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Dinoflagellate toxins in northern Baltic Sea phytoplankton and zooplankton assemblages

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The distribution of dinoflagellate toxins (OA, DTXs, PTXs, YTXs) in plankton communities was studied in the northern Baltic Sea during late summers of 2005 and 2006. The phycotoxins were analysed from samples that contained field collected microplankton in the size range of 20–76 μm, zooplankton collected with a net, and individually hand-picked copepods from open-sea and coastal sampling stations. Concentrations of pectenotoxin-2 (PTX2) present in the microplankton samples containing *Dinophysis* spp. were between 2.1 and 10.6 pg cell^{-1}. Zooplankton net samples were positive for pectenotoxin seco acid (PTX2SA) at 10 sampling sites, and for PTX2 at two stations. The zooplankton net material contained also *Dinophysis* spp. cells that were the likely origin of these toxins, corresponding to 7.7–17.5 pg PTX2 cell^{-1}. Individually picked copepods did not contain any of the phycotoxins that were included in the analyses. Yessotoxin (YTX) was found in two microplankton samples. Toxins from the okadaic acid group were not detected. Our results confirm previous observations from the study area that the presence of PTX2 is a recurrent phenomenon in microplankton communities, whereas okadaic acid presence is not.

Introduction

Dinoflagellates from the genus *Dinophysis* produce toxic compounds that can accumulate in filter feeding shellfish. Consumption of the contaminated shellfish may lead to a toxic syndrome in human consumers, known as the diarrhetic shellfish poisoning (DSP). Several chemically and toxicologically different toxins have previously been included in the diarrhetic shellfish (DS) toxin group, although they were not all confirmed to cause DSP. In this categorization, the first group, acidic toxins, includes okadaic acid (OA) and its derivatives dinophysistoxins (DTXs). The second group, the neutral toxins, includes pectenotoxins (PTXs) and the third group is the yessotoxins (YTXs). *Dinophysis* spp. are the most common producers of OA, DTXs and PTXs worldwide, whereas the production of YTXs is most often linked to the dinoflagellate *Protoceratium reticulatum* (Satake *et al*. 1997).

Pectenotoxins are cyclic polyether lactones that pollute shellfish in many coastal areas glo-

bally (Yasumoto *et al*. 1985, Draisci *et al*. 1996, James *et al*. 1999, Blanco *et al*. 2007). Pectenotoxins in *Dinophysis* spp. occur in the form of pectenotoxin-2 (PTX2) that is rapidly metabolized to other PTX-derivatives in filter-feeding shellfish. The primary product of this hydrolysis reaction in shellfish hepatopancreas is PTX2 seco acid (PTX2SA) (Lee *et al*. 1988, Draisci *et al*. 1996, MacKenzie *et al*. 2002, Miles *et al*. 2004b). The reason for grouping PTXs as DS toxins (DSTs) is their common co-occurrence with other DSTs in environmental samples (Burgess and Shaw 2001, Vale and de M. Sampayo 2002). However, recent studies suggest that the oral toxicity of pectenotoxins is limited (Miles *et al*. 2004b). Because of that, and since they have not been found to cause the common symptoms of DSP, their categorization as DS toxins has been criticized (Miles *et al*. 2004a). PTXs have been demonstrated to be hepatotoxic and cytotoxic to human cancer cell lines, and tumor promoters in animal testings (van Egmond *et al*. 1993, Draisci *et al*. 1996, Burgess and Shaw 2001). PTX2 has been found e.g. in *Dinophysis acuta* (James *et al*. 1999, Suzuki *et al*. 2003, Miles *et al*. 2004a), *Dinophysis acuminata* (Miles *et al*. 2004a, Kuuppo *et al*. 2006, Blanco *et al*. 2007) and *D. norvegica* and *D. rotundata* (Miles *et al*. 2004a).

Yessotoxins are lipophilic polyether compounds including yessotoxin (YTX) and a number of its analogues as well as homoyessotoxin. YTX was first isolated from scallops in Japan (Murata *et al*. 1987). The chemical structure and toxicological properties of YTXs differ from the main DS toxins. Unlike OA or DTXs, YTXs do not induce inflammatory gastrointestinal symptoms by acting as protein phosphatase inhibitors in metabolic processes. YTXs can however, cause ultra structural damage to heart tissue in mice when injected intraperitoneally, or by repeated oral exposure (Aune 2002, Franchini *et al*. 2004, Tubaro *et al*. 2008). The most commonly known producer of YTX is the dinoflagellate *Protoceratium reticulatum*. YTX production by *P. reticulatum* was first detected from cultures isolated from New Zealand (Satake *et al*. 1997). Besides *P. reticulatum*, also the dinoflagellates *Gonyaulax spinifera* (Rhodes *et al*. 2006) and *Lingulodinium polyedrum* (Tubaro 1998) have been confirmed to produce YTXs.

Dinophysis acuminata, *D. norvegica* and *D. rotundata* are common in the northern Baltic Sea (Kononen and Niemi 1984, Meyer-Harms and Pollehne 1998). In the study area, *Dinophysis* spp. typically occur in concentrations of 1000 to 5000 cells l^{-1} during late summer, but also higher concentrations in subsurface layers occur (Kuosa 1990, Carpenter *et al*. 1995, Setälä *et al*. 2005, Hällfors *et al*. 2011). Of the toxins produced by *Dinophysis* spp. in the Baltic Sea, okadaic acid has been detected in blue mussels and the common flounder (Pimiä *et al*. 1998, Sipiä *et al*. 2000), whereas PTX2, DTX1, DTX3 and PTX2 SA have been found in plankton samples both from open sea and coastal areas (Goto *et al*. 2000, Kozlowsky-Suzuki *et al*. 2006, Kuuppo *et al*. 2006). The presence of YTX was reported from the Finnish coastal waters in a survey in 2006 (Uronen 2007).

In a previous study (Setälä *et al*. 2009), we detected PTX2 in copepods that were incubated with *Dinophysis* spp. The present study was carried out to see whether dinoflagellate toxins were to be found in field collected zooplankton.

Material and methods

Study area and sampling

Plankton samples were collected during late summer of the years 2005 and 2006 from different parts of the northern Baltic Sea. In 2005, samples were collected from 13 stations (Fig. 1 and Table 1). Twelve of these were sampled once during a research cruise onboard r/v *Aranda* (former Finnish Institute of Marine Research). The only coastal station, Tvärminne Storfjärd (St. 3., 59°49´N, 23°17´E), was sampled four times using the Tvärminne Zoological Station vessel, *Saduria*. In 2006, material was collected from this station only.

The study area includes hydrographically different sampling stations. The deep (> 100 m) stations 4–9 in the Baltic Proper represent open sea conditions, while stations 1–3 in the Gulf of Finland are more sheltered and shallow (St. 3 only 32 m). Salinity in the study area varies depending on location and freshwater inflow.

At the northernmost station (St. 13) the surface salinity can be close to freshwater conditions (in 2005, 2.1), while the stations in the Gulf of Bothnia (e.g. St. 10) are as saline as the Baltic Proper stations (6–7 in 2005). Temperature and salinity profiles in this study were measured with CTD probes (onboard r/v *Aranda* with the SBE 911 Plus CTD and at station 3 with the SIS 100plus CTD). Overall, the water temperature was typical for the seasons varying between 16 and 19 °C at the surface. The thermocline was situated between 10 and 20 meters. The temperature and salinity profiles at station 3 are presented in Fig. 2.

The samples of 2005 included zooplankton that was collected with a 100 μm closable net (WP-2 cod end). At each station, 1–2 hauls were made from deep water layers (100–75 m) to the thermocline. Subsamples for toxin analyses (250–500 ml) and zooplankton counts were taken from the net material. Toxin samples were filtered onto glass fibre filters (Whatman GF/F) and frozen (–18 °C). Samples from the net material for microscopy were preserved in buffered formalin (4% final conc.). Zooplankton was identified and counted with a dissecting microscope (Leica MZ 7.5). For the *Dinophysis* cell counts, the net material was filtered through a 200 μm sieve to remove large zooplankton possibly masking microplankton cells. The number of *Dinophysis* species in the net material was counted under an inverted microscope (Leica DMIL) from 25 ml settled subsamples (Utermöhl 1958).

The year 2006 material contained samples of individually picked zooplankton and microplankton (concentrated 20–76 μm fraction) from station 3. Prior to isolation, net-collected zooplankton was kept in fresh seawater in a temperature-controlled room (+11 °C). The copepods were collected from the water using a 200 μm sieve, washed with GF/F (Whatman) filtered seawater onto petri dishes and adult females of *Eurytemora affinis* and *Acartia bifilosa* (24–100 indiv. per species; Table 2) were picked with forceps onto glass fiber filters (Whatman GF/F) and frozen. Water for the toxin analyses (15–25 l) of the microplankton fraction was collected below the thermocline. The water was filtered through a 76 μm net to remove zooplankton and large phytoplankton cells, and subsequently concentrated by inverse filtration through a 20 μm net. Finally the 20–76 μm fraction was filtered onto glass fiber filters and frozen.

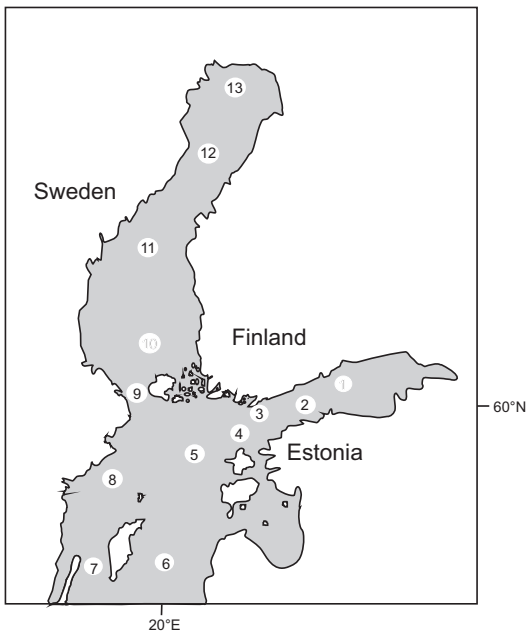

Fig. 1. The study area and sampling stations (1–13) in central and northern Baltic Sea.

Dinophysis spp. abundance at the sampling stations during the r/v *Aranda* cruise was quantified from integrated phytoplankton samples that were collected according to the Manual for Marine Monitoring in the COMBINE programme of HELCOM (Annex C-6; http://sea.helcom.fi/Monas/CombineManual2/contents.html). Water from the depths of 1, 2.5, 5, 7.5 and 10 m was mixed in equal amounts to obtain an integrated 200 ml subsample for quantitative phytoplankton counts. During every sampling occasion at station 3, the vertical distribution of *Dinophysis* spp. was estimated. Water samples were collected from 4–6 depths between 0–25 meters (Fig. 2) and from each depth a 200 ml subsample for microplankton counts was taken. The samples were preserved with 1% (final conc.) acid Lugol's solution (Hällfors *et al*. 1979) and *Dinophysis* cells were counted from settled 50 ml samples (Utermöhl 1958) with an inverted microscope (Leica DMIL, 100–400× magnifications).

Table 1. Zooplankton net samples, collected on 26 July–16 August 2005 and analyzed with LC-MS. Numbers of adult copepods and *Dinophysis* spp. in toxin samples, abundance of *Dinophysis* species at the sampling stations, toxin concentrations and cellular toxicity in total samples. The cellular toxin concentrations were calculated assuming that the toxins found in zpl net samples were located in *Dinophysis* spp. YTXs were not analyzed. + = positive signal (above limit of detection but below limit of quantification), * = no duplicates prepared for toxin analyses, n.a. = not analyzed. *Dinophysis* abundances (cells l^{-1}) from station 3 represent mean cell concentration of vertical samples taken between 0–10 meters, at other stations: cell concentrations integrated from samples (0–10 m).

Station	Copepods (indiv. sample^{-1})	*Dinophysis* spp. (cells sample^{-1})	*D. acuminata* (cells l^{-1})	*D. norvegica* (cells l^{-1})	*D. rotundata* (cells l^{-1})	PTX2 (ng sample^{-1})	PTX2 (pg cell^{-1})	PTX2SA +/–
1	214	275	3312	–	368	3.80*	13.8*	+
2	1840	75	4680	156	468	–	–	–
3	182	n.a.	3495	10	275	3.20 ± 0.02	n.a.	+
3	116	400	1727	–	120	6.00 ± 0.35	15.00 ± 0.80	+
3	120	730	1100	–	65	12.83 ± 1.37	17.56 ± 1.88	+
3	775	1000	1275	5	90	7.72 ± 1.52	7.72 ± 1.52	+
4	2300	200	2172	–	1810	–	–	+
5	5500	100	828	–	–	–	–	+
6	7750	–	–	–	–	–	–	+
7	7000	–	351	–	1380	–	–	+
8	4500	–	628	–	628	–	–	+
9	1975	50	1716	–	–	–	–	+
10	3800	50	692	–	173	–	–	+
11	817	–	178	–	–	–	–	–
12	700	–	–	–	–	–	–	–
13	129	–	–	–	–	–	–	+

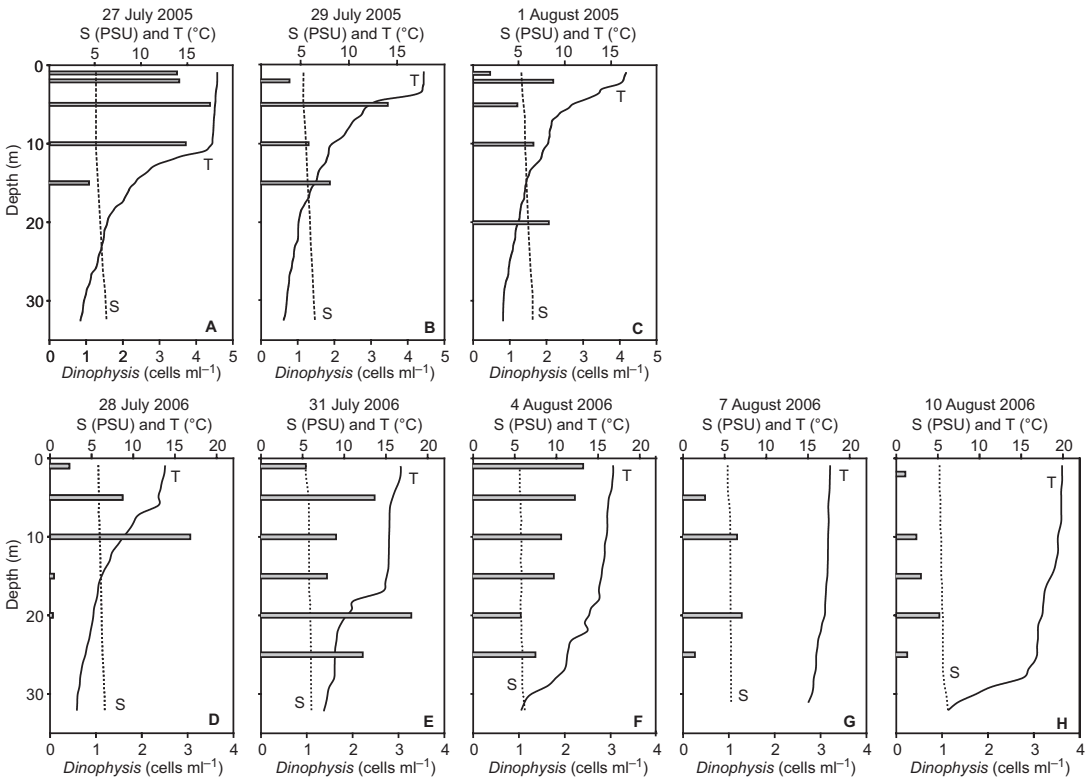

Fig. 2. Profiles of salinity (S, PSU), temperature (T, °C) and *Dinophysis* spp. vertical distribution (horizontal bars, cells ml^{-1}) at station 3 during sampling periods.

Toxin extraction and determination

Deionised water was used (Milli-Q, Millipore Eschborn, Germany). Formic acid (98%, p.a.) and ammonium formate (p.a.) were purchased from Acros Organics (Geel, Belgium). Acetonitrile (MeCN) and methanol were high-performance liquid chromatography (HPLC) grade (Acros Organics, Geel, Belgium). Standard solutions of lipophilic toxins (OA, PTX2 and YTX) were obtained from the NRC (Halifax, Canada) and of DTX1 from Bluebiotech (Kollmar, Germany).

Filters with the sample material were extracted with 1 ml of methanol:water (80:20) using an ultrasonic probe (Sonoplus GM 70 Bandelin, Berlin, Germany) for 30 s on ice. Prior to LC/MS/MS analysis, the suspension was centrifuged at 16–464× g and the supernatant was filtered through a 0.45 μm nylon micro filter into an auto sampler vial. There was a change in the analytical methods used to analyze the samples during the time this study was carried out. Consequently, some samples (not dependent on the year collected) were analysed with LC-MS (single quadrupole MS) while others with LC-MS/MS (triple quadrupole MS; introduced during the process) (Table 2). Measurements for the presence of YTXs were done only from the samples collected in 2006.

LC-MS analyses

The HPLC system consisted of a PE series 200 quaternary pump and a PE series 200 autosampler (Perkin Elmer, Ueberlingen, Germany). The mobile phases used in all chromatographic analyses consisted of a mixture of two solutions: (1) 10% MeCN–53 mM formic acid–5 mM ammonium formate, and (2) 90% MeCN–53 mM formic acid–5 mM ammonium formate. Chromatographic separation was achieved using a linear gradient from 0% to 70% B in 2 min, hold for 2 min, linear gradient to 100% B in 3 min,

Table 2. Samples from station 3 taken in 2006. Sample type, number of individually picked copepods and *Dinophysis* spp. cells. The toxin concentration of each filtered sample has been divided by the total number of *Dinophysis* cells, all species included. Positive signal but below limit of quantification = +, negative result = –. [a]Analyzed with LC-MS, [b]analyzed with LC-MS/MS.

Date	Sample	Material	Copepods (indiv. sample^{-1})	*Dinophysis* spp. (cells sample^{-1})	PTX2 (ng sample^{-1})	PTX2 (pg cell^{-1})	PTX2SA (ng sample^{-1})	YTX (ng sample^{-1})
28 July	Copepods[a]	*A. bifilosa*	100	–	–	–	–	–
31 July	Copepods[a]	*E. affinis*	100	–	–	–	–	–
4 Aug.	Copepods[a]	*A. bifilosa*	100	–	–	–	–	–
4 Aug.	Copepods[a]	*E. affinis*	73	–	–	–	–	–
5 Aug.	Copepods[a]	*E. affinis*	24	–	–	–	–	–
6 Aug.	Copepods[a]	*E. affinis*	50	–	–	–	–	–
7 Aug.	Copepods[a]	*E. affinis*	100	–	–	–	–	–
31 July	Plankton conc.[a]	20–76 µm	–	27200	72.61	2.67	0.60	–
3 Aug.	Plankton conc.[b]	20–76 µm	–	12120	25.92	2.14	–	–
4 Aug.	Plankton conc.[a]	20–76 µm	–	18800	199.00	10.64	1.00	11.5
5 Aug.	Plankton conc.[b]	20–76 µm	–	54288	186.20	3.43	–	–
7 Aug.	Plankton conc.[a]	20–76 µm	–	12312	71.42	5.80	+	+
10 Aug.	Plankton conc.[b]	20–76 µm	–	8886	32.92	3.70	+	–

followed by 5 min isocratic elution with 100% B. The conditions were then returned to initial (100% A) within 4 min followed by a re-equilibration time of 9 minutes. The total run time was 25 min. The flow rate was 300 µl min^{-1}, and the injection volume was 20 µl for both sample extracts and calibration solutions.

All measurements were performed using an API 165 SCIEX mass spectrometer with pneumatic-assisted atmospheric pressure ion (API) source (AB Applied Biosystems, Darmstadt, Germany). The electrospray ionisation interface operated in positive ion and negative ion modes. Selected ion monitoring (SIM) was performed using the following mass-to-charge (*m/z*) ratios: $[M + H]^+$, $[M + NH_4]^+$, $[M – H_2O + H]^+$, and $[M + Na]^+$ for OA (805.5; 822.5; 787.5; 827.5), DTX1 (819.5; 836.5; 801.5; 841.5).

For PTXs the following ion masses $[M + NH_4]^+$ and $[M + Na]^+$ were monitored: 876.5; 881.5 for PTX2 and 894.5 and 899.5 for PTX2 seco acid. PTX2 was quantified as ammonium adduct (*m/z* 876.5) in comparison with certified reference solution of PTX2. PTX2 seco acid was quantified in relation to PTX2 and the concentration is expressed as equivalents of PTX2. The limit of detection (LOD) of PTX2 was determined to 30 pg per injection (on column; s/n 3:1).

YTXs were analyzed in the negative mode $[M – H]^–$ with *m/z* 1141.5 for YTX, *m/z* 1157.5 for 45-OH-YTX, *m/z* 1155.5 for homo-YTX, and *m/z* 1171.5 for 45-OH-homo-YTX.

LC-MS/MS analyses

The LC-MS/MS determination of the different toxins was performed using a modification of the McNabb *et al*. (2005) method with slight modifications in mobile phase composition as described below. The chromatographic separation of lipophilic toxins was achieved by reverse-phase chromatography on a Luna C18(2) column 150 mm × 2.0 mm, particle size 5 µm (Phenomenex, Aschaffenburg, Germany) thermostated at 25 °C (Shimadzu CTO-10AS VP column oven).

A constant linear flow rate of 0.3 ml min^{-1} and constant injection volume of 20 µl were applied. Initial mobile phase conditions were 0% B, followed by a linear gradient to 60% B in

5 min, hold for 5 min, linear gradient to 100% B in 1 min, followed by 9 min isocratic with 100% B. The pump time program was then returned to initial conditions within 1 min, followed by a re-equilibration time of 24 min. The total analysis time was thus 45 min.

The mass spectrometric analyses were performed on an API 365 (Applied Biosystems, Darmstadt, Germany) triple quadrupole mass spectrometer equipped with an electrospray ionization interface coupled to a Shimadzu SCL-10A VP interface, a Shimadzu SIL-10AD VP auto injector and two Shimadzu LC-10AT pumps. Mass spectrometric detection was carried out either in the positive or negative mode with respective electrospray voltages of +5.2 kV and –4.5 kV. The turbo ion spray source was operated with nitrogen as the nebulizer gas (8 l min^{-1}; 450 °C for positive and 400 °C for negative modes respectively).

The positive mode was used for PTX2, PTX2SA, OA, DTX1 while the negative mode was applied with YTX. Multiple reaction monitoring (MRM) was used in both modes, and the analytes identified and quantified by using their following characteristic MRM transitions (precursor ion → fragment ion): *m/z* 822 → 769 and 822 → 751 (collision energy (CE): 25V) for OA; *m/z* 836 → 783, and 836 → 765 (CE: 25V) for DTX-1; *m/z* 876 → 823, 876 → 551 and 876 → 213 (CE: 40V) for PTX2; *m/z* 894 → 823 and 894 → 805 (CE: 40V) for PTX2SA, and *m/z* 1141 → 1061 (CE: –45V) for YTX. PTX2 was quantified by *m/z* 876 → 823 by comparison with certified reference solution of PTX2. PTX2SA acid was quantified based on calibration with PTX2 and its concentration was expressed as PTX2 equivalents.

In LC-MS/MS, retention times for the analyses were 23.8 min (PTX2SA), 23.5 min (OA), 24.7 min (PTX2), 25.7 min (DTX1) and 25.5 min (YTX). Illustrative LC-MS and LC-MS/MS chromatograms of analyses of standard mixtures are shown in Fig. 3. The limit of detection (LOD) of PTX2 was determined as 15 pg per injection (on column; s/n 3:1). No recovery data for the algal toxins analysed from the matrices of the present study are available. Signal (ion) suppression and enhancement in the MS analysis were tested by comparing responses from pure standard solutions against those from algae and zooplankton extracts spiked with known concentrations of pure phycototoxins.

Results

Dinophysis spp. distribution

Dinophysis spp. abundance in 2005 varied between 178 and 5304 cells l^{-1} in the topmost 10-m layer (Table 1). *Dinophysis acuminata* was the most common species while *D. norvegica* was found only at stations 2 and 3 in the Gulf of Finland (10–156 cells l^{-1}). The heterotrophic *D. rotundata* was present at abundances from 65 to 1810 cells l^{-1}. The highest total *Dinophysis* spp. abundance was found in the Gulf of Finland at station 2. In the northernmost part of the study area (Gulf of Bothnia) *Dinophysis* spp. were absent, or the abundances were low. In 2006, the total *Dinophysis* spp. abundances at station 3 ranged from 320 to 2180 cells l^{-1} (Fig. 2D). *Dinophysis acuminata* dominated the *Dinophysis* communities (94.5% ± 5.6%). The vertical distribution of *Dinophysis* spp. at station 3 differed between years (Fig. 2). During periods characterized by a distinct thermocline most of the *Dinophysis* spp. cells occurred at the uppermost 15 meters. When the thermocline was disrupted, the cells were more evenly distributed in the water column.

Copepod and *Dinophysis* spp. abundances and toxin concentrations in the net samples

In 2005, the zooplankton net material included copepods, cladocerans and rotifers. We left out cladocerans and rotifers from these analyses, since we assume that these groups most likely do not feed on the hard-shelled relatively large *Dinophysis*. The toxin samples that were prepared from this net material contained from 116 to 7750 adult copepod individuals and also *Dinophysis* spp. cells in abundances of 50–1000 cells $sample^{-1}$ (Table 1 and Fig. 4).

In 2006, the *Dinophysis* spp. numbers in the water samples (concentrated 20–76 μm fraction) varied from 8886 to 54 288 cells (Table 2).

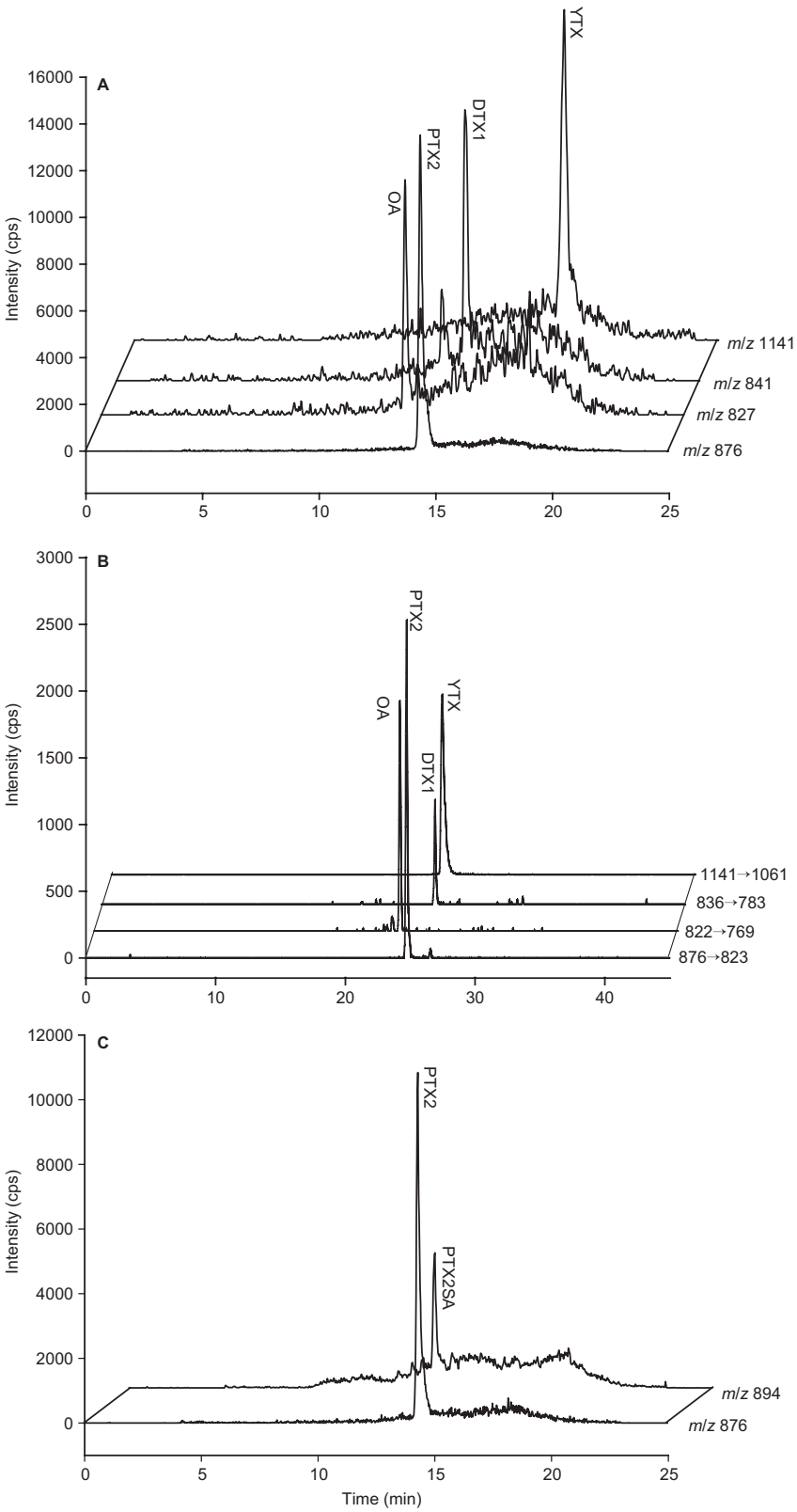

Fig. 3. (**A**) LC-MS/MS chromatogram of an 0.05 μg ml^{-1} standard mixture with PTX2 (RT 24.7 min), OA (RT 23.5 min), DTX1 (RT 25.7 min) with positive ionization, and YTX (RT 25.5 min) with negative ionization; 20 μl injection volume. (**B**) LC-MS chromatogram of 0.3 ng PTX2 (RT 14.2 min), 2.5 ng OA (RT 13.2 min), 2.5 ng DTX1 (RT 15.1 min) with positive ionization, and 0.25 ng YTX (RT 18.4 min) with negative ionization. (**C**) LC-MS chromatogram obtained by injection of PTX2SA (RT 13.1 min) pure extract and PTX2 (RT 14.2 min) 1:20 dilution; 20 μl injection volume.

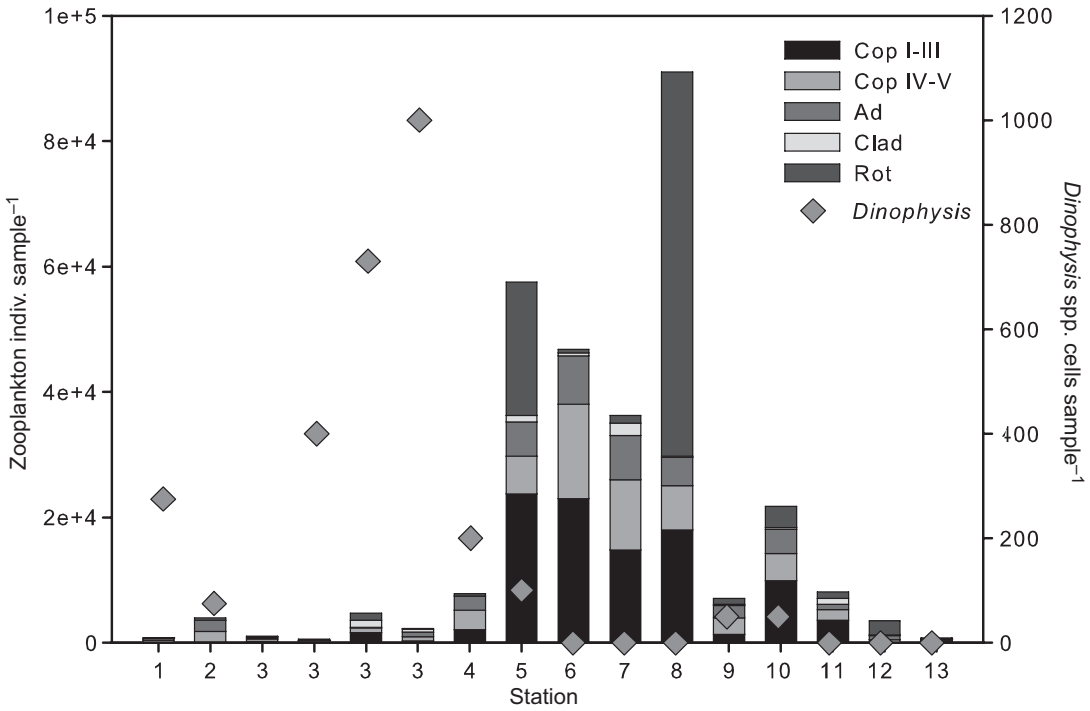

Fig. 4. The numbers of zooplankton (bars) and *Dinophysis* spp. (grey diamonds) in the toxin samples of the 2005 zooplankton net material from the stations 1–13. Cop I–III = copepodites of developmental stages I–III, Cop IV–V = copepodites of developmental stages IV–V, Ad = adult copepods, Clad = cladocerans, Rot= rotifers.

The 2005 toxin samples from stations 1 and 3 that were prepared from zooplankton net material contained 3.2–12.8 ng PTX2 per sample. These values were converted into *Dinophysis* cellular toxin concentrations on the basis of the number of *Dinophysis* cells found in these samples, resulting in concentrations of 7.7–17.5 pg PTX2 cell^{-1}. In addition, traces of (concentrations above the LOD) PTX-2SA were observed in the net material from 10 stations (Table 1), but these concentrations fell below the limit of quantification (signal-to-noise ratio of 5). Based on the spiking tests, ion suppression and enhancement in algae and zooplankton extracts were found insignificant.

Toxins in concentrated microplankton and isolated copepods

All the microplankton samples from station 3 in 2006 contained PTX2 at concentrations between 2.1 and 10.6 pg cell^{-1} (Table 2, all three species of *Dinophysis* included). Two samples from station 3 contained YTX (one sample 11.5 ng, not converted to cellular concentration, and one positive signal). All the samples of individually picked zooplankton were negative for phycotoxins analysed (Table 2).

Discussion

Toxins in the phytoplankton communities

Our results of cellular PTX2 concentrations in *Dinophysis* spp. (2.1–17.5 pg cell^{-1}) are close to the results of Kuuppo *et al*. (2006) from the same area (1.6–19.9 pg PTX2 cell^{-1}). We detected pectenotoxins, and also YTX in 2006, but no sign of toxins of the okadaic acid group. This is in accordance with Kuuppo *et al*. (2006), who detected pectenotoxins, but no OA in phytoplankton material or suspended organic matter. They, however, observed dinophysistoxins. Kozlowsky-Suzuki *et al*. (2006), found OA in the plankton communi-

ties of the southernmost Baltic Sea, but not in the central or northern parts of the basin. Dominance of pectenotoxins and absence of toxins from the OA group was also observed at the Chilean coast during a bloom of *D. acuminata* (Blanco *et al.* 2007). The lack of OA in the plankton communities of the northern Baltic Sea is interesting, since this seems to be a recurrent phenomenon, different from most marine environments where OA and its derivatives are the prominent DSTs, and especially since OA was measured from the Baltic Sea benthic fauna (Sipiä *et al.* 2000).

The dinoflagellates *Protoceratium reticulatum* (Satake *et al.* 1997), *Lingulodinium polyedrum* (MacKenzie *et al.* 1998) and *Gonyaulax spinifera* (Rhodes *et al.* 2006) are known to produce yessotoxins. Since the occurrence of these species in our samples was not examined at the time, it is unclear which of these species was responsible for the observed YTX. Uronen (2007) reported positive findings of yessotoxin from the Finnish coastal waters in summer 2006 but, like in this study, the information of the actual causative organism and its cellular toxin concentration is lacking. According to previous notes, both *P. reticulatum* and *G. spinifera* may occur in the phytoplankton communities of the study area during warm water periods, whereas the occurrence of *L. polyedrum* is restricted to the southernmost areas of the Baltic (Kuosa 1986, Hällfors 2004).

Pectenotoxins in zooplankton

Copepods can feed on *Dinophysis* spp. (Maneiro *et al.* 2000, Maneiro *et al.* 2002, Wexels Riser *et al.* 2003, Kozlowsky-Suzuki *et al.* 2006), but studies on toxin accumulation in the planktonic grazers are rare (Kozlowsky-Suzuki *et al.* 2006). Feeding on *Dinophysis* spp. cells may not lead to toxin accumulation in the grazer. In the study of Wexels Riser *et al.* (2003), intact *Dinophysis* cells were found in the faecal pellets of *Calanus helgolandicus*, and similar observations were reported for *Temora longicornis* (Maneiro *et al.* 2000, Maneiro *et al.* 2002). In the experiments of Kozlowsky-Suzuki *et al.* (2006), the copepods were actively feeding on *Dinophysis* spp., but no toxins were detected in the grazers after the incubations. The authors, however, gave estimates on OA retention and accumulation in the copepod *Centropages typicus* in the southern Baltic that were based on the measured ingestion rates on *D. norvegica*, and findings of OA in a field sample containing copepods. In our previous study (Setälä *et al.* 2009), we detected pectenotoxins in the copepods *Acartia bifilosa* and *Eurytemora affinis* that were incubated in microplankton suspension containing *D. acuminata*. To our knowledge these are the only findings concerning DST accumulation in copepods based on direct measurements. Since we did not find PTX2 in the field collected zooplankton individuals, the findings of PTX2 in copepods after the experiments cannot be used as proof for a toxin transfer route: algae–copepods–higher-trophic-level animals. In our incubation experiments, the number of *Dinophysis* spp. cells was higher than what is typically found in the study area, which may contribute to the accumulation of PTX2 in copepods during incubation.

Dinophysis spp. were present during the sampling periods in both years. At station 3, the cells were found almost throughout the water column, yet no toxins were found in zooplankton. All samples containing individually picked copepods from station 3 in 2006 were negative for toxins during the time when pectenotoxins were present in *Dinophysis* spp. communities. We did, however, detected PTX2 in the zooplankton net samples from two coastal stations in 2005. Since these samples also contained *Dinophysis* spp. cells, we consider now plausible that the toxins actually originated from these cells instead of zooplankton. This seems apparent since our estimates of cellular PTX2 concentrations in zooplankton samples (7.7–17.5 pg cell^{-1}) are close to what we found in the microplankton communities in the year 2006 (2.1–10.6 pg cell^{-1}) as well as to what was reported from the same area by Kuuppo *et al.* (2006).

In 2005, PTXSA was observed in the net samples from most of the stations, while PTX2 was detected only at two stations. PTX2SA is a known metabolite of PTX2 in shellfish that have been exposed to algae containing PTX2 (Suzuki *et al.* 2001), but no evidence exist that PTX2 metabolism in copepods would produce PTX2SA. It cannot, however, be excluded that

PTX2SA in the net samples may have originated from disrupted *Dinophysis* cells. Whatever the source of PTX2SA in the net samples is, the present results indicate that pectenotoxins can be found throughout the whole northern Baltic, even in the low-salinity areas in the Gulf of Bothnia.

Conclusions

The presence of pectenotoxins is a recurrent phenomenon in the northern Baltic Sea. PTX2 is produced in the *Dinophysis* spp. communities dominated by *D. acuminata*.

The origin of PTX2 and PTX2SA in our study was most likely *Dinophysis* spp., but the observations of YTX were not linked to a causative organism and need further studies. No phycotoxins occurred in copepods despite the simultaneous presence and abundance of PTX2-containing *Dinophysis* spp., therefore suggesting that the accumulation of DSTs in copepods of northern Baltic Sea is insignificant. However, the recurring presence of DSTs and the well-characterised abundance of other phycotoxins — especially peptide hepatotoxins — in the area would warrant inclusion of these contaminants into Baltic Sea chemical monitoring.

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