Allelopathic potential of the dinoflagellate *Alexandrium tamarense* on marine microbial communities

Astrid Weissbach a,⁎, Urban Tillmann b, Catherine Legrand a,b

aMarine Ecology section, School of Natural Sciences, Linnæus University, SE-39182 Kalmar, Sweden
bAlfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

1. Introduction

The adverse effects of harmful algal blooms (HABs) in marine ecosystems have focused attention on defining the role of factors that determine their abundance and distribution. Some marine dinoflagellates of the genus *Alexandrium* Halim (Balech) produce neurotoxins, which cause Paralytic Shellfish Poisoning (PSP), resulting in life-threatening human illness due to the consumption of contaminated seafood (Anderson, 1997). Blooms of toxic *Alexandrium* spp. occur worldwide, usually associated with embayments, estuaries or open coastal waters. *Alexandrium* spp. neurotoxins can also be transferred through the marine food web to higher trophic levels where they may affect zooplankton, fish, birds and marine mammals. *Alexandrium* species also release lytic compounds suppressing competitors (Fistarol et al., 2004; Tillmann et al., 2008). Although bacteria and phytoplankton are closely linked in coastal systems, little is known about their interactions in shaping the pelagic microbial communities (Riemann et al., 2000; Hay and Kubanek, 2002; Mayali and Azam, 2004). A few studies have shown that bioactive compounds produced by phytoplankton can inhibit bacteria (Ribelé et al., 2008; Doucette, 1995). However, experiments with natural communities showed higher bacterial abundances in presence of allelochemicals (Fistarol et al., 2004; Uronen et al., 2007). The flagellate *Prymnesium parvum* produces compounds that can lyse competitor cells and alter the functioning of the planktonic food web by increasing carbon transfer through the microbial loop (Uronen et al., 2007). Hence, phytoplankton may select for a specific phylogenetic and/or phenotypic composition of microbial communities in its surrounding environment. Many studies report the phylogenetic composition of bacterioplankton during *Alexandrium* blooms (Green et al., 2004; Wichels et al., 2004; Jasti et al., 2005; Sala et al., 2005). However, the mechanisms creating a specific type of phytoplankton-bacteria assemblage are unknown. The aim of this study was to examine the allelopathic effect of the formation of harmful algal blooms (Smayda, 1997). Chemical interactions among *A. tamarense* and other marine protists are an important biological factor for the dominance of *Alexandrium*, not solely restricted to the competition for nutrients (Fistarol et al., 2004; Tillmann et al., 2008). Although bacteria and phytoplankton are closely linked in coastal systems, little is known about their interactions in shaping the pelagic microbial communities (Riemann et al., 2000; Hay and Kubanek, 2002; Mayali and Azam, 2004). A few studies have shown that bioactive compounds produced by phytoplankton can inhibit bacteria (Ribelé et al., 2008; Doucette, 1995). However, experiments with natural communities showed higher bacterial abundances in presence of allelochemicals (Fistarol et al., 2004; Uronen et al., 2007). The flagellate *Prymnesium parvum* produces compounds that can lyse competitor cells and alter the functioning of the planktonic food web by increasing carbon transfer through the microbial loop (Uronen et al., 2007). Hence, phytoplankton may select for a specific phylogenetic and/or phenotypic composition of microbial communities in its surrounding environment. Many studies report the phylogenetic composition of bacterioplankton during *Alexandrium* blooms (Green et al., 2004; Wichels et al., 2004; Jasti et al., 2005; Sala et al., 2005). However, the mechanisms creating a specific type of phytoplankton-bacteria assemblage are unknown. The aim of this study was to examine the allelopathic effect of the...
dinoflagellate *Alexandrium* on bacterial and natural plankton communities using field samples from the North Sea and cultures of *A. tamarense*. Here, we compare the response of a plankton community to extracellular compounds produced by two strains of *A. tamarense* (lytic and non-lytic). This comparative approach was chosen to distinguish between the impact of the added *A. tamarense* suspension per se and the impact of lytic compounds, produced by *A. tamarense*, which cannot be separated from other algal exudates today.

2. Materials and methods

2.1. Algal cultures

*Alexandrium tamarense* strains (Alex2 and Alex5) were isolated from the east coast of Scotland (North Sea) in 2004 (Alpermann et al., 2009) and grown non-axenic in K-medium (Keller et al., 1987). These two clones were selected based on lytic capacity quantified by a *Rhodomonas* bioassay (Tillmann et al., 2009). Alex5 was found to have no lytic impact on *Rhodomonas* and will further be referred to as non-lytic *Alexandrium*. Alex2 had a high lytic capacity and is therefore called lytic *Alexandrium* in the further text.

Both *A. tamarense* and *Rhodomonas salina* (Kalmars Algal Collection, KAC 30) were grown in batch cultures in K-medium (salinity 32), prepared from sterile-filtered (VacuCap 0.2 μm Pall Life Sciences) North Sea water in 2000 ml Erlenmeyer flasks. Cultures were maintained under controlled conditions at 15 °C under cool-white fluorescent light (65 μmol m⁻² s⁻¹) on a 16 h light:8 h dark cycle. Prior to sampling, culture flasks were shaken gently by hand to allow for a homogenous cell distribution.

To quantify the lytic activity of *A. tamarense* on natural plankton communities, cell-free supernatant of *A. tamarense* cultures was used since filtration can remove up to 90% of the allelopathic activity probably due to absorption of the bioactive compounds on the filter (Tillmann et al., 2008). Aliquots (18 × 50 ml) of exponentially growing cultures of *A. tamarense* (lytic and non-lytic *Alexandrium*), 5000 cells ml⁻¹ were centrifuged for 15 min at 12,000 rpm. Aliquots of supernatant (30 ml) were stored in a glass bottle at 4 °C in the dark and used within 5 days. The lytic activity of the supernatant was checked prior to the experiment using a *Rhodomonas* bioassay (bioassay modified after Tillmann et al., 2009). Additionally, bacterial abundance and community composition in lytic and non-lytic *Alexandrium* supernatant and the North Sea water were measured.

2.2. Field sampling

A natural plankton community was collected during spring in May 2007 in the southern Helgoland Bight (North Sea) from the surface sample with a bucket. Seawater was filtered through 150 μm nylon nets to remove zooplankton and large detritus. Samples for bacterial abundance (in triplicates, 1.5 ml each) and production, chl a concentration (in triplicates, 300 ml each), phytoplankton (50 ml) and bacterial community composition (500 ml) were immediately taken. Nitrate (580 μM final concentration), phosphate (36 μM final concentration) and peptone (2 mg C l⁻¹) were added to ensure nutrient saturated conditions for both, phytoplankton and bacteria. Enriched seawater (400-495 ml) was placed in 11 glass bottles and incubated at 15 °C overnight prior to the experiment start.

2.3. Allelopathic effect of *Alexandrium* supernatant on microbial communities

Lytic and non-lytic *Alexandrium* supernatant were added, respectively, in three treatments (each in triplicates) corresponding to the lytic activity of 200 (low continuous addition), 1000 (high continuous addition) and 1000 (single addition) cells ml⁻¹, respectively.

The single addition corresponding to 1000 cells ml⁻¹ was added in 100 ml supernatant at T₀ to a final volume of 500 ml. Continuous additions corresponding to 300 (high continuous addition) and 50 (low continuous addition) cells ml⁻¹ were added daily in 30 and 5 ml of supernatant (T₀, T₂₄, T₄₈, T₇₂) to a final volume of 515 and 590 ml, respectively. We added K-medium to the control to obtain comparable nutrient conditions in control as well as *Alexandrium* treatments. Due to the different amounts of added supernatant, overall nutrient concentrations between treatments and controls may have slightly differed. However, since *A. tamarense* was cultivated in full K-medium, and the supernatant was produced during exponential growth, we assume that the amount of nutrients added to both the *Alexandrium* treatments as well as the controls exceeded the amount of nutrient that could possibly be used by phytoplankton and bacteria during the 96 h of incubation. 30 ml of K-medium were added daily (T₀, T₂₄, T₄₈, T₇₂) to triplicate control bottles to a final volume of 590 ml. All bottles were incubated for 96 h under similar conditions as the algal cultures. Samples for bacterial abundance were taken daily. After 96 h (Day 4), samples for bacterial abundance and production, chl a, phytoplankton and bacterial community composition were collected in treatments and controls. From T₂₄ and T₄₈, bacterial volumes in the treatments were calculated. Samples for bacteria counts were fixed in 2% formalin and placed at 4 °C. Whole water samples (20 ml) were filtered on glass fiber filters (Gelman A/E) and frozen at −20 °C prior to chi a analysis. Samples for identification and quantification of phytoplankton were preserved with Lugol’s solution. For extraction of bacterial community DNA, microbial biomass from 50 to 400 ml seawater was collected onto 0.2 μm-pore-size Supor 200 membrane filters by vacuum filtration at <150 mmHg. Filters were stored frozen at −20 °C in TE buffer (10 mM Tris, 1 mM EDTA).

2.4. Analytical procedures

2.4.1. *Rhodomonas* bioassay

A dilution series was made using different amounts of *Alexandrium* supernatant and whole cell culture corresponding to the following *Alexandrium* cell densities: 50, 75, 100, 150, 250, 375, 635, 937, 1250, 2500 cells ml⁻¹ in a total bioassay volume of 4 ml. K-medium (triplicate) served as control. *R. salina* was spiked to all samples to a final density of 12,500 R. salina cells ml⁻¹. After an incubation period of 3 h, samples were fixed with Lugol’s solution (2% final concentration) and counted with an inverted microscope (Zeiss Axiovert 35). A sub-area corresponding to at least 500 cells in the control was counted. Values of EC₅₀, defined as the *Alexandrium* cell concentration causing lysis of 50% of target cells, were calculated by using a sigmoidal curve fit function (Tillmann et al., 2009) in the mathematical programme Origin.

2.4.2. Chlorophyll a and enumeration of phytoplankton and nanoflagellates

Chlorophyll a concentration was measured fluorometrically (Turner AU-10 fluorometer) after 8 h extraction in the dark with 96% ethanol (Jespersen and Christoffersen, 1987). For each treatment, phytoplankton growth day⁻¹ was calculated by using the following Eq. (1):

\[
\mu = \frac{(LN(\text{Chl}_{96}) - LN(\text{Chl}_{0}))}{48}
\]
Except *Phaeocystis globosa*, phytoplankton was counted using a Zeiss Axiosvert inverted microscope and, when possible, identified at the species or genus level. The volume sedimented for the counts (5–50 ml) varied between different treatments. The total number of intact cells counted was always >200 per sample, except for the highest lytic *Alexandrium* treatments with >100 total counts per sample. *P. globosa* colonies were observed and counted in fresh samples fixed with Lugol’s solution a few hours after sampling. *P. globosa* total cells and nanoflagellates were counted at 1000 x using an Olympus BX50 inverted microscope and blue light epifluorescence. Samples fixed with Lugol’s solution were bleached with sodium iodide and stained with Lugol’s solution were bleached with sodium iodide and stained with Lugol’s solution, containing 10–50 ng DNA template, 100 μM of both primers, 22 μl PCR water and 25 μl Master mix (including 5U/ml Taq DNA polymerase, 400 μM dNTP’s, 3 μM MgCl₂). The template DNA was denatured in a thermal cycler for 5 min at 95 °C followed by PCR. PCR products were visualized by agarose gel electrophoresis with a molecular size standard in the gel. The PCR product was cleaned by using Sephadex and digested with the restriction enzyme Alu I. After an additional cleaning, the digested product was marked with the Map Marker 1000 and denatured. The following step, the capillary electrophoresis, separated the labelled fragments after their length. The data was visualised and analysed by using the applied biosystems software Gene mapper (applied biosystems). A statistical analysis in Past (Hammer et al., 2001) was performed to compare the different treatments. Non-metric multi dimensional scaling analysis was conducted to assess for the relationship between the different treatments. Non-metric multi dimensional scaling was performed with PAST software package [Hammer et al., 2001].
3. Results

3.1. Characterisation of A. tamarense cell-free supernatants

Lytic Alexandrium caused cell lysis of R. salina in a dose-dependent manner with an EC50 of 110 ± 50 A. tamarense cells ml−1. Cell-free supernatant was slightly less effective (EC50 = 180 ± 4 A. tamarense cells ml−1). Non-lytic Alexandrium supernatant or culture did not lyse R. salina at any concentration. Bacterial abundance was 4 times higher in lytic Alexandrium cultures (3.7 × 106 bacteria ml−1) compared to non-lytic cultures (0.9 × 106 bacteria ml−1). However, bacteria were on average twice as large in non-lytic Alexandrium culture (0.15 μm−1) compared to the lytic culture (0.07 μm−1) because the non-lytic culture contained large filamentous bacteria that were not observed in the lytic Alexandrium culture. Thus, bacterial biomass in the lytic Alexandrium culture (188 μg C l−1) was twice as high as in the non-lytic Alexandrium culture (95 μg C l−1). Bacterial production was significantly higher in lytic Alexandrium compared to non-lytic Alexandrium culture (228 ± 32 d−1 and 183 ± 12 d−1, respectively). However, production biomass−1 was lower in lytic Alexandrium compared to non-lytic Alexandrium culture (1.2 ± 0.2 d−1 and 1.9 ± 0.1 d−1, respectively). Centrifugation resulted in 90% (lytic Alexandrium) and 85% (non-lytic Alexandrium) reduction in bacterial numbers in the supernatant. Bacterial production in lytic and non-lytic Alexandrium supernatant was approximately 75% lower as in the corresponding cultures. Production biomass−1 was higher in Alexandrium supernatant compared to the Alexandrium cultures and equal in lytic and non-lytic Alexandrium supernatant.

3.2. Effect of A. tamarense on North Sea phytoplankton and nanoflagellates

Seawater was collected during the spring at a moderate chl a concentration of 3.7 μg Chl a l−1. In 2007, the spring phytoplankton was dominated by the colony forming P. globosa (Haptophyte) and cryptophytes (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Genus/species</th>
<th>Cells l−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinoflagellates</td>
<td>Gyrodinium spirale</td>
<td>7079</td>
</tr>
<tr>
<td></td>
<td>Scrippsiella spp.</td>
<td>6262</td>
</tr>
<tr>
<td></td>
<td>Protopero dinum spp.</td>
<td>4901</td>
</tr>
<tr>
<td></td>
<td>Dinophysis acuminata</td>
<td>4084</td>
</tr>
<tr>
<td></td>
<td>Pyrphacus horologium</td>
<td>2178</td>
</tr>
<tr>
<td></td>
<td>Procentrum micans</td>
<td>545</td>
</tr>
<tr>
<td></td>
<td>Alexandrium spp.</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>Polychiros schwartzii/kofoidii</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Ceratium lineatum</td>
<td>40</td>
</tr>
<tr>
<td>Cryptophytes</td>
<td>Teleaulax spp.</td>
<td>43224</td>
</tr>
<tr>
<td></td>
<td>Leucocryptos spp.</td>
<td>6372</td>
</tr>
<tr>
<td>Haptophytes</td>
<td>Phaeocystis globosa</td>
<td>971307</td>
</tr>
<tr>
<td>Diatoms</td>
<td>Pseudonitzschia spp.</td>
<td>7351</td>
</tr>
<tr>
<td></td>
<td>Leptocylindrus danicus</td>
<td>2723</td>
</tr>
<tr>
<td></td>
<td>Chaetoceros spp.</td>
<td>1260</td>
</tr>
<tr>
<td></td>
<td>Rhizosolenia spp.</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>Dityum brightwellii</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>Guinardia delicatula</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Coscinodiscus spp.</td>
<td>40</td>
</tr>
<tr>
<td>Ciliates</td>
<td>Laboea strobila</td>
<td>2040</td>
</tr>
<tr>
<td></td>
<td>Unidentified nanoflagellates &lt;10 μm</td>
<td>150720</td>
</tr>
<tr>
<td></td>
<td>Unidentified nanoflagellates &gt;10 μm</td>
<td>795467</td>
</tr>
</tbody>
</table>

Phytoplankton biomass, measured as chl a, increased during the experiment in all treatments at very different rates. The growth rate of the phytoplankton community, calculated from chl a measured initially and at the end of the experiment, was significantly lower in high lytic Alexandrium additions (t-test, p < 0.008; μ = 0.32 d−1 (single addition) and μ = 0.48 d−1 (high continuous addition)) compared to control treatments (μ = 0.72 d−1). All other treatments did not differ significantly from the control (t-test, p > 0.008).

The plankton community composition changed over the course of the experiment; cryptophytes disappeared and both diatoms and P. globosa became dominant in all treatments (Fig. 1 and Table 1).

Below, plankton community composition of the different treatments will be compared to the control treatments. Low continuous additions of Alexandrium supernatant did not affect phytoplankton and nanoflagellate groups. High continuous additions of lytic supernatant inhibited the abundance of P. globosa and large flagellates compared to the control. Chaetoceros spp. benefitted from high continuous additions of non-lytic Alexandrium supernatant. In single additions of lytic Alexandrium supernatant, P. globosa and Chaetoceros spp. > 10 μm were inhibited in comparison to the control treatments. Single additions of non-lytic Alexandrium supernatant increased the abundance of Chaetoceros spp. < 10 μm and pennate diatoms (independent samples t-test, p < 0.008).

When the treatments lytic and non-lytic Alexandrium were compared according to the amount of added supernatant, in low continuous additions the amount of nanoflagellates < 10 μm was higher in lytic Alexandrium treatments. In high continuous additions, abundances of Chaetoceros spp., P. globosa and small and large flagellates were elevated in non-lytic Alexandrium treatments whereas dinoflagellates were more abundant in lytic Alexandrium treatments. P. globosa, Chaetoceros spp., large flagellates and pennate diatoms were suppressed in single additions of lytic Alexandrium compared to non-lytic Alexandrium (independent samples t-test, p < 0.05).

3.3. Bacterial abundance

Abundance of heterotrophic bacteria ranged in between 0.6 and 0.7 × 106 bacteria ml−1 at the experimental start. Bacteria grew rapidly during the first 24 h of the experiment in all treatments (Fig. 2). Bacterial abundances in lytic Alexandrium supernatant high continuous and single additions were significantly higher than in any other treatments (multivariate test after repeated ANOVA, p < 0.05 Tukey HSD Post Hoc). The number of bacteria declined between 48 h and 76 h in all treatments. The magnitude of this reduction differed between the three groups: lytic Alexandrium supernatant high continuous and single additions (29 ± 3% loss); controls and low continuous lytic and non-lytic Alexandrium supernatant (45 ± 6% loss of bacteria) and non-lytic Alexandrium high continuous and single additions (62 ± 10% loss) (Tukey HSD Post Hoc test after One Way ANOVA, p < 0.05).

3.4. Bacterial morphology and production

The mean volume per bacterium was similar in all treatments after 24 h of incubation (One Way ANOVA, p = 0.221, data not shown). After 96 h, bacterial volume had decreased in the high continuous and single lytic Alexandrium supernatant treatments (from 0.056 to 0.043 μm3) whereas it increased in the other treatments (from 0.056 to 0.069 μm3). Bacterial biomass after 96 h was significantly lower in low continuous non-lytic Alexandrium additions and high continuous lytic Alexandrium additions compared to the control (Fig. 3). Bacterial production rates reached 44 μg C l−1 d−1 in the initial...
Fig. 1. Chl a concentrations and abundance of the dominant plankton groups, *Chaetoceros* spp. (<10 µm and >10 µm), Pennate diatoms, *Ditylum brightwellii*, dinoflagellates, nanoflagellates <10 µm and >10 µm and *Phaeocystis globosa* in initial North Sea water (May 2007), and after 96 h of incubation with different *Alexandrium* supernatant concentrations (lytic *Alexandrium*, non-lytic *Alexandrium*). Controls were incubated with K-medium: Mean (n = 3; ±1S.D.). Treatments significantly different from control are marked with a star. Lytic *Alexandrium* supernatant treatments significantly different from corresponding non-lytic *Alexandrium* supernatant treatments are marked with a triangle.
North Sea water. Bacterial production at the end of the experiment was elevated to approximately 100 μg C 1^{-1} d^{-1}. Bacterial production was significantly lower from the control in single non-lytic supernatant were unique and different (Fig. 4). After four days of similarity analysis showed that the bacterial communities in initial profiles of 16S rDNA fragments of duplicate samples. The genetic bacterial community composition was analyzed using DGGE (Fig. 3).

However, production bacteria^{-1} and production biomass^{-1} did not differ from the control with K-medium in any of the treatments (Fig. 3).

3.5. Bacterial community composition

For initial North Sea water, initial lytic and non-lytic *Alexandrium* supernatant and control and high single lytic and non-lytic *Alexandrium* additions (1000 cells ml^{-1}) after 96 h, bacterial community composition was analyzed using DGGE profiles of 16S rDNA fragments of duplicate samples. The genetic similarity analysis showed that the bacterial communities in initial North Sea water and in both lytic and non-lytic *Alexandrium* supernatant were unique and different (Fig. 4). After four days of incubation, profiles of the 16S rDNA fragments recovered from bacteria from all treatments were similar, and more similar to the initial North Sea community composition than either of the *A. tamarense* supernatant. However, control and non-lytic *Alexandrium* treatments showed a higher similarity to each other than with lytic *Alexandrium* treatments (Euclidean distance similarity in UPGMA).

Comparative analysis of TRFLP fingerprints was done on the basis of one replicate from the initial North Sea water and both initial *A. tamarense* supernatants, and all experimental treatments 96 h after incubation. Two replicates were analyzed in control treatments and high lytic *Alexandrium* treatments. Lytic and non-lytic *Alexandrium* supernatants differed from each other and the initial bacterial community (Fig. 5). Bacterial community composition changed in all treatments during the experiment, but still showed high similarities among the different treatments. However, treatments with high continuous additions of lytic and non-lytic *Alexandrium* differed more from the control than treatments with single additions of lytic and non-lytic *Alexandrium* (Fig. 5).

4. Discussion

Our results confirmed that allelopathic substances can shape the natural plankton community without the presence of the allelopathic cells, and also impact bacteria. The lytic *Alexandrium* supernatant inhibited biomass of phytoplankton and large nanoflagellates but benefitted bacteria, whereas the non-lytic *Alexandrium* supernatant provoked higher final abundances in several plankton species and benefitted nanoflagellates and bacteria.

Allelopathy is the impact of secondary metabolites, produced by phytoplankton, on growth and functions of other microbes (Cembella, 2003; Legrand et al., 2003; Gross, 2003; Granéli and Hansen, 2006; Ianora et al., 2006). According to this definition, both *A. tamarense* strains, lytic and non-lytic *Alexandrium*, exhibited allelopathy. However, adding a supernatant of any algal culture probably will show some “effects” on a community when compared to adding medium. Some of these observations may result from feedback mechanisms within the microbial community (i.e. an increase in bacterial biomass can explain an increase in heterotrophic nanoflagellate biomass). The beneficial effects of the non-lytic *Alexandrium* supernatant on *Chaetoceros* spp. may be explained by metal complexing properties of extracellular organic material, derived from phytoplankton, which can affect trace metal availability as well as seawater toxicity (Croot et al., 2000; Vasconcelos et al., 2002; Rijkenberg et al., 2008). In a laboratory study, growth of the diatom *Phaeodactylum tricornutum* was enhanced by algal exudates of different macro- and microalgae. The exudates supported nutrient uptake but also changed the intensity of production and nature of *P. tricornutum* own exudates (Vasconcelos and Leal, 2008).

Previous comparisons of both *Alexandrium* strains showed that only the lytic strain (*Alex2*) affected a whole range of different target species whereas the non-lytic strain (*Alex 5*) showed no negative effect (Tillmann and Hansen, 2009; Tillmann et al., 2009). Therefore, we interpret all changes caused by the lytic *Alexandrium* that differ from changes caused by the non-lytic strain in this study as due to lytic compounds.

High lytic *Alexandrium* supernatant additions inhibited the growth of *P. globosa*, whereas diatom abundance was not significantly negatively affected in comparison to the control (except *Chaetoceros* > 10 μm in the single addition). However, compared to non-lytic supernatant, lytic *Alexandrium* supernatant had a significantly negative effect on diatoms. In previous field experiments, several diatom species were only slightly inhibited by *A. tamarense* allelochemicals (Fistarol et al., 2004). When compared to other target species, *Thalassiosira weissflogii* was relatively resistant to lytic substances released from different *Alexandrium* species (Tillmann et al., 2008) supporting the hypothesis that diatoms are more resistant to the allelochemical substances produced by toxic dinoflagellates than ciliates and flagellates. However, recent co-incubation experiments of *A. tamarense* and 10 different phytoplankton species showed that both diatom species (*Skeletonema costatum*, *T. weissflogii*) were relatively affected, with *S. costatum* being amongst the most sensitive species (Tillmann and Hansen, 2009). Species-specific consequences of allelopathy are known from other allelopathic dinoflagellates such as *Karlodinium micrum* and *Karenia brevis* (Adolf et al., 2006; Kubanek et al., 2005). When exudates of the red-tide dinoflagellate *K. brevis* were tested on 12 different phytoplankton target species, only six of them were inhibited compared to the control (Kubanek et al., 2005). Among the diatoms tested by these authors, *S. costatum* and *T. weissflogii* were also among the most sensitive target species.

During the first 24 h, bacteria grew in all treatments due to the labile carbon added in form of peptone. Since PO_4 and NO_3 were
added in excess, bacteria were probably carbon limited when bacterial abundance stabilized after 24 h. Bacteria reached higher growth rates and bacterial yields in treatments containing high additions of supernatant. Highest bacterial yields were reached in lytic Alexandrium single additions. The release of DOM is usually an important source of labile dissolved organic carbon (DOC) for bacteria (Valiela, 1995). Dense algal cultures contain DOM due to phytoplankton exudates. The addition of supernatant provided an additional source for DOC, and therefore supported higher bacterial yields in both lytic and non-lytic Alexandrium supernatant treatments, compared to control treatments. However, DOM deriving from A. tamarense cultures was found to be essentially bacteria resistant (Chen and Wangersky, 1996). Additionally, lysis of plankton in lytic Alexandrium supernatant treatments may have increased the DOM pool even further. Mixed culture trials of R. salina and the allelopathic haptophyte P. parvum lead to a significant increase of DOC concentration after 30 min and an increase in bacterial biomass after 6–12 h (Uronen et al., 2007). We assume that DOC released from lysing cells was available to bacteria in lytic Alexandrium supernatant treatments.

Bacterial growth usually results in a slightly delayed growth of bacterivores; those have to adapt to the new nutrient conditions, but can reach high growth rates (4–6 h doubling time) (Weisse and Scheffelmoser, 1991; Jurgens et al., 2000). When bacterial grazers become abundant, bacteria decrease rapidly. We could observe this rapid decrease in all treatments after 48 h of incubation. However, bacterial decrease (a proxy for bacterivory) was lower in single and high continuous additions of lytic Alexandrium

<table>
<thead>
<tr>
<th>Initial</th>
<th>after 96 h of incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>low continuous addition</td>
</tr>
<tr>
<td></td>
<td>high continuous addition</td>
</tr>
<tr>
<td></td>
<td>single addition</td>
</tr>
<tr>
<td>North Sea water</td>
<td>lytic</td>
</tr>
<tr>
<td>non-lytic supernatant</td>
<td>lytic</td>
</tr>
<tr>
<td>lytic supernatant</td>
<td>non-lytic</td>
</tr>
</tbody>
</table>

Fig. 3. Bacterial abundance, biomass, production, production bacteria $^{-1}$ and production biomass $^{-1}$ ratios in initial North Sea water (May 2007), lytic and non-lytic Alexandrium supernatant, and after 96 h of incubation with addition of different Alexandrium supernatant concentrations. Controls were incubated with K-medium. Mean ($n = 3; ± 15\text{D}$). Treatments significantly different from control are marked with a star. Lytic Alexandrium supernatant treatments significantly different from corresponding non-lytic Alexandrium supernatant treatments are marked with a triangle.
supernatant (30%) compared to non-lytic additions (60%), suggesting that predation pressure was lower in treatments containing lytic compounds.

Nanoflagellate grazing accounts for most of the protistan bacterivory in plankton (Jürgens and Massana, 2008). Previous studies have shown that the presence of allelopathic substances could eliminate up to 80% of nanoflagellates over 3–4 days, and therefore contribute to a high bacterial abundance in algal filtrate treatments (Fistarol et al., 2003, 2004). Our study confirms these results, since the abundance of flagellates after 96 h of incubation was suppressed in lytic Alexandrium compared to non-lytic Alexandrium treatments.

A strong top down control in nutrient rich conditions may lead to a high production per bacterium but a low bacterial abundance (Ducklow et al., 2001). The larger size of bacteria and the relatively higher production per bacterium in non-lytic Alexandrium treatments could be explained by better growth caused by nutrient recirculation, a side effect of nanoflagellate grazing due to sloppy feeding. However, production biomass$^{-1}$ did not differ between lytic Alexandrium and all other treatments, indicating that bacterial production after continuous and single addition was not affected by A. tamarense supernatant after 96 h.

The effect of allelochemicals on bacterial abundance and production has been reported in a few studies (Nielsen et al., 1990; Fistarol et al., 2004; Uronen et al., 2007). In this study, the effect of allelochemicals on bacterial community in natural communities is reported for the first time. Studies on Alexandrium spp. blooms revealed a high abundance of bacteria that were repeatedly found in association to phytoplankton blooms (Wichels et al., 2004). Bacteria associated with diatom cultures revealed specific bacterial “satellite” communities (Schäfer et al., 2002; Grossart et al., 2005; Kaczmarska et al., 2005). In Alexandrium cultures, the presence of specific bacterial consortia may be explained by symbiotic relationships between bacteria and the dinoflagellate (Amaro et al., 2005; Jasti et al., 2005). Additionally, cell wall components, exudates, pigment composition and life cycle features of the algae may also select for specific bacterial consortia. However, bacterial communities associated to algae are not necessarily species specific, and strains of the same algae can harbour different bacterial communities (Sapp et al., 2007).

In this study, the initial composition of each bacterial “satellite” community was very different among the two Alexandrium supernatants and the North Sea water. After 96 h of incubation, the bacterial community shared a high similarity in all treatments and was closer to the initial North Sea water than to any Alexandrium supernatant. The development of the bacterial community towards a similar composition among the treatments may be due to the addition of nutrients, especially peptone, and the selection for copiotroph bacteria (Lauro et al., 2009). If bacterial “satellite” communities can be explained by their adaptation to...
algal released DOM (Sapp et al., 2007), an easily converted carbon source such as peptone may have made it irrelevant for bacteria to take up algal released DOM, and therefore, no strong bacterial selection could be observed.

In nature, allelochemicals are assumed to be continuously released into the surrounding seawater but not much is known about degradation of the compound or adaptation of target organisms. Allelochemicals of A. tamarense have been shown to be quite stable at room temperature and not degraded by the bacterium consortium present in the culture (Ma et al., 2009). Our results showed that addition of one high pulse of lytic A. tamarense supernatant affected the natural plankton community more than adding almost the same quantity of supernatant semi-continuously. Experimental observations of cell blistering and lysis in target species indicate that the compounds primarily seem to act upon the outer membrane of the target organisms (Tillmann et al., 2007). This binding probably leads to a removal of compounds from solution, as lysis of target species is not only depending on the concentration of donor cells but, interestingly, also depends on concentration of the cells of the targeted species (Tillmann, 2003; Tillmann et al., 2007). In addition, initial characterizations have shown a general “stickiness” of the compounds to all kind of surfaces, e.g. for filter material and for plastic containers compared to glass (Ma et al., 2009). Consequently, lytic substances binding to different matrices like growing P. globosa colonies, diatoms and organic surfaces may explain the lower efficiency of the continuous additions. Hence, as the amount of matrices increased over time, the impact of lytic substances did not accumulate but weakened in the microcosms. Furthermore, the phytoplankton community in treatments with continuous additions of A. tamarense supernatant might have adapted to the exposure of allelochemicals or might have metabolized or degraded allelopathic compounds.

Recently, allelopathy has been questioned as to its involvement in algal bloom formation (Jonsson et al., 2009). A meta-analysis of data from several experiments regarding allelopathy showed that donor organisms provoked inhibitory effects towards other species only at Chl a concentrations >5 μg l⁻¹ (bloom conditions). On the other hand, studies with low concentrations of donor organisms are scarce. In our study, the lower tested concentrations of lytic Alexandrium supernatant did not obviously impact the natural plankton community. However, motile phytoplankter, such as A. tamarense, reach highest densities in calm weather conditions and accumulate in horizontal layers, along thermoclines or the water surface (MacIntyre et al., 1997; Mouritsen and Richardson, 2003; Ryan et al., 2008). High concentrations of cells, and thus, secondary metabolites, similar to those in this experiment, can accompany marine diatoms colonies, diatoms and organic surfaces may explain the lower efficiency of the continuous additions. Hence, as the amount of matrices increased over time, the impact of lytic substances did not accumulate but weakened in the microcosms. Furthermore, the phytoplankton community in treatments with continuous additions of A. tamarense supernatant might have adapted to the exposure of allelochemicals or might have metabolized or degraded allelopathic compounds.

Weissbach, A., et al., Allelopathic potential of the dinoflagellate Alexandrium tamarense on marine microbial communities. Harmful Algae (2010), doi:10.1016/j.hal.2010.05.007


