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Allelopathic effects of Baltic Sea spring bloom dinoflagellates on co-occurring phytoplankton

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ABSTRACT: Dinoflagellate-dominated spring blooms are globally uncommon, but they regularly occur and are even increasing in the Baltic Sea, varying interannually in importance with diatom-dominated blooms. The success of dinoflagellates in the spring phytoplankton community has remained poorly understood, as they are expected to be inferior competitors due to their low growth rates and nutrient uptake capacities under nutrient-replete spring conditions. To prevail in the phytoplankton community, dinoflagellates must either be favored by specific environmental settings or possess adaptations, such as allelopathy, to compensate for their competitive disadvantage. Using batch cultures, we studied the allelopathic effects of 3 dominant vernal dinoflagellates — *Biecheleria baltica, Gymnodinium corollarium* and *Scrippsiella hangoei* — on 5 typical spring bloom diatoms and 1 cryptophyte. We also tested the effects of the dinoflagellates on each other. Three of the 5 diatoms — *Melosira arctica, Skeletonema marinoi* and *Thalassiosira baltica* — were significantly inhibited by cell-free filtrates or live cells of all dinoflagellates. *Chaetoceros* cf. *wighamii* and *Diatoma tenuis* were suppressed by *G. corollarium,* and *D. tenuis* was also suppressed by live cells of *S. hangoei*. In contrast, the cryptophyte *Rhodomonas* sp. was stimulated by all dinoflagellate species. The effects of dinoflagellate filtrates on other dinoflagellate species were mostly positive, but co-culturing tended to inhibit the growth of the respective target dinoflagellates. As some of the major players of the diatom spring bloom can be suppressed by co-occurring dinoflagellates in culture, we conclude that allelopathy may be one mechanism by which vernal dinoflagellates frequently outcompete diatoms and form intense spring blooms.

KEY WORDS: Allelopathy · Baltic Sea · Spring bloom · Dinoflagellate · *Biecheleria baltica* · *Gymnodinium corollarium* · *Scrippsiella hangoei* · Diatom

INTRODUCTION

In contrast to most temperate coastal ecosystems where diatoms form the main part of phytoplankton spring blooms, vernal phytoplankton communities in the Baltic Sea are characterized by co-occurrence, and often dominance, of cold-water dinoflagellates. There is high interannual variability in the Baltic spring blooms with diatoms and dinoflagellates alternating in dominance (cf. Kremp et al. 2008 and our Fig. 1). Typically the blooms start immediately after ice break-up in March/early April and last for up to 2 mo (Heiskanen 1993, Kremp & Heiskanen 1999). Due to their similar size and shape, the taxonomic affiliations of the Baltic spring bloom dinoflagellate species have long been unclear. Recent morphological and molecular analyses on cultured isolates revealed that 3 different species, inseparable by light microscopy, are associated with these blooms (Larsen et al. 1995, Kremp et al. 2005, Sundström et al. 2009). It was established that *Biecheleria baltica* Moestrup, Lindberg et Daugbjerg (= *Woloszynskia halophila* sensu Kremp et al. 2005) (Moestrup et al. 2009), co-occurs with *Scrippsiella hangoei* (Schiller) Larsen in

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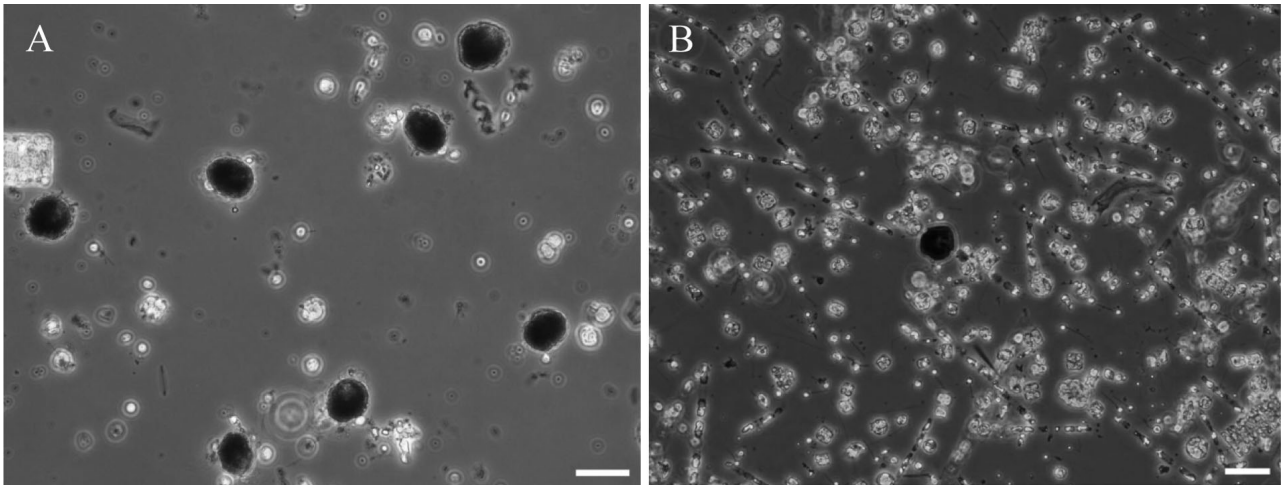

Fig. 1. Micrographs of 2 spring bloom communities in the northern Baltic Sea, dominated by (A) the dinoflagellate *Biecheleria baltica* in 2006, and (B) the diatom *Skeletonema marinoi* in 2008. Scale bars = 20 µm

the Gulf of Finland (Kremp et al. 2005), whereas a third recently described species, *Gymnodinium corollarium* A. M. Sundström, Kremp et Daugbjerg (Sundström et al. 2009), is most abundant in the Gulf of Bothnia and the open Baltic Proper. Other species typically belonging to the Baltic spring bloom community include the dinoflagellate *Peridiniella catenata* (Levander) Balech, and the diatoms *Chaetoceros* spp. Ehrenberg, *Melosira arctica* (Ehrenberg) Dickie, *Skeletonema marinoi* Sarno et Zingone, *Thalassiosira baltica* (Grunow) Ostenfeld, *T. levanderi* van Goor and *Achnanthes taeniata* Grunow (Niemi 1975, Hällfors 2004).

In some areas of the Baltic Sea, dinoflagellates have become increasingly dominant relative to diatoms in spring blooms during the last decades (Wasmund et al. 1998, Wasmund & Uhlig 2003, Klais et al. 2011). A concomitant decrease in terrestrial silicate discharges and an increase in other nutrients have been considered as a possible cause of such development, as they might lead to a deteriorating competitive position of co-occurring diatoms (Rahm et al. 1996). On the other hand, local physical conditions (e.g. mild winters, calm spring conditions) resulting from large-scale weather patterns, have been suggested to be more important than nutrients in explaining the shift towards dinoflagellate dominance (Wasmund et al. 1998, Klais et al. 2011).

Dinoflagellate-dominated spring blooms are considered a paradox, since dinoflagellates are regarded as inferior competitors relative to diatoms at high nutrient concentrations typical of spring in temperate areas. To counteract low nutrient uptake affinities compared with diatoms, other adaptive competitive strategies, such as motility, mixotrophy, allelopathy and antipredation defense mechanisms, have evolved in dinoflagellates (Smayda 1997). Allelopathy, i.e. the production and release of compounds that inhibit competitors, has been hypothesized to be an important competitive strategy of dinoflagellates under eutrophic conditions (Maestrini & Bonin 1981, Smayda 1997). Particularly, bloom-forming dinoflagellates seem to use allelopathy as a mechanism to outcompete sympatric phytoplankters (Rengefors & Legrand 2001) and to maintain their dense and nearly monospecific populations, many of which are toxic for a wide variety of organisms (Kubanek et al. 2005). Allelopathic properties have been reported from a number of dinoflagellate genera, including *Prorocentrum* spp., *Cochlodinium* spp., *Karenia* spp., *Karlodinium* spp., *Peridinium* spp. and *Alexandrium* spp. (Gentien & Arzul 1990, Windust et al. 1996, Wu et al. 1998, Arzul et al. 1999, Adolf et al. 2006, Tang & Gobler 2010). Additionally, lethal effects on other phytoplankton by direct cell contact have been detected in the genus *Heterocapsa* (Uchida et al. 1995).

Little is known of the mechanisms that allow the Baltic Sea spring dinoflagellates, which can be classified as red-tide organisms, to maintain their advantage, given the apparently superior competitive ability of co-occurring diatoms. Results from recent mesocosm experiments with natural spring phytoplankton indicated that initial dominance may be an important prerequisite for the later formation of dinoflagellate blooms: when dinoflagellates are established at high densities before the diatoms, such an

outcome of competition appears to be independent of nutrient or light conditions (Kremp et al. 2008). This suggests that a competitive strategy which is particularly effective at high densities is used by these dinoflagellates to control competitors and keep their abundances low.

Allelopathy could be a competitive adaptation that explains the success of dinoflagellates in building up populations that frequently dominate the phytoplankton spring community in the Baltic Sea, by allowing dinoflagellates to suppress their competitors once high-density populations are established. We investigated whether the 3 most important vernal dinoflagellate species in the Baltic Sea — *Biecheleria baltica, Gymnodinium corollarium* and *Scrippsiella hangoei* — are able to inhibit their competitors, and whether allelopathy can thus provide a way to support their dominance. We hypothesized that (1) *B. baltica, G. corollarium* and *S. hangoei* would produce allelochemicals that inhibit the growth of co-occurring diatom species, and (2) these effects would be enhanced at high cell densities.

MATERIALS AND METHODS

Algal cultures

For the cross-culture experiments, we selected 9 important species belonging to the phytoplankton community that annually constitutes the main part of the spring bloom biomass in the northern Baltic Sea (Niemi 1975, Fig. 1). As donors we used 3 cold-water dinoflagellates: *Gymnodinium corollarium* (strain GCTVB4, isolated from a bloom sample in the Northern Baltic Proper in May 2005), *Biecheleria baltica* and *Scrippsiella hangoei* (WHTVC1 and SHTV5, respectively, isolated from germinated resting cysts collected at the SW coast of Finland in March 2002). Target species included 1 cryptophyte, *Rhodomonas* sp. Karsten (Crypto07B1), and 5 diatoms, *Chaetoceros* cf. *wighamii* Brightwell (CWTVC1), *Melosira arctica* (MARTV), *Skeletonema marinoi* (SMATV), *Thalassiosira baltica* (TBTV) and *Diatoma tenuis* Agardh (DTTVB5). All diatoms were originally isolated from single cells at the SW coast of Finland in 2005. All strains are maintained at the Marine Research Centre, Finnish Environment Institute. In addition, we investigated the reciprocal effects of the 3 dinoflagellates.

All strains were grown in F/2 medium (Guillard 1975), prepared from GF/F filtered, autoclaved seawater (salinity 6), at +4 °C, in 150 µmol photons m^{-2} s^{-1}, and in a light:dark cycle of 12L:12D. The dinoflagellates were grown in F/2 medium without silicate addition. Before the start of the experiment, the donors had reached late exponential or stationary growth phase, whereas the targets were growing exponentially.

Experimental design

For the first set of experiments, we used one dense donor dinoflagellate concentration of ca. 10 000 cells ml^{-1}, which corresponds to bloom conditions in the field (e.g. Heiskanen 1993, Larsen et al. 1995, Jaanus et al. 2006, Spilling 2007). Starting concentrations of the target species were ca. 500 cells ml^{-1} for the large (*Melosira arctica, Thalassiosira baltica, Diatoma tenuis*, *Biecheleria baltica, Gymnodinium corollarium*, *Scrippsiella hangoei*) and 1300 cells ml^{-1} for the small (*Rhodomonas* sp., *Chaetoceros* cf. *wighamii, Skeletonema marinoi*) species. The concentrations were chosen so that counting of 1 ml samples produced statistically reliable results, and that the biovolumes of the different target species corresponded with each other. The effects of filtrates, as well as live cells of donor species, were compared to the control (described below, this subsection).

To check for linearity in the response, we conducted a second experiment where we compared the effects of 4 donor concentrations (1, 10, 50 and 100 % of the starting concentration) on *Skeletonema marinoi*. The starting (100 %) concentrations of donor dinoflagellates were 11 600 *Scrippsiella hangoei* cells ml^{-1}, 16 300 *Gymnodinium corollarium* cells ml^{-1} and 25 900 *Biecheleria baltica* cells ml^{-1}. Starting concentration of *S. marinoi* was ca. 3000 cells ml^{-1}. In this experiment, the effects of donor filtrates were tested; live cells were applied only in the case of *B. baltica*.

All experiments were conducted using batch cultures of target cells. The treatments were culture filtrate and live cells of donor species, and F/2 medium was used as the control. The filtrate was prepared by gently filtering the donor culture through a Whatman GF/F filter, with a pressure of <5 kPa to avoid breaking the dinoflagellate cells. Nutrients were added to the filtrate and the live donor cells in F/2 concentrations. To reach the desired donor filtrate or cell concentrations and starting target cell numbers, the experimental cultures were diluted with F/2 medium.

The experiments were conducted at +4 °C, in 50 ml tissue culture flasks, with an experimental volume of 40 ml and triplicates of each treatment. To avoid

settling of the diatoms, culture flasks were attached to a plankton wheel with a rolling velocity of ~1 rpm and variation in light intensity from 50 to 114 µmol photons m^{-2} s^{-1}. The *Rhodomonas* sp. and dinoflagellate cultures were grown without turbulence at 150 µmol photons m^{-2} s^{-1}. The light:dark cycle was 12L:12D for all target cultures.

To avoid effects of resource competition due to nutrient deficiency in the treatments with mixed donor and target cells, nutrients were added one or 2 times (once in 5 to 6 d) during the experiments. The additions were made in F/2 concentrations, with the exception that twice the F/2 concentration of phosphate was added to the treatments with *Scrippsiella hangoei* cells, because of larger P consumption by this species (Kremp et al. 2009).

Analyses

Samples (V = 1.8 ml) were taken every 1 to 4 d for fluorescence measurement, and 3 times during the experiment for cell counts. Fluorescence was immediately measured using Varian Cary Eclipse Fluorescence Spectrophotometer (excitation 430 nm, emission 680 nm) with a well-plate reader. Samples for cell counts were preserved with a drop of acid Lugol solution and counted using Sedgewick-Rafter cells (V = 1 ml) and an inverted microscope. A minimum of 400 counting units of each species were enumerated per sample, but if the total number was <400, all units were counted from the whole chamber area.

The experiments lasted from 11 to 14 d (with diatom and cryptophyte targets) to 18 d (dinoflagellate targets). On the last experimental day, the pH of the different treatments (filtrate and live cells) and the control was measured from 1 of the replicated flasks.

Statistical analysis

Repeated measures ANOVA was used to test for differences in cell numbers between the target cultures treated with filtrates or live cells of dinoflagellates and the control over the entire experimental period. Tukey's HSD post hoc test was used to find out which treatments significantly differed from the control and from each other. The data were tested for normality and homogeneity of variances. All tests were 2-tailed, with a significance level of p = 0.05. If the treatments differed according to the ANOVA, only the Tukey HSD results are indicated. The statistical analysed were performed using the software SPSS 15.0.1 for Windows.

RESULTS

Effects of dinoflagellates on diatom and cryptophyte growth

In general, the dinoflagellate treatments exerted significant negative effects on the cell number development of the 5 diatom species; by contrast, their effects on the cryptophyte *Rhodomonas* sp. were mostly positive (Fig. 2). Live dinoflagellate cells usually had a stronger negative effect on the target species than cell-free filtrate. The most affected diatom species were *Skeletonema marinoi,* which was significantly inhibited by filtrates and live cells of *Biecheleria baltica* (Tukey HSD, p = 0.021 and p < 0.001, respectively) and *Scrippsiella hangoei* (p ≤ 0.001 for both filtrate and live cells); *Melosira arctica,* which was decreased by live cells of *B. baltica* and *Gymnodinium corollarium* (p < 0.001 for both), and both filtrates and live cells of *S. hangoei* (p ≤ 0.001 for both); and *Thalassiosira baltica,* which was decreased by live cells, but not filtrates, of all 3 dinoflagellates (p < 0.001 for all).

Chaetoceros cf. *wighamii* was significantly decreased by live cells of *Gymnodinium corollarium* (p = 0.008), but increased by both filtrate and cells of *Scrippsiella hangoei* (p = 0.037 and p < 0.001, respectively). *Diatoma tenuis* was decreased by filtrate and live cells of *G. corollarium* (p = 0.001 for both) and cells of *S. hangoei* (p = 0.003), but increased by filtrate of *Biecheleria baltica* (p = 0.001) and *S. hangoei* (p = 0.012). *Rhodomonas* sp. was significantly stimulated by all dinoflagellate treatments (p ≤ 0.005 for all), except for live *S. hangoei* cells, which had no significant effect on its cell numbers.

In the fluorescence data (not shown), similar responses were observed, except for the significant negative effects of *Gymnodinium corollarium* filtrate on the fluorescence of *Chaetoceros* cf. *wighamii* (repeated measures ANOVA, $F_{1,4}$ = 73.89, p = 0.001), *Melosira arctica* ($F_{1,4}$ = 13.77, p = 0.021) and *Skeletonema marinoi* ($F_{1,4}$ = 16.27, p = 0.016), and the significant positive effect of *Biecheleria baltica* filtrate on *C.* cf. *wighamii* ($F_{1,4}$ = 17.85, p = 0.013). Effects of donors to target fluorescence could not be evaluated in mixed culture.

At the end of the experiment, the controls had an average pH of 8.60, filtrates 8.61 and live cell treatments 8.77 (Table 1).

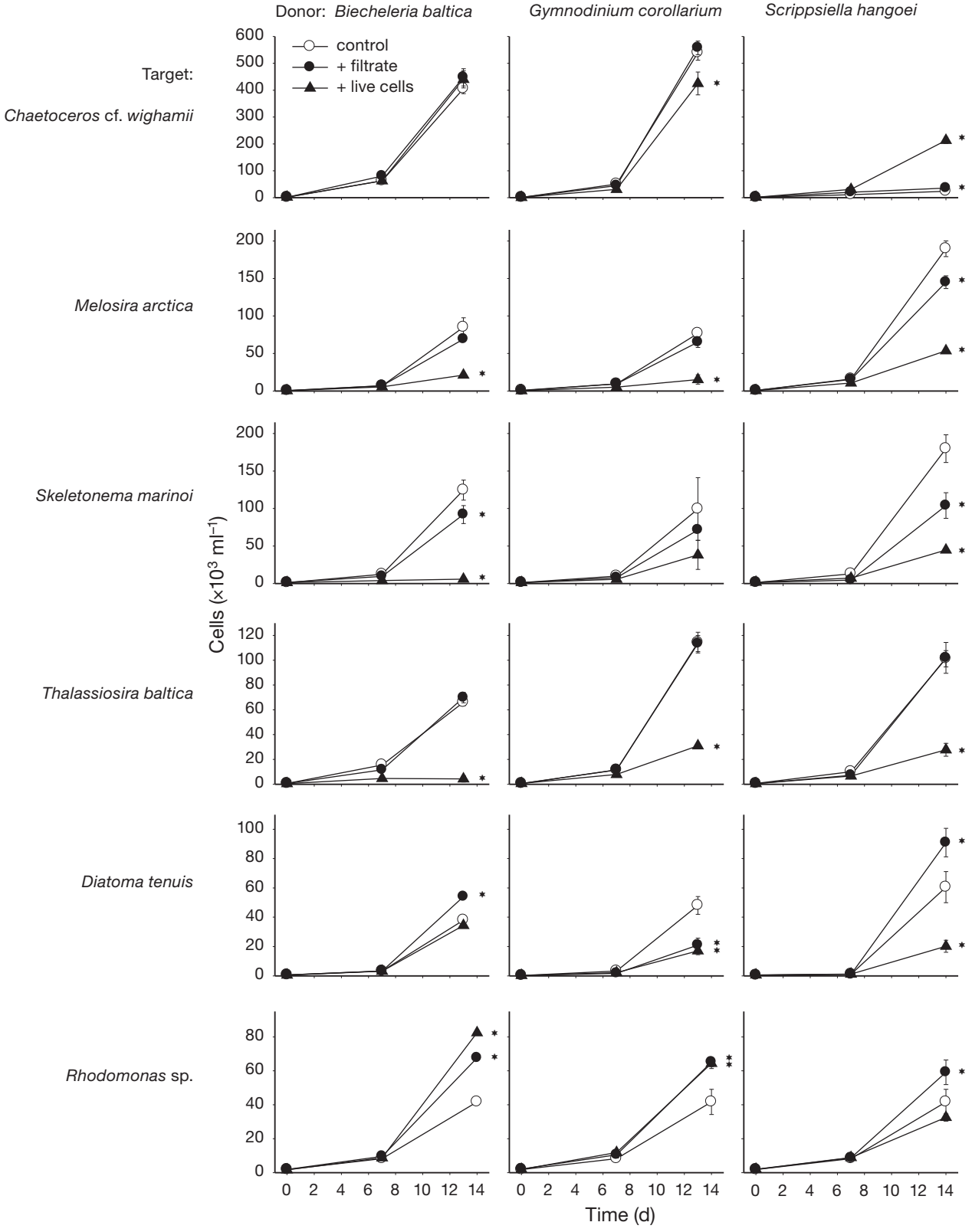

Fig. 2. Cell numbers (n = 3, mean ± SD) of the 5 target diatoms and 1 cryptophyte, exposed to control medium, as well as cell-free filtrates and live cells of the 3 dinoflagellates *Biecheleria baltica* (left), *Gymnodinium corollarium* (middle) and *Scrippsiella hangoei* (right). Treatments in which the cell numbers significantly differ from the control ($p < 0.05$) are marked with an asterisk

Table 1. pH of the 5 target diatom, 1 cryptophyte, and 3 dinoflagellate cultures, exposed to control medium, as well as one concentration (corresponding to ca. 10 000 cells ml^{-1}) of cell-free filtrates and live cells of *Biecheleria baltica, Gymnodinium corollarium* and *Scrippsiella hangoei*, on the first (start) and the last (end) day of the experiment

	Control	—*B. baltica*—		—*G. corollarium*—		—*S. hangoei*—	
		Filtrate	Cells	Filtrate	Cells	Filtrate	Cells
Start	8.94	8.91	8.97	9.21	9.55	9.35	9.55
Chaetoceros cf. *wighamii* (end)	9.01	9.94	9.75	9.16	8.95	8.09	9.65
Melosira arctica (end)	8.27	8.39	8.05	7.47	8.28	9.18	9.20
Skeletonema marinoi (end)	7.88	8.07	7.98	7.34	7.89	8.16	9.03
Thalassiosira baltica (end)	9.09	9.29	8.15	8.67	8.48	9.41	9.37
Diatoma tenuis (end)	8.20	8.61	8.69	7.22	8.23	9.24	9.62
Rhodomonas sp. (end)	9.15	9.05	8.81	8.74	8.64	8.96	9.05
B. baltica (end)	8.18			8.38	8.84	8.26	9.13
G. corollarium (end)	8.87	8.87	8.29			8.94	9.08
S. hangoei (end)	8.92	8.89	8.76	8.97	8.97		

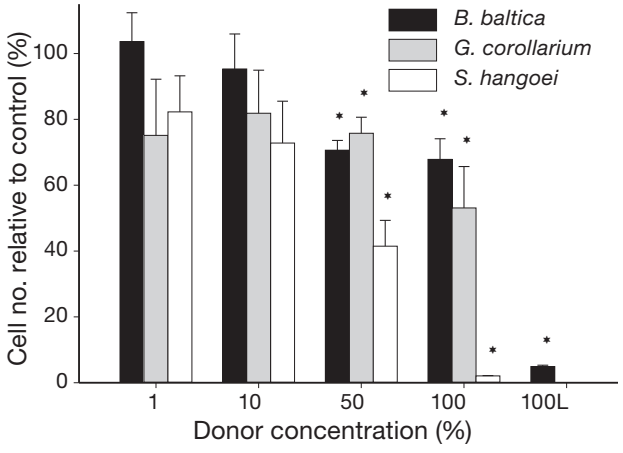

Fig. 3. *Skeletonema marinoi*. Cell number of the diatom relative to control (%) (n = 3, mean ± SD) on the last experimental day, exposed to 4 concentrations of cell-free filtrates of *Biecheleria baltica, Gymnodinium corollarium* and *Scrippsiella hangoei*. In addition to filtrates, live cells of *B. baltica* were applied (indicated by L). Treatments in which the cell numbers significantly differ from the control ($p < 0.05$) are marked with an asterisk

Effects of varying filtrate concentrations on *Skeletonema marinoi*

In the experiment where 4 different concentrations of dinoflagellate filtrates were added to *Skeletonema marinoi* cultures, the cell numbers of *S. marinoi* were negatively affected in the treatments with 50 and 100% concentrations of all 3 dinoflagellates (Fig. 3). The percentages corresponded with 13 000 to 26 000 *Biecheleria baltica* cells ml^{-1} (Tukey HSD, $p = 0.001$ and < 0.001, respectively), 8000 to 16 000 *Gymnodinium corollarium* cells ml^{-1} ($p = 0.013$ and < 0.001), and 6000 to 12 000 *Scrippsiella hangoei* cells ml^{-1} ($p < 0.001$ for both). Fluorescence of *S. marinoi* was also significantly reduced by the 10% (1200 cells ml^{-1}) *S. hangoei* filtrate ($p = 0.012$). In addition, live *B. baltica* cells (26 000 cells ml^{-1}) caused a significant ($p < 0.001$) and much stronger decrease in *S. marinoi* cell numbers than the cell-free filtrate prepared from the same cell concentration. On the last day of the experiment, the 50 and 100% *B. baltica* filtrates had caused on average 29 and 32% decrease in *S. marinoi* cell numbers, respectively. In contrast, live *B. baltica* cells had decreased *S. marinoi* cell numbers by 95% by the same date. The 50 and 100% *G. corollarium* filtrates reduced *S. marinoi* abundances by 24 and 47%, respectively, and *S. hangoei* by 59 and 98%.

At the end, pH was 8.19 (range: 8.02 to 8.36) in the control, 8.10 (8.04 to 8.22) in the 100% filtrates, and 8.27 in the *Biecheleria baltica* live cells treatment.

Reciprocal effects of dinoflagellates

The effects of dinoflagellate filtrates on the cell numbers of other dinoflagellate species were mostly positive (Fig. 4): *Biecheleria baltica* and *Scrippsiella hangoei* filtrates significantly increased the cell numbers of *Gymnodinium corollarium* (Tukey HSD, $p = 0.034$ and $p = 0.02$, respectively), and *G. corollarium* filtrate increased both *B. baltica* ($p < 0.001$) and *S. hangoei* ($p = 0.003$). The only significant positive effects on target fluorescence were caused by *G. corollarium* filtrate on *B. baltica* ($p < 0.001$) and *S. hangoei* ($p = 0.019$).

On the other hand, co-culturing with live dinoflagellate cells tended to inhibit the growth of other

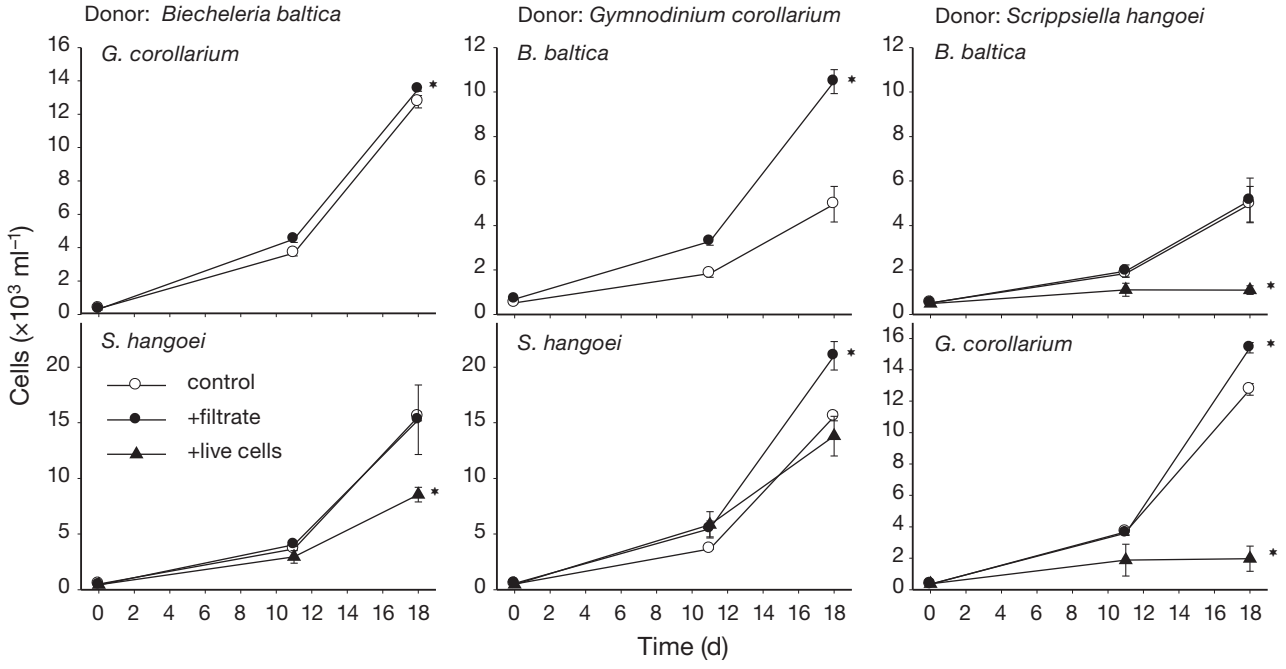

Fig. 4. *Biecheleria baltica*, *Gymnodinium corollarium* and *Scrippsiella hangoei*. Cell numbers (n = 3, mean ± SD) of the dinoflagellates exposed to control medium, as well as cell-free filtrates and live cells of the other dinoflagellates — left: *B. baltica*, middle: *G. corollarium* and right: *S. hangoei*. For *B. baltica* and *G. corollarium*, data from filtrate treatment vs. control only are presented. Treatments in which the cell numbers significantly differ from the control ($p < 0.05$) are marked with an asterisk

dinoflagellate species: presence of *Biecheleria baltica* cells significantly decreased cell numbers of *Scrippsiella hangoei* ($p = 0.002$), and *S. hangoei* cells inhibited both *B. baltica* ($p < 0.001$) and *Gymnodinium corollarium* ($p < 0.001$). Data on the reciprocal live cell effects of *B. baltica* and *G. corollarium* are not presented because these species could not be identified with certainty in mixed cultures.

On the last day, pH was on average 8.66 in the control, 8.78 in the filtrate treatments and 8.84 in the live cell treatments (Table 1).

DISCUSSION

Allelopathy is considered widespread among phytoplankton, especially in harmful algal bloom forming species that are also toxic to other aquatic organisms. However, toxicity and allelopathy are usually mediated by different compounds, e.g. in the harmful marine dinoflagellate genera *Alexandrium*, *Karenia* and *Prorocentrum* (Arzul et al. 1999, Sugg & VanDolah 1999, Kubanek et al. 2005). Extracellular substances with haemolytic effects have been found to act as allelochemicals in some dinoflagellates (Rengefors & Legrand 2001, Tillmann & John 2002, Yamasaki et al. 2011b). The present study is the first report on allelopathic effects of the brackish-water dinoflagellates *Biecheleria baltica*, *Gymnodinium corollarium* and *Scrippsiella hangoei* on co-occurring diatoms. These species form spring blooms that are dense enough to be regarded as red tides, although they have not been found harmful in previous studies, i.e. they do not produce any known toxins. In a preliminary study, however, haemolytic activity was observed in *S. hangoei* (S. Suikkanen & A. Kremp unpubl.), indicating that the species is potentially harmful to other aquatic organisms. This species has a genetically nearly identical sibling in Scandinavian freshwater habitats, *Peridinium aciculiferum*, which is known to be allelopathic (Rengefors & Legrand 2001, Logares et al. 2007).

In the present study, allelopathic effects were mostly visible after 4 to 7 d (based on the fluorescence data with a sampling interval of 1 to 2 d). This indicates that the effects were not primarily caused by haemolytic substances because cell lysis of target cells typically occurs within minutes to hours after exposure (Tillmann et al. 2008, Tang & Gobler 2010, Chang 2011). Since rapid cell lysis was not observed in our target species, it seems that the allelopathic effects of these dinoflagellates are mediated by less acute mechanisms, e.g. by lowering the photosynthetic efficiency and causing a delayed growth re-

sponse. Also, in some cases the dinoflagellate filtrates caused significant negative effects on target cell fluorescence, but not on cell numbers, indicating that the allelochemicals affected cell physiology, without reducing cell numbers. Also exudates of *Karenia brevis* have been found to lower photosynthetic efficiency and increase membrane permeability of competing phytoplankton (Prince et al. 2008). Allelochemicals have been characterized for only a few dinoflagellate species: karlotoxins in *Karlodinium veneficum* (Adolf et al. 2006), amphipathic lytic compounds in *Alexandrium tamarense* (Ma et al. 2009) and unstable, polar organic molecules in *K. brevis* (Prince et al. 2010). All of these substances cause effects in <48 h, so different active principles are probably involved in the allelopathic effects of the species examined in the present study.

The allelopathic effects were species-specific, i.e. depending on both the donor and the target species. *Gymnodinium corollarium* negatively affected 5, *Scrippsiella hangoei* 4, and *Biecheleria baltica* 3 of the 5 target diatoms. Also, the diatoms differed in their response to the dinoflagellate treatments. *Melosira arctica, Skeletonema marinoi* and *Thalassiosira baltica* were inhibited by all 3 dinoflagellates. *Diatoma tenuis* was inhibited by 2 dinoflagellates (*G. corollarium* and cells of *S. hangoei*), and stimulated by 2 (filtrates of *B. baltica* and *S. hangoei*), whereas *Chaetoceros* cf. *wighamii* was inhibited by 1 (*G. corollarium*) and stimulated by 1 (*S. hangoei*). Species-specific consequences of allelopathy, also reported for e.g. *Karenia brevis* (Kubanek et al. 2005) and *Alexandrium* spp. (Tillmann et al. 2008), could be due to either differences in sensitivity of the target species or nature of the allelochemicals. The target diatoms may be differently susceptible to the same compounds, e.g. due to differences in their growth stage or cell concentration, while each dinoflagellate may produce a cocktail of allelochemicals, some of which inhibit multiple competitors and others that are allelopathic only towards certain species (Poulson et al. 2010). To ensure a competitive advantage in a natural assemblage with multiple competitor species, it is probably beneficial to produce multiple allelochemicals.

It is rather surprising that we found such clear negative effects of vernal dinoflagellates on co-occurring diatoms, which could be assumed to have adapted to the compounds produced by the sympatric dinoflagellates. It is possible that diatoms are generally more sensitive to allelochemicals produced by dinoflagellates than other groups of phytoplankton, or that the allelochemicals of these dinoflagellates are especially targeted against diatoms. Diatoms have been widely used as target species in allelopathy studies, and especially the globally occurring *Skeletonema* spp., *Chaetoceros* spp. and *Thalassiosira* spp. seem to be sensitive to allelochemicals excreted by various dinoflagellates (e.g. Gentien & Arzul 1990, Arzul et al. 1999, Kubanek et al. 2005, Tillmann et al. 2007, 2009, Tameishi et al. 2009). On the other hand, diatoms may also produce allelochemicals (Yamasaki et al. 2011a), and in fact filtrates of some Baltic diatoms, used as targets in the present study, have been found to decrease the growth rates of some common vernal dinoflagellates, including *Biecheleria baltica* and *Scrippsiella hangoei* (Spilling 2008).

In contrast to diatoms, cell numbers of the cryptophyte *Rhodomonas* sp. were significantly increased by all 3 dinoflagellate species. In several studies, cryptophytes (together with diatoms) have been found most sensitive to allelochemicals produced by other phytoplankton, including dinoflagellates (e.g. Rengefors & Legrand 2007, Tillmann et al. 2007, Tang & Gobler 2010), and are therefore commonly used as test organisms to study mechanisms of allelopathy (Ma et al. 2009, Tillmann et al. 2009). On the other hand, filtrates and live cells of *Karenia brevis* also enhanced the growth of *Rhodomonas lens*, either as a result of stimulatory substances produced by *K. brevis* or a better ability of the cryptophyte to use nutrients in the form released by the dinoflagellate than in F/2 form when grown alone (Kubanek et al. 2005). Also in the present study, *Rhodomonas* sp. probably benefited from either organic matter released by the dinoflagellates or slightly higher nutrient concentrations in the dinoflagellate treatments compared with the control, whereas the lack of negative effects may again indicate that the allelochemicals produced by the Baltic vernal dinoflagellates have evolved to target specific competitors other than cryptophytes, e.g. diatoms.

As in the present study, co-culturing of donor and target species usually results in stronger allelopathic effects than the application of cell-free filtrate of the donor to the target (Tillmann & John 2002, Kubanek et al. 2005, Tang & Gobler 2010). In mixed cultures, allelopathic compounds are likely to be continually produced and released by live cells, and may even increase in concentration as the donor population density increases, whereas filtration may lead to the loss of some allelochemicals, and the substances that are left in the filtrates may decompose over periods of days to weeks (Kubanek et al. 2005). Other explanations for stronger inhibition of live cells compared to filtrates include the possible importance of cell–cell

contact (Uchida et al. 1995, 1999), or the induction of allelopathy only in the presence of competitors (Kubanek et al. 2005).

In mixed culture experiments, potentially harmful effects due to high pH must be taken into account because a pH exceeding 9 to 9.5 can affect the growth and survival of marine phytoplankton (Goldman et al. 1981, Hansen 2002). Such a high pH can evolve in a non-aerated batch culture with a dense phytoplankton population. In the present study, pH was elevated up to almost 10 in the *Chaetoceros* cf. *wighamii* cultures treated with *Biecheleria baltica* (filtrate treatment 9.94, live cells treatment 9.75). However, this high pH had no significant effect on the growth of *C.* cf. *wighamii* compared with the control, in which the pH also increased to 9.92. The high pH was probably due to the extremely high cell densities of *C.* cf. *wighamii* (up to 500×10^3 cells ml^{-1}). The only negative effect on *C.* cf *wighamii* was caused by live cells of *Gymnodinium corollarium*; however, high pH was an unlikely cause, because pH in the *G. corollarium* live cells treatment was even slightly lower (8.95) than in the control (9.05). In other donor-target combinations with significant negative effects, the pH either did not increase over 8.5, or the increase was even higher in the control, indicating that the negative effects of the treatments were not due to higher pH. The only exceptions were the treatments with live *Scrippsiella hangoei* cells, where significantly lower target cell numbers coincided with a higher pH (up to 9.62) compared with the control. Nevertheless, negative effects of *S. hangoei* cells with high pH were not consistent: *C.* cf. *wighamii* was even stimulated in co-culture with *S. hangoei* in pH 9.75. It is possible that this species generally tolerates a higher pH than the other species examined. A pH tolerance of up to 9 was reported for growth of *Chaetoceros didymus* (Hansen 2002).

In our first set of experiments, focusing on the effects of dinoflagellates on a variety of target organisms, bloom concentrations of dinoflagellate cells were applied. The treatments were prepared from dinoflagellate cultures that were in late exponential or early stationary growth phase because many dinoflagellate species, including the closely related *Peridinium aciculiferum,* have been found to be most allelopathic in stationary growth phase (Kayser 1979, Arzul et al. 1999, Rengefors & Legrand 2001). Thus, the results may relate mostly to established dinoflagellate blooms, which is why another experiment was conducted to examine the lowest effective cell concentration that produced significant allelopathic effects on one of the most sensitive target species, *Skeletonema marinoi*. The lowest cell concentrations that (as filtrates) significantly reduced *S. marinoi* cell numbers were 10 100 *Biecheleria baltica* cells ml^{-1} (based on the first experiment; Fig. 2), 8100 *Gymnodinium corollarium* cells ml^{-1} and 5800 *Scrippsiella hangoei* cells ml^{-1}. Fluorescence of *S. marinoi* was already suppressed by filtrate from 1200 *S. hangoei* cells ml^{-1}. Other studies have reported effective cell concentrations for dinoflagellate filtrates ranging from as low as 100 *Alexandrium tamarense* cells ml^{-1} (Arzul et al. 1999) up to several thousands of *Karenia brevis* cells ml^{-1} (Kubanek et al. 2005). In the present study, *S. hangoei* filtrate had the strongest allelopathic effects on *S. marinoi*, which was reduced by ca. 98% at the highest *S. hangoei* cell concentration examined: 12 000 cells ml^{-1}. In contrast, the highest concentrations of the other dinoflagellates, 26 000 *B. baltica* and 16 000 *G. corollarium* cells ml^{-1}, only caused 32 and 47% reductions in *S. marinoi* cell numbers, respectively. Only live *B. baltica* cells (26 000 cells ml^{-1}) caused a 95% reduction in *S. marinoi* cells, comparable to that of the strongest *S. hangoei* filtrate. It is probable that the effects of live dinoflagellate cells are already visible at lower concentrations than those of filtrates, since live cells tend to exert stronger negative effects on targets than filtrates, as discussed earlier in the 'Discussion' (previous page).

Filtrates of *Biecheleria baltica, Gymnodinium corollarium* and *Scrippsiella hangoei* either stimulated (in 4 out of 6 donor-target combinations) or had no effect on the growth of the other dinoflagellate species, but in mixed cultures the target species were either suppressed (in 3 out of 4 cases) or unaffected. This indicates that the allelochemicals produced by these dinoflagellates are not primarily targeted against the other sympatric dinoflagellate species, but the filtrates rather contain some organic material originating from the dinoflagellate cells that is directly useful for the other dinoflagellates. The beneficial effect was stronger than the possibly negative effect caused by allelochemicals. Growth of 2 species in mixed batch cultures often depends strongly on the ratio between cell numbers of the species inoculated at the start of the experiment, so that the species inoculated at a higher concentration usually prevails and suppresses the competitor. The suppression occurs mainly by nutrient competition in exponential stage, but at maximum cell densities an additional effect of inhibiting metabolic products can be involved (Kayser 1979). In the present study, we tried to avoid the effects of nutrient competition by regular inorganic nutrient additions to the mixed cultures, and thus the negative effects observed were probably due to some

other mechanism, e.g. competitor-induced allelopathy or inhibition through direct cell contact (Uchida et al. 1995, 1999).

CONCLUSIONS

The Baltic vernal dinoflagellates *Biecheleria baltica*, *Gymnodinium corollarium* and *Scrippsiella hangoei* have allelopathic effects that seem to be specifically targeted against co-occurring diatoms. The effects are both donor and target species-specific, so that some of the most important vernal diatom species such as *Skeletonema marinoi*, *Thalassiosira baltica* and *Melosira arctica* are severely inhibited, whereas some other species belonging to the same community, such as *Chaetoceros* cf. *wighamii* and *Diatoma tenuis* are less affected. The dinoflagellates may also suppress the growth of the other sympatric dinoflagellate species, but they seem to have no negative effects on the co-occurring cryptophyte *Rhodomonas* sp. The effects are probably not mediated by lytic compounds, but rather through an unknown mechanism, causing reductions in growth rate on a time scale of several days. The negative effects of *S. hangoei* may include elevation of pH to levels intolerable by certain phytoplankton species.

Collectively, these results indicate that dinoflagellates may use allelopathy to compensate for their lower growth rates compared to diatoms, although further studies are needed to elucidate the role of dinoflagellate allelochemicals in the natural spring phytoplankton community. However, the allelopathic effects are probably important only after the development and concentration of an adequate number of dinoflagellate cells in the surface layer (Maestrini & Bonin 1981, Jonsson et al. 2009), either under ice (Spilling 2007), or as a result of hydrographic conditions in early spring that favor dinoflagellates over diatoms, e.g. early stratification of the water column (Wasmund et al. 1998). In the northern Baltic Sea dinoflagellate blooms are typically initiated by such conditions. Stratification, e.g. under the ice, may lead to the build-up of a sizeable inoculum population well before the break-up of the ice cover and the rise of the spring bloom diatoms. Allelopathic substances released by such a dinoflagellate population immediately when conditions allow potentially competing diatoms to grow should be efficient enough to contribute to their suppression and the maintenance of dinoflagellate dominance, even if the effects are not immediate but manifest at a scale of days. On the contrary, diatoms grow rapidly in other years when the water column is initially more turbulent and gain dominance over dinoflagellates, possibly also producing chemicals that inhibit dinoflagellate growth.

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