et al. (2001)
<i>D. mitra</i>		10.0	Japan	Lee et al. (1989)
<i>D. rotundata</i>		101.0	Japan	Lee et al. (1989)
<i>D. tripos</i>		36.0	Japan	Lee et al. (1989)
<i>P. reticulatum</i>	81		New Zealand	MacKenzie et al. (2002)

In our samples, DTX-1 was measured only after mid-August, and its concentration in the suspended matter increased in September (cf. Table 1), when the environmental conditions (especially light) became suboptimal for growth. Hence, as the abundance of *Dinophysis* decreased simultaneously with increasing DTX-1 content of suspended matter, the calculated cellular concentration increased considerably (maximum 149 pg cell⁻¹; Table 1), exceeding the DTX-1 values measured elsewhere from *D. acuminata* (Table 3). The maximum was, however, lower than the values measured from *D. fortii* (Table 3).

Our results agree with experimental data by Johansson et al. (1996), who suggested that either nutrient limitation or growth limitation at stationary phase of growth increases the toxin content per *Dinophysis* cell. They found DTX-1 concentration in *D. acuminata* nonmeasurable in a sample from the Skagerrak, but in N-deficient growth conditions it increased to 0.2 pg cell⁻¹. In our study the cellular DTX-1 levels seemed to increase with deteriorating population of *Dinophysis* toward the autumn, and a similar pattern could be observed with PTX-2.

Kløpper et al. (2003) found extended presence of DSP toxins in suspended organic matter after the *Dinophysis* cell abundances had declined in the southern Baltic Sea. They postulated that it might have been caused by the DSP toxins remaining in the detritus and dead *Dinophysis* cells. In our study, the two measured toxins (PTX-2 and DTX-1) seemed to behave roughly similarly. When DTX-1 concentrations in the suspended matter increased and the PTX-2 remained at the same level, the cellular concentrations increased.

Sedimentation of DSP toxins—More DTX-1 toxin was found in the sedimenting organic matter compared with the PTX-2 toxin. The sedimentation rate of DTX-1 was two orders of magnitude higher (79 to 190 ng m⁻² d⁻², corresponding roughly to 1%, calculated for the 6-week period, of the DTX-1 suspended in the water column)

compared with PTX-1 (0.6 to 15.4 ng m⁻² d⁻¹, corresponding 0.01% 6 week⁻¹ of suspended PTX-2). This indicates that OA-derived toxins are perhaps more refractory in the detrital organic matter than the PTX-2 toxins. Furthermore, the finding of OA in the bottom fauna (Pimiä et al. 1997; Sipiä et al. 2000) also supports this assumption. Elimination of pectenotoxins is very rapid in mussels (Vale 2004). Especially PTX-2 is rapidly converted into its homologous seco acids in mussels and scallops (Suzuki et al. 2001). No PTX seco acids have been found from bottom fauna of the Baltic Sea. We did not find any derivatives of PTX-2 seco acid in the suspended matter or sedimenting organic matter either, even if the whole DSP profile was analyzed. This may be because of shorter retention time of PTX toxins in dead cells or decaying and sedimenting organic matter compared with DTX-1.

In the Baltic Sea, with the exception of the southern Baltic, there are no mussels hanging between the surface and the bottom, filtering *Dinophysis* cells directly from the water column. Instead, they are living on the bottom, and the only potential vector of DSP toxins for Baltic bottom fauna is sedimentation. This study is the first to measure the sedimentation of DSP toxins not only in the Baltic Sea, but also elsewhere. The rough estimate, which was based on direct sedimentation measurements (see calculation in “Material and methods”), is extremely low for PTX-2 (~0.01% of the concentration in suspended matter in 6 weeks), and low for DTX-1 (~1% of the concentration in suspended matter in 6 weeks). Even so, it seems that DTX-1 sedimentation is enough to end up with OA accumulation in the bottom fauna.

The low sedimentation rates also imply that the DSP toxins not sedimenting out from the pelagic ecosystem are either decomposed, transformed to other derivatives, or moved along the pelagic food web to higher trophic levels. According to results of Maneiro et al. (2000) from the coast of Spain, the Atlantic, large tintinnid ciliates (*Favella* sp.) are able to ingest *Dinophysis* cells. In the Baltic Sea,

however, tintinnids and other ciliates are much smaller than *Favella* and thus not likely to be able to ingest *Dinophysis* cells, which makes it unlikely for them to be a potential vector of DSP toxins in the planktonic food web.

In experiments carried out with copepods in the French Atlantic coast, *D. acuminata* containing OA acted as deterrent to some mesozooplankton grazers, but was ingested by *Acartia clausi* (Carlsson et al. 1995), which exists also in the Baltic Sea. Furthermore, it has been shown that some other copepod species can feed effectively on *Dinophysis*, and at least part of the cells appear in the fecal pellets of the copepods (e.g., Maneiro et al. 2002; Wexels Riser et al. 2003). These authors suggest that copepod fecal pellets are potential vectors for *Dinophysis* to transfer DSP toxins from pelagial to the bottom waters.

When examining microscopically the sedimented material in the trap samples, we found neither intact *Dinophysis* cells nor their remnants, but instead copepod fecal pellets varying in the range of 10–100 pellets mL⁻¹ 9-d sample⁻¹, which corresponds roughly to a sedimentation rate of 20–200 pellets m⁻² d⁻¹. These estimates are somewhat higher than the published values from the area (9–22 pellets m⁻² d⁻¹; Viitasalo et al. 1999). However, Viitasalo et al. concluded that less than 1% of copepod fecal pellets sedimented; most of them were probably broken up and decomposed in the water column. Previous studies on phytoplankton sedimentation in the area have not found *Dinophysis* cells in the trap material either (Heiskanen and Kononen 1994). It is therefore likely that the DSP toxins sedimented in the decaying organic matter or fecal pellets. It is also likely that at least part of the DSP toxins, ingested by the copepods, must be transferred to higher trophic levels in the planktonic food chain. Some indication of this has been gained in experiments, where PTX-2 was found in the copepod *Eurytemora affinis* incubated with a Baltic Sea *Dinophysis* community (Setälä et al. unpubl. data). The hypotheses of the food web transfer and sedimentation of DSP toxins have to be verified in the future with more detailed studies.

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