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Relative importance of the different negative effects of the toxic haptophyte *Prymnesium parvum* on *Rhodomonas salina* and *Brachionus plicatilis*

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ABSTRACT: The aim of this study was to determine the relative importance of the different processes/mechanisms by which the toxic haptophyte *Prymnesium parvum*, cultured under different nutrient conditions, affects non-toxic phytoplankton competitors and microzooplankton grazers. *P. parvum* was cultured under steady-state growth in different nutrient conditions: nitrogen depleted (–N), phosphorus depleted (–P) and balanced nitrogen and phosphorus (+NP). Cells from each nutrient condition and culture cell-free filtrates, alone and combined with non-toxic prey (*Rhodomonas salina*), were used as food for the rotifer *Brachionus plicatilis*. An additional experiment was carried out to test the effect of *P. parvum* cells and culture cell-free filtrate on *R. salina*. The highest haemolytic activity values were achieved by –P *P. parvum* cultures, followed by –N. However, the negative effect of *P. parvum* on *R. salina* and rotifers did not correlate with haemolytic activity but with the number of *P. parvum* cells. –N-cultured *P. parvum* were the most toxic for both *R. salina* and rotifers, followed by +NP. Therefore, haemolytic activity is not a good indicator of the total potential toxicity of *P. parvum*. The growth rate of *R. salina* was negatively affected by cell-free filtrates but the effect of *P. parvum* predation was greater. Rotifers fed on both toxic and non-toxic algae, indicating that they did not select against the toxic alga. The *P. parvum* cell-free filtrate had an effect on *B. plicatilis*, although this was weak. *B. plicatilis* was also indirectly affected by *P. parvum* due to the negative effects of the toxic alga on their prey (*R. salina*). However, the greatest negative effect of *P. parvum* on the rotifers was due to ingestion of the toxic cells. Therefore, the phytoplankton competitor *R. salina* is more affected by *P. parvum* predation and the grazer *B. plicatilis* is more affected by ingestion of the toxic cells, the effects of excreted compounds being secondary.

KEY WORDS: Haptophytes · *Prymnesium parvum* · Nutrient limitation · Toxicity · Haemolytic activity · Allelopathy · Rotifers · Zooplankton · Phytoplankton

INTRODUCTION

Blooms of toxic haptophytes are common phenomena in some areas (Scandinavia, Israel, Australia, North America) where their toxic effects are well documented (Shilo 1967, Edvarsen & Paasche 1998). *Prymnesium parvum* belongs to this group and produces exotoxins (prymnesins) with cytotoxic, neurotoxic and ichthyotoxic activity (Shilo 1967, Igarashi et al. 1998).

Most of the studies carried out with *Prymnesium parvum* have been concerned with the effects of abiotic factors on its toxicity. Light, salinity and tempera-

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ture were not found to affect toxicity (Larsen & Bryant 1998), but toxin production increases when *P. parvum* is grown under both nitrogen and phosphorus limitation (Johansson & Granéli 1999a,b). In the field, recurrent blooms of *P. parvum* with high toxicity associated with high N:P ratios have also been observed (Aure & Rey 1992, Lindholm 1998).

Toxicity is significantly reduced when bacteria are supplied to *Prymnesium parvum* cultured under nutrient limitation (Nygaard & Tobiesen 1993, Legrand et al. 2001). However, Skovgaard et al. (2003) found no difference in the feeding rate of *P. parvum* on *Rhodomonas baltica* when *P. parvum* was grown under nutrient deficient conditions (N and P). But despite the different culture conditions used in these experiments, all treatments had some degree of nutrient limitation, as shown by intracellular ratios.

Some studies have hypothesised that toxin production in haptophytes like *Prymnesium parvum* and *Chrysochromulina polylepis* is not directly coupled to nutrient limitation, but is a function of the physiological status of the cell (Johansson & Granéli 1999a,b, Legrand et al. 2001). However, Stolte et al. (2002) showed that toxins were produced in both *Alexandrium tamarense* and *P. parvum* in proportion to chlorophyll when the algae were grown in semi-continuous cultures, and they did not accumulate, as is seen in the stationary phase of batch cultures.

Studies of the allelopathic effect of *Prymnesium parvum* on non-toxic phytoplankton species have shown that *P. parvum* can inhibit the growth of these potential competitors by the excretion of allelochemicals into the water (Fistarol et al. 2003, Granéli & Johansson 2003a,b, Skovgaard & Hansen 2003). Other studies have shown that *P. parvum* can also ingest phytoplankton species (Martín-Cereceda et al. 2003, Skovgaard & Hansen 2003). Tillmann (2003) found that *P. parvum* can kill potential predators, such as the dinoflagellate *Oxyrrhis marina*, with allelochemicals and thereafter eat them.

Some micro- and mesozooplankton are negatively affected in the presence of *Prymnesium parvum*. Nejstgaard & Solberg (1996) demonstrated sublethal effects on the copepod *Acartia clausi* without ingestion of toxic cells. Koski et al. (1999) reported negative effects on reproduction and survival of the copepod *Eurytemora affinis* due to the ingestion of toxic *Prymnesium patelliferum* cells; however, mortality of the copepods was more closely related to *P. patelliferum* concentration in the medium rather than cell ingestion, which suggests a role for excreted toxins. Rosetta & McManus (2003) detected high mortality in ciliates fed with *P. parvum*, and sublethal effects have been observed for the ciliate *Euplotes affinis* in the presence of *P. parvum* (Granéli & Johansson 2003b).

Therefore, it is clear that *Prymnesium parvum* can produce toxic effects on plankton species via different processes/mechanisms, which may play an important role in structuring plankton communities: cell lysis by toxins and phagocytic activity towards bacteria and phytoplankton; and negative effects on zooplankton due to excreted toxins, ingestion of toxic cells and competition with zooplankton grazers for prey. The relative importance of these different toxic effects produced by *P. parvum* has never been studied.

The aim of this study was to determine the relative importance of the different negative effects of *Prymnesium parvum*, cultured under different nutrient conditions, on the population growth rates of a non-toxic phytoplankton species (*Rhodomonas salina*) and of the rotifer *Brachionus plicatilis*.

MATERIALS AND METHODS

Algal cultures. *Prymnesium parvum* (strain KAC39, Kalmar Algal Collection, University of Kalmar, 7 to 9 µm equivalent spherical diameter) was cultured in f/20 medium (Guillard & Ryther 1962) modified to N = 58 µM, P = 3.63 µM. *Rhodomonas salina* (6 to 9 µm equivalent spherical diameter), obtained from the Tvärminne Zoological Station culture collection, Finland (Hällfors & Hällfors 1992), was grown under the same conditions as *P. parvum*. The algal cultures were grown under a 14:10 h light:dark cycle at 16 (*P. parvum*) and 18°C (*R. salina*). Autoclaved and filtered (0.22 µm glass fibre filter) seawater from the Gulf of Finland (salinity 6 psu) was used to prepare the culture media.

Prymnesium parvum cultures were first grown as batch cultures until high biomass was reached, but the cells were still in exponential growth. Thereafter, 20% of the volume was replaced daily with fresh media, which produced steady-state growth conditions (Johansson & Granéli 1999a).

After steady-state growth was initially established for the *Prymnesium parvum* cultures, 3 different nutrient treatments were started in triplicate: nitrogen deficiency (–N), phosphate deficiency (–P) and balanced nitrogen and phosphate conditions (+NP). The concentrations and ratios of nutrients in the treatments were: –P (N:P = 80:1, 80 µM NO^{3-}, 1 µM PO), –N (N:P = 4:1, 16 µM NO^{3-}, 4 µM PO) and +NP (both nutrients corresponding to f/20 medium [Guillard & Ryther 1962] modified to N = 58 µM, P = 3.63 µM). Experiments were run during the last third of steady-state growth. Cell abundances were measured daily with an ELZONE electronic particle counter (Particle Data). Nutrient analyses were performed daily in order to ensure that cells were N- or P-depleted (P. Uronen et al. unpubl. data). The haemolytic activity of *Prymne-*

sium parvum cells and cell-free filtrate was measured daily in the cultures to determine cell toxicity.

Rotifer cultures. The SINTEF-strain (L-strain) of *Brachionus plicatilis* (Olsen et al. 1993) was fed with *Rhodomonas salina* and kept in culture for a few months before the experiments. Light and temperature conditions were the same as for *R. salina* cultures.

Experimental design. ***Effect of Prymnesium parvum on Rhodomonas salina growth:*** To test the effect of dissolved toxins on *R. salina* growth rate, *P. parvum* cultures were filtered through GF/F filters and the cell-free filtrate was added to 10×10^3 cells ml^{-1} *R. salina* suspensions at different percentages: 10, 25, 50 and 90%. To check the combined effect of phagotrophy and dissolved toxins (allelochemicals) on *R. salina* growth rate, different *P. parvum* concentrations (2, 10, 25 and 50×10^3 cells ml^{-1} final concentration) were added to 10×10^3 cells ml^{-1} *R. salina* suspensions. Experimental cell suspensions were kept in 25 ml beakers, with 7 replicates for each *P. parvum* nutrient condition (–P, –N, and +NP). The experiments were carried out over 24 h (10:14 h light:dark cycle, 18°C). Samples were taken at the beginning of the experiment and after 24 h, fixed with 4% paraformaldehyde and counted using flow cytometry.

Rotifer mortality and grazing experiments: For each *Prymnesium parvum* culture (–P, –N and +NP), we ran 5 different treatments with the rotifers: (1) rotifers and *P. parvum* alone (*Pry*); (2) rotifers with *P. parvum* and *Rhodomonas salina* (*Pry* + *Rho*); (3) rotifers with *P. parvum* cell-free filtrate and *R. salina* (*Rho* + F); (4) rotifers with filtered seawater in starvation conditions; and (5) rotifers with *R. salina* alone (*Rho*).

The final densities of *Prymnesium parvum* and *Rhodomonas salina* were 2, 50 and 100×10^3 cells ml^{-1} for *Pry* and *Rho* treatments, and 2 and 50×10^3 cells ml^{-1} in *Pry* + *Rho* and *Rho* + F treatments. *R. salina*: *P. parvum* cell ratios were always 1:1. The experiments were carried out with 15 replicates of 15 rotifers in 25 ml beakers. Rotifers were counted daily and transferred to new cell suspensions. The experimental conditions were 10:14 h light:dark cycle and 18 ± 1°C. In order to run all nutrient conditions and treatments simultaneously, we stopped the experiments when the first treatment lost the entire rotifer population (5 d, Treatment *Pry*, –N).

Growth and mortality of the rotifer population were estimated from the daily population counts. Growth rates were calculated according to $N = N_0 e^{rt}$, where N is the final rotifer density, N_0 is the initial rotifer density, r is the growth rate and t is time.

The ingestion rates were estimated on the second day of the experiment. For this purpose we used 10 beakers without grazers (controls) and the 15 replicate beakers containing rotifers per treatment. Samples were fixed initially and after 24 h with 4% paraformaldehyde. Cell numbers were counted using a flow cytometer. Cell abundances were converted to carbon estimates using 32 pg $cell^{-1}$ for *Prymnesium parvum* (Legrand et al. 2001) and 45 pg $cell^{-1}$ for *Rhodomonas salina* (Nejstgaard & Solberg 1996). Ingestion rates were calculated according to Frost's (1972) equations and negative values were considered as zero.

Analytical procedures. ***Haemolytic activity of Prymnesium parvum:*** Cells and cell-free filtrates were collected after filtration of 10 ml cultures onto GF/C glass microfibre filters. Toxins in the cells were extracted with 2 ml methanol (Igarashi et al. 1998). Extracellular toxicity was measured using a haemolytic test on the cell-free filtrates. The haemolytic activity of cell methanol extracts and cell-free filtrates on horse blood cells was measured in duplicate (Igarashi et al. 1998). Horse blood was washed and resuspended in isotonic phosphate buffer (IPB) (Fistarol et al. 2003). Cell methanol extract or cell-free filtrate was mixed with IPB at different ratios (0 to 100%) and 50 µl was added to 200 µl of horse blood cell suspension in microplates (Falcon, 96 wells). A standard curve was made with saponin (Sn). After 1 d dark incubation at room temperature, the microplates were centrifuged (3000 rpm [$32 \times g$] 5 min, Beckman AllegraTM centrifuge), and the supernatant (110 µl) was transferred to new microplates (black Costar®, 96 wells). The absorbance at 540 nm was read on a Fluostar 403 microplate reader. The results were expressed as µg Sn equivalent (Sneq) ml^{-1} *P. parvum* culture.

Phytoplankton enumeration: Phytoplankton cells were counted using a flow cytometer (FACSCalibur, Beckton-Dickson) which can distinguish between *Prymnesium parvum* and *Rhodomonas salina*. The ability to distinguish between the 2 similarly sized species is mainly due to detection by the FL-2 H fluorescence detector (yellow-orange fluorescence, 560 to 600 nm) and, to a lesser extent, by the FL-3 H detector (red fluorescence, 640 to 700 nm).

Statistical analyses. All statistical analyses were performed with SPSS 11.0 (2003: SPSS Headquarters).

RESULTS

Prymnesium parvum cultures

During steady-state growth, *Prymnesium parvum* cell densities were (mean ± SD) 368 000 ± 21 000, 310 000 ± 13 000 and 148 000 ± 15 000 for NP, –P and –N treatments, respectively. The cell densities between each nutrient condition during steady-state conditions were significantly different (analysis of covariance, [ANCOVA] with time as covariate, $F_{1,134} = 89.1$, $p < 0.001$).

The highest haemolytic activity was measured in *Prymnesium parvum* cells grown in –P cultures and the lowest was measured in +NP (Fig. 1) Haemolytic activity values were significantly different among nutrient conditions during the whole experiment (ANCOVA with time as covariate, $F_{1,42}$ = 19.1, p < 0.001). No haemolytic activity was detected in any of the *P. parvum* cell-free filtrates, although the small volumes analysed (5 to 100 µl) reduced the sensitivity of the assay. Thus, the presence of low levels of haemolytic substances cannot be excluded.

Effect of *Prymnesium parvum* on the growth rate of *Rhodomonas salina*

Both treatment with *Prymnesium parvum* cells and cell-free filtrates negatively affected the growth rate of *Rhodomonas salina* in all nutrient conditions (Figs. 2 & 3) An ANCOVA, taking the volume of the *P. parvum* cell-free filtrate as covariate (log-transformed) and the different nutrient conditions as factor, showed that the *R. salina* growth rate was significantly reduced as the volume of the cell-free filtrate added increased ($F_{1,74}$ = 57.5, p < 0.001, Fig. 2), and there were significant differences among the nutrient conditions ($F_{2,74}$ = 9.1 p < 0.001). As no haemolytic activity was detected in the cell-free filtrate, this might indicate that there are toxic compounds not detected by the haemolytic test, which negatively affect the growth rate of *R. salina*.

Although there is a negative relationship between *Rhodomonas salina* growth rate and both *Prymnesium*

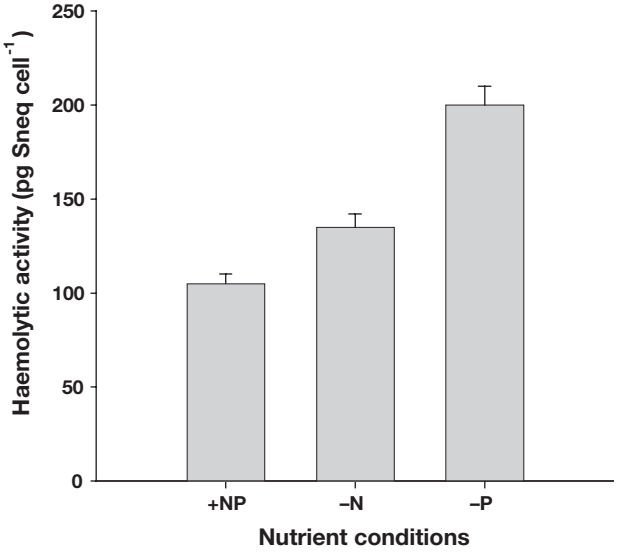

Fig. 1. *Prymnesium parvum*. Haemolytic activity in the –P, –N and +NP cultures during Days 16 to 26. Values are mean ± SE. Sneq: saponin equivalent

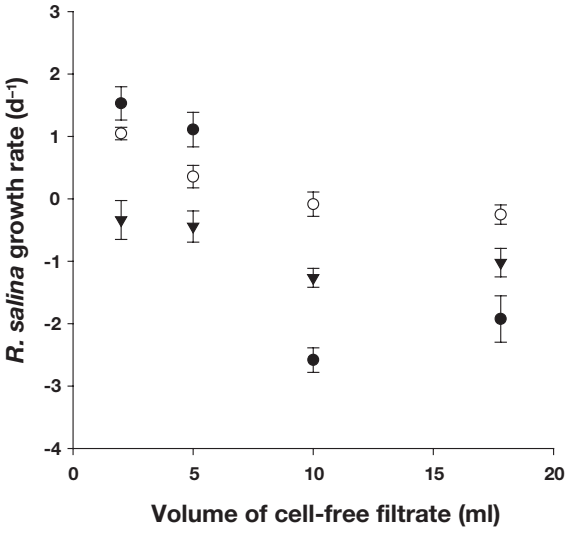

Fig. 2. *Rhodomonas salina*. Relationship between growth rate (mean ± SE, n = 7) and the volume of cell-free filtrate from *Prymnesium parvum* cultured under the different nutrient conditions +NP (●), –P (○) and –N (▼)

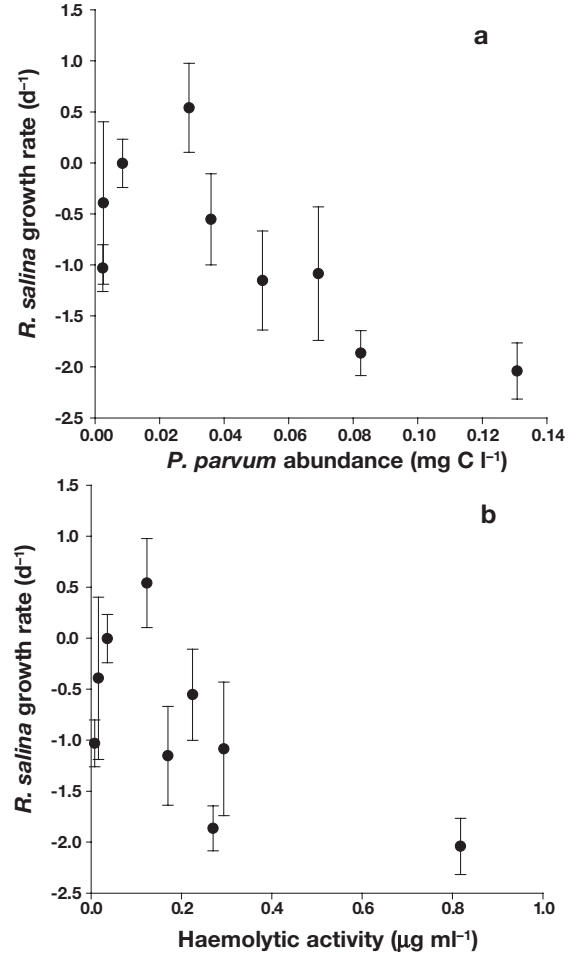

Fig. 3. *Rhodomonas salina*. Relationship between growth rate (mean ± SE, n = 7) and (a) *Prymnesium parvum* abundance, (b) haemolytic activity

parvum abundance (Fig. 3a) and haemolytic activity (Fig. 3b), a stepwise multiple regression showed that only *P. parvum* cell abundance (log-transformed) was significantly related to *R. salina* growth rate ($F_{1,179}$ = 83.7, r^2 = 0.32, p < 0.001), indicating that the effect of *P. parvum* predation on *R. salina* growth rate was greater than the haemolytic effect.

Ingestion rates

Rotifers fed on both toxic and non-toxic algae (Fig. 4). There were no significant differences between the rates of *Rhodomonas salina* and *Prymnesium parvum* ingestion in the mixed cultures (ANCOVA with each algal concentration as covariate, $F_{1,174}$ = 0.51, p = 0.47), indicating that the rotifers did not select against the toxic alga. When offered alone, *P. parvum* was ingested at higher rates than when offered mixed with *R. salina*. Rates of *R. salina* ingestion were lower than those of *P. parvum* in the treatments where both algae were offered alone (*Pry* and *Rho*). However, *R. salina* seemed to be ingested at a higher rate in the *Rho* + F treatments (except –P) than in *Rho*.

Effect of *Prymnesium parvum* on the growth rate of rotifers

For –P, –N and +NP, a stepwise multiple regression showed that there was a clear relationship between the growth rate of rotifers (G, in no. d^{-1}) and, *Rhodomonas salina* (R) and *Prymnesium parvum* (P) abundances (both in mg C ml^{-1}); the volume of cell-free filtrate (V, in ml) had a significant negative effect in –N and +NP treatments but not in the –P treatment:

$$\text{–P: } G = -0.04 + 0.51 \log(R+1) - 0.23 \log(P+1) \quad F_{2,161} = 299,\ r^2 = 0.79,\ p < 0.001 \tag{1}$$

$$\text{–N: } G = -0.09 + 0.78 \log(R+1) - 1.03 \log(P+1) - 0.11 \log(V+1) \quad F_{3,159} = 211,\ r^2 = 0.80,\ p < 0.001 \tag{2}$$

$$\text{+NP: } G = -0.02 + 0.50 \log(R+1) - 0.39 \log(P+1) - 0.03 \log(V+1) \quad F_{3,161} = 210,\ r^2 = 0.80,\ p < 0.001 \tag{3}$$

The *Rho* treatment produced a significantly greater rotifer growth rate than all other treatments under all nutrient conditions (with the exception of *Rho* + F in –P, which produced the same) (Table 1, Fig 5). Rotifer growth rate followed the pattern *Rho* + F > *Pry* + *Rho* > *Pry* under all nutrient conditions (Fig. 5). The starvation conditions produced lower growth rates than all other treatments except *Pry* (under all nutrient conditions) and *Pry* + *Rho* (in –N) (Fig. 5). These findings reveal that the abundance of *P. parvum* cells produced a greater toxic effect on rotifers than cell-free filtrates.

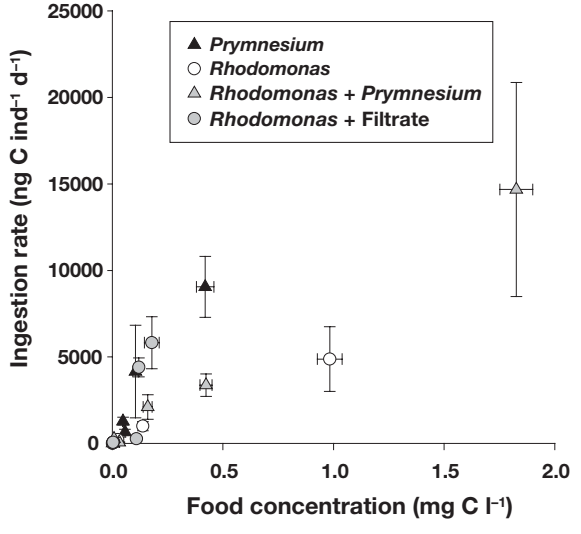

Fig. 4. *Brachionus plicatilis*. Relationships between total food concentration and total ingestion rate (mean ± SE, n = 15)

Table 1. *Brachionus plicatilis*. ANCOVA of the population growth rate with different treatments as factors and food concentration as covariate. Independent variables and covariate were log-transformed

Source of variation		df	*F*	p
+NP	*Pry* / *Pry* + *Rho*	1	70	<0.001
	Error	72		
	Total	75		
	Pry + *Rho* / *Rho* + F	1	17.2	<0.001
	Error	57		
	Total	60		
	Rho + F / *Rho*	1	70.1	<0.001
	Error	72		
	Total	75		
–N	*Pry* / *Pry* + *Rho*	1	59.4	<0.001
	Error	71		
	Total	74		
	Pry + *Rho* / *Rho* + F	1	42.5	<0.001
	Error	56		
	Total	59		
	Rho + F / *Rho*	1	122.6	<0.001
	Error	72		
	Total	75		
–P	*Pry* / *Pry* + *Rho*	1	63	<0.001
	Error	71		
	Total	74		
	Pry + *Rho* / *Rho* + F	1	39.7	<0.001
	Error	57		
	Total	60		
	Rho + F / *Rho*	1	0.36	0.55
	Error	72		
	Total	75		

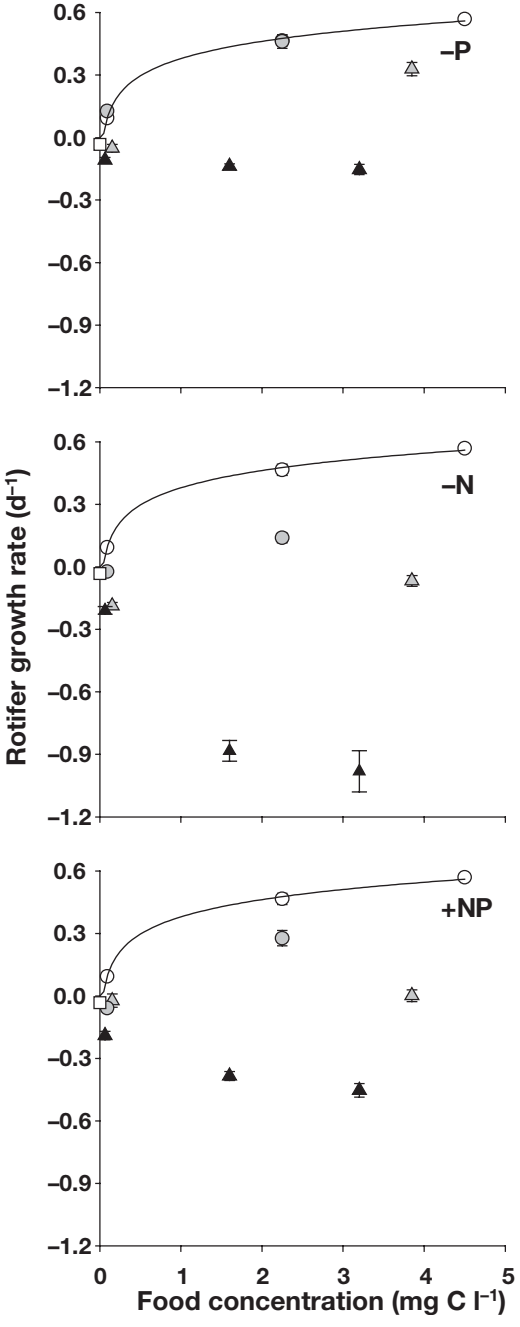

Fig. 5. *Brachionus plicatilis*. Relationship between total food concentration and population growth rate under the different treatments and nutrient conditions (mean ± SE, n = 15). □: starvation conditions; other symbols as in Fig. 4. The line represents the *Rho* control treatment

When comparing rotifer growth rate under the different nutrient conditions, the following pattern was observed: –P > +NP > –N (Table 2, Fig 5), indicating that the –N culture was most toxic for the rotifers, despite the fact that haemolytic activity was higher in –P (Fig. 2).

Table 2. *Brachionus plicatilis*. ANCOVA of the population growth rate with different nutrient conditions as factors and food concentration as covariate. Independent variables and covariate were log-transformed

Source of variation		df	*F*	p
Pry	–P / –N / +NP	2	81.4	<0.001
	Error	130		
	Total	134		
Pry + *Rho*	–P / –N / +NP	2	17.6	<0.001
	Error	85		
	Total	89		
Rho + F	–P / –N / +NP	2	24.7	<0.001
	Error	86		
	Total	90		

DISCUSSION

Fig. 6 summarises the effects of *Prymnesium parvum* on *Rhodomonas salina* and on the rotifers. The *P. parvum* cell-free filtrate had an effect on *Brachionus plicatilis*, although this was weak. *B. plicatilis* was also indirectly affected by *P. parvum* due to the negative effects of the toxic alga on their prey (*Rhodomonas salina*). However, the greatest negative effect of *P. parvum* on the rotifers was due to ingestion of the toxic cells (Fig. 5).

Previous studies have shown that excreted compounds of *Prymnesium parvum* are inhibitory to potential grazers, particularly ciliates (Fistarol et al. 2003), and even that *P. parvum* can kill and eat its predators

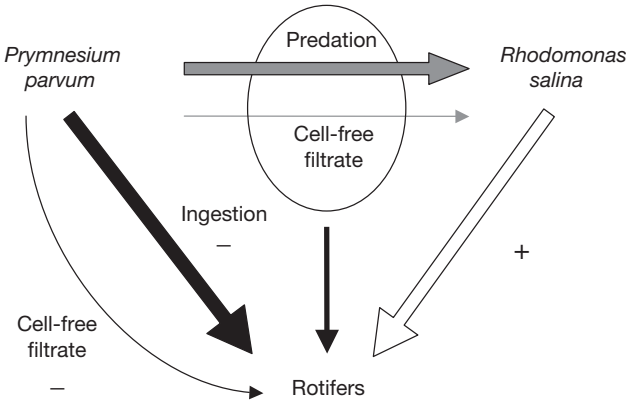

Fig. 6. *Prymnesium parvum*, *Rhodomonas salina* and *Brachionus plicatilis*. Negative (–) and positive (+) interactions. Black arrows indicate the effects of *P. parvum* on rotifers (solid line: direct effects; small black arrow: indirect effects), grey arrows the effects of *P. parvum* on *R. salina* and white arrow the effect of *R. salina* on rotifers. The thickness of the arrows indicates the importance of the interaction

(Tillmann 2003). Toxic effects on protist grazers have also been observed with the ichthyotoxic *Chrysochromulin polylepis* (Tobiesen 1991). Excreted toxins produced by *P. parvum* also decrease egg production in the copepod *Acartia clausi*, which does not feed on *P. parvum* (Nejstgaard & Solberg 1996). Similarly, in non-selective copepod species, such as *Eurytemora affinis*, mortality and egg production have been found to be more closely related to *P. parvum* cell density than to the number of ingested cells (Koski et al. 1999). Our results corroborate the hypothesis that compounds released by toxic species may function as a defence strategy by eliminating potential grazers. However, the negative effect produced by dissolved toxins on rotifers was not as important as the effect of *P. parvum* on their prey and the effect of toxic cell ingestion (Fig. 5).

Prymnesium parvum cell-free filtrate affected the growth rate of *Rhodomonas salina*, which is in agreement with other studies that have shown a negative effect of cell-free filtrates on phytoplankton species (Fistarol et al. 2003, Skovgaard & Hansen 2003). Compounds released by *P. parvum* may allow them to outcompete other phytoplankton species and, therefore, the role of allelopathic compounds should be taken into account in competition studies between phytoplankton species (Fistarol et al. 2003). However, the presence of *P. parvum* cells had a greater effect on *R. salina*, which might be explained by predation of *P. parvum* on *R. salina*. These findings are similar to those obtained by Tillmann (2003), who found that mortality of *Oxyrrhis marina* cells, although affected by cell-free filtrates, was strongly increased when *P. parvum* cells were present. Therefore, our results support the hypothesis that toxins produced by *P. parvum* may cause immobilisation and/or cell lysis of potential phytoplankton competitors and even predators, but the most important effect is associated with its own predatory behaviour. However, the lower effect of cell-free filtrates could also be caused by toxins being lost from the water during filtration processes due to the formation of toxin aggregates (Tillmann 2003). Furthermore, if these toxins are especially labile, rapid degradation of toxins when toxic algal cells are absent would also influence the results obtained in our and other studies.

This effect of *Prymnesium parvum* on *Rhodomonas salina* indirectly affects rotifers, potential predators of *P. parvum*, because the reduced amount of food is more important than the effect of dissolved compounds excreted by *P. parvum*. This indirect effect could also be extrapolated to mesozooplankton species.

The ingestion rate of rotifers offered mixed prey was the same for both *Prymnesium parvum* and *Rhodomonas salina*, indicating a non-selective feeding behaviour towards the toxic cells. This suggests that toxins produced by *P. parvum* did not act as feeding deterrents to the rotifers. However, toxins produced by the haptophyte *Chrysochromulina polylepis* deter grazing by the dinoflagellate *Oxyrrhis marin*a (John et al. 2002). The lack of feeding avoidance by the rotifer might be explained by the fact that the *Brachionus plicatilis* strain used in our study had not been exposed to *P. parvum* prior to our experiments. Feeding behaviour and resistance of the grazer to a specific toxic phytoplankton species have been shown to be affected by the exposure history of the grazer to that specific toxic alga (Colin & Dam 2002). Another possible explanation is that toxicity in *P. parvum* may not have evolved in order to directly prevent grazing. In this sense, *P. parvum* toxins differ from feeding deterrence toxins (endotoxins) because they are excreted and have a direct effect on both predators and competitors (allelopathic). In contrast, endotoxins such as those that cause paralytic shellfish poisoning (PSP) need to be ingested by predators to exert their feeding deterrence effect (Dutz 1998, Teegarden 1999, Frangópulos et al. 2000, Guisande et al. 2002).

Prymnesium parvum was ingested in higher amounts when offered as the only prey than when offered with *Rhodomonas salina*. This may be explained by non-selective feeding behaviour. When *R. salina* is also present, the total ingestion rate of algae would be expected to be shared between both species. *R. salina* was ingested at a higher rate in *Rho* + F treatments (except in −P, where there was no toxic effect) than in *Rho*. This could be due to a higher grazing activity of rotifers stressed by toxins or even to an underestimation of the number of grazing rotifers (overestimating the number of dead rotifers).

It has been suggested that, prior to the onset of toxicity and bloom formation, some ciliate species may exert grazing pressure on *Prymnesium parvum* (Rosetta & McManus 2003). *Brachionus plicatilis* fed on toxic cells; however, it is unlikely that grazing pressure on this harmful algal bloom species from rotifers contributes to the suppression or decline of *P. parvum* blooms, because rotifer mortality was very high when feeding on a monoculture of *P. parvum*. Although our results cannot be extrapolated to all microzooplankton species, we hypothesise that grazers are not able to control the proliferation of *P. parvum*.

In agreement with previous studies (Johansson & Granéli 1999a,b, Legrand et al. 2001), haemolytic activity of *Prymnesium parvum* cells was higher under nutrient-limiting conditions, and higher under phosphorus than under nitrogen limitation. In contrast, *P. parvum* cultured under +NP conditions produced intermediate negative effects on rotifer growth compared to −N and −P conditions. This indicates that haemolytic activity is not a good indicator of the total potential negative effect of *P. parvum* on *Brachionus*

plicatilis. Therefore, our findings reveal that *P. parvum* may produce deleterious compounds other than those detected with the haemolytic test.

Stepwise regressions also showed an important positive effect on rotifers due to the addition of *Rhodomonas salina* in the presence of *Prymnesium parvum* cells. This positive effect of non-toxic prey in the presence of *P. parvum* has already been observed for ciliates (Rosetta & McManus 2003), dinoflagellates *Oxyrrhis marina* (Tillmann 2003) and copepods (Nejstgaard & Solberg 1996). The negative effect of ingested *P. parvum* cells will be reduced when *R. salina* is added, because the total ingestion of *P. parvum* decreases. Moreover, toxins should be removed by binding to the membrane of *R. salina* cells, as suggested by Tillmann (2003) and Skovgaard & Hansen (2003), decreasing toxin concentration in the water. However, in natural plankton communities, the death of most ciliates in the presence of both *P. parvum* cell-free filtrates and non-toxic prey has been reported (Fistarol et al. 2003).

In summary, our findings reveal that *Rhodomonas salina* may be primarily affected by predation of *Prymnesium parvum* cells and that *Brachionus plicatilis* may be primarily affected by ingestion of the toxic cells, the effects of compounds excreted into the water by *P. parvum* being secondary. Some studies have also found a strong negative influence of the presence of *P. parvum* cells on other predators, like the dinoflagellate *Oxyrrhys marina* (Tillmann 1998, 2003). On the other hand, other studies (Nejstgaard & Solberg 1996, Fistarol et al. 2003, Rosetta & McManus 2003, Skovgaard & Hansen 2003) suggest that our conclusions should not be generalised to other phytoplankton competitors and predators.

From our results and previous studies, it is clear that *Prymnesium parvum* is unbeatable in biotic interactions (competition and predation) and its 'Achilles' heel' is probably in the abiotic factors that control its dominance in plankton communities.

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