The toxic dinoflagellate *Alexandrium ostenfeldii* promotes incapacitation of the calanoid copepods *Eurytemora affinis* and *Acartia bifilosa* from the northern Baltic Sea

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Dense localized blooms of the toxic dinoflagellate *Alexandrium ostenfeldii* are a recent phenomenon in the low salinity waters of the Baltic Sea. This study reports results from laboratory experiments investigating the interaction between PSP toxin producing strains of *A. ostenfeldii* and two copepod species, *Eurytemora affinis* and *Acartia bifilosa*, from the northern Baltic Sea. Copepod grazing rates were studied by incubating the copepods with mixed cultures of *A. ostenfeldii* isolated from a bloom area. The effect of *A. ostenfeldii* on copepod condition and behavior was studied by exposing copepods to the mixed cultures and cell-free filtrates. Grazing on *A. ostenfeldii* cells was negligible for both copepod species. The 24 h incapacitation incubations indicated that the condition of both copepod species was negatively affected by the presence of *A. ostenfeldii*. The behavioral changes observed took place during the first hours of exposure to *A. ostenfeldii* cells, and little or no change was observed in the condition of the copepods after 6 h compared to 24 h. Similar observations were made for *E. affinis* incubated in cell-free filtrates of *A. ostenfeldii*, while the response of *A. bifilosa* was less pronounced. Our study shows that the copepods do not graze on *A. ostenfeldii* due to rapid behavioral disturbance and incapacitation by the algal cells and their exudates. This represents the first observation of a negative effect of the *A. ostenfeldii* on co-occurring biota in the northern Baltic Sea.

KEYWORDS: Baltic Sea; *Alexandrium ostenfeldii*; Harmful Algal Blooms; copepod; toxicity; incapacitation

INTRODUCTION

Harmful algal blooms (HABs) are a recurrent problem in coastal areas around the world. Globally, most of the bloom events are caused by dinoflagellates, a group of unicellular microalgae that can produce highly potent toxins. In the Baltic Sea, dinoflagellates have so far been considered minor HAB organisms compared to...
cyanobacteria, which regularly form extensive toxic blooms and dominate the second peak of the seasonal production cycle in late summer. However, recent evidence suggests a growing role of dinoflagellates in the Baltic ecosystem: particularly the late summer phytoplankton community harbors a number of potentially toxic species, some of which occur at high cell concentrations and may even become dominant (Hajdu et al., 2005; Kremp et al., 2009). Several studies have reported dinoflagellate toxins from the summer phytoplankton community and shown that such toxins can be transferred in the food web (Sipia et al., 2000; Sopanen et al., 2006; Setälä et al., 2009). One of the toxic species that is presently expanding in the Baltic Sea is *Alexandrium ostenfeldii*, a toxin producing dinoflagellate that can be recognized by a conspicuous ventral pore in the hypotheca. In shallow embayments of the eastern and northern coasts, this species now frequently forms dense blooms that can reach cell concentrations of $10^5$ to $>10^6$ cells $L^{-1}$ (Kremp et al., 2009). Such abundances are exceptional for *A. ostenfeldii*. In most regions of the world, the species occurs at low numbers in bloom communities typically dominated by other *Alexandrium* species (Brown et al., 2010; Touzet et al., 2011). In the Baltic Sea, *A. ostenfeldii* is the only species of the genus, and bioluminescent blooms have been reported from the Finnish, Swedish and Polish coasts.

*Alexandrium ostenfeldii* can produce neurotoxins such as paralytic shellfish toxins (PSTs) and spirolides (Hansen et al., 1992; Mackenzie et al., 1996; Cembella et al., 2000a). While spirolides are the dominant toxins in the European and North Atlantic *A. ostenfeldii* populations (Cembella et al., 2000b; Gribble et al., 2003; Ciminiello et al., 2006; Touzet et al., 2008), all Baltic populations investigated so far were found to produce only PSTs (STX and GTX2/3) (Kremp et al., 2009; Suikkanen, unpublished data).

PSTs are of concern because they can be transferred through the grazing food chain to consumers, e.g. shellfish (Teegarden and Cembella, 1996; Mons et al., 1998; Campbell et al., 2005), to higher trophic level such as fishes (White, 1981; Beaulieu et al., 1996), sea birds (Nisbet, 1983; Shumway et al., 2003) and mammals (Durbin et al., 2002).

The interactions of PST-producing algae and their zooplankton grazers have been investigated extensively. Studies have addressed the feeding behavior of copepods grazing on toxic *Alexandrium* species, including the mechanisms behind reduced grazing (Colin and Dam, 2003), resistance to toxicity (Colin and Dam, 2004, 2007) and grazer-induced toxin production in dinoflagellates (Bergqvist et al., 2008). The observed effects on zooplankton are diverse and vary among algae and zooplankton species, and are often species- and site-specific. *Alexandrium* spp. may affect the grazing rates of copepods by feeding deterrents (Turriff et al., 1995; Teegarden and Cembella, 1996; Shaw et al., 1997) and/or toxic compounds (Ives, 1987; Teegarden et al., 2008). Feeding deterrents are waterborne compounds that are detected by the copepod and cause the grazer to cease feeding. Toxic effects of *Alexandrium* spp. are caused by the ingestion of toxin-producing cells (Colin and Dam, 2003). Copepods feeding on toxic dinoflagellates may accumulate PST toxins in their tissues and thus possibly act as vectors in the planktonic food web (Turner et al., 2000). Alternately, they may act as sinks for these toxins because of their low toxin retention efficiencies (Teegarden et al., 2003). Interestingly, toxicity of PST-producing dinoflagellates often leads to sub-lethal effects on copepod grazers. Such effects include changes in behavior and reduction in fecundity (Teegarden and Cembella, 1996; Dutz, 1998; Turner et al., 1998).

This paper investigates the effects of toxic *Alexandrium ostenfeldii* on two copepod species, *Eurytemora affinis* and *Acartia bifilosa*, likely to co-occur with *A. ostenfeldii* in the coastal northern Baltic. *Acartia bifilosa* is a common member of the zooplankton community throughout the whole Baltic Sea, both in the open sea and coastal areas (Lindqvist, 1959; Hernroth and Ackefors, 1979; Viitasalo et al., 1994). *Eurytemora affinis*, the second most abundant species in the northern Baltic Sea (Viitasalo et al., 1994), is considered as a eurythermic brackish water species that is more confined to the warm water than *A. bifilosa*. While *A. bifilosa* maintains low abundances during winter and begins to reproduce in early spring, *E. affinis* communities develop later in spring (Viitasalo et al., 1995). Both species are also found at the Aland site where the extensive blooms of *A. ostenfeldii* occur. Both these copepod species have been shown to accumulate cyanobacterial and dinoflagellate toxins (Lehtiniemi et al., 2002; Karjalainen et al., 2005a, b; Kozlowsky-Suzuki et al., 2006; Setälä et al., 2009; Sopanen et al., 2009).

The high magnitude blooms of the PST-producing *Alexandrium ostenfeldii* are a fairly recent phenomenon in the northern Baltic, and potential effects on local zooplankton consumers are not known. A further expansion of the *A. ostenfeldii* distribution would create a new environmental threat to areas, without a history of toxic dinoflagellate blooms. In this study, we exposed individuals of *E. affinis* and *A. bifilosa* under controlled experimental conditions to toxic mixtures of *A. ostenfeldii* isolates and documented grazing and behavioral responses of the animals. The aim was to better understand the interactions of the new toxic bloom species and
co-occurring zooplankton, and estimate potential pathways of toxins in the coastal system of the northern Baltic Sea. When the initial grazing experiments revealed that copepods did not ingest A. ostenfeldii due to rapid incapacitation upon exposure to cell suspensions, experiments were performed to further elucidate the mechanisms triggering the observed behavioral responses.

**METHOD**

**Copepods and algal cultures**

The copepods used in the experiments were collected between July and October from Tvärminne Storfjärd, SW coast of Finland (59°50’N, 23°15’E). Samples were taken with vertical hauls (25–0 m) of a 100 µm plankton net and placed in a temperature controlled room set to in situ temperature (10–12°C). Adult females were hand-picked from the net material and transferred to GF/F (Whatman) filtered seawater for ca. 24 h acclimation to experimental conditions. Animals were caught within 1–3 days before the start of the experiment.

All experiments were conducted with two A. ostenfeldii strains (AOTV-A1 and AOTV-A4) isolated from a bloom in the Åland archipelago, northern Baltic Sea in 2004. Mixtures were used instead of monocultures to account for potential intraspecific diversity of the population. Cultures were maintained in F/2-Si enriched sea water (Guillard and Ryther, 1962) of local salinity (5–6 PSU) at 17°C, 14:10 L:D cycle at 100 µmol photons m⁻² s⁻¹. For the experiments, mixed inoculum cultures were grown to stationary phase in 500 mL tissue culture flasks.

Samples for confirmation of the algal toxin content were taken from the algal mixtures shortly before the experiments. Duplicate 50 mL aliquots of the culture were filtered onto glass fiber filters (Whatman GF/F) and freeze-dried (Savant Super Modyulo-230, Thermo Electron Corporation). PSTs were measured using the HPLC/FD method of Diener et al. (Diener et al., 2006) outlined in Kremp et al. (Kremp et al., 2009).

**Grazing experiments**

To assess the grazing rates of *E. affinis* and *A. bifilosa* on *A. ostenfeldii*, copepods were placed in algal suspensions prepared from stationary phase mixed *A. ostenfeldii* cultures (AOTV-A1 and AOTV-A4). Experimental cell concentrations of 560 cells mL⁻¹ for *Eurytemora affinis* and 300 cells mL⁻¹ for *Acartia bifilosa* were achieved by respective dilution of the *A. ostenfeldii* culture with filtered sea water. The cell concentrations used represent intermediate natural cell densities that occur during the growth period of the *A. ostenfeldii* population in August (10⁸–10⁹ cells L⁻¹) (Kremp et al., 2009). Before addition of the copepods, samples for initial cell counts needed to calculate ingestion rates were taken from the experimental suspensions counts, and preserved with 1% (final conc.) acid Lugol’s solution. Cell counts were performed according to Utermöhl (Utermöhl, 1958) using a Leica DMIL (Leica, Wetzlar, Germany) inverted microscope.

Six healthy acclimated animals were added to three replicate 130 mL transparent glass bottles containing the *A. ostenfeldii* suspensions. Replicate bottles containing algal suspension without copepods served as grazing controls. To account for potential effects of starvation, additional controls where animals were incubated in algae free filtered sea water were prepared. Experimental bottles were filled completely to avoid air bubbles, sealed and placed on a plankton wheel rotating at a speed of 1 rpm at +11°C, 12:12 h L:D cycle in dim light. After 24 h of incubation, the content of each bottle was carefully poured through a 200 µm sieve, copepods were washed into Petri dishes where they were counted and evaluated for their condition. The procedure followed the methods used previously and described in, for example, Sopanen et al. (Sopanen et al., 2008) and Setälä et al. (Setälä et al., 2009). The remaining *A. ostenfeldii* suspension was preserved with Lugol’s solution and the cell numbers were determined as described above. Ingestion was calculated according to Frost (Frost, 1972).

Toxin accumulation in the copepods was determined by measuring toxin content of copepods after incubations. The animals were picked from the Petri dishes, washed carefully with filtered seawater and placed onto glass fiber filters. Freeze-dried filters were analyzed for toxin content as described above. In order to collect enough material for the toxin analyses, the copepods from the replicate bottles were pooled for a single toxin sample.

**Incapacitation experiments**

The behavioral responses of *Eurytemora affinis* and *Acartia bifilosa* to *Alexandrium ostenfeldii* were examined by observing the animals and documenting their condition during and after their exposure. Three different aspects were studied: (i) the effect of *A. ostenfeldii* cell concentration on copepod condition; (ii) the role of exposure duration on copepod condition; and (iii) the effect of substances excreted by *A. ostenfeldii* cells (cell-free treatments).

All incapacitation experiments were conducted in tissue culture plates where copepods (3–4 copepods/well) were incubated in 5 mL of experimental water (toxic treatments containing *A. ostenfeldii* cell suspensions...
or cell-free filtrates) or in 5 mL of GF/F filtered seawater (non-toxic controls). The plates were maintained under controlled light and temperature conditions (12°C, LD cycle of 14:10 h in 50 µE m⁻² s⁻¹). The lid of the wells was not sealed to prevent oxygen deficiency from developing. A total of 18–20 animals were incubated in each toxic treatment or in non-toxic controls. The copepod condition was evaluated by their behavior and movement and the copepods were ranked into four categories (0–3): 0 = dead, 1 = immobile (alive but not moving), 2 = disturbed swimming behavior (ceased/erratic movement, still able to escape reaction when agitated), 3 = normal. Average condition indexes were calculated from all individuals observed in one treatment at one time point to assess the general change in the copepod condition. For statistical analyses, the condition categories 1 and 2 were combined to represent one group of impaired individuals.

To evaluate effects of *A. ostenfeldii* cell concentration on copepod condition, animals were incubated at different *A. ostenfeldii* cell concentrations ranging between approximately 50 and 1200 cells mL⁻¹. The concentrations, including the very high concentrations, were chosen to cover the abundance range documented from the bloom sites (Kremp et al., 2009). The copepod condition was visually assessed at the beginning of the experiment and after 24 h under a stereo microscope (6.3–50× magnification, Leica MZ 7.5), and ranked accordingly (categories 0–3).

To study the effects of exposure time on copepod condition, animals were inspected repeatedly over a time period of 24 h: several times during the first 6 h and finally at 24 h. Time course observations were carried out at 3 selected *A. ostenfeldii* concentrations covering the range mentioned above. At the end of the incubations, copepods were collected, washed and picked on GF/F filters for toxin analyses. All animals exposed to the same concentration of *Alexandrium* were pooled to ensure enough material for analyses.

To test whether the observed changes in copepod condition were caused by direct interaction of the animals with the *A. ostenfeldii* cells, or by dissolved substances excreted by the cells into the water, copepods were incubated for 24 h in cell-free *A. ostenfeldii* filtrates. The filtrates were made by filtering highly concentrated *A. ostenfeldii* cell suspensions (1000 cells mL⁻¹) gently through a GF/C filter (Whatman). Again, copepod condition was evaluated during 0–6 h and after 24 h.

**Recovery test**

Reversibility of incapacitation was tested in a recovery experiment with *Eurytemora affinis*. Copepods were incubated as described above with four different experimental treatments: (i) filtered sea water, serving as a control, (ii) a low density *A. ostenfeldii* suspension (50 cells mL⁻¹), (iii) a medium density *A. ostenfeldii* suspension (600 cells mL⁻¹) and (iv) a filtrate made from the 600 cells mL⁻¹ *A. ostenfeldii* suspension. After 24 h, the same animals were transferred to filtered seawater and incubated for another 24 h. At 48 h, they were transferred to a suspension containing the green alga *Brachionus submarina* (TV22; culture collection of Tvarminne Zoological Station), to feed on food known to be non-toxic (Koski and Kuosa, 1999; Koski et al., 1999) for yet another 24 h. The condition of copepods was evaluated at 0, 24, 48 and 72 h.

**Statistical analyses**

Simple and multiple regression analyses with dummy variables were used to assess the effects of concentration (24 h, 50–1200 mL⁻¹) and time and concentration (0–24 h, 100–1200 cells mL⁻¹), respectively, on the relative portions (%) of organisms in each condition category. Two dummy variables encoding the condition categories, concentration, time (excl. 24 h experiment) and all the cross-product terms were included as independent variables and a stepwise method was used to eliminate the non-significant variables (probability of F to remove: 0.10). The simple regression analysis of the 24 h *E. affinis* incapacitation experiment was run with the 3–4 replicates but in the *A. bifilosa* 24 h experiment, 6 replicates per concentration were reduced to 2 by summing in order to achieve a better fit of the model. For the same reason, in the time-concentration experiments, the replicates were summed together. In cases where the constant was not significant for the model, the regression model was forced through the origin. The results are described with $R^2$ (goodness-of-fit of the model), probability for rejecting a true null hypothesis (i.e. no effect) ($P$-value) and degrees of freedom (df). All the procedures were performed in PASW Statistics 18.

**RESULTS**

Toxin analyses confirmed that the cellular suspensions of *Alexandrium ostenfeldii* used in this study were toxic. Cellular toxicities of the suspensions used in the different experiments ranged between 1.47 and 2.26 pg cell⁻¹ GTX2, 0.59–1.15 pg cell⁻¹ GTX3 and 0.39–0.64 pg cell⁻¹ STX.

The calculated ingestion rates based on the Frost equation varied between −26.0 and 8.4 cells h⁻¹ ind⁻¹ for *E. affinis* (−0.055–0.018 µg C ind⁻¹) and −23.2
and 8.1 cells h$^{-1}$ ind.$^{-1}$ ($-0.049$–$0.17$ µg C ind.$^{-1}$) for *A. bifilosa* (Fig. 1.). The corresponding clearance rates (mean ± SD) were $-0.004 ± 0.023$ mL ind.$^{-1}$ h$^{-1}$ for *E. affinis* and $0.009 ± 0.059$ mL ind.$^{-1}$ h$^{-1}$ for *A. bifilosa*, respectively. Most copepods were inactive when retrieved from the experimental bottles at the end of the experiments, while the animals in the control units were healthy. Toxin analyses revealed no PSTs in either of the copepod species after the incubation with toxic cells.

The 24 h incapacitation incubations showed that the condition of both copepod species was negatively affected by the presence of *A. ostenfeldii* (Fig. 2). The number of impaired *E. affinis* individuals increased logarithmically with increasing *A. ostenfeldii* concentrations while the proportion of normal individuals had an

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**Fig. 1.** Ingestion of *A. ostenfeldii* cells by the copepods *A. bifilosa* and *E. affinis* during 24 h incubation. Error bars represent standard deviation.

**Fig. 2.** Condition of the copepods *E. affinis* and *A. bifilosa* (shown as percentage of all copepods in each experiment) after 24 h exposure to *A. ostenfeldii* cell suspensions at different cell concentrations (50–1200 cells mL$^{-1}$). The copepod condition is ranked based on observations on their behavior and movement.

**Fig. 3.** Condition of the copepods *E. affinis* and *A. bifilosa* exposed to *A. ostenfeldii* cell suspensions at different concentrations through time. The copepod condition is ranked based on observations on their behavior and movement. The condition ranks (0–3) used are: 0 = dead, 1 and 2 = impaired, 3 = normal. Dotted line: animals incubated in sea water controls. Error bars represent standard deviation.
exponentially decreasing trend ($R^2 = 0.79$, $P = 0.000$, df = 92). The proportion of dead *E. affinis* was not dependent on the concentration. As in the *E. affinis* experiment, the proportion of normal *A. bifilosa* individuals decreased and impaired individuals increased with increasing *A. ostenfeldii* concentration but the relationships were linear ($R^2 = 0.73$, $P = 0.000$, df = 48). In contrast to *E. affinis*, the proportion of dead *A. bifilosa* individuals was dependent on the concentration and their proportion increased at the same rate as that of impaired individuals, although at a substantially lower level. In the control treatments without *A. ostenfeldii* cells, 100% of the *E. affinis* individuals were swimming normally after 24 h, whereas some dead *A. bifilosa* (8–11%) were found in the incubations in filtered sea water.

The time course experiments showed that the observed behavioral changes took place during the first hours of exposure to *A. ostenfeldii* cells, and little or no change was observed in animal condition after 6 h compared to 24 h (Fig. 3). Due to the large variation in the percentage individuals per condition class at each concentration, concentration was a non-significant variable for the *E. affinis* regression model ($R^2 = 0.83$, $P = 0.000$, df = 93). The proportion of impaired and normal individuals was significantly dependent only on time, whereas the proportion of dead individuals was stable. For the regression model of *A. bifilosa* ($R^2 = 0.91$, $P = 0.000$, df = 114), both time and concentration were significant variables in the cross-product terms but time had much stronger impact than concentration. The proportion of normal individuals decreased to zero with increasing time and concentration, whereas the proportion of impaired and dead individuals had opposite trends.

Toxins were measured in 3 of the 14 pooled samples, each containing 18–20 copepods. Toxin levels per individual (0.149–0.244 ng GTX2 ind.\(^{-1}\), 0.014 and 0.054 ng GTX3 ind.\(^{-1}\)) did not reflect the concentrations of *A. ostenfeldii* in which the respective animals had been incubated. No toxins were measured in any of the *A. bifilosa* samples prepared for toxin analyses.

When incubated in cell-free filtrates, *E. affinis* showed similar responses as in the cell suspensions, with most animals becoming inactivated within a few hours of exposure (Fig. 4A). In *A. bifilosa*, the response to filtrates was different from the cell suspension: the copepod did not show clear signs of lowered condition during the
first hours. However, a slight decrease in condition was observed after 24 h in all treatments (Fig. 4B).

The recovery experiment performed with *E. affinis* showed that the inactivation caused by exposure to *A. ostenfeldii* cells and filtrates was reversible when copepods were transferred from the toxic *A. ostenfeldii* cell suspensions or cell-free filtrates to fresh filtered seawater and non-toxic food (Fig. 5). Recovery was complete (no difference to control copepods) in the animals incubated at *A. ostenfeldii* low densities. Also *E. affinis* individuals exposed to medium *A. ostenfeldii* concentrations and the respective filtrates regained their motility.

**DISCUSSION**

Our experiments suggest that *A. ostenfeldii* was not grazed by the copepods as a result of a rapid behavioral disturbance and incapacitation caused by the algal cells and their exudates. Such obvious inhibition of copepod grazing represents the first observation of a negative effect of the presently expanding *A. ostenfeldii* on co-occurring zooplankton in the northern Baltic Sea. In order to estimate the effects of *A. ostenfeldii* in natural conditions with co-occurring algal species and various grazers, more experiments need to be carried out, for example with multiple prey.

The negligible ingestion rates found in the grazing experiments reflect what has been observed earlier for some zooplankton grazers when offered toxic algae as food. Other *Alexandrium* species have been shown to have toxic (Huntley et al., 1986; Ives, 1987) and deterrent effects on copepod grazers (Turriff et al., 1995; Teegarden and Cembella, 1996). The toxic effects of an algal species are easy to recognize when leading to grazer mortality. However, algal toxicity does not inevitably lead to the death of the grazer, but can be reflected by suppressed grazing, egg production and population growth (Dutz, 1998; Frangopulos et al., 2000; Colin and Dam, 2007). Such sublethal toxic effects can easily be mixed with the effects of feeding deterrents which lower the grazers feeding activity toward the algae. The latter help the grazer to actively avoid toxic algae and are based on chemosensory mechanisms (Wolfe, 2000). When grazers detect feeding deterrents, i.e. compounds produced by the algae that signal toxicity or danger, they cease feeding or select against that particular algal species (Shaw et al., 1997).

In the case of the two Baltic copepod species tested, incapacitation was triggered by both cell suspensions as well as cell-free filtrates of *A. ostenfeldii*. This, together with the fact that toxic cells were not ingested in significant amounts, suggests that the effect was not primarily caused by ingested cellular PSP toxins, but by extracellular compounds released by *A. ostenfeldii* cells into the water. The incapacitation process, which was at first seen as erratic swimming motion and later lead to a total loss of motility or, in some cases, death, took place within a few hours and thus effectively suppressed copepod feeding on *A. ostenfeldii*. It is, however, possible that ingested cells play a role in this incapacitation, even if only one or two cells would have been ingested. This may be the reason why the proportion of dead animals was actually higher in the lowest cell concentration for *E. affinis* compared to the next concentration that was double the first one (Fig. 2). If the animals tolerated the lowest cell concentration somewhat better, they could have been able to ingest a few cells, which may have then caused their death. For *A. biflora*, the lowest cell concentration was not lethal during the 24 h incubation, but already at concentration of 100 cells mL⁻¹, dead animals were found. Similar effects of toxic algae have earlier been reported for the pelagic harpacticoid *Euterpina acutifrons* (Bagøien et al., 1996) which responded at all developmental stages with reduced motility and even death to the toxic dinoflagellate *Alexandrium minutum*. Hansen et al. (Hansen et al., 1992) showed that tintinnids were able to feed on toxic algae at low prey cell densities, and were only affected by the exudates produced by *Alexandrium tamarense*. Likewise, Tillmann and John (Tillmann and John, 2002) reported how cell suspensions and cell-free culture media of *Alexandrium* spp. immobilized and lysed heterotrophic dinoflagellates. Since the observed responses in their study were also triggered by non-toxic strains, they concluded that the effective compounds of these interactions were allelochemicals other than the actual phycotoxins.

Toxins have rarely been measured directly from cell-free filtrates and hence it remains unresolved to what extent water-soluble PSTs are involved in allelopathic interactions such as the ones observed in our experiments with *A. ostenfeldii* filtrates. When analyzing filtrates from cell suspensions prepared in conjunction with a separate toxin distribution study for PSTs, significant amounts of GTX2/3 were detected. Although the density of the culture (25 000 cells mL⁻¹) from which PST-containing filtrates were obtained by different filtering methods (GF/F, GF/C and 10 μm plankton net) was an order of magnitude higher than natural and experimental cell concentrations, this finding indicates that PSTs could have been present in the experimental filtrates and affected the copepod response (Suikkanen, unpublished data).

It is also possible that the filtrates used in our experiments contained PSTs that might have been involved in the incapacitation caused by the *A. ostenfeldii* filtrates.
Since filtrates had been prepared from stationary phase cultures in our experiments, it is possible that the toxins were, if not actively excreted, released from decaying cells. Despite the rapid and severe immobilization of the copepods, in some of the incapacitation experiments small amounts of GTX2 and GTX3 were found in *Eurytemora affinis*. It is possible that the copepods were accumulating toxins during the 24 h incubation period from the dissolved phase. Such a mechanism of toxin accumulation (nodularin) in *E. affinis* has previously been reported by Sopanen et al. (Sopanen et al., 2009) and Karjalainen et al. (Karjalainen et al., 2005a), and is also consistent with the findings of Teegarden and Cembella (Teegarden and Cembella, 1996) who observed that the copepod *Eurytemora herdmani* accumulated saxitoxins without significant feeding.

Although *E. affinis* suffered from the presence of *A. ostenfeldii*, the incapacitated individuals were able to recover completely within a few days when transferred to sea water without *Alexandrium ostenfeldii* cells or exudates and fed with a suitable diet. This could mean that the incapacitation itself protects the animals from more severe damage that might be caused if they were feeding on the algae. Immobile animals may also escape blooms that are normally concentrated in the productive top layer of the water column by simply sedimenting down. The incapacitation process also prevents the accumulation of large amounts of toxins in the copepod grazers which thus cannot act as vectors for the toxins in the food web.

The effects of *A. ostenfeldii* on ingestion rates and motility were more severe in *Eurytemona affinis* than in *Acartia bifilosa*. The share of normal *E. affinis* individuals decreased clearly with the algal concentration used and the share of impaired individuals increased, while in *A. bifilosa* the dependence on the dose was not obvious. *Acartia bifilosa* appears to be more resistant to *A. bifilosa* than *E. affinis* which may also be seen as differences in individual responses to the exposure. Since the animals responded already at the lowest cell concentrations and we thus do not have a cell concentration where the animals would have tolerated the toxins, or likewise suffered all at a high concentration, no dose-dependence (e.g. a case where 50% of the animals could be affected) could be estimated. Baltic copepods have been shown earlier to respond differently to algal toxins, e.g. of the haptophyte *Prymnesium parvum* (cf. *P. parvum*), which can be tolerated by some species despite prolonged exposure but lead to elevated mortalities in others (Koski et al., 1999). Species-specific differences in the interactions between toxic alga and grazers are common and have been reported repeatedly (Turner and Tester, 1997; Colin and Dam, 2003).

Studies on the interaction of toxic algae and grazers suggest that active avoidance of and resistance to toxic prey is usually a result of a common history, i.e. co-evolution of the respective algae and a grazer (Ives, 1985; Colin and Dam, 2002). It is thus possible that the disturbed behavior and the resulting grazing inhibition are a reflection of the lack of common history of *A. ostenfeldii* and the two copepod species in the northern Baltic. Recurrent blooms of *A. ostenfeldii* are a relatively new phenomenon here and interaction of this dinoflagellate with zooplankton has been limited so that resistance has not evolved. In fact, the copepods used in our experiments were collected from a site, where *A. ostenfeldii* is rare and bloom formation has never been reported. The strong response of copepods to *A. ostenfeldii*, possibly reflecting a lack of resistance, might have consequences for system processes in the coastal Northern Baltic, where the blooms occur. When copepod feeding is inhibited, the biomass produced by the *A. ostenfeldii* blooms might not be transferred through the zooplankton, which may reduce the energy available to higher trophic levels. Such consequences of HABs have been reported earlier (Sunda et al., 2006). Inhibition of copepod grazing, on the other hand, will restrict top-down control of the toxic *Alexandrium* population and thereby contribute to bloom formation. Future studies, going beyond the laboratory approach, need to address such potential ecological consequences.

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