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Feeding by ciliates on two harmful algal bloom species, *Prymnesium parvum* and *Prorocentrum minimum*

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Abstract

We have focused on ciliates as potential grazers on toxic phytoplankton because they are major herbivores in aquatic food webs. Ciliates may exert top down control on toxic phytoplankton blooms, potentially suppressing or shortening the duration of harmful algal blooms (HABs). We measured the growth rates of several ciliate species on uni-algal and mixed diets of both HAB and non-HAB algae. The tintinnids *Favella ehrenbergii*, *Eutintinnus pectinis* and *Metacylis angulata* and the non-loricate ciliates *Strombidinopsis* sp. and *Strombidium conicum* were isolated from Long Island Sound (LIS), and fed HAB species including the prymnesiophyte *Prymnesium parvum* (strain 97-20-01) and the dinoflagellate *Prorocentrum minimum* (strains Exuv and JA 98-01). Ciliates were fed algal prey from cultures at various growth phases and at varying concentrations. We observed no harmful effects of *P. minimum* (Exuv) on any of the ciliates. However in a comparison of strains, *P. minimum* (Exuv) supported high growth rates, whereas *P. minimum* (JA 98-01) supported only nominal growth. *P. parvum* was acutely toxic to ciliates at high concentrations (2×10^4–3×10^4 cells ml^{-1}). At low concentrations (5×10^3–1×10^4 cells ml^{-1}), or in culture filtrate, ciliates survived for at least several hours. In mixed diet experiments, as long as a non-toxic alga was available, ciliates survived and at times grew well at concentrations of *P. parvum* (5×10^2–3×10^4 cells ml^{-1}) that would otherwise have killed them. The present study suggests that prior to the onset of toxicity and bloom formation ciliates may exert grazing pressure on these HAB species, potentially contributing to the suppression or decline of *P. minimum* and *P. parvum* blooms.
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1. Introduction

Phytoplankton blooms may be dispersed by physical factors or removed from the water column due to sinking, while mortality of individual cells within blooms may be effected by autolysis, viruses, predatory bacteria, or grazing zooplankton (Needler, 1949; Stoecker et al., 1981; Watras et al., 1985; Sellner and Brownlee, 1990; Imai et al., 1993; Banse, 1994; Jeong and Latz, 1994; Nagasaki et al., 1994a,b; Brussaard et al., 1995, 1996; Buskey et al., 1997; Turner and Tester, 1997; Yih and Coats, 2000; Lawrence et al., 2001). Microzooplankton grazing of harmful algal bloom species (HABs) is important in two ways; first, grazing may serve to prevent blooms or lessen their magnitude; second, for toxin producing species, grazers may accumulate and subsequently transfer toxins

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to higher trophic levels (Turner et al., 2000; Maneiro et al., 2000).

It is well established that the microzooplankton (diameter 20–200 μm) are quantitatively the most important grazers of phytoplankton biomass (e.g. Lee and Capriulo, 1990; Gifford, 1991; Cyr and Pace, 1992; McManus and Ederington-Cantrell, 1992; Pierce and Turner, 1992; Breteler et al., 1999; Montagnes and Lessard, 1999). This size class is primarily composed of metazoan larvae and heterotrophic or mixotrophic protists, and contains a biomass comparable to that of larger zooplankton size fractions. Protists and other microzooplankters generally have higher biomass-specific metabolic rates than larger zooplankton and thus would be expected to have higher community ingestion and respiration rates. Moreover, many organisms in the microzooplankton size fraction have growth rates commensurate with those of their phytoplankton prey and hence can respond rapidly to higher phytoplankton growth with higher growth rates of their own (Strom and Morello, 1998). Thus, populations of microzooplankton and the phytoplankton they feed on should be more closely coupled than populations of larger phytoplankton and their correspondingly larger metazoan grazers (Malone, 1971; Walsh, 1976).

The observation of closely matched growth rates between small grazers and small phytoplankton leads to the prediction that blooms of smaller algal species would be more difficult to initiate and sustain unless they can avoid grazing by microzooplankton. This prediction is upheld in coastal and estuarine waters at mid-latitudes, where annual spring blooms of larger, chain-forming diatoms mostly sink to the benthos ungrazed and smaller, relatively constant levels of microflagellate biomass dominate through late spring and summer. These latter populations are usually more productive per unit biomass and are grazed by microzooplankton at a similarly high rate (Capriulo, 1982; Capriulo and Carpenter, 1983; Capriulo and Carpenter, 1980; McManus and Ederington-Cantrell, 1992).

The specific role of microzooplankton in grazing blooms of harmful or nuisance phytoplankton is poorly known, though it has been suggested that top down control by zooplankton grazers may prevent bloom formation or shorten the duration of blooms (Banse, 1994; Buskey et al., 1997; Turner and Tester, 1997). Several studies have reported high grazer concentrations in blooms with concomitant losses of phytoplankton biomass (Stoecker et al., 1981; Watras et al., 1985; Sellner and Brownlee, 1990; Jeong and Latz, 1994). For example, the ciliate microzooplankter *Favella ehrenbergii* has been credited with grazing down a *Gonyaulax* (*Alexandrium*) *tamarense* bloom in the Bay of Fundy (Needler, 1949). Additionally it is reported that ciliates attain optimal reproduction and growth rates when fed some HAB species (Gifford, 1985; Stoecker et al., 1986; Kamiyama, 1997; Jeong et al., 1999).

The production by phytoplankton of toxins (such as okadaic acid, domoic acid and prymnesins) or grazing deterrents (such as dimethylsulfide (DMS) or acrylic acid) by phytoplankton, may have lethal or sublethal effects on planktonic consumers and subsequently alter the role of grazers, possibly facilitating bloom formation (Igarashi et al., 1996; Plumley, 1997; Smayda, 1997; Turner and Tester, 1997; Arzul et al., 1999; Wolfe, 2000; Strom et al., 2003a,b). This is demonstrated by the acutely toxic effects of some *Prymnesium* species on cladocerans, crustaceans, isopods and amphipods (Nejstgaard, 1997), compared to the sublethal effects on copepod fecundity and feeding (Nejstgaard and Solberg, 1996; Koski et al., 1999). On the other hand, some zooplankton, including ciliates and copepods, are able to graze on several species of red tide or toxic dinoflagellates, such as *Alexandrium* spp., *Gymnodinium* spp., *Prorocentrum minimum* and *Heterocapsa circularisquama*, without any apparent harmful effects (Hansen, 1989; Hansen et al., 1992; Jeong et al., 1999; Montagnes and Lessard, 1999; Kamiyama, 1997; Colin and Dam, 2002; Frangópulos et al., 2000). It has been suggested that the production of grazing deterrent compounds is widespread among the phytoplankton and that these compounds may reduce interspecfic competition and grazing pressure, thereby facilitating bloom formation (Smayda, 1997; Turner and Tester, 1997; Arzul et al., 1999; Wolfe, 2000; Strom et al., 2003a,b).

We examined the effects of two HAB species, *Prorocentrum minimum* and *Prymnesium parvum* on representative microzooplankton grazers. The mixotrophic dinoflagellate *P. minimum* is a common component of the summer phytoplankton assemblage in North American coastal waters. Blooms of it contribute to poor survival, growth and development of oysters and clams as well as mortality of scallops (Wikfors and Smolowitz, 1993, 1995; Li

et al., 1996). Several *Prorocentrum* species, including *P. minimum*, reportedly synthesize okadaic acid and are rich in dimethylsulfoniopropionate (DMSP) both of which can function as grazing deterrents (Zhou and Fritz, 1994; Wolfe, 2000). *P. parvum*, a euryhaline species that has been implicated in recent and historic fish kills around the world exhibits both ichthyotoxic and molluscotoxic properties (Parnas, 1963; Johnsen and Lein, 1989; Hallegraeff, 1993). The toxins of *P. parvum* (prymnesin 1 and prymnesin 2) have cytolytic, hemolytic and neurotoxic effects (Igarashi et al., 1996, 1999), and toxicity is reported to be variable within and among strains (Johnsen and Lein, 1989; Moestrup and Larsen, 1992; Aure and Rey, 1992; Igarashi et al., 1996; Koski et al., 1999). Blooms typically occur in eutrophic brackish water, and may be subsequently exported into coastal waters (Edvardsen and Paasche, 1998; Moestrup and Larsen, 1992). Blooms can reach concentrations as high as 10^9 cells ml^{-1} and cause fish kills at concentrations as low as 10^3 cells ml^{-1} (Aure and Rey, 1992).

For grazers we used large, commonly abundant spirotrich ciliates. Several genera where isolated from Long Island Sound (LIS), USA, and their growth and mortality responses to the HABs *P. parvum* and *P. minimum*, were examined. In addition, we examined the effects of two different strains of *P. minimum* (Exuv and JA 98-01) on the growth rates of ciliate grazers. Experiments were performed to examine three aspects of *P. parvum* toxicity to grazers: (a) acute toxic effects, (b) effects of algal growth stage on toxicity, and (c) growth of grazers when non-toxic food was available along with *P. parvum*.

2. Materials and methods

2.1. Organisms

We utilized two strains of *P. minimum*, the Exuv strain which was isolated from Great South Bay, NY, by Irma Pinter in 1958 and the JA 98-01 strain which was isolated from the Choptank River, Chesapeake Bay, MD, by Patricia Glibert and Jennifer Alix in 1998 (G. Wikfors pers com). Besides originating in a different geographic region than JA 98-01, Exuv has been in culture for over 40 years, and it is not uncommon for algal toxicity to decrease over time in culture (Cembella and Therriault, 1998; Burkholder et al., 2001). Although this clone is still toxic to bivalves, we wanted to compare its effect on grazers with that of the more recently isolated and reportedly more toxic strain JA 98-01 (Wikfors and Smolowitz, 1993, 1995). Both strains of *P. minimum* were cultured in 25 ml or 250 ml tissue culture flasks in F/2 growth medium (Guillard, 1975) at 20 °C and 100 μmol photons m^{-2} s^{-1} in a 12:12 light:dark cycle.

The *P. parvum* (strain 97-20-01) used in our experiments was isolated in 1997 from a bloom in Boothbay Harbor, ME, by R.R.L. Guillard (G. Wikfors pers com). *P. parvum* (97-20-01) cultures were grown axenically in 8-L Fernbach flasks containing 1500 ml of culture in F/2 growth medium (Guillard, 1975) in a temperature and light controlled incubator at 20 °C, 100 μmol photons m^{-2} s^{-1}, 12:12 light:dark cycle. For experiments where cultures at different growth phases were used, a growth curve was determined by counting sub-samples of a culture every 2 days for 28 days. Subsequently, separate cultures were incubated for 2 days for logarithmic growth phases, 12 days for early stationary, and 28 days for late stationary. The cultures were inoculated on different days so they would reach these growth phases and could be harvested on the same day. Because the toxins of Prymnesiophytes have not been well characterized, toxicity to juvenile bay scallops, *Argopecten irradians*, was determined to verify toxin production in the *P. parvum* cultures used in our ciliate grazing experiments (Wikfors et al., 2000).

Quantitatively, the two most important subclasses of planktonic spirotrichs are the Choreotrichia, consisting of the tintinnids and several non-loricate genera, principally *Strombidinopsis* and *Strobilidium*, and the Oligotrichia, dominated by the genus *Strombidium*. Altogether five different spirotrich species (ciliates) were isolated from LIS, USA, for this study. Species isolated included the tintinnids (O. Choreotrichida) *Favella ehrenbergii*, *Eutintinnus pectinis*, and *Metacylis angulata* and the non-loricate ciliates *Strombidinopsis* sp. (O. Choreotrichida) and *Strombidium conicum* (O. Oligotrichida). The ciliates were picked either directly from seawater or from a 63 μm mesh plankton net concentrate and brought into laboratory culture using methods described by Gifford (1985). Tintinnids were identified using lorica morphology including observations on shape and measurements of

oral diameter and maximal width and length of loricas (Gold and Morales, 1975). *Strombidinopsis* sp. and *S. conicum* were identified by cell morphology and behavior (Montagnes and Lynn, 1991; Montagnes and Taylor, 1994; Montagnes, 2001). Ciliates were maintained in 10 ml culture well plates with F/20 growth medium (Guillard, 1975) at 30 psu salinity and 20 °C at 100 μmol photons $m^{-2} s^{-1}$ on a 12:12 light:dark cycle. Ciliate culture diets consisted of a mixture of algal species, principally *Isochrysis galbana* (Tiso; ca. 1×10^3 cells ml^{-1}) and *Rhodomonas lens* (Rhodo; ca. 1×10^4 cells ml^{-1}). These species have been shown to support ciliate growth (Taniguchi and Kawakami, 1983). After preliminary experiments indicated that ciliates grew well on it, *P. minimum* (Exuv) was added to the standard diets at 1×10^4 cells ml^{-1}. Algal species used in mixed-food experiments, included the dinoflagellate *P. minimum* (Exuv), the prymnesiophytes *Pavlova lutheri*, *Pavlova gyrans* and *I. galbana*, and the cryptophyte *R. lens*. All algal cultures were obtained from the National Marine Fisheries Service Laboratory in Milford, CT.

3. Experiments

Experiments to evaluate the effects of the harmful algal bloom species on ciliate grazers included: (a) *acute toxicity tests*, where mortality or altered behavior of ciliates was observed directly when ciliates were exposed to bloom-level concentrations of *P. parvum*, (b) *alternative food experiments*, where ciliate growth was measured on combinations of algae including treatments of only HAB species, a mix of HAB and non-toxic algae, or only non-toxic algae, (c) *numerical response experiments*, where the growth of ciliates was determined at a range of concentrations of *P. minimum* (strains Exuv and JA 98-01). Data were analyzed using a two-way analysis of variance (ANOVA) with interactions. If interactions were not significant the model was sub-divided into one-way ANOVAs in order to examine effects of food concentration or composition on ciliate growth or survival. Whenever ANOVA's showed significant effects, the significant differences among treatments was determined using a Tukey–Kramer post-hoc test; an α of 0.05 was assumed for all statistical analyses.

3.1. Acute toxicity tests

Initial acute toxicity tests were conducted on *F. ehrenbergii*, *Strombidinopsis* sp. and *S. conicum* using *P. parvum* in logarithmic, early stationary, and late stationary growth phases at a concentration of 4.8×10^4 cells ml^{-1}. Five to 10 ciliates were placed in each of three 5 ml replicate wells containing filtered seawater (FSW), the alga, or culture filtrate. Culture filtrate was obtained by using an aliquot of the highest concentration of algae and filtering through a 0.45 μm syringe filter. Subsequent toxicity tests utilized *P. parvum* at a range of concentrations, but in late stationary growth phase only. Concentrations used were within the range 5×10^2–3×10^4 cells ml^{-1} while controls included FSW and culture filtrate. Ciliate mortality, determined when the ciliate lysed, was examined at ten-minute time intervals for 2 h and again after 24 h. For all experiments reported here, toxicity of the *P. parvum* cultures was verified using a scallop bioassay. Five bay scallops (*Argopecten irradians*) were exposed to the alga at bloom level concentrations (10^5 cells ml^{-1}). Responses of the scallops to toxicity, including shell clapping, changes in filtering, feces and pseudofeces production, were monitored visually at 10 min intervals for 2 h (Wikfors et al., 2000). Scallop mortality was determined after an overnight incubation.

3.2. Alternative food experiments

The effects on ciliate growth of uni-algal and mixed diets containing *P. parvum* were determined. Experimental diets consisted of mixtures of *P. parvum* ranging from 0 to 100% and reciprocal concentrations of a non-toxic species known from preliminary experiments to support growth in ciliates. In treatments where algal species were not the same size, treatment concentrations were normalized by cell volume estimated from cell dimensions (Tomas et al., 1996). Food levels were determined by the concentrations at which the non-toxic food supported good growth rates in preliminary experiments. The species used included *P. minimum*, *P. gyrans*, *P. lutheri*, *R. lens* and *I. galbana*. Additionally, in several alternative food experiments, the effect of algal growth phase on toxicity was examined. For those experiments, *P. parvum* was harvested from cultures at logarithmic, early stationary, and late stationary growth phases.

The experiments were conducted in six-well culture plates with three or four replicate wells for each treatment. Five to 10 ciliates were transferred individually from stock cultures into each of the 10 ml wells containing an experimental mixed diet and incubated at 20 °C in dim light (<10 μmol photons m^{-2} s^{-1}) on a 12:12 light:dark cycle. Well plates were examined daily to determine the survival of ciliates and to aid in deciding when to terminate the experiment. After 3–5 days, the ciliates were preserved using 10% acid lugols solution and counted using inverted light microscopy using methods described by Gifford and Caron (2000). Growth rates were calculated assuming exponential growth from the initial populations using the equation

$$\mu = \ln \frac{[(Ct_f + 1)/Ct_0]}{t} \qquad (1)$$

where μ is the growth rate (time^{-1}), C the number of individuals in wells at times t_0 and t_f and t the duration of the experiment in days. A value of one was added to the final number of individuals to avoid ln[0] when all ciliates died.

3.3. Numerical response experiments

Numerical response experiments were conducted using *P. minimum* strains Exuv and JA 98-01 at concentrations ranging from food limiting (ca. 500 cells ml^{-1}) to saturating (ca. 1.5×10^4 cells ml^{-1}). Ciliates were acclimated overnight to different food treatments. Five to 10 ciliates were then transferred into triplicate 10 ml wells containing the experimental diets and incubated at 20 °C, 100 μmol photon m^{-2} s^{-1} on a 12:12 light:dark cycle. Cultures were checked daily to verify that ciliates were alive and swimming actively. After 3–5 days, the ciliates were preserved using 10% acid lugols and counted using inverted light microscopy using methods described by Gifford and Caron (2002). Growth rates were calculated assuming exponential growth from the initial populations using Eq. (1). Growth rate data were fit to a modified Michaelis–Menten function, using SigmaPlot (Montagnes and Lessard, 1999).

$$\mu = \mu_{max} \frac{C - x}{[k + (C - x)]} \qquad (2)$$

where μ is the growth rate (time^{-1}), C the algal concentrations (cells ml^{-1}), x the threshold concentration at which no growth occurs (cells ml^{-1}), k the concentration (cells ml^{-1}) at which $\mu = (1/2)\mu_{max}$ and μ_{max} the maximum growth rate (time^{-1}).

4. Results

4.1. Acute toxicity tests

P. parvum offered to *S. conicum* at 4.8×10^4 cells ml^{-1} was significantly more toxic in early and late stationary growth phases than in logarithmic growth phase, culture filtrate or filtered seawater (FSW) (Tukey–Kramer post-hoc test comparing treatments, $P < 0.0001$; Fig. 1). Ciliates survived in both filtered seawater and culture filtrate from all growth phases of *P. parvum* (Tukey–Kramer post-hoc test comparing concentration, $P > 0.05$; Fig. 1). In subsequent experiments using only late stationary phase cells at concentrations of *P. parvum* ranging from 2×10^4–3×10^4 cells ml^{-1}, all ciliates died within 40 min (Tukey–Kramer post-hoc test comparing concentrations, $P < 0.0001$; Fig. 2). Only *F. ehrenbergii* had significantly higher mortality in culture filtrate compared to FSW controls (Tukey–Kramer post-hoc test, $P < 0.0003$).

At low concentrations of *P. parvum* (5×10^3–1×10^4 cells ml^{-1}), *Strombidinopsis* sp. and *S. conicum* did not have increased mortality compared to culture filtrate or FSW during the first 40 min (Tukey–Kramer post-hoc test, $P > 0.05$, Fig. 2). However, the mortality of *F. ehrenbergii* increased at 5×10^3 cells ml^{-1} compared to FSW and filtrate and it exhibited 100% mortality at 1×10^4 cells ml^{-1} (Tukey–Kramer post-hoc test comparing 5×10^3–1×10^4 cells ml^{-1} for *F. ehrenbergii*, $P < 0.0001$). All ciliates died more quickly in wells containing whole cells of *P. parvum* at high concentrations (2×10^4 cells ml^{-1}) compared to those with culture filtrate and FSW controls (Tukey–Kramer post-hoc test, $P < 0.05$), and exhibited behavioral responses including reverse beating of ciliary membranelles, immobility and erratic swimming behavior. After 24 h, all ciliates showed 100% mortality at all concentrations of *P. parvum*, but survived in culture filtrate and the control FSW.

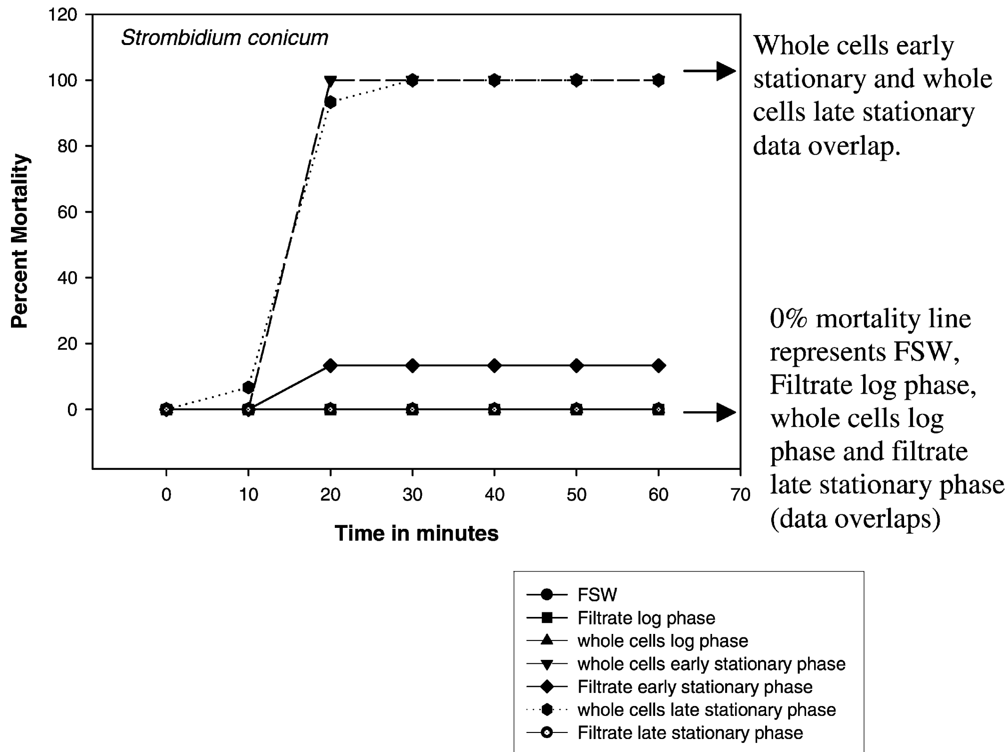

Fig. 1. Percent mortality of *S. conicum* on *P. parvum* at 4.8×10^4 cells ml^{-1} at different growth phases. Line at 0% mortality represents FSW, filtrate log phase, whole cells log phase and filtrate late stationary phase (data overlaps).

4.2. Alternative food experiments

The results of alternative food experiments using *F. ehrenbergii*, *Strombidinopsis* sp. and *S. conicum* are shown in Figs. 3–7. All three ciliates exhibited 100% mortality at high concentrations of *P. parvum* ($(3–5) \times 10^4$ cells ml^{-1}), as expected based on the results of the acute toxicity experiments. Responses to lower concentrations ($(0.5–1.5) \times 10^4$ cells ml^{-1}) varied according to species and are discussed below.

When *F. ehrenbergii* was offered a mixed diet of *P. parvum* and *P. lutheri* (Fig. 3), the proportion of *P. parvum* relative to *P. lutheri* had a significant effect on ciliate growth (one-way ANOVA, $P < 0.0001$). In experiment 1 at low and intermediate concentrations of *P. parvum* (0.5×10^4, 1.5×10^4 and 2×10^4 cells ml^{-1}), fewer ciliates survived than initially placed in experimental wells. Thus, growth rates were negative and not significantly different from each other ($\mu_{(5\times10^3)} = -0.05$ per day, $\mu_{(1.5\times10^4)} = -0.09$ per day and $\mu_{(2\times10^4)} = -0.10$ per day; Tukey–Kramer post-hoc test, $P > 0.05$, Fig. 3). In experiment 2 at the same concentrations, ciliate growth rates were slightly higher and not significantly different from each other ($\mu_{(5\times10^3)} = 0.16$ per day, $\mu_{(1.5\times10^4)} = 0.17$ per day and $\mu_{(2\times10^4)} = 0$ per day; Tukey–Kramer post-hoc test, $P > 0.05$, Fig. 3). In experiment 1 at 3×10^4 cells ml^{-1} (∼86% *P. parvum*) there was no survival, however, at the same cell concentrations in experiment 2, a few individuals survived ($\mu_{(3\times10^4)} = -0.14$ per day). When offered only *P. parvum* (5×10^4 cells ml^{-1}) there was no survival. In both experiments, treatments containing only *P. lutheri* (5×10^4 cells ml^{-1}) had positive growth rates ($\mu_{\text{exp1}} = 0.25$ per day and $\mu_{\text{exp2}} = 0.52$ per day).

In experiments with *P. parvum* at different growth phases and *P. minimum* (Exuv) as the non-toxic food the effect of algal growth phase on growth rate of *F. ehrenbergii* was not significant (one-way ANOVA,

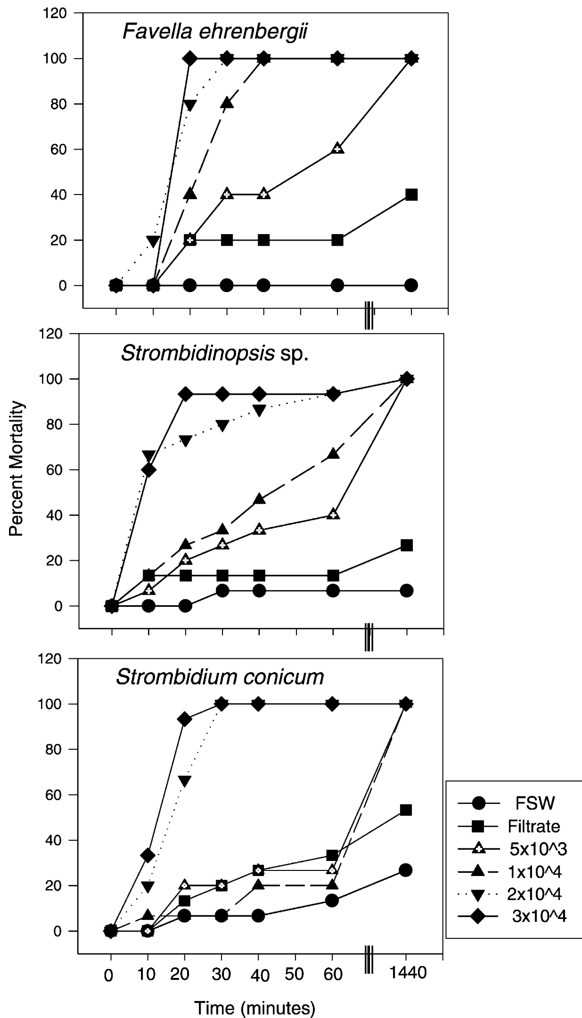

Fig. 2. Percent mortality of the ciliates *F. ehrenbergii*, *Strombidinopsis* sp. and *S. conicum* at a range of *P. parvum* concentrations. The *x*-axis represents time in minutes, however the last point is the sample taken at $t = 1440$ min (24 h).

$P = 0.67$; Fig. 4). The concentration of *P. parvum* showed a significant effect on the growth rates of *F. ehrenbergii* (one-way ANOVA, $P < 0.001$, Table 1). This ciliate experienced 100% mortality at high concentrations of *P. parvum* (4.5×10^4 and 5×10^4 cells ml^{-1}), as in acute toxicity experiments. At low to intermediate concentrations of *P. parvum* (0.5×10^4 and 2.5×10^4 cells ml^{-1}), ciliate growth rates were similar to and at times higher than those on 100% *P. minimum* as shown in Table 1.

When *Strombidinopsis* sp. was offered a mixed diet of *P. parvum* and *P. lutheri* the ratio of the concentration of *P. parvum* relative to *P. lutheri* showed a significant effect on growth rate (one-way ANOVA, $P < 0.0001$). The ciliate attained positive growth rates only in treatments containing 100% *P. lutheri* ($\mu_{exp1} = 0.35$ per day; $\mu_{exp2} = 0.13$ per day; Fig. 5). In all treatments containing *P. parvum* at concentrations ranging from 1.5×10^4 to 5×10^4 cells ml^{-1}, ciliate mortality was 100% (Tukey–Kramer post-hoc test, $P \geq 0.05$). At the lowest concentration of *P. parvum* (0.5×10^4 cells ml^{-1}) there was some survival of the ciliates, however, there was no net growth (Tukey–Kramer post-hoc test, $P < 0.0001$).

When *Strombidinopsis* sp. was offered a mixed diet of *P. parvum* and *P. gyrans*, it exhibited 100% mortality at high to intermediate concentrations of *P. parvum* (1.5×10^4, 2×10^4 and 3×10^4 cells ml^{-1}; Fig. 6A). At low concentrations of *P. parvum* (0.5×10^4 cells ml^{-1}) there was some ciliate growth ($\mu = 0.03$ per day Tukey–Kramer post-hoc test, $P < 0.0001$; Fig. 6A). *Strombidinopsis* sp. had growth rates of 0.4 per day when *R. lens* was used as the non-toxic food, and did not exhibit 100% mortality in any of the mixed diets (Fig. 6B). Growth rates at low concentrations of *P. parvum* in mixtures were positive, compared to 100% mortality when fed only *P. parvum* (5×10^4 cells ml^{-1}; Tukey–Kramer post-hoc test comparing treatments $P < 0.0001$). At low to intermediate concentrations of *P. parvum* (0.5×10^4, 1.5×10^4 and 3.5×10^4 cells ml^{-1}), growth rates were not significantly different from those achieved on 100% *R. lens* as shown in Table 2.

The results of *S. conicum* feeding on a mixed diet of *P. parvum*, at different growth phases, with *R. lens* as the non-toxic alga, are shown in Fig. 7. The ratio of *P. parvum* to *R. lens* had a significant effect on growth rates (one-way ANOVA, $P < 0.0001$). Algal growth phase had no significant effect on toxicity ($P = 0.49$). In treatments containing intermediate and high concentrations of *P. parvum* (2.4×10^4, 4.3×10^4 and 4.8×10^4 cells ml^{-1}), *S. conicum* exhibited 100% mortality. However, in the treatments containing low concentrations of *P. parvum* (0.5×10^4 cells ml^{-1}), *S. conicum* exhibited higher growth rates on the mixed diet (average $\mu = 0.59$ per day), than on only *R. lens* (3×10^4 cells ml^{-1}, average $\mu = 0.46$ per day) (Table 3).

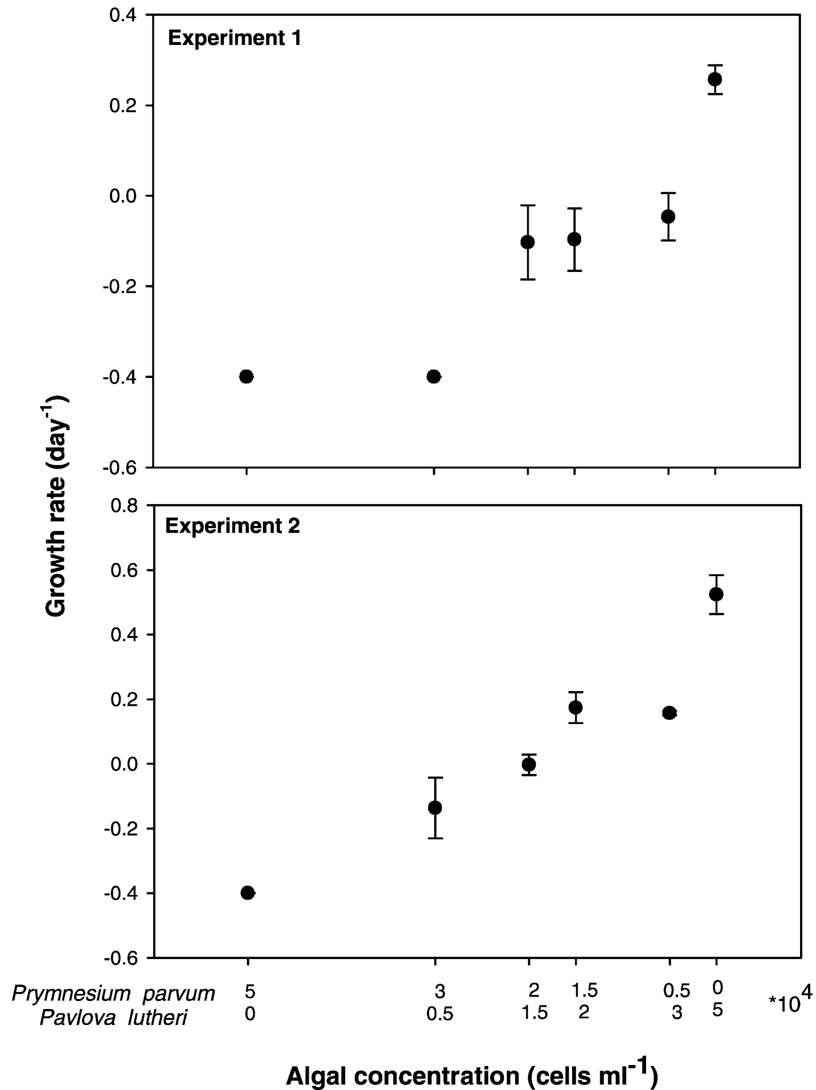

Fig. 3. Alternative food experiments 1 and 2, *F. ehrenbergii* feeding on a mixed diet of *P. parvum* and *P. lutheri*. Mixed diet algal concentrations total 3.5×10^4 cells ml^{-1} whereas uni-algal treatments are 5×10^4 cells ml^{-1}. Growth rate of 0.4 per day = 100% mortality as 1 was added to ciliate abundance to avoid ln[0]. Points represent means ± 1 S.E. ($n = 3$).

4.3. Numerical response experiments

Fig. 8 shows the results of the experiments in which the ciliates, *F. ehrenbergii*, *Strombidinopsis* sp., *M. angulata* and *E. pectinis* were fed a range of concentrations of *P. minimum* strains Exuv and JA 98-01. A two-way ANOVA showed a significant difference in the effect of Exuv and JA 98-01 on growth rates for the ciliates *F. ehrenbergii*, *Strombidinopsis* sp. and *M. angulata* ($P < 0.05$). Algal concentration also exhibited a significant effect on ciliate growth rates ($P < 0.0001$).

As shown in Fig. 8A, *F. ehrenbergii* grew well on Exuv ($\mu_{max(Exuv)} = 0.51$ per day), but only achieved positive growth at the highest concentrations (1.5×10^4 cells ml^{-1}) of JA 98-01 ($\mu_{max(JA\ 98-01)} = 0.22$ per

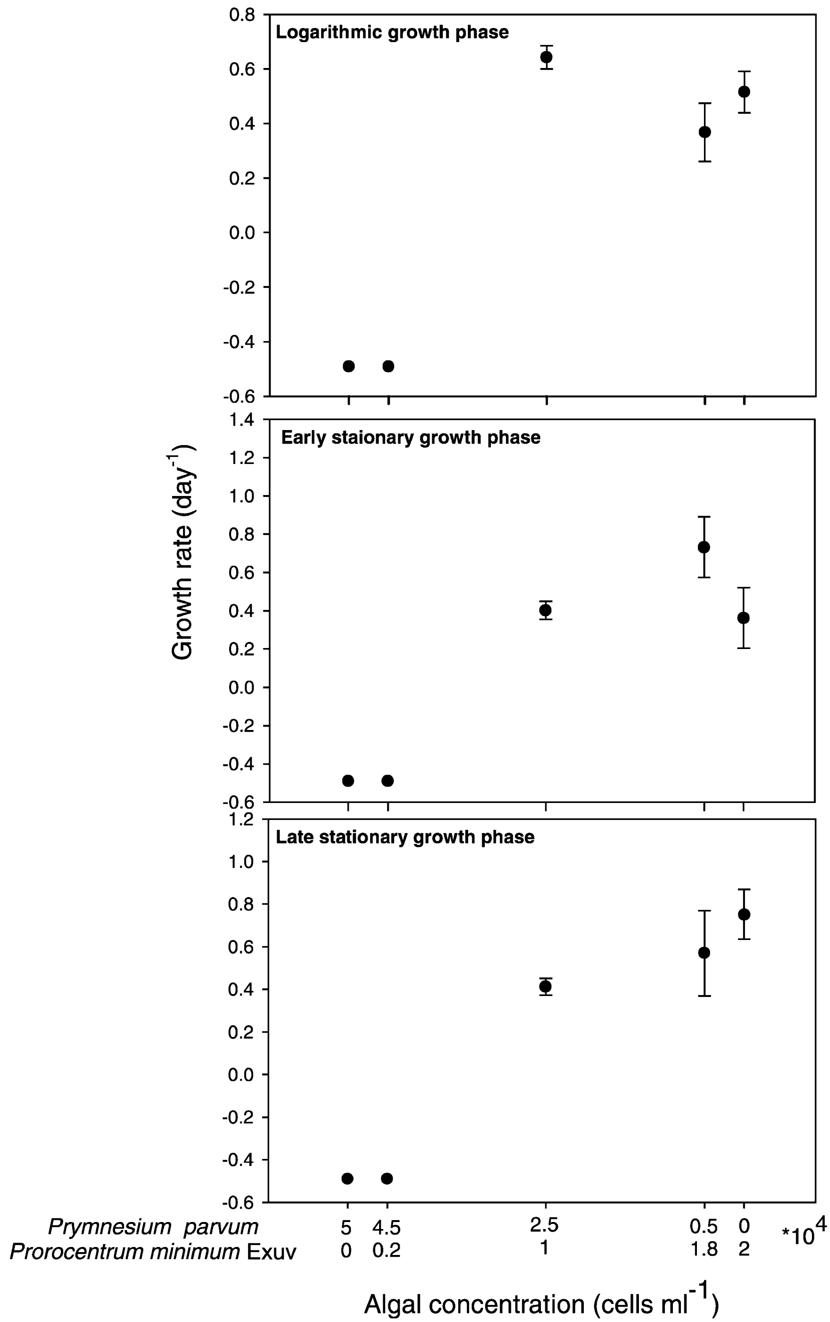

Fig. 4. Alternative food experiments with *F. ehrenbergii* feeding on a mixed diet of *P. parvum* (at different growth phases) and *P. minimum* (Exuv). Concentrations are normalized to cell volumes. Growth rate of 0.5 per day = 100% mortality as 1 was added to ciliate abundance to avoid ln[0]. Points represent means $\pm$ 1 S.E. ($n = 4$).

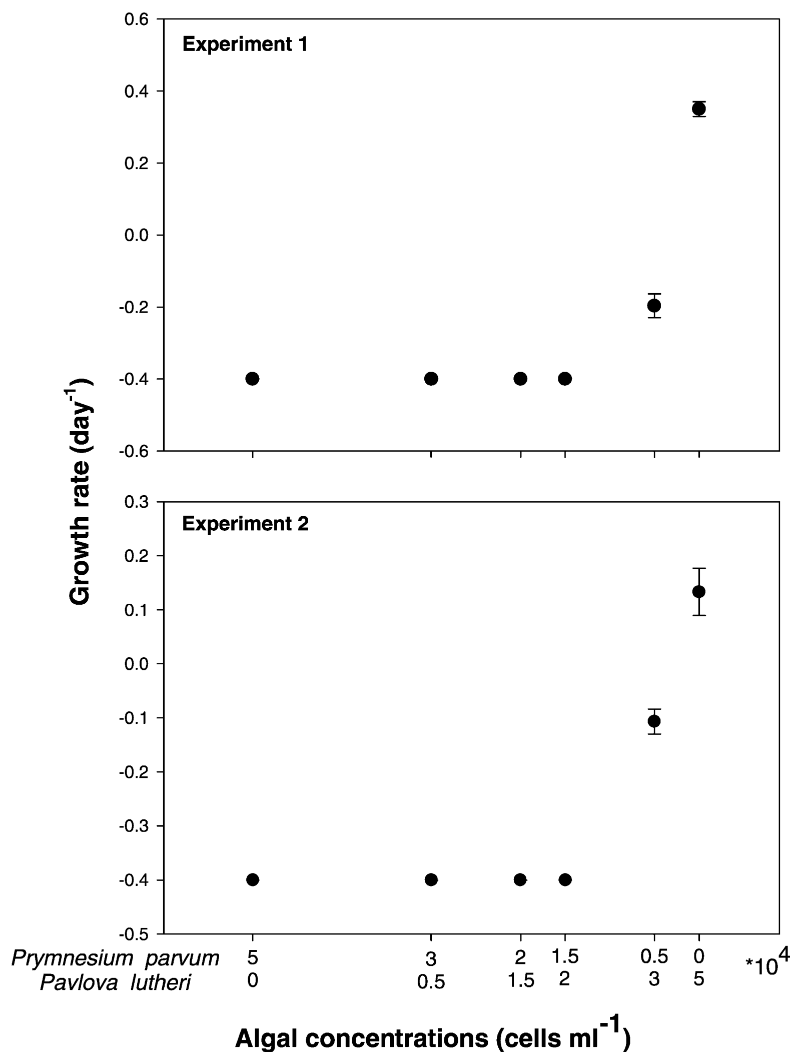

Fig. 5. Alternative food experiments 1 and 2, *Strombidinopsis* sp. feeding on a mixed diet of *P. parvum* and *P. lutheri*. Mixed diet algal concentrations total 3.5×10^4 cells ml^{-1} whereas uni-algal treatments are 5×10^4 cells ml^{-1}. Growth rate of 0.4 per day = 100% mortality as 1 was added to ciliate abundance to avoid ln[0]. Points represent means ± 1 S.E. ($n = 3$).

Table 1
Growth rates (per day) of *F. ehrenbergii* at different concentrations of *P. parvum* (in either logarithmic, early and late stationary growth phase) relative to *P. minimum*

Concentration (cells ml^{-1})		Growth rate of *F. ehrenbergii* (per day)		
P. parvum	*P. minimum*	Logarithmic	Early stationary	Late stationary
2.5×10^4	1×10^4	0.64 NS	0.40 NS	0.41 NS
0.5×10^4	1.8×10^4	0.37 NS	0.73**	0.57 NS
0	2×10^4	0.51 NS	0.36 NS	0.75 NS

NS: not significantly different. Overall there was no significant difference among growth phases (ANOVA).
** Significant $P = 0.01$, Tukey–Kramer post-hoc test comparing growth phases.

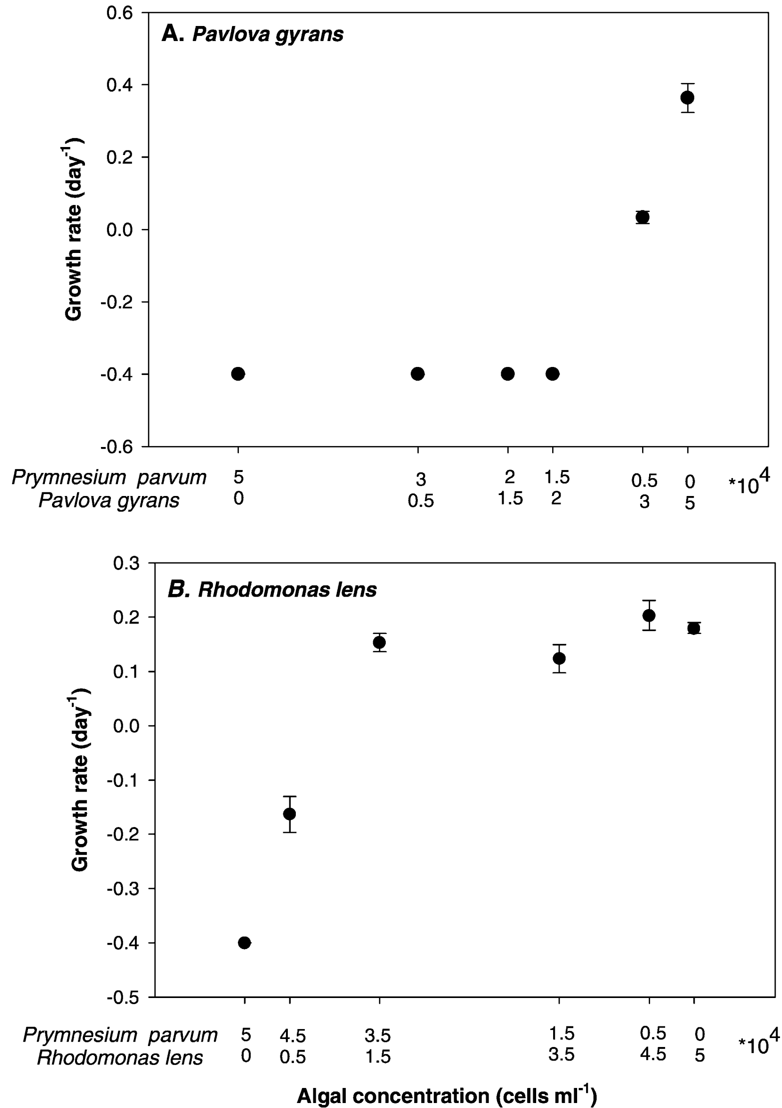

Fig. 6. Alternative food experiments with *Strombidinopsis* sp. feeding on a *P. parvum* mixed with *P. gyrans* (A), or *R. lens* (B). In (A) mixed diet algal concentrations total 3.5×10^4 cells ml^{-1} whereas uni-algal treatments are 5×10^4 cells ml^{-1}. Growth rate of 0.4 per day = 100% mortality as 1 was added to ciliate abundance to avoid ln[0]. Points represent means ± 1 S.E. ($n = 3$).

day; ANOVA comparing algal strains, $P = 0.0015$). *F. ehrenbergii* growth rates on Exuv increased with algal concentration (Tukey–Kramer post-hoc test comparing concentrations, $P < 0.05$). The strain JA 98-01 did not support growth of *F. ehrenbergii* at low concentrations (500 cells ml^{-1}; Tukey–Kramer post-hoc test $P > 0.05$).

Strombidinopsis sp. attained excellent growth rates on both strains of *P. minimum* ($\mu_{\max(\text{Exuv})} = 1.64$ per day and $\mu_{\max(\text{JA 98-01})} = 1.39$ per day; one-way ANOVA, $P < 0.05$; Fig. 8B). Growth rates were positive even at the lowest concentration of algae (500 cells ml^{-1}). Ciliate growth rates decreased slightly at high concentrations (3×10^4 cells ml^{-1}) of both

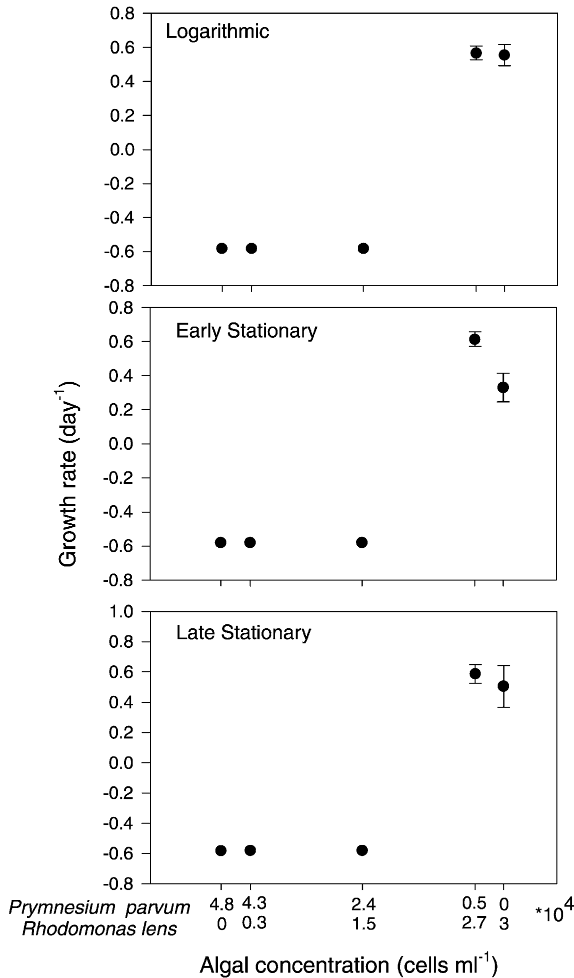

Fig. 7. Alternative food experiments with *S. conicum* feeding on a mixed diet of *P. parvum* (at different growth phases) and *R. lens*. Concentrations normalized for cell volume. Growth rate of 0.6 per day = 100% mortality as 1 was added to ciliate abundance to avoid ln[0]. Points represent means ± 1 S.E. ($n = 4$).

Table 2
Growth rates (per day) of *Strombidinopsis* sp. on a mixed diet of *R. lens* and *P. parvum*

Concentration (cells ml^{-1})		Growth rate (per day)
P. parvum	*R. lens*	*Strombidinopsis* sp.
3.5×10^4	1.5×10^4	0.15
1.5×10^4	3.5×10^4	0.12
0.5×10^4	4×10^4	0.20
0	5×10^4	0.18

There were no significant differences among treatments (ANOVA).

Exuv and JA 98-01, and the two strains were not significantly different from each other (Tukey–Kramer post-hoc test, $P = 0.0933$).

Growth rates of *M. angulata* were higher on Exuv ($\mu_{max(Exuv)} = 0.45$ per day) compared to JA 98-01 ($\mu_{max(JA\ 98-01)} = 0.20$ per day), and there was a significant difference in growth between the strains (one-way ANOVA, $P = 0.0014$; Fig. 8D). Growth rates increased with the concentration of both algal strains (Tukey–Kramer post-hoc test $P < 0.05$). However, for strain JA 98-01 no growth occurred at concentrations exceeding 1×10^4 cells ml^{-1} (Tukey–Kramer post-hoc test, $P < 0.05$). *E. pectinis* did not grow on either strain of *P. minimum* (Fig. 8C).

5. Discussion

We found that the ciliates *F. ehrenbergii* and *M. angulata* grew better on the Exuv strain of *P. minimum* than the JA 98-01 strain, although there were no acute toxic effects of either strain. This is consistent with results showing that JA 98-01 is more toxic than Exuv to molluscs (G. Wikfors pers com), as well as reports that algae in culture for a long time may decrease in toxicity (Cembella and Therriault, 1998; Burkholder et al., 2001), though we cannot rule out nutritional differences between strains. *Strombidinopsis* sp. grew equally well on both strains of *P. minimum*. In the present study *Eutintinnus* sp. did not grow on either strain, however, in a previous study (data not shown) this ciliate grew well on the Exuv strain ($\mu_{max} = 0.7$ per day). This may be attributed to variability between ciliate strains isolated from Long Island Sound at different times. The ciliate growth rates we measured were similar to those reported in other studies using *P. minimum* (Table 4).

All ciliates examined (*F. ehrenbergii*, *Strombidinopsis* sp., and *S. conicum*) exhibited acute toxic responses to the prymnesiophyte *P. parvum* at concentrations of this alga found in natural blooms (10^3–10^9 cells ml^{-1}) (Aure and Rey, 1992). Ciliates exhibited behavioral responses consistent with those described in the literature, including reverse beating of ciliary membranelles, sinking to the bottom of experimental wells, altered swimming behavior and ultimately dying (Capriulo, 1990; Buskey and Stoecker, 1988; Taniguchi and Takeda, 1988; Buskey and Stoecker,

Table 3
Growth rates (per day) of *S. conicum* at the lowest concentration of *P. parvum* to *R. lens* and 100% *R. lens* at logarithmic, early and late stationary growth phases

Concentration (cells ml^{-1})		Growth rate of *S. conicum* (per day)		
P. parvum	*R. lens*	Logarithmic	Early stationary	Late stationary
0.5×10^4	2.7×10^4	0.57 NS	0.61**	0.59 NS
0	3×10^4	0.55 NS	0.33**	0.50 NS

NS: not significantly different.
** Significant $P = 0.0003$, Tukey–Kramer post-hoc test comparing treatments.

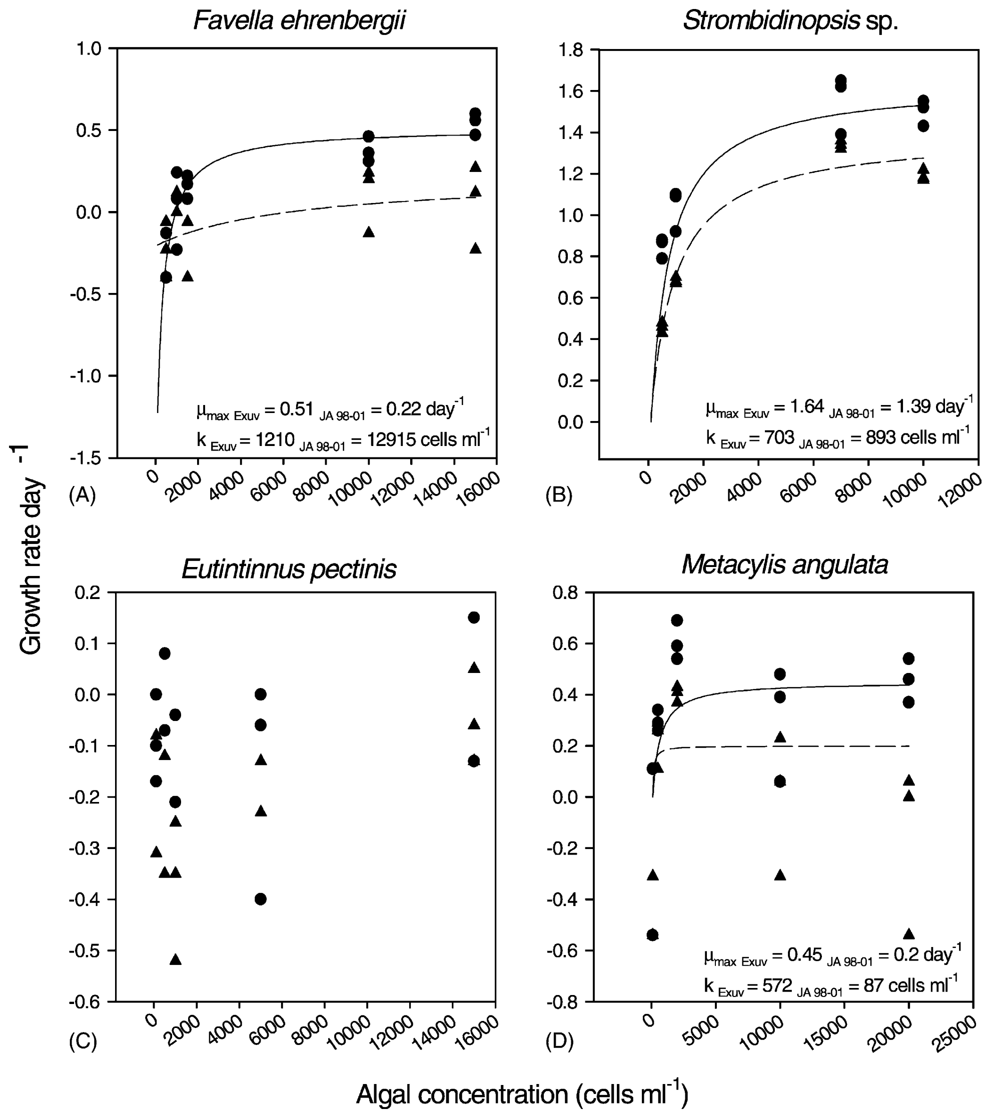

Fig. 8. (A–D) Growth of ciliates on *P. minimum* strains (●, —) Exuv and (▲, – – –) JA 98-01 at a range of concentrations. Points represent means ± 1 S.E. ($n = 3$). *E. pectinis* did not grow on either strain and a curve was not fit to the data.

Table 4
Growth rates of ciliates (μ per day) on *P. minimum* Exuv and JA 98-01 compared to reported growth rates on *P. minimum*

Ciliate species	$\mu_{(Exuv)}$ this study (per day)	$\mu_{(JA\ 98-01)}$ this study (per day)	μ reported (per day)	Reference
Favella sp.	0.55	0.10	0.71	Taniguchi and Kawakami, 1983
Strombidinopsis sp.	1.55	1.34	1.06	Jeong et al., 1999
Eutintinnus sp.	0.03	0.03	0.53	Taniguchi and Kawakami, 1983
M. angulata	0.61	0.40	Not reported	

1989; Stoecker et al., 1995). In the acute toxicity tests ciliates died more quickly in treatments containing whole cells of *P. parvum* than in treatments of culture filtrate; thus ingestion of the alga is necessary for the toxic response.

Consistent with previously published results, *P. parvum* was most toxic in early or late stationary growth phase (Hansen, 1989; Jiawan et al., 1996). However, when *P. parvum* was offered in mixed diet experiments, the same phenomenon was not observed. Instead, ciliates survived and at times grew well at concentrations of *P. parvum* which given alone would kill them. This decrease in toxicity may be attributed to the presence of non-toxic algal species, nutrient enrichment from experimental media or a shift in algal growth phase during the experiment, but it is most likely due to ciliates avoiding ingesting the *P. parvum* when non-toxic food is available.

As long as a non-toxic alga was available, ciliates survived and at times grew well at concentrations of *P. parvum* (5×10^2–3×10^4 cells ml^{-1}) that would otherwise have killed them. Ciliates may select against *P. parvum* in the presence of non-toxic algal prey, or low concentrations of *P. parvum* may provide some nutritional benefit in mixed diets. *Strombidinopsis* sp. and *S. conicum* actually grew slightly better on a mixture of *R. lens* and *P. parvum* than on *R. lens* alone.

The different degrees to which the two HAB species are toxic to ciliates may provide some insight into the role of grazing in bloom formation. For example, *P. minimum* and *P. parvum* appear to form blooms through different mechanisms. *P. minimum* is molluscotoxic, mixotrophic, and has high growth rates. It blooms in shallow estuarine bays and populations decline as they are exported to deeper water where grazing by ciliates is more important than that of molluscs and other benthic suspension feeders (Li et al., 1996; Stoecker et al., 1997; Manoharan et al., 1999).

P. parvum, on the other hand, appears to reduce microzooplankton grazing pressure directly and may inhibit competition from other algal species by the secretion of phycotoxic compounds (Parnas, 1963; Aure and Rey, 1992; Moestrup and Larsen, 1992; Hallegraeff, 1993; Igarashi et al., 1996; Koski et al., 1999). In addition, *P. parvum* is able to grow heterotrophically on dissolved organic matter, giving it a competitive advantage over autotrophic algae for nutrients (Johnsen and Lein, 1989). When concentrations of *P. parvum* exceeded 10^4 cells ml^{-1}, ciliate grazers died in our experiments. In the field, this mortality would reduce grazing pressure and allow *P. parvum* to increase in density, thus starting a positive feedback loop, which would facilitate bloom formation. Our findings are similar to reports of bloom development and persistence due to disturbance of grazer populations by *Aureococcus anophagefferens*, and the *Emiliania huxleyi* and *Alexandrium* spp. blooms (Smayda and Villareal, 1989a,b; Buskey et al., 1997; Bricelj and Lonsdale, 1997; Vance et al., 1998; Gobler et al., 2002).

Many aspects of trophic interactions within HABs remain to be addressed. Extrapolation of laboratory experiments such as ours can be enhanced if observations if HAB grazing in situ can be quantified. Methods of addressing this include using labeled (fluorescent or radioactive) algae and antibody or immunochemical techniques (Rublee and Gallegos, 1989; Ohman et al., 1991; Putt, 1991; Li et al., 1996; Kamiyama, 2000). Also, more detailed studies of the complexity of trophic interactions in blooms are needed (Nejstgaard, 1997). Studies examining the effects of larger zooplankton, such as copepods, on microzooplankton assemblages as well as interactions between zooplankton and organisms at higher

trophic levels, such as ctenophores, fish and shellfish will elucidate the food web dynamics of planktonic consumers and harmful algal species.

Toxicity of HAB species to ciliate grazers (e.g. *P. parvum* in our experiments) may enhance bloom formation; but lack of toxicity (e.g. *P. minimum* in our experiments) may also be important if it leads to the transfer of toxins to higher trophic levels. White (1979) reported that *Favella* sp. contained the majority of saxitoxin measured in a 64 μm sample during peak *Gonyaulax excavata* toxicity. Also, copepods were observed capturing *Favella* sp. containing ingested *Gonyaulax* cells. It is therefore necessary to continue to examine toxin accumulation and transfer via planktonic grazer populations (White, 1979; White, 1981; Smayda and Villareal, 1989a,b; Vance et al., 1998; Gifford, 1991; Buskey et al., 1997; Bricelj and Lonsdale, 1997; Turner and Tester, 1997; Maneiro et al., 2000; Haley, 2002). Since toxicity of algal species in culture may decrease over time (Cembella and Therriault, 1998; Burkholder et al., 2001) and cultured ciliates reportedly differ in feeding responses compared to ciliates caught in the field (Capriulo, 1990), experiments using field-collected ciliate grazers and harmful algal species should be performed. Colin and Dam (2002) reported a biogeographical component in the ability of copepods to graze the harmful alga *Alexandrium* spp. Perhaps this phenomenon also occurs in ciliates and naïve species are more susceptible to harmful effects. There is still a relative paucity of information on the formation and fate of harmful algal blooms. Future research both in natural blooms and in the laboratory on all facets of HABs will lead to a better understanding of blooms and allow for their management and mitigation.

6. Summary

- Both strains (Exuv and JA 98-01) of *P. minimum* support ciliate growth and show no acute toxic effects but growth on Exuv was higher.
- *P. parvum* is toxic to ciliates at concentrations exceeding 10^4 cells ml^{-1}, however at low concentrations of *P. parvum* and culture filtrate ciliates survived.
- In mixed diets, as long as a non-toxic alga was available, ciliates survived and at times grew well at concentrations of *P. parvum* that could otherwise kill them.
- Ciliates may select against *P. parvum* in the presence of non-toxic algal prey, or low concentrations of *P. parvum* may provide a nutritional benefit.

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References

Arzul, G., Seguel, M., Guzman, L., Erard-LeDenn, E., 1999. Comparison of allelopathic properties in three toxic *Alexandrium* species. J. Exp. Mar. Biol. Ecol. 232, 285–295.

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.

Banse, K., 1994. Grazing and zooplankton production as key controls for phytoplankton production in the open ocean. Oceanography 7, 13–20.

Breteler, W.C.M.K., Schogt, N., Baas, M., Schouten, S., Kraay, G.W., 1999. Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids. Mar. Biol. 135, 191–198.

Bricelj, V.M., Lonsdale, D.J., 1997. *Aureococcus anophagefferens*: causes and ecological consequences of brown tides in U.S. mid-Atlantic coastal waters. Limnol. Oceanogr. 42, 1023–1038.

Brussaard, C.P.D., Reigman, R., Noordeloos, A.A.M., Cadee, G.C., Witte, H., Kop, A.J., Nieuwland, G., Van Duyl, F.C., Bak, R.P.M., 1995. Effects of grazing, sedimentation and phytoplankton cell lysis on the structure of a coastal pelagic food web. Mar. Ecol. Prog. Ser. 123, 259–271.

Brussaard, C.P.D., Gast, G.J., Van Duyl, F.C., Riegman, R., 1996. Impact of phytoplankton bloom magnitude on a pelagic microbial food web. Mar. Ecol. Prog. Ser. 144, 211–221.

Burkholder, J.M., Glasgow, H.B., Deamer-Melia, N., 2001. Overview and present status of the toxic Pfiesteria complex (Dinophyceae). Phycologia 40, 186–214.

Buskey, E.J., Stoecker, D.K., 1988. Locomotory patterns of the planktonic ciliate *Favella* sp.: adaptations for remaining within food patches. Bull. Mar. Sci. 43, 783–796.

Buskey, E.J., Stoecker, D.K., 1989. Behavioral responses of the marine tintinnid *Favella* sp. to phytoplankton: influence of chemical, mechanical and photic stimuli. J. Exp. Mar. Biol. Ecol. 132, 1–16.

Buskey, E.J., Montagna, P.A., Amos, A.F., Whitledge, T.E., 1997. Disruption of grazer populations as a contributing factor to the initiation of the Texas brown tide algal bloom. Limnol. Oceanogr. 42, 1215–1222.

Capriulo, G.M., 1982. Feeding of field-collected tintinnid micro-zooplankton on natural food. Mar. Biol. 71, 73–86.

Capriulo, G.M., 1990. Feeding-related ecology of marine protozoa. In: Capriulo, G.M. (Ed.), Ecology of Marine Protozoa. Oxford University Press, New York, pp. 86–259.

Capriulo, G.M., Carpenter, E.J., 1980. Grazing by 35 to 202 um micro-zooplankton in Long Island Sound. Mar. Biol. 56, 319–326.

Capriulo, G.M., Carpenter, E.J., 1983. Abundance, species composition and feeding impact of tintinnid microzooplankton in central Long Island Sound. Mar. Ecol. Prog. Ser. 10, 277–288.

Cembella, A.D., Therriault, J.C., 1998. Comparative toxicity of cultured isolates and natural populations of *Protogonyaulax tamarensis* (Lebour) Taylor from the St. Lawrence. J. Shellfish Res. 7, 152.

Colin, S.P., Dam, H.G., 2002. Latitudinal differentiation in the effects of the toxic dinoflagellate *Alexandrium* spp. on the feeding and reproduction of populations of the copepod *Acartia hudsonica*. Harmful Algae 1, 113–125.

Cyr, H., Pace, M.L., 1992. Grazing by zooplankton and its relationship to community structure. Can. J. Fish. Aquat. Sci. 49, 1455–1465.

Edvardsen, B., Paasche, E., 1998. Bloom dynamics and physiology of *Prymnesium* and *Chrysochromulina*. In: Anderson, S.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms. Springer-Verlag, New York, pp. 193–208.

Frangópulos, M., Guisande, C., Maniero, I., Riveiro, T., Franco, J., 2000. Short term and long-term effects of the toxic dinoflagellate *Alexandrium minutum* on the copepod *Acartia clausi*. Mar. Ecol. Prog. Ser. 175, 87–96.

Gifford, D.J., 1985. Laboratory culture of marine planktonic oligotrichs (Ciliophora, Oligotrichidia). Mar. Ecol. Prog. Ser. 23, 257–267.

Gifford, D.J., 1991. The protozoan-metazoan trophic link in pelagic ecosystems. J. Protozool. 38, 81–86.

Gifford, D.J., Caron, D.A., 2000. Sampling, preservation, enumeration and biomass of marine protozooplankton. ICES Zooplankton Methodology Manual. Academic Press, San Diego, CA, pp. 193–221.

Gobler, C.J., Renaghan, M.J., Buck, N.J., 2002. Impacts of nutrients and grazing mortality on the abundance of *Aureococcus anophagefferens* during a New York brown tide bloom. Limnol. Oceanogr. 47, 129–141.

Gold, K., Morales, E.A., 1975. Seasonal changes in lorica sizes and the species of Tintinnida in the New York Bight. J. Protozool. 22, 520–528.

Guillard, R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Changley, M.H. (Eds.), Culture of Marine Invertebrate Animals. Plenum Press, New York, pp. 29–60.

Haley, S.T., 2002. Toxin dynamics in populations of the copepod *Acartia hudsonica* resistant and non-resistant to the harmful dinoflagellate *Alexandrium fundyense*. Masters Thesis. University of Connecticut, pp. 1–40.

Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. Phycologia 32, 79–99.

Hansen, P.J., 1989. The red tide dinoflagellate *Alexandrium tamarense*: effects on behavior and growth of a tintinnid ciliate. Mar. Ecol. Prog. Ser. 53, 105–116.

Hansen, P.J., Cembella, A.D., Moestrup, O., 1992. The marine dinoflagellate *Alexandrium ostenfeldii*: paralytic shellfish toxin concentration, composition, and toxicity to a tintinnid ciliate. J. Phycol. 28, 597–603.

Igarashi, T., Satake, M., Yasumoto, T., 1996. Prymnesin-2: a potent ichthytoxic and hemolytic glycoside isolated from the red tide alga *Prymnesium parvum*. J. Am. Chem. Soc. 118, 479–480.

Igarashi, T., Satake, M., Yasumoto, T., 1999. Structures and partial stereochemical assignments for Prymnesin-1 and Prymnesin-2: potent hemolytic and ichythytoxic glycosides isolated from the red tide alga *Prymnesium parvum*. J. Am. Chem. Soc. 121, 8499–8511.

Imai, I., Ishida, Y., Hata, Y., 1993. Killing of marine phytoplankton by a gliding bacterium *Cytophaga* sp., isolated from the coastal sea of Japan. Mar. Biol. 116, 173–185.

Jeong, H.J., Latz, M.I., 1994. Growth and grazing rates of the heterotrophic dinoflagellates *Protoperidinium* spp. on red tide dinoflagellates. Mar. Ecol. Prog. Ser. 106, 173–185.

Jeong, H.J., Shim, J.H., Lee, C.W., Kim, J.S., Koh, S.M., 1999. Growth and grazing rates of the marine planktonic ciliate *Strombidinopsis* sp. on red-tide and toxic dinoflagellates. J. Euk. Microbiol. 46, 69–76.

Jiawan, H., Chen, M., Zhenrong, H., 1996. Isolation and characterization of toxins from the phytoflagellate *Prymnesium parvum*. Acta Hydrobiol. Sinica 20, 41–48.

Johnsen, T.M., Lein, T.E., 1989. *Prymnesium parvum* Carter (Prymnesiophyceae) in association with macroalgae in Ryfylke, southwestern Norway. Sarsia 74, 227–281.

Kamiyama, T., 1997. Growth and grazing responses of tintinnid ciliates feeding on the toxic dinoflagellate *Heterocapsa circularisquama*. Mar. Biol. 128, 509–515.

Kamiyama, T., 2000. Application of a vital staining method to measure feeding rates of field ciliate assemblages on a harmful alga. Mar. Ecol. Prog. Ser. 197, 299–303.

Koski, M., Rosenberg, M., Viitasalo, C., Tanskanen, S., Sjölund, U., 1999. 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.

Lawrence, J.E., Chan, A.M., Suttle, C.A., 2001. A novel virus (HaNIV) causes lysis of the toxic bloom-forming alga *Heterosigma akashiwo* (Raphidophyceae). J. Phycol. 37, 216–222.

Lee, J., Capriulo, G., 1990. The ecology of marine protozoa: an overview. In: Capriulo, G.M. (Ed.), Ecology of Marine Protozoa. Oxford University Press, New York, pp. 3–45.

Li, A., Stoecker, D.K., Coats, D.W., Adam, J.A., 1996. Ingestion of fluorescently labeled and phycoerythrin-containing prey by mixotrophic dinoflagellates. Aquat. Microb. Ecol. 10, 139–147.

Malone, T.C., 1971. The relative importance of nanoplankton and netplankton as primary producers in the California current system. Fish. Bull. 69, 799–820.

Maneiro, I., Frangópulos, M., Guisande, C., Fernández, M., Reguera, B., Riveiro, I., 2000. Zooplankton as a potential vector of diarrhetic shellfish poisoning toxins through the food web. Mar. Ecol. Prog. Ser. 201, 155–163.

Manoharan, K., Lee, T.K., Cha, J.M., Kim, J.H., Lee, W.S., Chang, M., Park, C.W., Cho, J.H., 1999. Acclimation of *Prorocentrum minimum* (Dinophyceae) to prolonged darkness by use of an alternative carbon source from triacylglycerides and galactolipids. J. Phycol. 35 (2), 287–292.

McManus, G.B., Ederington-Cantrell, M.C., 1992. Phytoplankton pigments and growth rates, and microzooplankton grazing in a large temperate estuary. Mar. Ecol. Prog. Ser. 87, 77–85.

Moestrup, Ø., Larsen, J., 1992. Potentially toxic phytoplankton 1. Haptophyceae (Prymnesiophyceae). In: Lindley, J.A. (Ed.), ICES Identification Leaflets for Phytoplankton. NERC Plymouth Marine Laboratory, Plymouth, England, pp. 1–11.

Montagnes, D.J.S., 2001. © The Planktonic ciliate project, guide to UK coastal planktonic ciliates. The University of Liverpool, Port Erin Marine Laboratory, Port Erin, Isle of Man, British Isles IM9 6JA. http://www.liv.ac.uk/ciliate/.

Montagnes, D.J.S., Lynn, D.H., 1991. Taxonomy of Choreotrichs, the major marine ciliates, with emphasis on the aloricate forms. Mar. Micro. Food Webs. 5, 59–74.

Montagnes, D.J.S., Taylor, F.J.R., 1994. The salient features of five marine ciliates in the class spirotrichea (Oligotrichia), with notes on their culturing and behavior. J. Euk. Microbiol. 41, 569–586.

Montagnes, D.J.S., Lessard, E.J., 1999. Population dynamics of the marine planktonic ciliate *Strombidinopsis multiaris*: its potential to control phytoplankton blooms. Aquat. Microb. Ecol. 20, 167–181.

Nagasaki, K., Ando, M., Imai, I., Itakura, S., Ishida, Y., 1994a. Virus-like particles in *Heterosigma akashiwo* (Raphidophyceae): a possible red tide disintegration mechanism. Mar. Biol. 119, 307–312.

Nagasaki, K., Ando, M., Itakura, S., Imai, I., Ishida, Y., 1994b. Viral mortality in the final stage of *Heterosigma akashiwo* (Raphidophyceae) red tide. J. Plankton Res. 16, 1595–1599.

Needler, A.B., 1949. Paralytic shellfish poisoning and *Gonyaulax tamarensis*. J. Fish. Res. Bd. Can. 7, 490–504.

Nejstgaard, J.C., 1997. Food web dynamics in flagellate-dominated marine environments. Trophic interactions between haptophytes, calanoid copepods and microzooplankton. Doctoral Thesis 1997. University of Bergen, Norway, pp. 5–52.

Nejstgaard, J.C., Solberg, P.T., 1996. Repression of copepod feeding and fecundity by the toxic haptophyte *Prymnesium patelliferum*. Sarsia 81, 339–344.

Ohman, M.D., Theilacker, G.H., Kaupp, S.E., 1991. Immunochemical detection of predation on ciliate protists by larvae of the northern anchovy (*Engraulis mordax*). Biol. Bull. 181, 500–504.

Parnas, I., 1963. The toxicity of *Prymnesium parvum*. Israel J. Zool. 12, 15–23.

Pierce, R.W., Turner, J.T., 1992. Ecology of planktonic ciliates in marine food webs. Rev. Aquat. Sci. 6, 139–181.

Plumley, G.F., 1997. Marine algal toxins biochemistry, genetics, and molecular biology. Limnol. Oceanogr. 42, 1252–1264.

Putt, M., 1991. Development and evaluation of tracer particles for use in microzooplankton herbivory studies. Mar. Ecol. Prog. Ser. 77, 27–37.

Rublee, P.A., Gallegos, C.L., 1989. Use of fluorescently labeled algae (FLA) to estimate microzooplankton grazing. Mar. Ecol. Prog. Ser. 51, 221–227.

Sellner, K.G., Brownlee, D.C., 1990. Dinoflagellate-microzooplankton interactions in Chesapeake Bay. In: Granéli, E., Sundström, B., Edler, L., Anderson, D.M. (Eds.), Toxic Marine Phytoplankton. Elsevier, New York, pp. 221–226.

Smayda, T.J., 1997. What is a bloom? A commentary. Limnol. Oceanogr. 42, 1132–1136.

Smayda, T.J., Villareal, T.A., 1989a. The 1985 "brown tide" and the open phytoplankton niche in Narragansett Bay during summer. In: Cosper, E.M., Bricelj, V.M., Carpenter, E.J. (Eds.), Novel phytoplankton blooms: causes and impacts of recurrent brown tides and other unusual blooms. Springer-Verlag, New York, pp. 159–187.

Smayda, T.J., Villareal, T.A., 1989b. An extraordinary, noxious brown-tide in Narragansett Bay. Part I. The organism and its dynamics. In: Okaichi, T., Anderson, D.M., Nemoto, T. (Eds.), Red Tides Biology, Environmental Science and Toxicology. Elsevier, New York, pp. 129–132.

Stoecker, D.K., Guillard, R.R.L., Kavee, R.M., 1981. Selective predation by *Favella ehrenbergii* (Tintinnia) on and among dinoflagellates. Biol. Bull. 160, 36–145.

Stoecker, D.K., Cucci, T.L., Hulburt, E.M., Yentsch, C.M., 1986. Selective feeding by *Balanion* sp. (Ciliata: Balanionidae) on phytoplankton that best support its growth. J. Exp. Mar. Biol. Ecol. 95, 113–130.

Stoecker, D.K., Gallager, S.M., Langdon, C.J., Davis, L., 1995. Particle capture by *Favella* sp. (Ciliata, Tintinnina). J. Plankton Res. 17, 1105–1124.

Stoecker, D.K., Li, A., Coats, D.W., Gustafson, D.E., Nannen, M.K., 1997. Mixotrophy in the dinoflagellate *Prorocentrum minimum*. Mar. Ecol. Prog. Ser. 152 (1), 1–12.

Strom, S.L., Morello, T.A., 1998. Comparative growth rates and yields of ciliates and heterotrophic dinoflagellates. J. Plankton Res. 20, 571–584.

Strom, S.L., Wolfe, G., Holmes, J., Stecher, H., Shimeneck, C., Lambert, S., Moreno, E., 2003a. Chemical defense in the microplankton I: feeding and growth rates of heterotrophic protists on the DMS-producing phytoplankter Emiliania huxleyi. Limnol. Oceanogr. 48, 217–229.

Strom, S.L., Wolfe, G., Slajer, A., Lambert, S., Clough, J., 2003b. Chemical defense in the microplankton II: inhibition of protist feeding by β-dimethylsulfoniopropionate (DMSP). Limnol. Oceanogr. 48, 230–237.

Taniguchi, A., Kawakami, R., 1983. Growth rates of ciliate *Eutintinnus lususundae* and *Favella taraikaensis* observed in laboratory culture experiments. Bull. Plankton Soc. Jpn 30, 33–40.

Taniguchi, A., Takeda, Y., 1988. Feeding rate and behavior of the tintinnid ciliate *Favella taraikaensis* observed with a high speed VTR system. Marine Microb. Food Webs. 3, 21–34.

Tomas, C.R., Hasle, G.R., Syvertsen, E.E., Steidinger, K.A., Tanger, K., Throndsen, J., Heimdal, B.R., 1996. In: Tomas, C. (Ed.), Identifying Marine Phytoplankton. Academic Press, San Diego, CA.

Turner, J.T., Tester, P.A., 1997. Toxic marine phytoplankton, zooplankton grazers and pelagic food webs. Limnol. Oceanogr. 42, 1203–1214.

Turner, J.T., Doucette, G.J., Powell, C.L., Kulis, D.M., Keafer, B.A., Anderson, D.M., 2000. Accumulation of red tide toxins in larger size fractions of zooplankton assemblages from Massachusetts Bay, USA. Mar. Ecol. Prog. Ser. 203, 95–107.

Vance, T.C., Schumacher, J.D., Stabeno, P.J., Baier, C.T., Wyllie-Echeverria, T., Tynan, C.T., Brodeur, R.D., Napp, J.M., Coyle, K.O., Decker, M.B., Hunt Jr., G.L., Stockwell, D., Whitledge, T.E., Jump, M., Zeeman, S., 1998. Aquamarine H_2O recorded for the first time in eastern Bering Sea. EOS 79, 121–126.

Walsh, J.J., 1976. Herbivory as a factor in patterns of nutrient utilization in the sea. Limnol. Oceanogr. 21, 1–13.

Watras, C.J., Garcon, V.C., Olson, R.J., Chisholm, S.W., Anderson, D.M., 1985. The effect of zooplankton grazing on estuarine blooms of the toxic dinoflagellate *Gonyaulax tamarensis*. J. Plankton Res. 7, 891–908.

White, A.W., 1979. Dinoflagellate toxins in phytoplankton and zooplankton fractions during a bloom of *Gonyaulax excavata*. In: Taylor, D.L., Seliger, H.H. (Eds.), Toxic Dinoflagellate Blooms. Elsevier, New York, pp. 381–384.

White, A.W., 1981. Marine zooplankton can accumulate and retain dinoflagellate toxins and cause fish kills. Limnol. Oceanogr. 26, 103–109.

Wikfors, G.H., Smolowitz R.M., 1993. Detrimental effects of a *Prorocentrum* isolate upon hard clams and bay scallops in laboratory feeding studies. In: Smayda, T.J., Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam, pp. 447–452.

Wikfors, G.H., Smolowitz, R.M., 1995. Experimental and histological studies of four life-history stages of the eastern oyster, *Crassostrea virginica*, exposed to a cultured strain of the dinoflagellate *Prorocentrum minimum*. Biol. Bull. 188, 313–323.

Wikfors, G.H., Alix, J.H., Smolowitz, R.M., 2000. Responses of bay scallops, at several life-history stages, to cultures of potentially-harmful marine microalgae. J. Shellfish. Res. 19, 584.

Wolfe, G.W., 2000. The chemical defense ecology of marine unicellular plankton: constraints, mechanisms and impacts. Biol. Bull. 198, 225–244.

Yih, W., Coats, D.W., 2000. Infection of *Gymnodinium sanguineum* by the dinoflagellate *Amoebophyra* sp.: effect of nutrient environment on parasite generation time, reproduction and infectivity. J. Eukaryot. Microbiol. 47, 504–510.

Zhou, J., Fritz, L., 1994. Okadaic acid antibody localizes to chloroplasts in the DSP-toxin-producing dinoflagellates *Prorocentrum lima* and *Prorocentrum maculosum*. Phycologia 33, 445–461.