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Prymnesium parvum exotoxins affect the grazing and viability of the calanoid copepod *Eurytemora affinis*

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ABSTRACT: The calanoid copepod *Eurytemora affinis* from the northern Baltic Sea was exposed to cell-free filtrates of the toxic haptophyte *Prymnesium parvum* as well as to cell mixtures of *P. parvum* and *Rhodomonas salina*. To test the effects of *P. parvum* exudates and allelopathy on selective grazers, copepods were incubated (1) in increasing concentrations of cell-free filtrates of *P. parvum* in the presence of good food (*R. salina*), (2) in 1:1 cell mixtures at 2 cell concentrations of *P. parvum* and *R. salina* and (3) in *R. salina* cell suspension, which was used as a control for good-quality food. *P. parvum* cultures were grown in nutrient-balanced (+NP) or limited (–N or –P) media to obtain different levels of toxicity. Survival, ingestion, faecal pellet production rates and egg production were measured over 3 d, together with measurements of *P. parvum* toxicity (hemolytic activity) (HA). Most of the copepods incubated in high-filtrate concentrations died or became severely impaired, although (HA) in filtrates was under the detection limit. Further, the ingestion and faecal pellet production rates were suppressed in the highest filtrate concentrations in nutrient-limited treatments. Higher cell density in cell mixtures resulted in significantly lower faecal pellet production, although survival remained high. Our results show that HA is not a good overall indicator of the total harmful effects of *P. parvum* on grazers. Besides monospecific *P. parvum* diets, filtrates and cell mixtures have negative effects on grazers, and these effects are stronger under nutrient-depleted conditions; however, the presence of good-quality food lowers harmful effects for copepods. The negative effects caused either by direct intoxication or by food limitation following from strong allelopathic effects of *P. parvum* on other components of nano- and microplankton suggest that *P. parvum* blooms have a realistic potential to be deleterious for copepod secondary production, irrespective of the presence of alternative food sources.

KEY WORDS: *Prymnesium parvum* · Exotoxins · Hemolytic activity · *Eurytemora affinis* · Viability · Feeding · Faecal pellet production · Egg production rate

INTRODUCTION

The haptophyte *Prymnesium parvum* (Carter 1937) is a common member of coastal plankton communities worldwide. This species causes noxious blooms with deleterious effects on diverse marine organisms (Richardson 1997), affecting coastal marine ecosystems and causing economic loss for commercial aquaculture. For example, in Norway *P. parvum* was found to destroy fish gills, thus causing mass mortality of farmed fish (Aure & Rey 1992). In the Baltic Sea, *P. parvum* belongs to the natural plankton community and may, in suitable environmental conditions, bloom with harmful effects (Lindholm & Virtanen 1992, Lindholm et al. 1999).

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The coastal areas in the whole Baltic Sea have eutrophied during recent decades (HELCOM 2004). The main nutrient sources include diffuse loading, aerial deposition (especially of N), fish farming and municipal wastewaters (Helminen et al. 1998, HELCOM 2004). Although the frequency and intensity of other harmful algal blooms, especially those of cyanobacteria, have increased (Finni et al. 2001), only few *Prymnesium parvum* blooms with high fish mortality have so far been recorded in Finnish coastal waters (Lindholm & Virtanen 1992, Lindholm et al. 1999). Toxic *P. parvum* bloom events in Finland have been related to reduced water exchange, calm weather, high oxygen saturation and high N:P ratio with phosphorus limitation (Lindholm et al. 1999). Fish farms in Finland are usually situated in sheltered bays, which make them potentially vulnerable for *P. parvum* blooms.

Prymnesium parvum excretes toxic compounds with ichthyotoxic, cytotoxic and neurotoxic activity into the surrounding water (Johansson & Granéli 1999). The presence of a potent ichthyotoxin, prymnesin, was first reported by Shilo & Rosenberger (1960). Later on, Igarashi et al. (1995) isolated prymnesin-1 (PRM1) and prymnesin-2 (PRM2), which both are potent in haemolytic activity (HA). These compounds increase cell membrane permeability leading to disruption of the cellular ion balance, which causes an increase in cell turgor and finally cell lysis (Shilo & Rosenberger 1960, Igarashi et al. 1995). This may harm organisms ranging from protozoans to fish (Edvardsen & Paasche 1998). The toxic compounds excreted by *P. parvum* have severe allelopathic effects on many phytoplankton groups (Fistarol et al. 2003, Legrand et al. 2003, Skovgaard et al. 2003, Uronen et al. 2005) and noxious effects to ciliates (Fistarol et al. 2003, Granéli & Johansson 2003) and rotifers (Barreiro et al. 2005) as well as copepods (Nejstgaard & Solberg 1996, Koski et al. 1999b, Sopanen et al. 2006). Thus, a bloom of this species is likely to have adverse effects on large parts of the plankton community.

Interactions between zooplankton and toxin-producing phytoplankton are species-specific, and the relative potency of toxins is not always the major determinant of zooplankton response (Turner & Tester 1997). The effect of toxic algae on zooplankton is dependent on, for instance, the nature of the toxin, the ability of the zooplankton species to avoid feeding on the toxic algae and the tolerance for the toxin in question. If the algae is endotoxic and needs to be ingested for harmful effects to occur, species that graze on a selective basis may gain benefit. On the other hand, in the presence of exotoxic species, such as *Prymnesium parvum*, tolerance for toxins becomes crucial, although compounds that are released into the water are rapidly diluted. Therefore, the exudates should be potent enough or occur in sufficiently high concentrations to counteract the dilution. Reports of negative correlation between the abundance of toxic microalgae and other aquatic organisms in the field have shown that co-occurring species may detect substances released by microalgae (Nielsen et al. 1990). For example, due to a heavy subsurface *Chrysochromulina polylepis* bloom, copepods were virtually absent from the pycnocline, but higher concentrations of copepods were found both immediately above and below the pycnocline (Nielsen et al. 1990).

To our knowledge, there are no studies that have focused on the direct effect of *Prymnesium parvum* exudates on copepods, although these exudates are likely to be the main pathway affecting selective grazers, such as copepods, during a *P. parvum* bloom. In the present study, we investigated the direct and indirect effects of *P. parvum* exudates on copepods. As nutrient limitation has been shown to affect the toxicity of *P. parvum* (Johansson & Granéli 1999, Uronen et al. 2005), we used cultures grown in balanced (+NP) or nutrient-limited (–N, –P) conditions to cover the range of *P. parvum* toxicity. The main objectives were (1) to study the mortality, feeding, egestion and egg production in copepods exposed to the cell-free filtrates originating from nutrient-stressed and nutrient-balanced *P. parvum* in the presence of good-quality food (i.e. are there direct effects that depend on the amount of toxin?) and (2) to study if *P. parvum* cells or exudates interfere with copepod feeding, either via direct effects on the grazer or via allelopathic effects on the alternative food species. The overall hypothesis was that *P. parvum* exudates are harmful to copepods already at relatively low concentrations and that N- and P-deficient cells/exudates of *P. parvum* are more harmful to copepods than NP-balanced conditions.

MATERIALS AND METHODS

Copepds and algae. All experiments were conducted with adult females of the calanoid copepod *Eurytemora affinis* (Poppe), which is among the most common copepod species in the northern Baltic Sea. Three types of experiments were set up: adult females were incubated (1) with 4 different concentrations of cell-free *Prymnesium parvum* (grown in –N, –P and +NP media) filtrates (Filt) in the presence of a saturating concentration of the high-quality food *Rhodomonas salina* (>400 µg C l^{-1}; (Berggreen et al. 1988), (2) with ca. 1:1 mixtures (Mix) of *P. parvum* (–N, –P and +NP) and *R. salina* and (3) with *R. salina* in saturating food concentration (Table 1).

Copepods for the experiments were collected with vertical hauls (30 to 0 m) using a 200 µm plankton-net from the Tvärminne Storfjärd, SW coast of Finland

Table 1. *Prymnesium parvum* and *Rhodomonas salina*. Cell concentrations (10^3 cells ml^{-1}) and carbon (µg C l^{-1}) in incubations with filtrates (filt), food mixtures (mix) and *R. salina* control (control *Rho*) (mean ± SE). (–N): Nitrogen-limited cultures; (–P): phosphorus-limited cultures; and (+NP): nutrient-balanced cultures. In filtrate treatments, 1 to 4 correspond to increasing *P. parvum* (*Pry*) carbon and cell concentrations in cultures; 4 corresponds to the total cell concentration in cultures, whereas 1 to 3 were diluted from the total filtrate (see 'Materials and methods'). In mixtures, 1 and 2 indicate the 2 different total cell concentrations. (–): not measured

Treatment	Cell conc. *Pry*	Carbon *Pry*	Cell conc. *Rho*	Carbon *Rho*
Control Rho			11.4 ± 0.7	480 ± 30
Filt 1 –N	5	160	14.2 ± 1.3	596 ± 55
Filt 2 –N	10	320	14.9 ± 1.5	629 ± 63
Filt 3 –N	50	1600	13.5 ± 3.7	566 ± 155
Filt 4 –N	127 ± 4.6	4060 ± 150	12.6 ± 2.0	531 ± 83
Filt 1 –P	5	160	13.9 ± 0.9	585 ± 39
Filt 2 –P	10	320	14.5 ± 0.8	607 ± 35
Filt 3 –P	50	1600	14.4 ± 1.2	603 ± 49
Filt 4 –P	294 ± 9.8	9408 ± 300	–	–
Filt 1 +NP	5	160	14.9 ± 0.4	625 ± 15
Filt 2 +NP	10	320	16.0 ± 0.8	676 ± 34
Filt 3 +NP	50	1600	15.0 ± 0.9	629 ± 38
Filt 4 +NP	354 ± 2.2	11330 ± 70	–	–
			Total cell conc.	Total carbon
Mix 1 –N			25.6 ± 2.8	947 ± 104
Mix 2 –N			113 ± 3.7	4172 ± 136
Mix 1 –P			30.7 ± 4.0	1138 ± 150
Mix 2 –P			115 ± 22.8	4263 ± 845
Mix 1 +NP			30.8 ± 3.6	1140 ± 133
Mix 2 +NP			107 ± 16.9	3970 ± 624

(59° 49′ N, 23° 17′ E). Salinity and surface temperature at the sampling station were ca. 6 psu and 16°C, respectively. In the laboratory, adult females were picked up individually, using a binocular microscope, and placed in 64 µm filtered seawater at the experimental temperature (15°C) for ca. 24 h prior to the start of the experiment.

The haptophyte *Prymnesium parvum* (strain KAC 39) (ESD ca. 7 to 9 µm) was obtained from the Kalmar Algal Collection (University of Kalmar). *P. parvum* was cultured in modified f/20 media (Guillard & Ryther 1962) under NP-balanced (+NP: 58 µM NO_3 and 3.6 µM PO_4), nitrogen-deficient (–N: 16 µM NO_3 and 4 µM PO_4) and phosphorus-deficient (–P: 80 µM NO_3 and 1 µM PO_4) conditions. We used semi-continuous cultures, which were diluted daily by 20% with fresh medium to maintain the cells in a steady exponential growth phase. Cultures were aerated gently, and the temperature was kept at 16°C. The light:dark cycle was 14 :10 h and irradiance was 330 µmol photons m^{-2} s^{-1} outside the containers on the bright side and <100 µmol m^{-2} s^{-1} on the dark side (measured with a Li-Cor LI-1000 Data Logger). Detailed descriptions of *P. parvum* cultures as well as their growth and toxicity during the experiments are presented in (Uronen et al. 2005).

The cryptomonad *Rhodomonas salina* (ESD ca. 7 to 9 µm) was used as a control for good-quality food. It was obtained from the culture collection (TV 22/4) of the Tvärminne Zoological Station (University of Helsinki) and cultured in 5 l batches in a modified f/20 medium at 18°C with a 14:10 h light:dark cycle. Irradiance was 75 µmol photons m^{-2} s^{-1}.

The cell concentrations of both algae cultures were monitored using an ELZONE electronic particle counter (Particle Data). The food media was prepared assuming a C content of 32 pg $cell^{-1}$ for *Prymnesium parvum* (Legrand et al. 2001) and 42 pg $cell^{-1}$ for *Rhodomonas salina* (Koski et al. 1998).

Experimental set-up. Cell-free filtrates of *Prymnesium parvum* were prepared by gently GF/C filtering (pore size 1 µm) the cell suspensions taken from –N, –P or +NP *P. parvum* cultures. No cells or debris were observed under light microscopy after filtration. Filtrates were kept 0.5 to 1 h in cold and dark before use. Filtrates were diluted with 0.2 µm filtered seawater to obtain appropriate concentrations (equivalent *P. parvum* cells, Table 1). *Rhodomonas salina* suspension was mixed with the filtrates shortly before the start of the experiments.

Additions of *Prymnesium parvum* filtrates (Filt 1 to 3) corresponded to *P. parvum* concentrations of ca. 160, 320 and 1600 µg C l^{-1} (Table 1). The non-diluted, total filtrate concentrations (Filt 4) corresponded to ca. 4060 ± 150, 9408 ± 300 and 11330 ± 70 µg C l^{-1} in Filt –N, –P and +NP, respectively (Table 1). The filtrate concentrations used were chosen to cover densities observed during *P. parvum* blooms (Edvardsen & Paasche 1998).

Two different food concentrations were used in mixtures. Total cell concentration (*Rhodomonnas salina* + *Prymnesium parvum*) corresponded to ca. 1075 ± 64 in Mix 1 and 4135 ± 87 µg C l^{-1} in Mix 2, respectively (Table 1), with a ca. 1:1 ratio of both species (in C) at the start of the experiments.

The experiments were carried out in 130 ml glass bottles that were placed in a rotating plankton wheel (1 rpm) in a thermostated water bath (16°C). All treatments consisted of 6 replicate bottles containing 6 to 10 copepods $bottle^{-1}$. The copepod C content corresponded to ca. 30% of the algae C in experimental bottles. In addition, 4 grazing controls without animals per each treatment were used. The experiments lasted for 3 d, consisting of 3 subsequent 24 h incubations with

the same animals. After every 24 h incubation period, the condition of the copepods was checked, the animals were gently transferred to a new food suspension, and the water was preserved with 4% acidic Lugol's solution for total faecal pellet and phytoplankton counts.

Analyses. Algal cell concentrations were counted at the start and end of each 24 h incubation. The ingestion and filtration rates of *Eurytemora affinis* on *Rhodomonas salina* in filtrates were calculated, according to (Frost 1972) on the basis of cell disappearance, separately for each day of the incubation. Because the particle counter cannot differentiate between similarly sized *R. salina* and *P. parvum* cells in mixtures, only faecal pellet production was measured in these treatments. Since *P. parvum* effectively lyses *Rhodomonas* sp. (Skovgaard & Hansen 2003, Uronen et al. 2005), its effect on cell numbers had to be distinguished from the grazing effect. This was done by comparing the controls without copepods in the filtrate experiment to *R. salina* controls.

HA was assessed daily from *Prymnesium parvum* cultures and from the cell-free *P. parvum* filtrates with an HA assay (Igarashi et al. 1998, Johansson & Granéli 1999, Fistarol et al. 2003, Uronen et al. 2005). Cell-free filtrates were collected by GF/C filtering (glass microfibre filters, pore size 1 µm) 10 ml of the cultures (–N, –P and +NP). Haemolytic substances in the *P. parvum* cells were extracted with 2 ml of methanol from the GF/C filters. After 24 h incubation, with or without copepods, 10 ml of filtrate was collected from experimental bottles. The HA of both cell methanol extract (cultures) and GF/C filtrate (filtrates) on horse blood cells was measured in duplicate. The horse blood was washed and resuspended in an isotonic phosphate buffer. Thereafter, 200 µl of horse blood cell suspension in the isotonic phosphate buffer and 50 µl of the cell methanol extract or the GF/C filtrate was pipetted into microplate wells (Falcon, 96 wells) in different ratios (1 to 100%). A standard curve was made with saponin (Sn). After 1 d incubation at room temperature in the dark, the microplates were centrifuged ($1107 \times g$, 5 min, Beckman Allegra™ S2096 centrifuge), and the supernatant (110 µl) was transferred to new microplate wells (Black Costar®, 96 wells). The absorbance at 540 nm was read on a Fluorstar 403 microplate reader. The results were expressed as saponin equivalent (SnEq) µg ml^{-1} and calculated further to SnEq pg $cell^{-1}$.

Faecal pellets were counted from 3 to 6 replicates after filtration on a 50 µm net, using a binocular microscope. Faecal pellet volume was estimated from 3 replicates in each treatment by measuring the length and width of ca. 30 pellets. To estimate egg production, females were incubated in individual vials (in 0.2 µm filtered seawater) after the initial 3 d experiments. The number of eggs and nauplii were counted after a 48 h incubation. Egg production was estimated using:

$$p = N_e / (N_f D),$$

where p = egg production (eggs $ind.^{-1}$ d^{-1}), N_e = no. of eggs, N_f = no. of females, and D = development time of the eggs. According to our previous studies, egg production rate of *Eurytemora affinis* reflects experimental conditions already after 3 d exposure, which is recognized as a low egg production rate (range, 0 to 2 egg $ind.^{-1}$ d^{-1}), in the GF/F filtered seawater control (Koski et al. 1999a, Sopanen et al. 2006).

The development time of the eggs was calculated to be 2.06 d at 16°C according to Andersen & Nielsen (1997). The weight-specific egg production rate was calculated assuming the C content of females to be 3.6 µg C $ind.^{-1}$ and the C content of eggs to be 0.03 µg C egg^{-1} (Koski 1999). Gross growth efficiency (GGE% d^{-1}) of the copepods was calculated from the weight-specific egg production rate divided by their average weight-specific ingestion during the 3 d incubation.

Differences in *Rhodomonas salina* growth rates among the filtrates, mixtures and control were tested with a 1-way ANOVA or with a *t*-test. *Eurytemora affinis* survival on day 3 (percentages arcsine transformed) was tested for differences among treatments with a 1-way ANOVA. Grazing and faecal pellet production rates of *E. affinis* were tested with a 2-way ANOVA, using day and cell-free filtrate concentration (Filt) or cell density (Mix) as independent variables. If conditions for the ANOVA (normality and equality of variances) were not met, non-parametric methods were used. The Holm-Sidak method (1- and 2-way ANOVA) or Dunn's method (Kruskal-Wallis ANOVA) were used for all pairwise comparisons. The analyses were performed with SigmaStat for Windows 3. 0. 1 (SPSS) software.

RESULTS

HA and allelopathic effects of *Prymnesium parvum*

The *Prymnesium parvum* cultures were in steady state and were all haemolytic during the experiments (for details of the cultures, see Uronen et al. 2005). Cell density of *P. parvum* in cultures varied between 130×10^3 in –N media and 350×10^3 cells ml^{-1} in +NP media with a PON: POP ratio (mol:mol) between ca. 12 (–N) and ca. 38 (–P). HA was significantly higher, ca 50%, in the –P culture compared to the –N or +NP cultures (ANOVA: $F_{2, 8} = 13.1$; $p < 0.01$, Fig. 1). In the filtrates, HA was below the detection limits of HA analysis.

Generally, the growth rates of *Rhodomonas salina* in mixtures were significantly lower compared to the growth rates in the *R. salina* control (Kruskal-Wallis ANOVA: $H_6 = 74.3$; $p < 0.001$ in all but Mix 1 –N). Moreover, the negative effect was connected to cell concentration of *Prymnesium parvum*, as the significant difference between Mix 1 and Mix 2 (see Table 1 and Fig. 2) was found in –P treatments (Mann-Whitney rank sum test: $T_8 = 99$; $p < 0.001$) and in –N treatments (*t*-test: $t_{14} = 12.63$; $p < 0.001$). As a rule, ca. 30% of the *R. salina* cells in mixtures disappeared during the experiments, both from the controls (without grazers) and from the replicates (with grazers), indicating a strong allelopathic effect. The direct allelopathic effect caused by the filtrates of *P. parvum* on *R. salina* was weaker compared to cell mixtures and hence only 5 to 10% of the cells disappeared during the incubations. However, the growth rates of *R. salina* in filtrates were significantly lower compared to the *R. salina* control in Filt 3 –P, Filt 3 and 4 –N and Filt 2 +NP, respectively (ANOVA: $F_{10, 118} = 3.9$; $p < 0.001$).

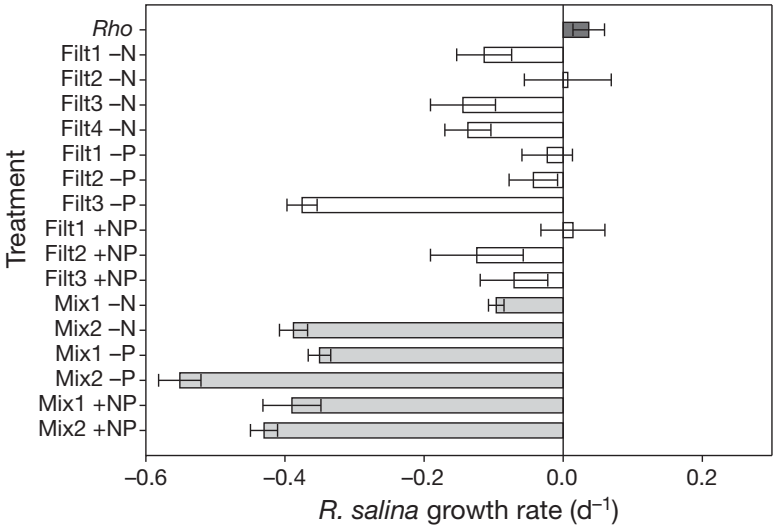

Fig. 2. *Rhodomonas salina*. Growth rate (d^{-1}, mean ± SE) in *R. salina* control (*Rho*, dark grey bar), in different concentrations of *Prymnesium parvum* filtrates (Filt –N, –P and +NP, white bars) and in 2 different mixture concentrations (Mix –N, –P and +NP, grey bars). For concentrations, see Table 1

Survival of *Eurytemora affinis*

The survival of copepods was inversely related to the initial concentration of *Prymnesium parvum* cells in the filtrates, although HA in the cell-free *P. parvum* filtrates was below detection. Both in the control and at low *P. parvum* filtrate concentrations (Filt 1 and 2: 5 and 10×10^3 cells ml^{-1}, respectively) the copepod survival remained high, >80% during the experiments. The copepods were also looking healthy and active in these treatments. On the contrary, exposure to higher *P. parvum* filtrate concentrations (Filt 3 and 4: $>50 \times 10^3$ cells ml^{-1}) resulted in significantly lower survival (Kruskal-Wallis ANOVA: $H_{12} = 54.3$; $p < 0.001$). In Filt 4 (–P and +NP nutrient treatments) the mortality was 100% during the first 24 h incubation period. The survival of copepods in Filt 4 (–N) was also low (36.4 ± 13%) and significantly lower compared to *Rhodomonas salina* control (Kruskal-Wallis ANOVA: $H_4 = 13.8$; $p < 0.01$) (Fig. 3).

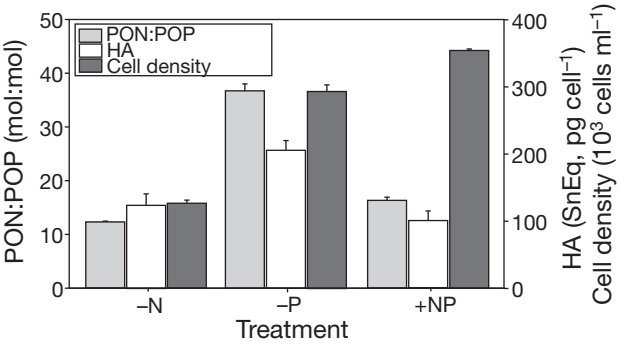

Fig. 1. Particulate organic nutrient ratios PON:POP, haemolytic activity (HA; saponin equivalent, SnEq) and *Prymnesium parvum* cell density in semi-continuous cultures during the 3 d experiments (mean ± SE)

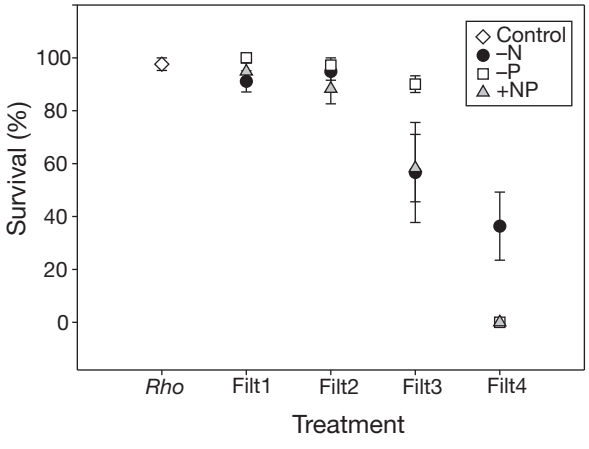

Fig. 3. *Eurytemora affinis*. Survival (%) of the copepod in *Rhodomonas salina* (*Rho*) control (◇) and in 4 *Prymnesium parvum* filtrates equivalent to increasing haptophyte concentration in cultures (Filt 1 to Filt 4, see Table 1) on Day 3 (mean ± SE). Different symbols indicate *P. parvum* nutrient treatments: (●) N-limited, (□) P-limited, (△) NP-balanced

In the cell mixtures of *Prymnesium parvum* and *Rhodomonas salina*, the copepods were active and their survival remained high in all treatments throughout the experiments. The average survival percentage (mean ± SE) in mixtures was always >90% (range, 91.4 to 100%), comparable to the *R. salina* control, in which the survival was 98 ± 2.4%. *P. parvum* concentrations in mixtures (from ca. 13 to 57.5×10^3 cells ml^{-1} depending on the treatment) were comparable to Filt 2 and 3, the latter of which resulted in significant adverse effects on copepods.

Clearance rates and ingestion in filtrates

Average clearance rates of *Eurytemora affinis* on *Rhodomonas salina* in Filt 1 to 3 were 3.5 to 6.9 (–N), 4.6 to 7.0 (–P) and 3.8 to 5.4 (+NP) ml ind.$^{-1}$ d^{-1}. Mean weight-specific ingestion rates (0.4 to 1.1 µgC µgC^{-1} d^{-1}) were comparable to the average ingestion rate in the *R. salina* control (1.0 ± 0.1) (Fig. 4). Significant differences between 3 successive d in ingestion rates were not detected in the *R. salina* control (ANOVA: $p > 0.05$). In contrast, the ingestion rates in the filtrates varied significantly among days in all nutrient treatments: highest ingestion rates were observed on Day 3 in –N and –P and on Day 2 in +NP. In addition, filtrate concentration had a significant effect on the ingestion rates in nutrient-limited filtrates: the highest filtrate concentrations (Filt 3 and 4, –N, and Filt 3, –P) resulted in lower ingestion on Day 3. Significant interactions between days and filtrate concentration were found in –P and +NP nutrient treatments (Table 2). We were not able to measure ingestion in Filt 4 (–P and +NP) because all animals died in these treatments during the first 24 h incubation.

Egestion

The average faecal pellet production rate of the copepods in filtrates was ca. 20 pellets ind.$^{-1}$ d^{-1}, which reflected *Eurytemora affinis* feeding rates well during the experiments (Fig. 5), as also suggested by the significant regression between faecal pellet production

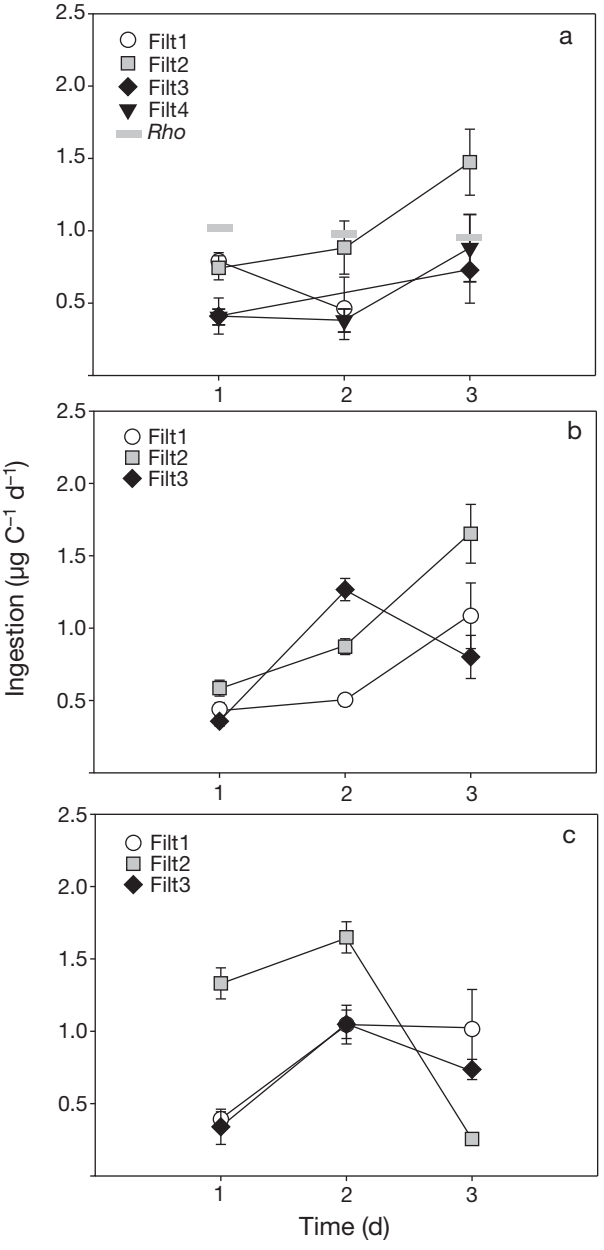

Fig. 4. *Eurytemora affinis*. Weight-specific ingestion by the copepod of *Rhodomonas salina* in (a) N-limited, (b) P-limited and (c) NP-balanced filtrates (mean ± SE). Different symbols indicate different filtrate concentrations (see Table 1). *Rho*: *R. salina* control. Filt 3 –N (Day 2) and Filt 1 –N (Day 3) are missing

Table 2. Weight-specific ingestion of *Eurytemora affinis*. Two-way ANOVA results for filtrates in different nutrient treatments (–N, –P and +NP) using day (d) and cell concentration (c) as independent variables. The Holm-Sidak method was used for multiple comparisons. –: interactions not tested because of missing data

Treatment	df_d	df_c	$df_{d \times c}$	df_{total}	F_d	F_c	$F_{d \times c}$	p_d	p_c	$p_{d \times c}$
Filt –N	2	3	–	49	10.3	7.6	–	<0.001	<0.001	–
Filt –P	2	2	4	48	29.5	7.5	7.9	<0.001	0.002	<0.001
Filt +NP	2	2	4	41	11.4	0.7	13.7	<0.001	0.5	<0.001

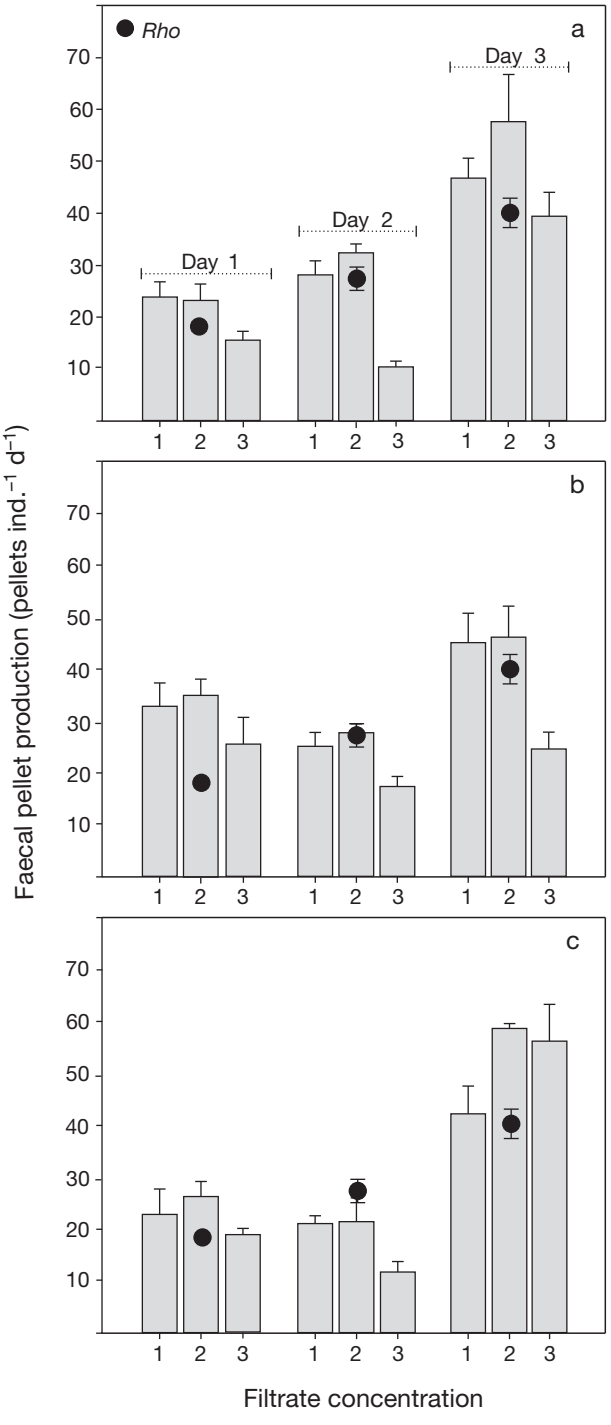

Fig. 5. *Eurytemora affinis*. Faecal pellet production (mean ± SE) of the copepod in increasing concentrations of *Prymnesium parvum* filtrates (1, 2 and 3; see Table 1) during the 3 d experiments. (a) N-limited, (b) P-limited and (c) NP-balanced. (●) *Rho*: *Rhodomonas salina* control

and ingestion — ingestion = −0.28 + (0.032 × egestion); $R^2 = 0.64$, n = 9, $p < 0.05$. Moreover, it was comparable to the faecal pellet production (ca. 29 ± 2 pellets ind.$^{-1}$ d^{-1}) in the *Rhodomonas salina* control. Significant differences in egestion were found both between days and filtrate concentrations: the faecal pellet production on day 3 was significantly higher compared to previous days in all nutrient treatments. High filtrate concentrations (Filt 3; –P, –N and +NP) always resulted in lower production rates compared to Filt 1 and 2 (Table 3). No interactions between days and filtrate concentrations were found.

Average faecal pellet size (mm^3 ± SE × 10^{-4}) was 1.2 ± 0.1 in Filt –P, 0.8 ± 0.08 in Filt –N, 0.7 ± 0.06 in Filt +NP, 0.9 ± 0.4 in the control *Rhodomonas salina* and not significantly different among treatments (Kruskal-Wallis ANOVA: $p > 0.05$). The faecal pellet production, expressed in volume (mm^3 adult^{-1} d^{-1}), ranged between 0.003 and 0.01 in Filt 1 to 3 (–N), 0.004 and 0.007 in Filt 1 to 3 (–P) and 0.005 and 0.009 in Filt 1 to 3 (+NP). The variation among days became smaller when looking at pellet volumes, but differences between filtrate concentrations were still unaffected.

Average faecal pellet production in mixtures was 24.4 and 14.5 pellets ind.$^{-1}$ d^{-1} in Mix 1 and 2, respectively. Like in filtrates, the highest production rates of faecal pellets were detected on day 3 in all treatments (Fig. 6, Table 3). The total cell density affected the faecal pellet production rate: higher total cell density resulted in significantly lower production rates in Mix 2 (–P and +NP), even though the food concentration also increased. The faecal pellet production in Mix 1 indicated similar ingestion rates as in filtrates, while the ingestion rate in Mix 2 was ca. 40% lower, assuming that the pellet production reflected ingestion similarly in both treatments. If estimated from the ingestion:faecal pellet production ratio of the filtrate experiments (see regression above), the ingestion in Mix 1 was ca. 0.5 µgC µgC^{-1} d^{-1}, whereas the ingestion in Mix 2 was ca. 0.2 µgC µgC^{-1} d^{-1}.

Average faecal pellet size (mm^3 ± SE × 10^{-4}) was 0.5 ± 0.04 in Mix 1 and 2 (–N), 0.7 ± 0.07 in Mix 1 and 2 (–P) and 0.6 ± 0.07 in Mix 1 and 2 (+NP). Similar to filtrate experiments, differences among faecal pellet size were non-significant among the nutrient treatments (ANOVA: $p > 0.05$). The pellet production, expressed in volume (mm^3 adult^{-1} d^{-1}), varied between 0.008 and 0.01 in Mix 1 and 2 (–N), 0.007 and 0.01 in Mix 1 and 2 (–P) and 0.006 and 0.02 in Mix 1 and 2 (+NP), respectively. Like in filtrates, the difference among days became smaller in the mixtures, but differences among treatments were unchanged.

Reproduction

In general, egg production rate varied unrelated to the treatment. The average egg production rate of *Eury-*

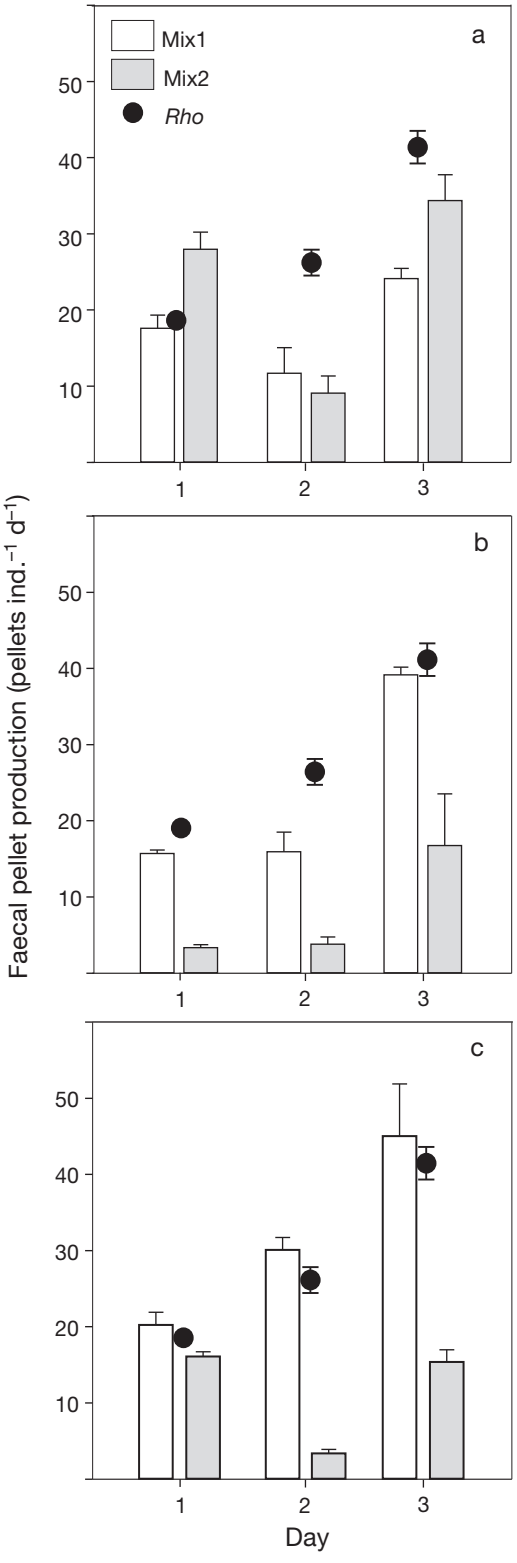

Fig. 6. *Eurytemora affinis*. Faecal pellet production (mean ± SE) of the copepod in mixtures of *Rhodomonas salina* and *Prymnesium parvum*. Mix 1 and Mix 2 represent the 2 food concentrations (see Table 1). (a) N-limited, (b) P-limited and (c) NP-balanced. Other abbreviations as in Fig. 5

temora affinis ranged from 1.1 to 4 eggs ind.$^{-1}$ d^{-1} in the filtrates and from 1.6 to 4.9 in the mixtures. The egg production rate in *Rhodomonas salina* control (3.6 eggs ind.$^{-1}$ d^{-1}) was at the same level as in filtrates and mixtures without significant differences among treatments (ANOVA: $p > 0.05$). Hatching was generally relatively high (>60%) in all treatments, whereas the gross growth efficiency was low (<8%) (Table 4).

DISCUSSION

Allelopathy

All *Prymnesium parvum* cultures were haemolytic during the present study, but in phosphorus-limited cultures HA was ca. 50% higher compared to nitrogen-limited or nutrient-balanced cultures. Thus, we can presume that *P. parvum* cells, as well as the cell-free filtrates in all nutrient treatments, had a potential to cause harmful effects.

Differences in the growth rates of *Rhodomonas salina* revealed that allelopathic effects were weaker in the cell-free filtrates compared to the mixtures, which is in accordance with Uronen et al. (2007). In

Table 3. Pellet production of *Eurytemora affinis*. Two-way ANOVA results for filtrates (filt) and mixtures (mix) in different nutrient treatments (treat.; –N, –P and +NP) using day (d) and cell concentration (c) as independent variables. The Holm-Sidak method was used for multiple comparisons

Treat.	df_d	df_c	df_{total}	F_d	F_c	p_d	p_c
Filt –N	2	2	30	40	11.8	<0.001	<0.001
Filt –P	2	2	47	9.4	7.5	<0.001	0.002
Filt +NP	2	2	25	73.9	3.4	<0.001	0.057
Mix –N	2	1	16	25.6	7.5	<0.001	0.02
Mix –P	2	1	17	24.3	39.9	<0.001	<0.001
Mix +NP	2	1	17	24.5	120.5	<0.001	<0.001

Table 4. *Eurytemora affinis*. Reproduction in filtrates (filt –N, –P and +NP), in mixtures (mix –N, –P and +NP) and in *Rhodomonas salina* control (*Rho*). Egg production rate (mean ± SE), hatching percentage (mean ± SE) and gross growth efficiency (GGE) are shown. No. of observed females and no. of eggs included in hatching estimates are indicated in parentheses. (–) missing data

Treatment	Eggs (ind.$^{-1}$ d^{-1})	Hatch (%)	GGE (% d^{-1})
Filt –N	1.1 ± 0.8 (8)	100 ± 0 (18)	3.8
Filt –P	4.0 ± 0.8 (21)	67 ± 11 (67)	5.6
Filt +NP	2.5 ± 1.0 (12)	100 ± 0 (61)	3.3
Mix –N	4.9 ± 2.0 (8)	89 ± 11 (80)	
Mix –P	–	–	
Mix +NP	1.6 ± 0.7 (14)	63 ± 15 (46)	
Rho	3.6 ± 0.9 (11)	–	7.7

mixtures, the cell density decreased ca. 30% during the incubations. In the study by Uronen et al. (2005), the decrease of the *R. salina* density was in the same range: 35 to 50% after 23 h incubation. However, in the present study, the allelopathic effect of *Prymnesium parvum* was probably underestimated, since we did not count damaged or lysing cells but only the disappearance of *R. salina* cells. The stronger allelopathic effect in the mixtures compared to the filtrates may be due to continuous release of allelopathic substances by *P. parvum* cells.

Effects on viability

Although HA was below detection limit in the cell-free filtrates, it was evident that the filtrates had severe negative effects on the viability of the copepods. All individuals that were incubated in the total filtrates died during the first 24 h incubation period, and those that survived were severely impaired and inactive. Although the mortality was most pronounced in total filtrates, increased mortality responses were also observed in lower filtrate concentrations corresponding to $>50 \times 10^3$ cells ml^{-1}.

Fistarol et al. (2003) found that *Prymnesium parvum* cell-free filtrates corresponding to 13.5×10^3 cells ml^{-1} have deleterious effects on ciliates. In addition, they found that while the cell-free filtrate was haemolytic at the start of the experiment, HA decreased below detection after 48 h. These results, together with our observations, indicate that *P. parvum* exudates can be harmful to other organisms while not haemolytic. Hence, HA may not be a good indicator of the toxicity of *P. parvum* to aquatic animals (Johansson & Granéli 1999), but the harmful effects are a consequence of a set of compounds that have different effects on organisms, such as ichthyotoxic, cytotoxic or HA.

In contrast to the filtrates, in cell mixtures of *Prymnesium parvum* and *Rhodomonas salina* no lethal effects were detected, and the majority of the copepods stayed active throughout the experiment. This was the case even though *P. parvum* concentrations were sufficient to induce adverse effects on the copepods (Sopanen et al. 2006). In a previous study by Koski et al. (1999b), the mortality of *Eurytemora affinis* was similarly insignificant in the mixtures of *Prymnesium patelliferum* and good-quality food *Pseudopedinella elastica* or *Brachiomonas submarina* but clearly elevated in *P. patelliferum* treatments.

One possible explanation for the better survival of copepods in mixtures is the tendency of the *Prymnesium parvum* exudates to bind to particles such as other phytoplankton cells (Uronen et al. 2005). In the mixtures, the target cell *Rhodomonas salina* densities were higher compared to filtrates. This might enable the copepods to escape the toxic effects. Secondly, several studies have shown that *P. parvum* is mixotrophic and able to prey on bacteria as well as on protists of a wide size range (Legrand et al. 2001, Skovgaard et al. 2003). In addition, mixotrophy, for example ingestion of bacteria, may release *P. parvum* from cellular stress, and thus decrease its toxicity (Legrand et al. 2001, Uronen et al. 2007). Skovgaard et al. (2003) showed that the feeding activity of *P. parvum* increased with greater predator density, so that at 50×10^3 *P. parvum* cells ml^{-1} ca. 40 to 45% of the cells were feeding on *Rhodomonas baltica* or contained food vacuoles. In our cell mixtures, allelopathic effects by *P. parvum* were strong and probably promoted feeding, which may have decreased the toxicity and therefore copepod mortality.

Results from other studies done with different organisms are controversial. Barreiro et al. (2005) found that the mortality of the rotifer *Brachionus plicatilis* was lower in the cell-free filtrates compared to the mixtures of *Prymnesium parvum* and *Rhodomonas salina* or sole *P. parvum*. They speculated that the mortality was connected to *P. parvum* ingestion rather than to dissolved compounds, because the rotifers did not select between toxic *P. parvum* and non-toxic *R. salina*. However, in our previous study, *Eurytemora affinis* did not ingest *P. parvum* and became inactive in sole *P. parvum* treatments (Sopanen et al. 2006). Thus, organisms that are able to avoid toxic cells may deal better in the mixtures of toxic and non-toxic food compared to situations where selection is not possible (in monocultures) or in situations where harmful substances are continuously released into the water.

Cell concentration of *Prymnesium parvum* in blooms usually varies between 50×10^3 and 100×10^3 cells ml^{-1}, but extreme concentrations, between 800×10^3 and 1600×10^3cells ml^{-1}, have been observed in Great Britain and Denmark (Edvardsen & Paasche 1998). Thus, our highest filtrate concentrations (Filt 3 and 4) as well as Mix 2 represent a normal bloom event of *P. parvum*. Adverse effects caused by dissolved compounds are most pronounced in sheltered areas where water turbulence is low. Since turbulence causes rapid dilution of dissolved toxins, as shown for nodularin (Henriksen 2005), it is not clear if the effects of dissolved toxins shown in laboratory studies (e.g. Fistarol et al. 2003, present study) apply in nature. Although field and laboratory studies have revealed that *Chrysochromulina polylepis* inhibited the activity of heterotrophic bacteria and heterotrophic protists, as well as copepods, and that exudates of this species also have allelopathic effects on other phytoplankton species (Nielsen et al. 1990, Schmidt & Hansen 2001), it remains to be investigated if similar effects of toxic exudates may be observed during a *P. parvum* bloom.

Effects on feeding and egestion

Both ingestion and faecal pellet production rates indicated that *Eurytemora affinis* was feeding actively on *Rhodomonas salina* both in the cell-free filtrates and in the mixtures of *Prymnesium parvum* and *R. salina*. The concentration of *P. parvum* filtrate, however, had a slight effect on feeding. As a result, the faecal pellet production rates in the high-filtrate concentrations were lower. Moreover, the ingestion rates in the filtrates, as well as the faecal pellet production rates in the filtrates, mixtures and in the *R. salina* control, were higher on day 3 compared to previous days. Since this was found irrespective of the manipulations, it is possible that this variation was due to acclimatization or variation in copepod metabolism during the experiments.

Faecal pellet production rate was significantly lower in higher total cell concentrations in Mix 2 (–P and +NP) compared to Mix 1. There are 2 possible reasons for this: firstly, exudates from *Prymnesium parvum* may have directly affected the overall activity of *Eurytemora affinis,* reflected in feeding rates, especially in the higher food concentrations. Secondly, the availability of intact *Rhodomonas salina* cells for copepods was probably lower than what was initially measured since ca. 30% of the cells disappeared during the 24 h incubations due to the allelopathic effects of *P. parvum*.

In the study of Uronen et al. (2005), ca. 30 to 50% of the *Rhodomonas salina* cells disappeared and ca. 25 to 45% of the *R. salina* cells were damaged after a 23 h incubation in mixtures of *Prymnesium parvum* and *R. salina* (5000 + 5000 cells ml^{-1}). In addition, when total *P. parvum* and *R. salina* cultures were mixed >90% of the *R. salina* cells disappeared after a 12 h incubation (Uronen et al. 2007). Based on these studies, it is possible to estimate that only a minor proportion (from 15 to 45%) of the *R. salina* cells were intact and remained available in our mixtures, and consequently *Eurytemora affinis* became food-limited. When the advantages of selective feeding are considered during *P. parvum* blooms, the allelopathic effects of *P. parvum* on alternative food sources should be taken account. Although monospecific phytoplankton blooms are rare, *P. parvum* blooms may in reality be close to monospecific if the allelopathic effects on other potential food items of copepods are strong.

Effects on reproduction

We did not find clear trends on egg production rates induced by *Prymnesium parvum* filtrates or mixtures. Although the lowest values were clearly lower compared to *Rhodomonas salina*, values above the control were also detected, with the highest values comparable to general egg production of sac-spawning copepods (Kiørboe & Sabatini 1995). However, in contrast to our results, Koski et al. (1999b) observed that *Eurytemora affinis* produced more eggs in mixtures of *Prymnesium patelliferum* and a control alga compared to the single-species control diet. They concluded that the copepods may have gained nutritional benefit from food mixtures, as shown by Schmidt & Jónasdóttir (1997). However, as no significant differences in egg production among treatments were observed in the present study, *E. affinis* presumably did not get any advantage by feeding on mixtures of *R. salina* and *P. parvum*. Hatching success was relatively high irrespective of the treatment. Turner et al. (2001) showed, when they studied the effects of diatom and dinoflagellate diets on copepods productivity, that long-term effects on hatching success could be observed in longer incubations (>1 wk). The hatching in our experiments only reflects immediate effects.

CONCLUSIONS

Our results revealed that filtrates clearly decrease the viability of copepods in all nutrient treatments, although HA was not detected. Therefore, high mortality might not be dependent on HA only, but on other non-measurable substances that *Prymnesium parvum* may excrete into the water. Hence HA alone may not be responsible for the total potential harmful effects of *P. parvum* on grazers and should not be used as an indicator for the harmfulness of this alga. Our results indicate that, besides monospecific *P. parvum* diets (Sopanen et al. 2006), filtrates and cell mixtures also have indirect negative effects on grazers. In addition, the higher *Rhodomonas salina* density in mixtures clearly buffered the harmful effects of *P. parvum* on copepods. The negative effects caused either by direct intoxication or by food limitation, following strong allelopathic effects of *P. parvum* on other components of nano- and microplankton, suggest that *P. parvum* blooms have a realistic potential to be deleterious for copepod secondary production, irrespective of the presence of alternative food sources. These harmful effects, together with heavy allelopathy towards competing phytoplankton species, may substantially accelerate the bloom formation of *P. parvum*.

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LITERATURE CITED

Andersen CM, Nielsen TG (1997) Hatching rate of the egg-carrying estuarine copepod *Eurytemora affinis.* Mar Ecol Prog Ser 160:283–289

Aure J, Rey F (1992) Oceanographic conditions in the Sandsfjord system, western Norway, after a bloom of the toxic Prymnesiophyte *Prymnesium parvum* Carter in August 1990. Sarsia 76:247–254

Barreiro A, Guisande C, Maneiro I, Lien TP and others (2005) Relative importance of the different negative effects of the toxic haptophyte *Prymnesium parvum* on *Rhodomonas salina* and *Brachionus plicatilis.* Aquat Microb Ecol 38: 259–267

Berggreen U, Hansen B, Kiørboe T (1988) Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. Mar Biol 99:341–352

Edvardsen B, Paasche E (1998) Bloom dynamics and physiology of *Prymnesium* and *Chrysochromulina*. In: Anderson DM, Cembella AD, Hallegraeff GM (eds) The physiology and ecology of harmful algal blooms. Springer-Verlag, Heidelberg, p 193–208

Finni T, Kononen K, Olsonen R, Wallström K (2001) The history of cyanobacterial blooms in the Baltic Sea. Ambio 30: 172–178

Fistarol GO, Legrand C, Granéli E (2003) Allelopathic effect of *Prymnesium parvum* on a natural plankton community. Mar Ecol Prog Ser 255:115–125

Frost BW (1972) Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod *Calanus pacificus.* Limnol Oceanogr 17:805–815

Granéli E, Johansson N (2003) Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N- or P-deficient conditions. Harmful Algae 2:135–145

Guillard RR, Ryther JH (1962) Studies of marine planktonic diatoms. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

HELCOM (Helsinki Commission) (2004) The fourth Baltic Sea pollution load compilation (PLC-4). Balt Sea Environ Proc No. 93

Helminen H, Juntura E, Koponen J, Laihonen P, Ylinen H (1998) Assessing of long-distance background nutrient loading to the Archipelago Sea, northern Baltic, with a hydrodynamic model. Environ Model Softw 13:511–518

Henriksen P (2005) Estimating nodularin content of cyanobacterial blooms from abundance of *Nodularia spumigena* and its charasteristic pigments — a case study from the Baltic entrance area. Harmful Algae 4:167–178

Igarashi T, Oshima Y, Murata M, Yasumoto T (1995) Chemical studies on prymnesins isolated from *Prymnesium parvum.* In: Lassus P, Arzul G, Erard Le Denn E, Gentien P, Marcaillou Le Baut C (eds) Harmful marine algal blooms. Lavoisier, Paris, p 303–308

Igarashi T, Aritake S, Yasumoto T (1998) Biological activities of prymnesin-2 isolated from a red tide alga *Prymnesium parvum.* Nat Toxins 6:35–41

Johansson N, Granéli E (1999) Influence of different nutrient conditions on cell density, chemical composition and toxicity of *Prymnesium parvum* (haptophyta) in semi-continuous cultures. J Exp Mar Biol Ecol 239:243–258

Kiørboe T, Sabatini M (1995) Scaling of fecundity, growth and development in marine planktonic copepods. Mar Ecol Prog Ser 120:285–298

Koski M (1999) Carbon:nitrogen ratios of Baltic Sea copepods — indication of mineral limitation? J Plankton Res 21:1565–1573

Koski M, Klein Breteler W, Schogt N (1998) Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). Mar Ecol Prog Ser 170:169–187

Koski M, Engström J, Viitasalo M (1999a) Reproduction and survival of the calanoid copepod *Eurytemora affinis* fed with toxic and non-toxic cyanobacteria. Mar Ecol Prog Ser 186:187–197

Koski M, Rosenberg M, Viitasalo M, Tanskanen S, Sjölund U (1999b) Is *Prymnesium patelliferum* toxic for copepods? Grazing, egg production and egestion of the calanoid copepod *Eurytemora affinis* in mixtures of 'good' and 'bad' food. ICES J Mar Sci 56:131–139

Legrand C, Johansson N, Johnsen G, Borsheim KY, Granéli E (2001) Phagotrophy and toxicity variation in the mixotrophic *Prymnesium patelliferum* (Haptophyceae). Limnol Oceanogr 46:1208–1214

Legrand C, Rengefors K, Fistarol GO, Granéli E (2003) Allelopathy in phytoplankton — biochemical, ecological and evolutionary aspects. Phycologia 42:406–419

Lindholm T, Virtanen T (1992) A bloom of *Prymnesium parvum* Carter in a small coastal inlet in Dragsfjärd, southwestern Finland. Environ Toxicol Water Qual 7:165–170

Lindholm T, Öhman P, Kurki-Helasmo K, Kincaid B, Meriluoto J (1999) Toxic algae and fish mortality in a brackish-water lake in Åland, SW Finland. Hydrobiologia 397: 109–120

Nejstgaard JC, Solberg PT (1996) Repression of copepod feeding and fecundity by the toxic haptophyte *Prymnesium patelliferum.* Sarsia 81:339–344

Nielsen TG, Kiørboe T, Bjørnsen PK (1990) Effects of a *Chrysocromulina polylepis* subsurface bloom on the planktonic community. Mar Ecol Prog Ser 62:21–35

Richardson K (1997) Harmful or exceptional phytoplankton blooms in the marine ecosystem. In: Blaxter JHS, Southward AL (eds) Advances in marine biology , Vol 31. Academic Press, San Diego, CA, p 302–385

Schmidt LE, Hansen PJ (2001) Allelopathy in the prymnesiophyte *Chryschromulina polylepis*: effect of cell concentration, growth phase and pH. Mar Ecol Prog Ser 216:67–81

Schmidt K, Jónasdóttir SH (1997) Nutritional quality of two cyanobacteria: How rich is 'poor' food? Mar Ecol Prog Ser 151:1–10

Shilo M, Rosenberger RF (1960) Studies on the toxic principles formed by the chrysomonad *Prymnesium parvum* Carter. Ann N Y Acad Sci 90:866–876

Skovgaard A, Hansen PJ (2003) Food uptake in the harmful alga *Prymnesium parvum* mediated by excreted toxins. Limnol Oceanogr 48:1161–1166

Skovgaard A, Legrand C, Hansen PJ, Granéli E (2003) Effects of nutrient limitation on food uptake in the toxic haptophyte *Prymnesium parvum.* Aquat Microb Ecol 31: 259–265

Sopanen S, Koski M, Kuuppo P, Uronen P, Legrand C, Tamminen T (2006) Toxic haptophyte *Prymnesium parvum* affects grazing, survival, egestion and egg production of the calanoid copepods *Eurytemora affinis* and *Acartia bifilosa.* Mar Ecol Prog Ser 327:223–232

Turner JT, Tester PA (1997) Toxic marine phytoplankton, zooplankton grazers and pelagic food webs. Limnol Oceanogr 42:1203–1214

Turner JT, Ianora A, Miralto A, Laabir M, Esposito F (2001) Decoupling of copepod grazing rates, fecundity and egg-hatching success on mixed and alternating diatom and dinoflagellate diets. Mar Ecol Prog Ser 220:187–199

Uronen P, Lehtinen S, Legrand C, Kuuppo P, Tamminen T (2005) Haemolytic activity and allelopathy of the haptophyte *Prymnesium parvum* in nutrient-limited and balanced growth conditions. Mar Ecol Prog Ser 299: 137–148

Uronen P, Kuuppo P, Legrand C, Tamminen T (2007) Allelopathic effects of toxic haptophyte *Prymnesium parvum* lead to release of dissolved organic carbon and increase in bacterial biomass Microb Ecol: 54:183–193

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