

Toxic haptophyte *Prymnesium parvum* affects grazing, survival, egestion and egg production of the calanoid copepods *Eurytemora affinis* and *Acartia bifilosa*

Sanna Sopanen^{1,*}, Marja Koski², Pirjo Kuuppo¹, Pauliina Uronen^{1,3},
Catherine Legrand⁴, Timo Tamminen¹

¹Finnish Environment Institute (SYKE), PO Box 140, 00251 Helsinki, Finland

²Danish Institute for Fisheries Research, Kavalergården 6, 2920 Charlottenlund, Denmark

³Tvärminne Zoological Station, 10900 Hanko, Finland

⁴Marine Sciences Department, University of Kalmar, 39182 Kalmar, Sweden

ABSTRACT: Nitrogen- and phosphorus-depleted or NP-balanced toxic haptophyte *Prymnesium parvum* was fed to 2 dominant copepod species of the northern Baltic Sea (*Eurytemora affinis* and *Acartia bifilosa*), and their ingestion, egg and faecal pellet production rates and mortality were measured. The copepods were incubated in 5 different cell concentrations of *P. parvum* for 3 consecutive days; the cryptophyte *Rhodomonas salina* was used as a control for non-toxic, nutritionally high-quality food. Toxicity (haemolytic activity) of *P. parvum* was measured before and after the incubations. The haemolytic activity of *P. parvum* was the highest in cultures grown under nutrient deficiency. The toxicity decreased after 1 d incubation in all treatments, in both the presence and absence of copepods. Neither of the copepod species ingested *P. parvum*, irrespective of the nutrient treatment (toxicity) or cell concentration, and the pellet and egg production rates were correspondingly low. Although there was no significant increase in mortality in *P. parvum* treatments, copepods that were exposed to *P. parvum* in any concentration or nutrient treatment soon became inactive. It was evident that the toxicity of even nutrient-replete *P. parvum* had an indirect and sublethal influence on copepods, although this could not be measured as short-term increased mortality. Our results suggest a strong reduction in secondary production of copepods in an event of a *P. parvum* bloom.

KEY WORDS: *Prymnesium parvum* · Feeding · Toxicity · Food quality · Nutrient limitation · *Acartia bifilosa* · *Eurytemora affinis* · Copepods

—Resale or republication not permitted without written consent of the publisher—

INTRODUCTION

Blooms of toxic haptophytes are well-known nuisances in several areas around the world (Richardson 1997). Harmful blooms of *Prymnesium parvum* and *Chrysochromulina polylepis* have been reported from coastal brackish water ecosystems, e.g. in the Kattegat–Skagerrak area (Nielsen et al. 1990), in Norwegian fjord systems (Kaartvedt et al. 1991, Aure & Rey 1992), in Australian estuaries (Hallegraeff 1992), and in fish ponds in China (Guo et al. 1996). Globally, these blooms are hazardous to coastal planktonic organisms

and cause great economic loss to commercial aquaculture. Prymnesiophytes are common members of the summer phytoplankton community in the Baltic Sea, but so far only a few toxic-bloom incidents have been reported in Finnish coastal waters (Lindholm & Virtanen 1992, Lindholm et al. 1999). *P. parvum* is a truly euryhaline species that usually blooms in fairly shallow eutrophic areas, where water exchange is limited (Edvardsen & Paasche 1998).

The toxicity of *Prymnesium parvum* blooms is caused by actively excreted compounds (Shilo 1971) that are haemolytic, ichthyotoxic and cytotoxic (Shilo

*Email: sanna.sopanen@environment.fi

1971, Igarashi et al. 1998). Furthermore, several studies have shown that both nitrogen and phosphorus limitation enhance the toxicity of *P. parvum* (Shilo 1971, Johansson & Granéli 1999, Uronen et al. 2005).

Interactions between toxic algae and zooplankton are complex. For example, some copepod species can ingest toxic phytoplankton cells without any immediate effect, while other species suffer high mortality after feeding on toxic algae (Turner & Tester 1997). Ingestion of toxic algae may repress copepod egg production or hatching success (Uye 1996, Schmidt & Jónasdóttir 1997, Dutz 1998, Koski et al. 1999b). Ingestion of toxic cells or exposure to toxic-cell exudates may also cause physiological stress with lethal or sublethal effects (Uye & Takamatsu 1990, Carlsson et al. 1995). Rejection of toxic algae can lead to starvation if alternative food is scarce (Teegarden 1999, Guisande et al. 2002), or the ingestion of nutritionally high-quality food can be perturbed in the presence of toxic algae, with the same result (Koski et al. 1999b). In addition, allelopathic effects of the toxins on other phytoplankton may reduce the availability of suitable food, which can affect resource competition within the grazer community (Fistarol et al. 2003, Granéli & Johansson 2003).

Toxic effects of prymnesiophytes on zooplankton have been sparsely studied. Nejstgaard & Solberg (1996) studied the effect of *Prymnesium patelliferum* (currently considered to be the same species as *Prymnesium parvum*; Larsen 1999) on the copepod *Acartia clausi*, and they found depressed feeding and egestion rates already at moderate algal concentrations, but no increase in mortality. In contrast, Koski et al. (1999b) showed that *Eurytemora affinis* was able to feed on *P. patelliferum* in single-species experiments and in algal mixtures. However, the positive feeding response resulted in increased mortality, abnormally low pellet production and suppressed fecundity of *E. affinis*. Furthermore, the presence of *P. patelliferum* in mixtures with nutritionally high-quality algal food decreased the copepod feeding rate on the high-quality alternative. Similar controversy exists in microzooplankton studies. For example, elevated mortality and low feeding of the ciliate *Euplotes affinis* on *P. parvum* has been demonstrated (Fistarol et al. 2003, Granéli & Johansson 2003), but Barreiro et al. (2005) observed similar ingestion rate of the rotifer *Brachionus plicatilis* on *P. parvum* as on *Rhodomonas salina*, even though the mortality of *B. plicatilis* increased in the presence of *P. parvum*.

Because previous studies on the relationships between copepods and prymnesiophytes (Nejstgaard & Solberg 1996, Koski et al. 1999b) are controversial, and because they have not taken into account the nutritional status of *Prymnesium parvum*, we present

responses of 2 dominant copepod species in the Gulf of Finland (Baltic Sea), *Eurytemora affinis* and *Acartia bifilosa*, to toxic prymnesiophyte *P. parvum* or non-toxic cryptomonad *Rhodomonas salina*. In our experiments, we also tested whether the nutritional status of *P. parvum* (N-depleted, P-depleted or NP-balanced) modifies its toxicity and effects on copepods. Our main questions were: (1) Does the presence of toxic *P. parvum* have an effect on the survival and activity of *E. affinis* or *Acartia bifilosa*? And, if so, does it depend on the concentration or nutritional status of *P. parvum*? (2) What are the feeding responses (grazing and pellet-production rate) of the 2 copepod species to the toxic *P. parvum*? (3) Does ingestion of *P. parvum* reduce the fecundity of the copepods?

MATERIALS AND METHODS

Cultures. The cryptomonad *Rhodomonas salina* (Rho; equivalent spherical diameter [ESD] ca. 7 µm) was used as a control of non-toxic food. It was obtained from the culture collection (TV 22/4) of the Tvärminne Zoological Station, University of Helsinki, Finland, and grown in batches in f/2 medium (Guillard & Ryther 1962) at 15°C under a 14:10 h light:dark cycle. The haptophyte *Prymnesium parvum* (Pry; ESD ca. 7 to 9 µm; strain KAC 39) was obtained from the Kalmar Algal Collection, University of Kalmar, Sweden. *P. parvum* was grown either under NP-balanced conditions (modified f/20 media, Guillard & Ryther 1962) or under nitrogen (-N) or phosphate (-P) deficiency in semi-continuous cultures, where 20% of the culture was removed daily and the same amount of new media was added (Johansson & Granéli 1999). Cultures were aerated gently, and the temperature was kept at ca. +16°C. Both species are oval/spherical flagellates commonly occurring in the Baltic Sea and of a suitable size range for the copepods used in the present study (e.g. Berggreen et al. 1988). Detailed description of culturing methods and *P. parvum* growth and toxicity are presented in Uronen et al. (2005).

Experiments. All experiments were conducted with adult females of *Eurytemora affinis* or *Acartia bifilosa*, which are the dominant copepods in the Gulf of Finland, Baltic Sea. The copepods were collected 1 d prior to the experiments using a 200 µm plankton net on the SW coast of Finland, where salinity was around 6 psu and temperature ca. 15°C (for a description of the study area, see Niemi 1975). To collect *E. affinis*, portions of the net haul were gently narcotized with a few drops of 5% MS-222 (tricaine methanesulfonate; Sigma-Aldrich) according to Dussart & Defaye (2001) and adult females were collected using a stereo microscope. The copepods recovered quickly after they

were transferred to 0.2 µm-filtered seawater. For *A. bifilosa* no narcotics were used. Before the experiments, the copepods were placed in 64 µm-filtered seawater for ca. 24 h to prevent starvation and to adapt to the experimental temperature (16°C). All experiments were conducted during a 2 wk period in July 2002.

Eurytemora affinis and *Acartia bifilosa* were incubated (1) with a cell suspension of *Rhodomonas salina* (as non-toxic control treatment); (2) with 0.2 µm-filtered seawater (as starvation treatment); and (3) with a cell suspension of *Prymnesium parvum* (as toxic food treatments). *P. parvum* was grown in 3 different growth media: (1) phosphorus-limited (Pry -P; 80 µM NO₃ and 1 µM PO₄); (2) nitrogen-limited (Pry -N; 16 µM NO₃ and 4 µM PO₄); and (3) NP-balanced medium (Pry +NP; 58 µM NO₃ and 3.6 µM PO₄) (see Uronen et al. 2005). In the experiments with *E. affinis*, both the control-food and toxic-food treatments had 5 target prey cell concentrations: 2000, 5000, 10 000, 50 000 and 100 000 cells ml⁻¹, whereas in the experiments with *A. bifilosa*, the median cell concentration (10 000 cells ml⁻¹) was used (Table 1). The cell suspensions were prepared prior to the incubations by diluting the stock cultures with fresh f/20 medium with the adjusted N:P ratios. Biomass ratios between the copepods and their food were adjusted by increasing the number of copepod individuals per bottle with increasing cell concentration of *P. parvum* and *R. salina*, in order to enable the measurement of grazing at high food concentrations (Table 1). Experiments were carried out with 10 experimental units per treatment (6 treatments with copepods + 4 control treatments). Each experiment lasted for 3 d, consisting of 3 subsequent 24 h incubations with the same copepods.

The experiments were carried out in 130 ml glass bottles, which were filled completely avoiding air bubbles and placed in a plankton wheel in a thermostated water bath (rotating at a speed of 1 rpm, temperature 16°C, dim light). After every 24 h incubation period, the contents of each bottle were poured into a large Petri dish, the condition of the copepods was checked, and actively swimming copepods were picked into a fresh food suspension. From the 'old' cell suspension, faecal pellets and the eggs of *Acartia bifilosa* were collected onto a 50 µm net, and a 100 ml subsample was fixed with 4% acidic Lugol's solution for phytoplankton counts. The rest of the suspension was used for the measurement of haemolytic activity, which was done from 3 replicate samples and 3 grazing controls after the first 24 h incubation period.

Cell concentrations of *Rhodomonas salina* or *Prymnesium parvum* in incubation bottles were analyzed in the beginning and at the end of each 24 h incubation period with an electronic particle counter (Elzone, Particle Data). Grazing (filtration and ingestion rates) was

calculated according to Frost (1972) for each 24 h period from the disappearance of the food cells from the experimental bottles with copepods, in comparison to the control bottles without copepods. In the *P. parvum* treatments, most of the grazing values were negative or not significantly different from zero. These negative findings most probably were due to higher algal growth in the presence of copepods, which enhanced nutrient regeneration, compared with the controls without copepods. The values from the highest cell concentration levels (100 000 cells ml⁻¹) were omitted, because the grazer:food carbon ratio turned out to be too low (<10%) to give a reliable grazing signal. Similarly, the replicates where >30% of the copepods died during the incubation were omitted from the analysis.

Faecal pellets were counted from 3 to 6 replicates using a stereo microscope (Zeiss Stemi SV 11). Faecal pellet volume was estimated from 3 replicates in each treatment by measuring the length and width of ca. 30 pellets. Their carbon content was calculated using the conversion factor of 0.057×10^{-9} mg C µm⁻³ (Gonzales & Smetacek 1994).

Table 1. Concentrations of target prey cells ($\times 10^3$ cells ml⁻¹), initial cells ($\times 10^3$ cells ml⁻¹) and carbon (µg C l⁻¹; mean \pm SE), as well as number of copepods (Zpl) and percentage of copepod carbon (%C) of phytoplankton carbon, in *Eurytemora affinis* and *Acartia bifilosa* experiments

| Treatment | Target density | Real density | Carbon content | Zpl | %C |
|----------------------------------|----------------|-----------------|----------------|-----|----|
| <i>Eurytemora affinis</i> | | | | | |
| Rho 1 | 2 | 3.5 \pm 0.4 | 150 \pm 17 | 2 | 40 |
| Rho 2 | 5 | 6.3 \pm 0.6 | 265 \pm 25 | 3 | 30 |
| Rho 3 | 10 | 11.4 \pm 0.7 | 480 \pm 30 | 6 | 35 |
| Rho 4 | 50 | 49.2 \pm 1.1 | 2070 \pm 45 | 10 | 10 |
| Rho 5 | 100 | 98.9 \pm 1.2 | 4150 \pm 490 | 10 | 7 |
| Pry -N1 | 2 | 2.5 \pm 0.2 | 80 \pm 4 | 2 | 70 |
| Pry -N2 | 5 | 5.1 \pm 0.9 | 160 \pm 30 | 3 | 50 |
| Pry -N3 | 10 | 11.0 \pm 1.4 | 350 \pm 45 | 6 | 45 |
| Pry -N4 | 50 | 45.5 \pm 0.9 | 1455 \pm 30 | 10 | 20 |
| Pry -N5 | 100 | 91.6 \pm 0.7 | 2930 \pm 85 | 10 | 9 |
| Pry -P1 | 2 | 2.6 \pm 0.4 | 85 \pm 10 | 2 | 65 |
| Pry -P2 | 5 | 5.1 \pm 0.4 | 160 \pm 10 | 3 | 50 |
| Pry -P3 | 10 | 10.8 \pm 0.8 | 350 \pm 25 | 6 | 50 |
| Pry -P4 | 50 | 51.4 \pm 1.6 | 1650 \pm 50 | 10 | 15 |
| Pry -P5 | 100 | 105.5 \pm 6.0 | 3370 \pm 190 | 10 | 8 |
| Pry +NP1 | 2 | 3.0 \pm 0.8 | 95 \pm 25 | 2 | 60 |
| Pry +NP2 | 5 | 5.2 \pm 0.4 | 165 \pm 10 | 3 | 50 |
| Pry +NP3 | 10 | 9.2 \pm 0.6 | 300 \pm 20 | 6 | 55 |
| Pry +NP4 | 50 | 50.4 \pm 0.8 | 1600 \pm 25 | 10 | 15 |
| Pry +NP5 | 100 | 104.2 \pm 0.9 | 3340 \pm 30 | 10 | 8 |
| <i>Acartia bifilosa</i> | | | | | |
| Rho 3 | 10 | 13.5 \pm 0.5 | 570 \pm 20 | 6 | 15 |
| Pry -N3 | 10 | 9.6 \pm 0.2 | 310 \pm 5 | 6 | 25 |
| Pry -P3 | 10 | 11.5 \pm 0.4 | 370 \pm 10 | 6 | 20 |
| Pry +NP3 | 10 | 10.1 \pm 0.3 | 3245 \pm 10 | 6 | 20 |

The eggs produced by the broadcast spawning *Acartia bifilosa* were counted from 3 to 6 replicate samples using the same stereo microscope after each 24 h incubation period. To measure the egg production of the egg-carrying *Eurytemora affinis*, females were incubated in individual vials (in 0.2 µm-filtered seawater) for 48 h after the initial 3 d experiments. The number of eggs and nauplii were then counted and egg production estimated using:

$$P = N_e / (N_f D)$$

where P = egg production (eggs ind.⁻¹ d⁻¹), N_e = number of eggs, N_f = number of females and D = temperature-dependent development time of eggs (estimated to be 2.06 d at 16°C; Andersen & Nielsen 1997).

Weight-specific egg production was calculated assuming carbon contents of 3.6 µg C ind.⁻¹ for *Eurytemora affinis* and 1.5 µg C ind.⁻¹ for *Acartia bifilosa* (Koski 1999). The carbon content of eggs (0.04 µg C egg⁻¹) was estimated from an average egg diameter of 82 µm (Katajisto et al. 1998) and a carbon content of 0.14×10^{-6} µg C µm⁻³ (Kjørboe & Sabatini 1995). 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.

Haemolytic activity. Haemolytic activity of the *Prymnesium parvum* cultures, as an estimate of their toxicity, was measured daily from the initial cultures (Uronen et al. 2005) and on the first experimental day from all experimental treatments. For the haemolytic activity assay, 10 ml of *P. parvum* suspension was filtered onto GF/C glass-fibre filters, and haemolytic activity of the samples was measured according to the method described in Igarashi et al. (1998), Johansson & Granéli (1999) and Uronen et al. (2005).

Statistical analyses. Differences in the grazing, survival (percentages arcsine transformed) and egestion of *Eurytemora affinis* were tested with a 2-way ANOVA, using cell concentration and algal treatment (*Rhodomonas salina*, *Prymnesium parvum* -N, -P, +NP) as independent variables. The differences in the egg production of *E. affinis* were tested using a 1-way ANOVA. Grazing, egestion and egg production of *Acartia bifilosa* were tested with a 2-way ANOVA, using day and algae treatment as independent variables. Survival on Day 3 was tested with a 1-way ANOVA. If conditions for the ANOVA (normality and equality of variances) were not met, a non-parametric Kruskal-Wallis test was used instead. The Holm-Sidak method (2-way ANOVA), Tukey test (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

Toxicity

At the beginning of the experiments, the cultures of *Prymnesium parvum* had reached a steady state, and they were toxic independent of the N:P ratio of the culture medium (Uronen et al. 2005). The highest toxicity (haemolytic activity per cell) was measured in the N- and P-depleted cultures, in which the particulate N:P ratios were 12 and 33 (mol:mol), respectively, whereas in the +NP culture, in which the particulate N:P ratio was close to the balanced Redfield ratio (Table 2; Redfield 1958), the toxicity was ca. 20 to 40% lower. The cell concentration of *P. parvum* varied between 1.5×10^3 (-N) and 3.8×10^3 (+NP) cells ml⁻¹ (Table 2).

During the grazing experiments, the toxicity of *Prymnesium parvum* decreased in all treatments, both with and without copepods (Table 3). In the *Eurytemora affinis* experiments, the decrease was ca. 51 and 44% with and without animals, respectively, while the corresponding decrease in the *Acartia bifilosa* experiments was ca. 70 and 58%. However, differences in the toxicity between the copepod units and the controls were not statistically significant.

Grazing

The functional response of *Eurytemora affinis* with *Rhodomonas salina* was typical: ingestion rate increased with increasing food concentration (2-way ANOVA: $df_{\text{conc.,total}} = 4, 53$; $F_{\text{conc.}} = 3.7$; $p = 0.01$), and filtration rate remained stable or decreased slightly (Fig. 1). In contrast to the treatments with *R. salina*, no statistical differences between the ingestion rates at different *Prymnesium parvum* (-N, -P, +NP) cell concentrations were found. *E. affinis* ingested *R. salina* significantly more (from 20 to 240% of the body carbon d⁻¹) compared with *P. parvum*, which was ingested at only $\leq 2.5\%$ of body carbon d⁻¹ (2-way ANOVA: $df_{\text{algae,total}} = 3, 42$; $F_{\text{algae}} = 4.6$; $p = 0.01$). At the lowest cell concentration (2000 cells ml⁻¹) in both -N and +NP treatments, the filtration rate on *P. parvum* was, how-

Table 2. Average (\pm SE) cell concentration ($\times 10^3$ cells ml⁻¹), cellular N:P ratio (PON:POP, mol:mol) and haemolytic activity (HA) (SnE pg cell⁻¹) in semi-continuous cultures of *Prymnesium parvum* in a steady state

| | Cell conc. | PON:POP | HA |
|-----|----------------|--------------|----------------|
| -N | 148 \pm 11.3 | 12 \pm 0.9 | 136 \pm 7.2 |
| -P | 308 \pm 5.8 | 33 \pm 4.4 | 200 \pm 10.4 |
| +NP | 378 \pm 7 | 16 \pm 1.2 | 105 \pm 5.4 |

Table 3. Haemolytic activity (\pm SE) (SnE pg cell⁻¹) in the *Eurytemora affinis* and *Acartia bifilosa* grazing experiments with different nutrient treatments (-N, -P, +NP). Start: initial values; End_{control}: values after 24 h incubation without copepods; End_{copepods}: with copepods; -: data missing

| | Start | End _{control} | End _{copepods} |
|---------------------------|-------|------------------------|-------------------------|
| <i>E. affinis</i> | | | |
| -N | 164 | 126 \pm 19 | 117 \pm 6.7 |
| -P | 158 | 48 \pm 5.6 | 77 \pm 7.8 |
| +NP | 109 | 43 \pm 2 | 52 \pm 6.7 |
| <i>A. bifilosa</i> | | | |
| -N | 157 | - | - |
| -P | 179 | 67 \pm 3.5 | 72 \pm 7.2 |
| +NP | 73 | 16 \pm 0 | 32 \pm 2.3 |

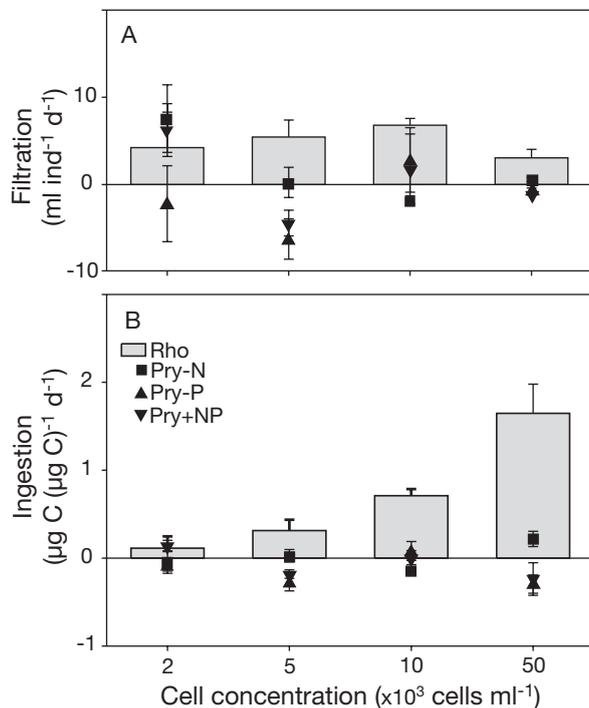


Fig. 1. Average (\pm SE) (A) filtration and (B) weight-specific ingestion of *Eurytemora affinis* feeding on *Rhodomonas salina* (Rho) or *Prymnesium parvum* (Pry -N, -P, +NP)

ever, slightly higher (-N, 7.5 \pm 3.8 ml ind.⁻¹ d⁻¹; +NP, 6.2 \pm 3 ml ind.⁻¹ d⁻¹) compared to *R. salina* (4.2 \pm 2 ml ind.⁻¹ d⁻¹; Fig. 1A), indicating that *E. affinis* ingested *P. parvum* cells in small amounts in these treatments (Fig. 1B).

Acartia bifilosa had lower filtration rates on *Rhodomonas salina* compared with *Eurytemora affinis* at a cell concentration of 10 \times 10³ cells ml⁻¹ (Fig. 2A). However, because *A. bifilosa* is smaller than *E. affinis*, the ingestion rates (Fig. 2B) were comparable, correspond-

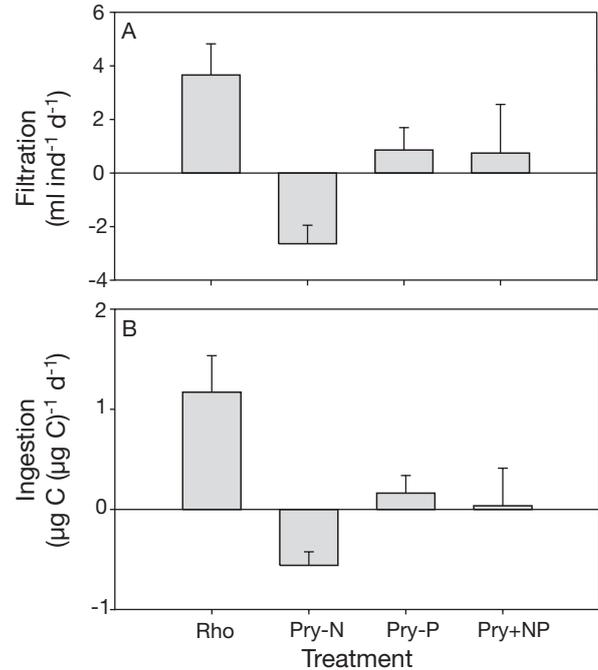


Fig. 2. Average (\pm SE) (A) filtration and (B) weight-specific ingestion of *Acartia bifilosa* feeding on *Rhodomonas salina* (Rho) or *Prymnesium parvum* (Pry -N, -P, +NP)

ing to ca. 120% of body carbon d⁻¹. The filtration rates on *Prymnesium parvum* cells were clearly lower compared with those on *R. salina*, being always less than 20% of body carbon d⁻¹ (Fig. 2A). Consequently, *P. parvum* treatments differed significantly from *R. salina* treatment during the experiments (2-way ANOVA: df_{day,total} = 2, 54; $F = 38$; $p \leq 0.001$).

Egestion

The production of faecal pellets by *Eurytemora affinis* reflected its feeding during the experiment. Weight-specific pellet production (μ g C (μ g C)⁻¹ d⁻¹) was extremely low both in the filtered seawater treatment and when *E. affinis* was feeding on *Prymnesium parvum* (corresponding to ca. 0.3 to 0.8 pellets ind.⁻¹ d⁻¹), irrespective of the cell concentration or the nutrient treatment (Fig. 3). In contrast, the average weight-specific pellet production with the *Rhodomonas salina* diet was 50- to 200-fold higher, corresponding to 29 pellets ind.⁻¹ d⁻¹ (2-way ANOVA: df_{conc.,algae,interact.,total} = 4, 3, 12, 59; $F_{conc.} = 0.9$, $F_{algae} = 117.8$, $F_{interact.} = 0.1$; $p_{algae} \leq 0.001$; Fig. 3).

The average weight-specific pellet production of *Acartia bifilosa* was similarly higher with the *Rhodomonas salina* diet (corresponding to ca. 15 pellets ind.⁻¹ d⁻¹), compared with the *Prymnesium parvum* or fil-

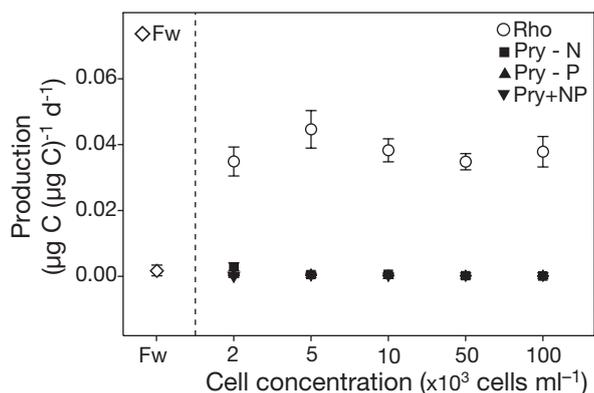


Fig. 3. Average (\pm SE) weight-specific pellet production of *Eurytemora affinis* in 0.2 μ m-filtered seawater (Fw) and at different concentrations of *Rhodomonas salina* (Rho) and *Prymnesium parvum* (Pry -N, -P, +NP)

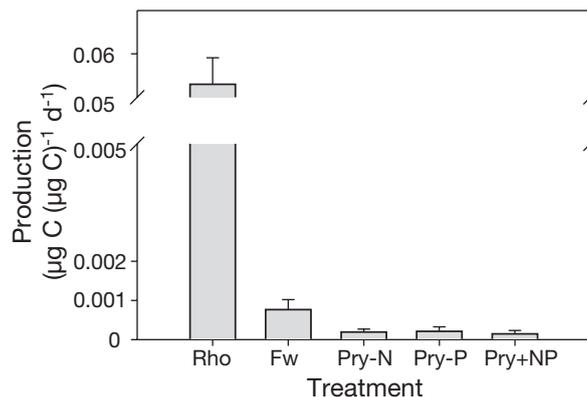


Fig. 4. Average (\pm SE) weight-specific pellet production of *Acartia bifilosa* in 0.2 μ m-filtered seawater (Fw) or with *Rhodomonas salina* (Rho) and *Prymnesium parvum* (Pry -N, -P, +NP) diets

tered seawater treatments (<0.5 pellets ind. $^{-1}$ d $^{-1}$; Fig. 4). The pellet production rates were significantly different during all incubation periods. Weight-specific pellet production varied between days with the *R. salina* diet, but it was always ca. 200-fold compared with the *P. parvum* treatments, in which pellet production was low (2-way ANOVA: $df_{\text{day,algae,interact.,total}} = 2, 4, 8, 88$; $F_{\text{day}} = 76$, $F_{\text{algae}} = 744$, $F_{\text{interact.}} = 78$; $p \leq 0.001$ for all).

Activity and survival

The individuals of *Eurytemora affinis* moved actively in the *Rhodomonas salina* suspension and in the 0.2 μ m-filtered seawater, whereas in the *Prymnesium parvum* treatments, the copepods were inactive and displayed reduced pipette avoidance. Although average survival percentage of *E. affinis* in the *P. parvum* treatments on Day 3 was slightly lower (26 to 58% for -N, 46 to 63% for -P, 31 to 66% for +NP) compared with that in the *R. salina* treatments (43 to 86%) and in the 0.2 μ m-filtered seawater (60%; Fig. 5), the differences were not statistically significant (2-way ANOVA: $df_{\text{conc.,algae,interact.,total}} = 4, 4, 16, 143$; $F_{\text{conc.}} = 0.5$, $F_{\text{algae}} = 1.0$, $F_{\text{interact.}} = 0.7$). Fairly high copepod mortality was found once in the first incubation period in the *R. salina* treatment, but due to an unknown cause. The visually observed end condition of copepods incubated with *P. parvum* was, however, noticeably worse than the actual survival rates indicated (Fig. 5).

Accordingly, the individuals of *Acartia bifilosa* moved more actively in the *Rhodomonas salina* suspension and in the 0.2 μ m-filtered seawater compared with individuals in the *Prymnesium parvum* treatments. In spite of that, only minor differences in sur-

vival between the treatments could be found. While 100 ± 0 SE % ($n = 6$) of the individuals in the *R. salina* treatments were alive at the end of the experiment, the corresponding percentages (\pm SE) in the 0.2 μ m-filtered seawater and in the *P. parvum* (-N, -P, +NP) treatments were 89 ± 3.9 ($n = 6$), 84 ± 5.5 ($n = 6$), 72 ± 15 ($n = 6$) and 71 ± 15 % ($n = 6$), respectively (Fig. 5). These differences were not statistically significant (ANOVA on ranks, $p = 0.1$).

Reproduction

The average egg production rate of *Eurytemora affinis* was highest in the *Rhodomonas salina* treatments (3.2 ± 1.2 eggs ind. $^{-1}$ d $^{-1}$, $n = 17$); in the *Prymnesium parvum* -P (0.6 ± 0.45 ; $n = 14$) and +NP (1.9 ± 0.9 ; $n = 16$) treatments and the 0.2 μ m-filtered seawater treatment (0 ± 0 ; $n = 2$), the egg production was significantly

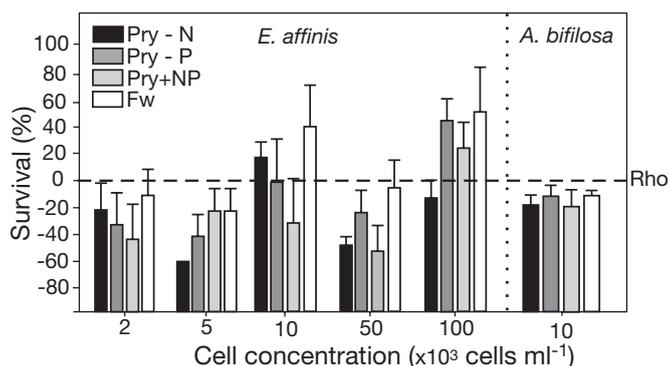


Fig. 5. Survival (\pm SE) of *Eurytemora affinis* and *Acartia bifilosa* on Day 3 (% of survival with the *Rhodomonas salina* (Rho) diet [zero reference line]) in the *Prymnesium parvum* (Pry -N, -P, +NP) and 0.2 μ m-filtered seawater (Fw) treatments

lower (ANOVA on ranks, $p < 0.001$, performed with pooled cell concentrations). The copepods in the $-N$ treatments were severely impaired, and thus egg production could not be measured. Correspondingly, the GGEs were higher with the *R. salina* diet ($21.5\% \text{ d}^{-1}$) compared with the different *P. parvum* diets (1.3 to $19\% \text{ d}^{-1}$).

Likewise, *Acartia bifilosa* produced higher amounts of eggs with the *Rhodomonas salina* diet compared with the *Prymnesium parvum* ($-N$, $-P$, $+NP$) and $0.2 \mu\text{m}$ -filtered seawater treatments (2-way ANOVA: $\text{df}_{\text{algae, total}} = 4, 89$; $F_{\text{algae}} = 39$; $p \leq 0.001$). Average egg production rate was 2.9 ± 0.6 with the *R. salina* diet, 0.5 ± 0.1 with $0.2 \mu\text{m}$ -filtered seawater, and 0.9 ± 0.2 , 0.6 ± 0.2 and 0.6 ± 0.2 with *P. parvum* $-N$, $-P$, and $+NP$ diets, respectively. GGEs of *A. bifilosa* were higher with the *R. salina* diet ($15.3\% \text{ d}^{-1}$) compared to the *P. parvum* treatments ($3.3\% \text{ d}^{-1}$ in the $-P$, and $1.5\% \text{ d}^{-1}$ in the $+NP$). The highest egg production rates (3.8 to 8.4 eggs $\text{ind.}^{-1} \text{ d}^{-1}$) were detected on the last incubation day with the *R. salina* diet (2-way ANOVA: $\text{df}_{\text{day, total}} = 2, 89$; $F_{\text{day}} = 19.2$; $p \leq 0.001$). In contrast, the egg production rate remained low or even decreased in the *P. parvum* and $0.2 \mu\text{m}$ -filtered seawater treatments.

DISCUSSION

Activity and survival of copepods

Eurytemora affinis and *Acartia bifilosa* were active in the *Rhodomonas salina* treatments and in the $0.2 \mu\text{m}$ -filtered seawater. In addition, *E. affinis* showed obvious functional response in weight-specific ingestion to the concentration of non-toxic food (Fig. 1B). In contrast, in the cell suspensions of *Prymnesium parvum* ingestion was low or undetectable at all cell concentrations. Copepods were also severely impaired and reacted slowly to mechanical disturbance (some of the individuals were only vibrating slightly). This finding is consistent with video observations of *E. affinis* by Koski et al. (1999b).

Haemolytic activity assays indicated that all our *Prymnesium parvum* cultures used in the experiments were toxic. In the balanced nutrient cultures ($+NP$) the toxicity was, however, ca. 45% lower compared with the N - and P -limited ones (Uronen et al. 2005). The observed degrees of *P. parvum* toxicity were manifested systematically in the survival, activity and feeding of the copepods, suggesting that even the least toxic cultures were potent enough to deter the grazers.

Because the copepods were active in the $0.2 \mu\text{m}$ -filtered seawater during the experiments, their inactivity was most likely caused by the toxin or other deleterious effects by *Prymnesium parvum*, rather than due

to starvation. For example, Meldahl & Fonnum (1995) studied how purified toxin extracted from *P. patelliferum* affects rat brain synaptosomes. They found that the algal extract increased the permeability to Na^+ , Ca^+ and K^+ in the synaptosomal membrane and that membrane depolarization and inhibition of the net uptake of L -glutamate also occurred. This kind of general effect on the cell membranes can possibly be seen as paralyzing symptoms in the copepods. Despite the obvious influence on the condition and performance of the copepods, the toxicity was not fully reflected in their mortality, probably partly due to the reasonably short duration of the experiments.

There are only a few studies on acute toxicity of *Prymnesium parvum* on copepods. In one of the earliest investigations, Valkanov (1964) demonstrated that *P. parvum* has toxic effects on a large number of organisms. However, the copepods (*Cyclops* sp. and *Calanipeda* sp.) did not show any signs of acute mortality or inactivity, even after 3 d exposure to *P. parvum* (1.1×10^8 cells l^{-1}). In accordance with this study, Nejstgaard et al. (1995) found no mortality or visible effect on the swimming activity of *Calanus finmarchicus* and *Acartia clausi* after 2 d exposure to *P. patelliferum* in concentrations of 10^9 cells l^{-1} . In contrast, Koski et al. (1999b) found elevated mortality when copepods were fed with *P. patelliferum* (cell concentration range 13 to 29×10^6 cells l^{-1}).

These controversial results of mortality and incapacitation of copepods suggest that responses to *Prymnesium parvum* toxins can be species specific. Copepod species that are intolerant to the toxins of *P. parvum* may thus become easily inactive in a bloom situation, which further enhances the bloom development. It has been shown, however, that the toxicity of *P. parvum* varies significantly due to culturing conditions (Johansson & Granéli 1999, Uronen et al. 2005). If one strain can modify its toxicity, it is far from certain that the results obtained with different strains are directly comparable. Therefore, it is obvious that verification and quantification of the prey culture toxicity at the time of the experiments is necessary in order to make a comparative evaluation of the tolerance of different grazers.

Grazing and egestion

In our experiments both copepod species fed effectively on *Rhodomonas salina* and mostly avoided *Prymnesium parvum*. Even though the average ingestion rate on *P. parvum* usually did not differ significantly from zero, the filtration rates indicated that *Eurytemora affinis* occasionally ingested small amounts of *P. parvum*. This was most pronounced at

the lowest *P. parvum* concentration (2000 cells ml⁻¹). However, the feeding of *E. affinis* on *P. parvum* was too low to allow observation of any functional responses to increasing cell concentration. *Acartia bifilosa* responded to *P. parvum* with low or negative ingestion rates, and no relationships between the nutrient treatment and the ingestion rates were found.

The results of this study are in accordance with Nejstgaard et al. (1995), who found that *Calanus finmarchicus* had generally very low feeding rates on *P. patelliferum* (daily food to body carbon ratio always less than 1%). However, Koski et al. (1999b) found that ingestion rates of *Eurytemora affinis* on *P. patelliferum* (0.3 to 0.6 µg C (µg C)⁻¹ d⁻¹) were higher than those in our results. In their study, the copepods were acclimated to the experimental food for 5 d, and *P. patelliferum* cultures were not limited by essential nutrients, which may have affected their toxicity. The copepods in our study were inactive and clearly incapacitated in the presence of *Prymnesium parvum* during the 3 d experimental period, which makes it very improbable that they would have started to feed on *P. parvum* after a longer exposure.

Even if it has been suggested that top-down control by zooplankton grazers may prevent bloom formation or shorten the duration of blooms (Buskey et al. 1997, Turner & Tester 1997, and references therein), our results, as well as previous studies with the common copepods of the North Sea and the Baltic Sea, show that copepods avoid feeding on *Prymnesium parvum* or ingest it only in small amounts (Nejstgaard et al. 1995, Nejstgaard & Solberg 1996, this study). The low faecal pellet production of *Eurytemora affinis* and *Acartia bifilosa* supports this view.

Reproduction

Eurytemora affinis and *Acartia bifilosa* showed reduced fecundity on a diet of *Prymnesium parvum* compared with *Rhodomonas salina*. In the non-toxic food treatments, the egg production of *E. affinis* and *A. bifilosa* was well within the range (3 to 7 eggs ind.⁻¹ d⁻¹, maximum 8 eggs ind.⁻¹ d⁻¹) reported by Koski et al. (1999a, 2002), who used another good-quality algae, *Brachiomonas submarina*.

The difference between non-toxic and toxic food was clearly seen in our experiments with *Acartia bifilosa*: egg production increased constantly during the incubations with *Rhodomonas salina*, whereas the *Prymnesium parvum* diet caused constantly low egg production rate. As egg production of *Eurytemora affinis* was calculated on the basis of 48 h incubation after the 3 d experiment, no corresponding trend could be found.

The reduced fecundity was probably caused by low feeding rates on *Prymnesium parvum* together with the inactivation of copepods. This is consistent with earlier studies that showed decreased egg production because copepods avoided feeding on prymnesiophytes, e.g. *P. patelliferum* (Nejstgaard & Solberg 1996) or *Chrysochromulina polylepis* (Nielsen et al. 1990). In situations where copepods avoid feeding on algae because of taste, toxicity (Nielsen et al. 1990, Nejstgaard & Solberg 1996, Dutz 1998), shape/size (Infante & Abella 1985, Berggreen et al. 1988) or low nutritional value (Jónasdóttir 1994, Schmidt & Jónasdóttir 1997, and references therein), it is beneficial to allocate energy reserves to survival rather than reproduction. In our experiments, the lower GGEs found with *P. parvum* diet support this hypothesis.

CONCLUSIONS

In our experiments, *Prymnesium parvum* had immediate negative effects on copepods. In all nutrient treatments (-P, -N, +NP) and at all cell concentrations, both *Eurytemora affinis* and *Acartia bifilosa* became inactive and mostly avoided feeding on the algae. Due to low feeding rates, exceptionally low pellet production and egg production rates were observed. Despite the obvious influence on the condition and performance of the copepods, the toxicity of *P. parvum* was not fully reflected in their mortality, probably due to the reasonably short duration of the experiments. Copepod grazing could not control the growth of *P. parvum*. Inactivation of copepods, followed by low feeding rates, can instead promote development of *P. parvum* blooms.

Acknowledgements. We thank the Tvärminne Zoological Station (University of Helsinki) for providing the laboratory facilities and the FATE team for the congenial atmosphere during the experiments. We especially thank S. Lehtinen for helping with algae dilutions and J. Hagström and L. T. Phuong for toxin analyses. We also thank 4 anonymous reviewers for their helpful comments, which significantly improved the manuscript. This work was funded by the European commission through the FATE project 'Transfer and Fate of Harmful Algal Bloom (HAB) Toxins in European Marine Waters' (EVK3-CT2001-00055) as part of the EC-EUROHAB cluster. M.K. and S.S. were funded by the Academy of Finland. Culturing facilities at Tvärminne Zoological Station were partly funded by the Academy of Finland.

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 Sands-

- fjord 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 5 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
- Buskey EJ, Montagna PA, Amos AF, Whitedge TE (1997) Disruption of grazer populations as a contributing factor to the initiation of the Texas brown tide algal bloom. *Limnol Oceanogr* 42:1215–1222
- Carlsson P, Granéli E, Finenko G, Maestrini SY (1995) Copepod grazing on a phytoplankton community containing the toxic dinoflagellate *Dinophysis acuminata*. *J Plankton Res* 17:1925–1938
- Dussart BH, Defaye D (2001) Introduction to the copepoda. In: Dumont HJF (ed) *Guides to the identification of the microinvertebrates of the continental waters of the world*, Vol 16. Backhuys Publishers, Leiden, p 4–11
- Dutz J (1998) Repression of fecundity in the neritic copepod *Acartia clausi* exposed to the toxic dinoflagellate *Alexandrium lusitanicum*: relationship between feeding and egg production. *Mar Ecol Prog Ser* 175:97–107
- Edvardsen B, Paasche E (1998) Bloom dynamics and physiology of *Prymnesium* and *Chrysochromulina*. In: Anderson DM, Cembella AD, Hallegraeff GM (eds) *Physiological ecology of harmful algal blooms*. NATO ASI Series, Vol G 41. Springer-Verlag, Berlin, p 193–208
- Fistarol GO, Legrand C, Granéli E (2003) Allelopathic effect of *Prymnesium parvum* on a natural plankton community. *Mar Ecol Prog Ser* 225:115–125
- 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
- Gonzales HE, Smetacek V (1994) The possible role of the cyclopoid copepod *Oithona* in retarding vertical flux of zooplankton faecal material. *Mar Ecol Prog Ser* 113:233–246
- Granéli E, Johansson N (2003) Effects of the toxic haptophyte *Prymnesium parvum* on the survival and feeding of a ciliate: the influence of different nutrient conditions. *Mar Ecol Prog Ser* 254:49–56
- Guillard RR, Ryther JH (1962) Studies of marine planktonic diatoms. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Can J Microbiol* 8:229–239
- Guisande C, Frangópulos M, Carotenuto Y, Maneiro I, Riveiro I, Vergara AR (2002) Fate of paralytic shellfish poisoning toxins ingested by the copepod *Acartia clausi*. *Mar Ecol Prog Ser* 240:105–115
- Guo M, Harrison PJ, Taylor FJR (1996) Fish kills related to *Prymnesium parvum* N. Carter (Haptophyta) in the People's Republic of China. *J Appl Phycol* 8:111–117
- Hallegraeff GM (1992) Harmful algal blooms in the Australian region. *Mar Pollut Bull* 25:186–190
- 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
- Infante A, Abella SEB (1985) Inhibition of *Daphnia* by *Oscillatoria* in Lake Washington. *Limnol Oceanogr* 30:1046–1052
- 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
- Jónasdóttir SH (1994) Effects of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations. *Mar Biol* 121:67–81
- Kaartvedt S, Johnsen TM, Aksnes DL, Lie U (1991) Occurrence of the toxic phytoflagellate *Prymnesium parvum* and associated fish mortality in Norwegian fjord system. *Can J Fish Aquat Sci* 48:2316–2323
- Katajisto T, Viitasalo M, Koski M (1998) Seasonal occurrence and hatching of calanoid eggs in sediments of the northern Baltic Sea. *Mar Ecol Prog Ser* 163:133–143
- 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, 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
- Koski M, Schmidt K, Engström-Öst J, Viitasalo M, Jónasdóttir SH, Repka S, Sivonen K (2002) Calanoid copepods feed and produce eggs in the presence of toxic cyanobacteria *Nodularia spumigena*. *Limnol Oceanogr* 47:878–885
- Larsen A (1999) *Prymnesium parvum* and *Prymnesium patelliferum* (haptophyta)—one species. *Phycologia* 38:541–543
- 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-Helasma K, Kincaid B, Merilüoto J (1999) Toxic algae and fish mortality in a brackish-water lake in Åland, SW Finland. *Hydrobiologia* 397:109–120
- Meldahl AS, Fonnum F (1995) The effects of purified toxic extract of *Prymnesium patelliferum* on transport of ions through the plasma membrane of synaptosomes. *Toxicol* 33:1071–1086
- Nejstgaard JC, Solberg PT (1996) Repression of copepod feeding and fecundity by the toxic haptophyte *Prymnesium patelliferum*. *Sarsia* 81:339–344
- Nejstgaard JC, Båmstedt U, Bagøien E, Solberg PT (1995) Algal constraints on copepod grazing. Growth state, toxicity, cell size, and season as regulating factors. *ICES J Mar Sci* 52:347–357
- Nielsen GT, Kiørboe T, Bjørnsen PK (1990) Effect of *Chrysochromulina polylepsis* subsurface bloom on the planktonic community. *Mar Ecol Prog Ser* 62:21–35
- Niemi Å (1975) Ecology of phytoplankton in the Tvärminne area, SW coast of Finland II. Primary production and environmental conditions in the archipelago and the sea zone. *Acta Bot Fenn* 105:1–73
- Redfield AC (1958) The biological control of chemical factors in the environment. *Am Sci* 46:205–221
- Richardson K (1997) Harmful or exceptional phytoplankton blooms in the marine ecosystem. In: Blaxter J, Southward A (eds) *Advances in marine biology*, Vol 31. Academic Press, New York, p 302–385
- Schmidt K, Jónasdóttir SH (1997) Nutritional quality of two cyanobacteria: how rich is 'poor' food? *Mar Ecol Prog Ser* 151:1–10

- Shilo M (1971) Toxins of chrysophyceae. In: Kadis S, Ciegler A, Ajl SJ (eds) Microbial toxins: a comprehensive treatise, Vol VII. Algal and fungal toxins. Academic Press, New York, p 67–103
- Teegarden GJ (1999) Copepod grazing selection and particle discrimination on the basis of PSP toxic content. Mar Ecol Prog Ser 181:163–176
- Turner JT, Tester PA (1997) Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs. Limnol Oceanogr 42:1203–1214
- Uronen P, Lehtinen S, Legrand C, Kuuppo P, Tamminen T (2005) Haemolytic activity and allelopathy of the hapto-phyte *Prymnesium parvum* in nutrient-limited and balanced growth conditions. Mar Ecol Prog Ser 299: 137–148
- Uye S (1996) Introduction of reproductive failure in the planktonic copepod *Calanus pacificus* by diatoms. Mar Ecol Prog Ser 133:89–97
- Uye S, Takamatsu K (1990) Feeding interactions between planktonic copepods and dinoflagellates from Japanese coastal waters. Mar Ecol Prog Ser 59:97–107
- Valkanov A (1964) Untersuchungen über *Prymnesium parvum* Carter und seine toxische Einwirkung auf die Wasserorganismen. Kiel Meeresforsch 20:65–81

Editorial responsibility: Howard Browman (Associate Editor-in-Chief), Storebø, Norway

*Submitted: November 15, 2005; Accepted: May 11, 2006
Proofs received from author(s): November 17, 2006*