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Growth and grazing on *Prochlorococcus* and *Synechococcus* by two marine ciliates

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Abstract

The two most abundant marine autotrophic prokaryotes, *Prochlorococcus* and *Synechococcus,* often have different distributions in the ocean. For example, *Synechococcus* is restricted to the first 100 m, whereas *Prochlorococcus* extends much deeper in oligotrophic waters. This is in part explained by differences in adaptation to nutrient and light regimes. However, they could also be subjected to different predation rates. To explore this hypothesis, we compared the consumption of these two picoplankters by an algivorous ciliate, *Strombidium sulcatum,* and a bactivorous ciliate, *Uronema* sp. For both ciliate species, removal rates were higher, by a factor of 3 to 10, for *Synechococcus* compared to *Prochlorococcus* when prey items were presented alone or together. The growth of the two ciliates fed *Synechococcus* and/or *Prochlorococcus* also differed. *S. sulcatum* grew well on both prey items, whether alone or together, whereas *Uronema* sp. grew slowly when fed *Synechococcus* and very poorly when fed *Prochlorococcus* either alone or with *Synechococcus.* Our results suggest that *Prochlorococcus* may be less subject to ciliate predation than *Synechococcus.*

Prokaryotic picoplankton often dominate phytoplankton assemblages in marine systems (Platt et al. 1983; Olson et al. 1985; Blanchot and Rodier 1996). For many open oceans, the contribution of one picophytoplankton group, *Synechococcus,* in terms of abundance and contribution to primary productivity has been recognized for nearly 20 yr (Johnson and Sieburth 1979; Waterbury et al. 1979; Morris and Glover 1981). The existence of *Prochlorococcus* was established relatively recently using flow cytometry, and it appears to have a significance, in terms of carbon fixation, comparable to that of *Synechococcus* (Chisholm et al. 1988).

The relative importance of *Prochlorococcus* differs among oceanic regions and often seems to vary inversely with that of *Synechococcus* (Campbell and Vaulot 1993; Li 1995; Landry et al. 1996; Partensky et al. 1996). In oligotrophic open waters, *Prochlorococcus* populations are more abundant and extend deeper in the water column than *Synechococcus* throughout most of the year (Olson et al. 1985; Chisholm et al. 1988; Campbell and Vaulot 1993; Campbell et al. 1994). The distinct distributions of *Synechococcus* and *Prochlorococcus* are generally thought to reflect adaptations to different nutrient and light regimes. For example, maximal *Prochlorococcus* concentrations have been reported to occur in nitrate-depleted layers (Lindell and Post 1995; Blanchot and Rodier 1996), whereas *Synechococcus* can be abundant in transition areas where nitrate is present (Chisholm et al. 1988; Glover et al. 1988*a*,*b*; Campbell and Vaulot 1993; Campbell et al. 1994). *Prochlorococcus* appears better adapted for growth at low light intensities relative to *Synechococcus* (Moore et al. 1995). However, it is worthwhile to point out that the observed distributions, usually attributed to different growth capacities, are the sum of both growth and mortality.

Chroococcoid cyanobacteria have long been observed in the food vacuoles of nanoplanktonic protists (Johnson et al. 1982), but their contribution to protist nutrition is uncertain. In culture studies, *Synechococcus* has been described as a poor food item for protists (Verity and Villareal 1986; Caron et al. 1991), while field populations of *Synechococcus* can apparently support rapid growth of some ciliates (Simek et al. 1995; Pérez et al. 1996; Simek et al. 1996). Data on the growth rate of *Prochlorococcus* are relatively abundant (e.g., Goericke and Welschmeyer 1993; Moore et al. 1995; Vaulot et al. 1995) compared to the little existing information on grazing losses (Liu et al. 1995; Reckermann and Veldhuis 1997). To our knowledge, there are no data on the food value of *Prochlorococcus.* The question arises then as to whether or not *Synechococcus* and *Prochlorococcus* are exploited similarly by protist grazers.

There are reasons to suspect that, although *Prochlorococcus* and *Synechococcus* are roughly similar in size, the two may be removed at different rates. Selective ingestion of picoplankton-sized particles by flagellates (Epstein and Shiaris 1992; Sherr et al. 1992; Jürgens and DeMott 1995) and ciliates (Turley et al. 1986; Sanders 1988; Simek et al. 1994; Christaki et al. 1998) has been reported. The ingestion of picoplankton can be affected by quality and motility of prey as well as small differences in prey size and the physiological state of the grazer (Sanders 1988; Snyder 1991; Christaki et al. 1998). Furthermore, even if a prey type is removed efficiently by grazers, it may not experience high grazing pressure over extended periods of time if it is an inadequate food source for the grazer.

Given these considerations, we thought it of interest to compare *Synechococcus* and *Prochlorococcus* as prey items for planktonic ciliates. We compared consumption of *Prochlorococcus* and *Synechococcus* by an algivorous ciliate, *S. sulcatum,* and a bactivorous ciliate, *Uronema* sp. Short-term

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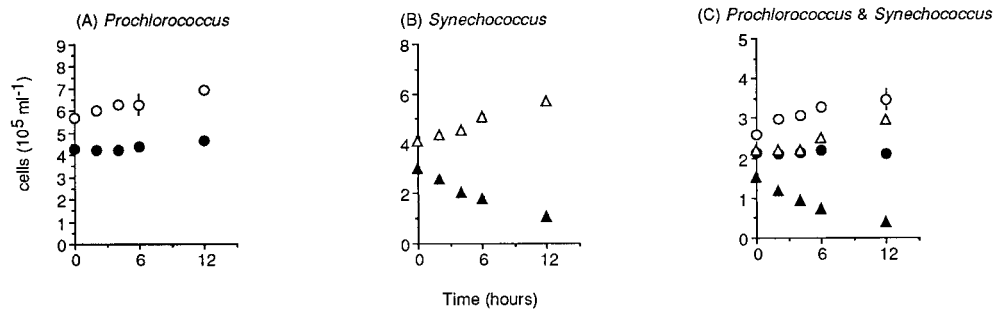

Fig. 1. *Strombidium sulcatum* ingestion: changes in cell concentrations of *Prochlorococcus, Synechococcus* in experiments with *S. sulcatum. S. sulcatum* culture with addition of (A) *Prochlorococcus* SS120, (B) *Synechococcus* WH8103, and (C) mixed SS120 and WH8103. Open symbols show prey concentrations in control solutions. Error bars show the range of duplicate cultures. Where error bars are not shown, the range is smaller than the symbol.

experiments were used to estimate ingestion rates and possible differential removal of *Prochlorococcus* and *Synechococcus.* Long-term experiments compared *Prochlorococcus* and *Synechococcus* as food sources for the two ciliates.

Materials and methods

Culture conditions—Prochlorococcus SS120 (Chisholm et al. 1992), approximately 0.65 μm in diameter, and *Synechococcus* WH8103 (Waterbury et al. 1986), originally isolated from Sargasso Sea and approximately 1.0 μm in length, were grown in 500-ml sterile flasks in n K/10—Cu medium in aged seawater as described in Scanlan et al. (1996). The two well-characterized strains (Moore et al. 1995) are typical of oligotrophic provinces of the open ocean (Campbell and Iturriaga 1988; Goericke and Welschmeyer 1993). Cultures of both populations were acclimated for 3 weeks to experimental conditions. Cultures were grown at 20 $\pm$ 0.5°C in a temperature-regulated room under continuous light (15 μE m^{-2} s^{-1}), provided by a pair of cool-white fluorescent bulbs wrapped in blue filter (Lee filter, band-pass at 475 nm). Neither *Prochlorococcus* nor *Synechococcus* cultures were axenic. The cultures used for the experiments were in exponential growth phase, with background heterotrophic bacterial densities of approximately 1×10^6 bacteria ml^{-1} compared to 1×10^7 autotrophs ml^{-1}.

S. sulcatum and *Uronema* sp., originally isolated from the bay of Villefranche-sur-Mer (Mediterranean Sea), were maintained in stock cultures on a bacterized wheat-grain medium (Rivier et al. 1985). To obtain exponentially growing cultures, protozoa inocula from stock cultures were transferred into bacterized yeast extract media (0.015–0.030 g liter^{-1}, *see* Christaki et al. 1998 for details).

Ingestion experiments—In short-term experiments, we estimated ingestion rates of *S. sulcatum* and *Uronema* sp. cultures feeding on (1) *Prochlorococcus* SS120, (2) *Synechococcus* WH 8103, or (3) mixed *Prochlorococcus* and *Synechococcus.* Ciliates were removed from late exponential growing cultures when the concentration was 0.25 and 1.0 $\times$ 10^3 ml^{-1} for *S. sulcatum* and *Uronema* sp., respectively. Fifty-milliliter aliquots of ciliate cultures were spiked with exponentially growing *Prochlorococcus* and/or *Synechococcus* cultures, yielding a final total concentration of prokaryotic autotrophs of approximately 5×10^5 ml^{-1}. The concentration of a particular picoautotroph was 5×10^5 ml^{-1} when offered alone and 1.5–2.5 $\times$ 10^5 ml^{-1} when offered with the other picoautotroph. In the ingestion experiment, heterotrophic bacteria from the ciliate and picoautotroph cultures were present in concentrations of about 7.5×10^6 ml^{-1}. Control solutions of picoautotrophs were prepared by adding the same concentration of autotrophs to 50 ml of 0.2-μm–fil-

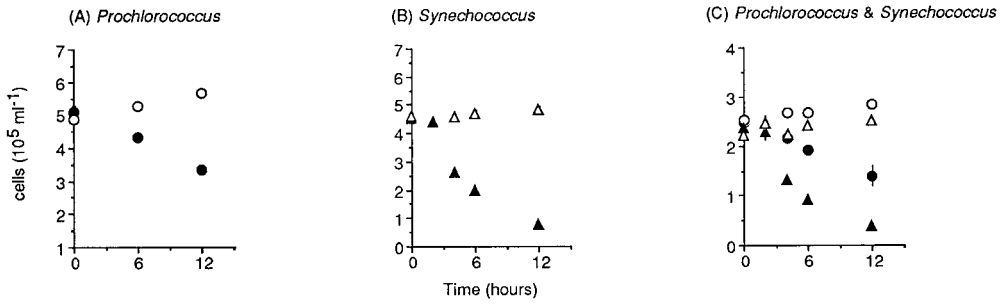

Fig. 2. *Uronema* sp. ingestion: changes in cell concentrations of *Prochlorococcus, Synechococcus* in ingestion experiments with *Uronema* sp. *Uronema* culture with addition of (A) *Prochlorococcus* SS120, (B) *Synechococcus* WH8103, and (C) mixed SS120 and WH8103. Open symbols show prey concentrations in control cultures. Error bars show the range of duplicate cultures. Where error bars are not shown the range is smaller than the symbol.

Table 1. Parameters from the ingestion experiment with *Strombidium sulcatum* feeding on *Prochlorococcus* and *Synechococcus,* calculated over 0–12 h.

	Prochloro-cococcus	*Synecho-coccus*	*Prochlorococococcus* + *Synechococcus* mixed culture	
Growth rate (h^{-1})*	0.017	0.02	0.015	0.02
Grazing rate (h^{-1})	0.010	0.105	0.012	0.130
Clearance rate (nl ciliate^{-1} h^{-1})	45.3	515	52.3	568
Average prey concentration (10^5 ml^{-1})	4.46	1.88	2.18	0.82
Average ciliate concentration (ml^{-1})	221	204	237	237
Ingestion (cells ciliate^{-1} h^{-1})	20.0	96	11	264
Specific clearance (10^4 body volume h^{-1})	0.29	3.29	0.34	3.64

* Growth rate of picoautotrophs in the control.

tered ciliate culture. All experimental and control bottles were prepared in duplicate (total of 12 bottles for each ciliate), and the bottles were incubated under the same light conditions as the original prokaryote cultures. Samples were removed for counts of picoautotrophs (2 ml) every 2 h over the first 6 h and at time 12 h. Samples for ciliate enumerations (5 ml) were taken at times 0 and 12 h.

Growth experiments—In a second series of experiments, we studied growth of *S. sulcatum* and *Uronema* sp. on exponentially growing cultures of (1) *Prochlorococcus* SS120, (2) *Synechococcus* WH 8103, and (3) a mixture of both *Prochlorococcus* and *Synechococcus.* The initial concentration of the picoautotrophs in these experiments was about 2.8×10^7 cells ml^{-1} for *Prochlorococcus* offered alone, 8×10^6 cells ml^{-1} for *Synechococcus* alone, and 1.8×10^7 autotrophs ml^{-1} when offered together. The initial abundances of heterotrophic bacteria from the ciliate and autotroph cultures were about 5×10^6 cells ml^{-1}. Concentrations of the ciliate inocula removed from stationary stock cultures were 10–20 cells ml^{-1} and 150 cells ml^{-1} for *S. sulcatum* and *Uronema* sp., respectively. Controls were prepared by adding, to the autotroph cultures, an equivalent volume (10–15 ml) of 0.2-μm–filtered ciliate culture. All experimental and control bottles were prepared in duplicate (total of 12 bottles for each ciliate species). Samples were taken every 6 or 12 h over 54 h from each of the flasks for protozoa cell counts (5 ml) and every 6 h (2-ml samples) for picoautotroph counts.

Flow cytometry analysis—Samples for picoplankton counts were processed similarly for both sets of experiments. Samples were divided into two aliquots. The first one, for autotrophic prokaryotes, was analyzed fresh by flow cytometry after dilution in 0.2-μm–filtered seawater. The second aliquot, for counts of heterotrophic bacteria, was preserved with paraformaldehyde fixation (1% final concentration), frozen in liquid nitrogen (modified from Vaulot et al. 1989), and stored at -80°C (Marie et al. 1997). For these analyses, the protocol of Marie et al. (1997) was employed. Briefly, the preserved samples were thawed and then stained with SYBR Green I (Molecular Probes).

A FACSort flow cytometer (Becton Dickinson) was used to analyze samples. The device provides two light scatter signals, corresponding to forward (FALS) and right-angle light scatters (RALS), and three fluorescence signals referred to as ''green'' (530 $\pm$ 15 nm), ''orange'' (585 $\pm$ 21 nm), and ''red'' (>650 nm), respectively, linked to DNA-dye fluorescence, phycoerythrin, and chlorophyll content of cells. Seawater, 0.2-μm–filtered, was used as the sheath fluid. Autotrophic populations were discriminated on the basis of RALS and the fluorescence of chlorophyll and of phycoerythrin for *Synechococcus.* Heterotrophic bacteria were discriminated on the basis of RALS vs. green-DNA fluorescence. All cellular parameters were normalized to the values measured for 0.95-μm beads (Polyscience). Acquisition was performed at a high rate (85–90 μl min^{-1}) for the unfixed samples and at a medium rate (40–50 μl min^{-1}) for bacterial counting. Data were collected in list mode files and then

Table 2. Parameters from the ingestion experiment with *Uronema* sp. feeding on *Prochlorococcus* and *Synechococcus* calculated over 0–12 h.

	Prochloro-cococcus	*Synecho-coccus*	*Prochlorococococcus* + *Synechococcus* mixed culture	
Growth rate (h^{-1})*	0.013	0.003	0.009	0.01
Grazing rate (h^{-1})	0.048	0.154	0.056	0.16
Clearance rate (nl ciliate^{-1} h^{-1})	44	148.2	54.5	154.6
Average prey concentration (10^5 ml^{-1})	4.16	2.09	1.89	1.09
Average ciliate concentration (ml^{-1})	1,087	1,040	950	950
Ingestion (cells ciliate^{-1} h^{-1})	18	31	10	17
Specific clearance (10^4 body volume h^{-1})	5.4	17.9	6.6	18.7

* Growth rate of picoautotrophs in the control.

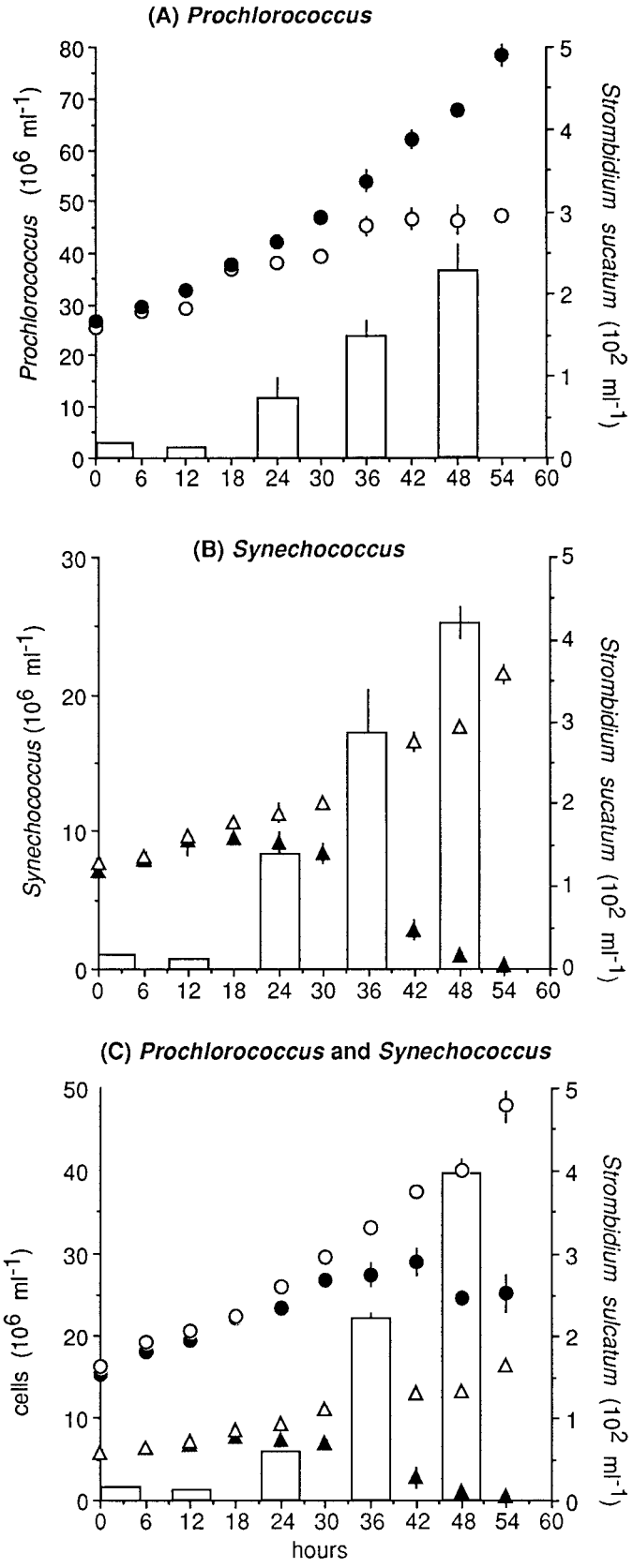

Fig. 3. *S. sulcatum* growth experiment: changes in cell concentrations of *S. sulcatum* and its prey (A) *Prochlorococcus* SS120 culture, (B) *Synechococcus* WH8103 culture, and (C) SS120 and WH8103 mixed culture. Error bars show the range of duplicate cultures. Where error bars are not shown, the range is smaller than the symbol.

analyzed using the Windows CYTOWIN freeware of Vaulot (1989), available through anonymous ftp server at ftp.sb-roscoff.fr/pub/cyto.

Ciliate abundance and data analysis—Samples for ciliate enumerations were fixed in Lugol's fixative (2% final concentration). Cell concentrations were determined with an inverted microscope by examining 2-ml aliquots in the baseplates of sedimentation chambers. Growth and grazing were calculated using the equations devised by Frost (1972) and modified by Heinbokel (1978). Biovolume of ciliates was estimated from linear dimensions as prolate spheroids (Verity et al. 1992).

Results

Ingestion experiments—Both ciliate species ingested *Synechococcus* and *Prochlorococcus*, and both showed an apparent preference for *Synechococcus* cells. A reduction in picoautotroph cell concentrations was evident after only 2–4 h of incubation (Figs. 1, 2) and was more pronounced for *Synechococcus*. The ciliates in these experiments were feeding on picoautotrophs in the presence of heterotrophic bacteria in the ciliate cultures. The concentration of heterotrophic bacteria was 0.5 and 1.0 $\times$ 10^7 ml^{-1} for *S. sulcatum* and *Uronema* sp. experiments, respectively, while that of the picoautotrophs was 10^5 ml^{-1} (Tables 1, 2; Figs. 1, 2). The grazing parameters were calculated for the time period 0–12 h, during which the prey decrease was linear.

Ciliate concentrations in experimental flasks did not change significantly over the 12-h period. The growth rates of both *Synechococcus* and *Prochlorococcus* in the experimental control bottles (to which filtered *S. sulcatum* culture fluid was added) were almost equivalent to growth in standard stock picoautotroph cultures (0.02 h^{-1}), while picoautotrophs with *Uronema* sp. culture fluid added grew at significantly lower rates (0.003–0.01 h^{-1}).

Both ciliate species cleared *Synechococcus* at much higher rates than *Prochlorococcus*. For *S. sulcatum*, clearance rates differed by an order of magnitude; rates estimated for the clearance of *Synechococcus* were on the order of 500 nl h^{-1} $cell^{-1}$ compared to 45 nl h^{-1} $cell^{-1}$ for the clearance of *Prochlorococcus* (Table 1). Nearly equivalent rates were estimated in the treatment in which the two picoautotrophs were offered together.

Uronema sp. ingested *Synechococcus* at rates approximately three times those estimated for *Prochlorococcus* (Table 2). Similar to results obtained with *S. sulcatum*, rate estimates differed little between treatments in which a single or both picoautotrophs were offered to the ciliates. In terms of specific clearance, there were large differences between the two ciliates. *Uronema* sp. cleared picoplankton at volume-specific rates of about an order of magnitude higher (10^4 – 10^5 body volumes h^{-1}) than *S. sulcatum* (10^3 – 10^4

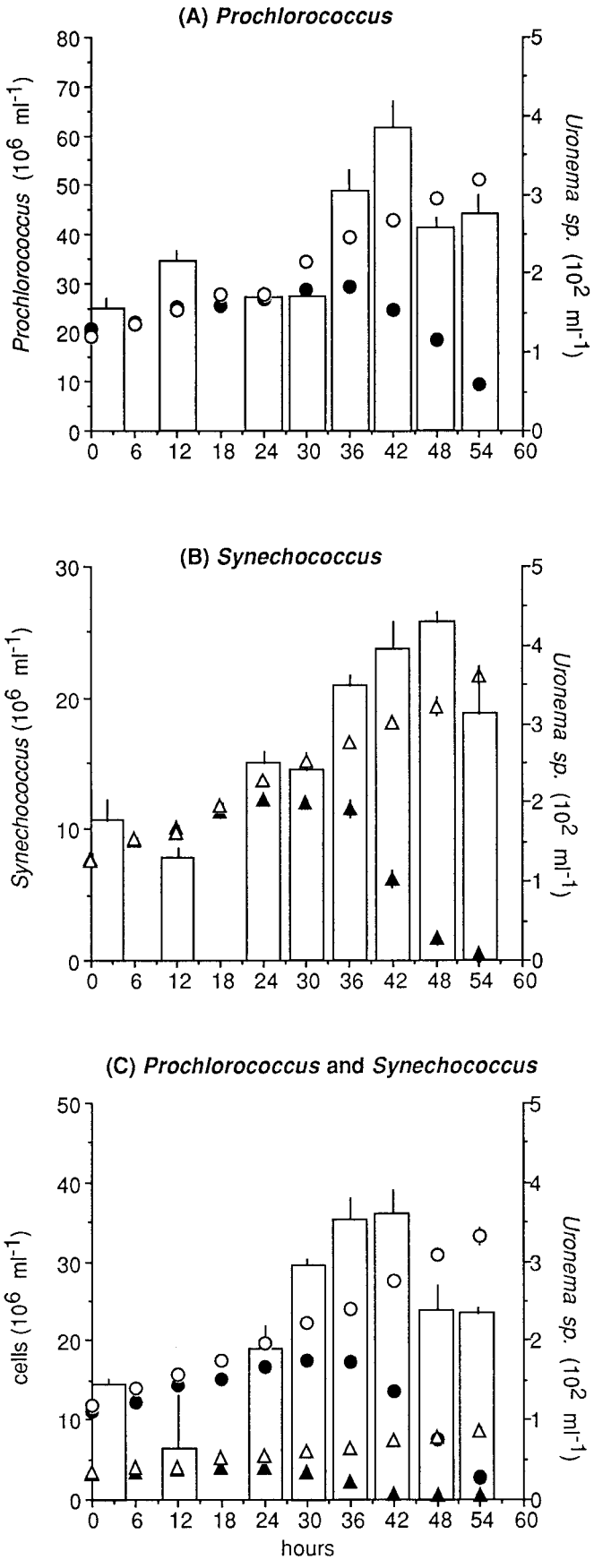

Fig. 4. *Uronema* sp. growth experiment: changes in cell concentrations of *Uronema* sp. and its prey (A) *Prochlorococcus* SS120 culture, (B) *Synechococcus* WH8103 culture, and (C) SS120 and Wh8103 mixed culture. Error bars show range of duplicate cultures. Where error bars are not shown, duplicate values are smaller than the size of the symbol.

body volumes h^{-1}) for *Synechococcus* and *Prochlorococcus*, respectively (Tables 1, 2).

Growth experiments—For both ciliate species, grazing pressure on the picoautotrophs was clearly evident after approximately 24 h of incubation when the concentration of prey in the experimental bottles started to decrease markedly. The different growth and grazing parameters for this experiment were calculated for the first 36 h to avoid artifacts due to possibly insufficient food concentrations after 36 h (Figs. 3, 4). The experiments revealed large differences between *S. sulcatum* and *Uronema* sp. While both ciliates grazed *Prochlorococcus* and *Synechococcus*, only the nanoplanktivore *S. sulcatum* grew well on these picoautotrophs (Figs. 3, 4; Tables 3, 4).

In the control cultures of autotrophs alone, growth rates were similar to those in stock algal cultures (μ = 0.02 h^{-1}), indicating that at least for the time of the incubation, the addition of ciliate culture solution probably did not have any significant stimulating or inhibitory effect on the growth of the picoautotrophs. *S. sulcatum* grew well on both prey items (Table 3) with generation times of 11 and 8.5 h, grazing on *Prochlorococcus* and *Synechococcus*, respectively. However, the grazing rate of *S. sulcatum* on *Synechococcus* was higher than the rate on *Prochlorococcus*, and this was also evident when the two picoautotrophs were offered together (Fig. 3; Table 3).

Uronema sp. ingested picoautotrophs at high rates; however, its growth rate was modest, particularly in the presence of *Prochlorococcus*. Growth rates of ciliates fed *Synechococcus* and *Prochlorococcus* were 0.025 h^{-1} and 0.018 h^{-1}, respectively, compared to 0.08 h^{-1} in stock cultures grown on heterotrophic bacteria. The cell volume of the ciliate, measured at 36 h of growth, did not show the same trend; biovolume was highest in the *Synechococcus* diet (775 μm^3) and lowest in the *Prochlorococcus* diet (442 μm^3). From microscopic counts of *Uronema* sp. grown on *Prochlorococcus*, we observed a high frequency of dividing *Uronema* sp. cells from time 12 h. Surprisingly, cell concentrations did not accordingly increase. We sampled *Uronema* sp. every 6 h instead of at 12 h to follow closely changes in the concentration of cells; cell numbers did increase (μ = 0.018–0.026 h^{-1}), but growth was not typically exponential (Fig. 4). Moreover, *Uronema* sp. cell numbers decreased at 48 h in *Prochlorococcus* culture and at 54 h in the *Synechococcus* culture. When *Uronema* sp. is grown on heterotrophic bacteria, exponential growth at rates of about 0.08 h^{-1} generally continues for up to 96 h (Christaki et al. 1998).

Prochlorococcus and *Synechococcus* cultures were not axenic. However, calculations indicate heterotrophic bacteria were a minor portion of available prey. In terms of carbon,

Table 3. Parameters from growth experiment with *Strombidium sulcatum* feeding on *Prochlorococcus* and *Synechococcus.*

	Prochloro-cococcus	*Synecho-coccus*	*Prochlorococococcus* + *Synechococcus* mixed culture	
Picoautotrophs (control)				
Growth rate (h^{-1})	0.02	0.02	0.02	0.02
Strombidium sulcatum				
Growth rate (h^{-1})	0.064	0.082	0.075	0.075
Grazing rate (h^{-1})	0.004	0.025	0.004	0.025
Clearance rate (nl ciliate^{-1} h^{-1})	65	272	48	273
Average prey concentration (10^5 ml^{-1})	33.3	4.2	19.3	3.3
Average ciliate concentration (ml^{-1})	78	122	115	115
Ingestion (cells ciliate^{-1} h^{-1})	2,164	1,142	926	900
Specific clearance (10^4 body volume h^{-1})	0.41	1.73	0.31	1.74

heterotrophic bacteria in the autotroph cultures were mostly small cells, probably containing about 20 fg cell^{-1} (Lee and Fuhrman 1987) compared to approximately 250 fg cell^{-1} for cultured *Synechococcus* (Kana and Glibert 1987) or 50 fg cell^{-1} for *Prochlorococcus* (Calliau et al. 1996). Using these values, background concentrations of heterotrophic bacteria equalled 5–7% of total prokaryotic carbon in the cultures. However, this relatively low percentage represented 10^6 ml^{-1} bacterial numbers in the cultures. Such concentrations are exploitable by the ciliates; heterotrophic bacteria concentrations decreased in cultures where the ciliates were added. More bacteria were consumed by *Uronema* sp. than by *S. sulcatum;* however, calculations using carbon content values cited above suggest the bacterial carbon was probably <5% of the total carbon ingested by either *Uronema* sp. or *S. sulcatum.* Specifically, for *Uronema* sp., the bacterial carbon ingested was 4.8 and 3.2% of the total carbon ingested in *Prochlorococcus* and *Synechococcus* cultures, respectively. For *S. sulcatum,* these values were 2.2 and 1.6%, respectively, indicating that most of the biomass consumed by the ciliates was in the form of the autotrophs.

In comparing the ingestion rate and clearance rates in the two sets of experiments, it should be noted that prey concentrations varied over three orders of magnitude. For both ciliate species, the clearance rate on *Synechococcus* increased with decreasing prey concentration, while clearance of *Prochlorococcus* remained relatively constant (Fig. 5).

Discussion

Abundances of *Synechococcus* or *Prochlorococcus* are often in the range of mid -10^4 to low 10^5 ml^{-1} (Glover et al. 1986; Chisholm et al. 1988; Campbell et al. 1994) compared to the concentrations of 10^5 ml^{-1} used in the ingestion experiments. Our results show, then, that both ciliates ingested picoautotrophs at prey concentrations similar to those in the field and indicate that planktonic ciliates can likely exploit autotrophic picoplankton encountered in oceanic waters (Table 5). Thus, some of the primary production of prokaryotic picoplankton could be transferred directly to the microplankton community and made available for consumption by higher trophic levels. Supporting evidence of a such a direct trophic link has been found in field studies. Kudoh et al. (1990) examined *Synechococcus* grazing losses in different size

Table 4. Parameters from growth experiment of *Uronema* sp. feeding on *Prochlorococcus* and *Synechococcus.*

	Prochloro-cococcus	*Synecho-coccus*	*Prochlorococococcus* + *Synechococcus* mixed culture	
Picoautotrophs (control)				
Growth rate (h^{-1})	0.02	0.02	0.02	0.018
Uronema sp.				
Growth rate (h^{-1})	0.018	0.025	0.026	0.026
Grazing rate (h^{-1})	0.011	0.011	0.007	0.023
Clearance rate (nl ciliate^{-1} h^{-1})	47.6	46	30	100
Average prey concentration (10^6 ml^{-1})	24.8	9.5	13.3	4.0
Average ciliate concentration (ml^{-1})	223	230	230	230
Ingestion (cells ciliate^{-1} h^{-1})	1,180	437	399	400
Specific clearance (10^4 body volume h^{-1})	10	5.9	6	22
	(442 μm^3)*	(775 μm^3)*	(496 μm^3)*	(496 μm^3)*

* Biovolume of the ciliate at time 36 h, growing on picoautotrophs.

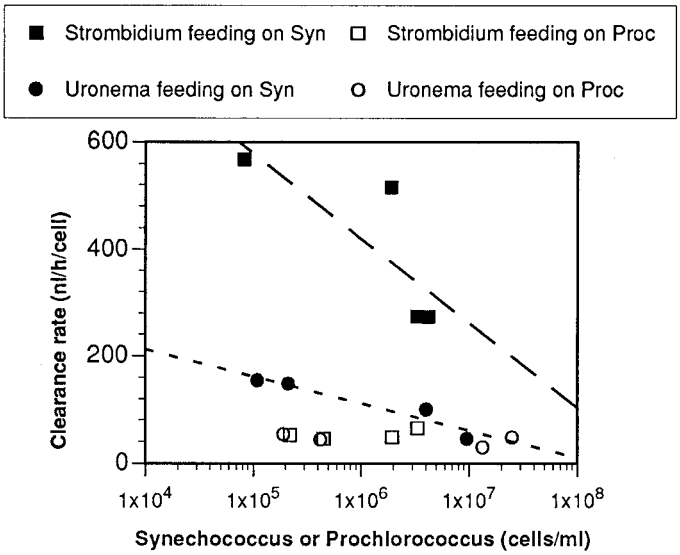

Fig. 5. The relationship between clearance rate and concentration of the picoautotrophs *Synechococcus* (Syn) and *Prochlorococcus* (Proc) for *S. sulcatum* and *Uronema* sp.

fractions of natural plankton communities and found that grazing by small ciliates was higher than grazing by an assemblage of flagellates. These authors concluded that more than two-thirds of the grazing mortality of *Synechococcus* spp. could be due to ciliates in waters of the North Pacific.

However, *Synechococcus* could suffer very different grazing losses from ciliates compared to *Prochlorococcus.* Grazing rates, in terms of clearance or specific clearance, were much higher for *Synechococcus* than for *Prochlorococcus,* whether the picoplankters were presented alone or together. Plotting clearance rates of *S. sulcatum* or *Uronema* sp. against prey concentration (Fig. 5) indicated that clearance of *Synechococcus* was sensitive to *Synechococcus* concentration, with higher clearance rates at lower *Synechococcus* concentrations. In contrast, for both ciliates, clearance of *Prochlorococcus* showed little variability with the concentration of *Prochlorococcus.*

The basis of the apparent discrimination is unclear. In a recent study, Christaki et al. (1998), using the same ciliate species and a variety of picoplankton-sized prey analogs, found clearance rates to vary with prey size and prey surface characteristics, as well as the physiological state of the ciliate grazer. There is a distinct difference in size between *Synechococcus* and *Prochlorococcus.* However, for *S. sulcatum,* the difference in clearance rates, a factor of 10 between *Prochlorococcus* and *Synechococcus* cells of about 0.65 and 1.0 μm in diameter, respectively, is much greater than the differences in rates that Christaki et al. estimated using fluorescent microspheres between 0.5 and 1 μm in diameter. Thus, the difference between the clearance of *Prochlorococcus* and *Synechococcus* by *S. sulcatum* is difficult to ascribe to size alone. Similarly, for *Uronema* sp., the large differences in rates estimated for *Prochlorococcus* and *Synechococcus* are in contrast to the small differences in clearance rates found with microspheres between 0.5 and 1.0 μm in diameter reported by Christaki et al. Furthermore, for *Synechococcus,* in contrast to *Prochlorococcus,* clearance rates changed with prey concentration (Fig. 5). Therefore, for both ciliate species, the differences in clearance rates are difficult to ascribe to size or volume-related contact rates alone. The mechanism involved is more likely one of differences in surface characteristics of the two picoplankters.

While picophytoplankton probably contribute to the diet of ciliate communities, from our data, it is uncertain that they, in general, constitute a high quality food for these consumers. We found that both *Synechococcus* and *Prochlorococcus* could yield high growth rates in the algivorous-bacterivorous *S. sulcatum* (Fig. 3; Table 3). These findings concerning an oligotrich parallel those of Simek et al. (1995), who showed that cyanobacteria might supply most of the carbon of a pelagic ciliate community dominated by oligotrichs and that freshwater oligotrichs can survive on a diet of picoplankton (Simek et al. 1996). However, in *Uronema* sp., *Synechococcus* yielded moderate growth, and *Prochlorococcus* may be a poor food (Fig. 4; Table 4). Such results with a bacteriovorous ciliate and *Synechococcus* are similar to those of previous studies. Johnson et al. (1982) found that a *Uronema* sp. grew on a mixed diet of chroococcoid cyanobacteria and heterotrophic bacteria, and Caron et al. (1991) showed that cyanobacterial prey alone yielded growth in a hymenostome and a scuticociliate but that growth rates, in all cases, were lower than those on bacterial prey alone.

Our data on the growth response of *Uronema* sp. to *Prochlorococcus* are intriguing. *Uronema* sp. grew poorly (barely significant changes in cell concentrations), despite significant ingestion (up to 1×10^3 cells ciliate^{-1} h^{-1}; Table 4) and even when ingesting *Synechococcus* as well. It is possible that *Prochlorococcus* may have inhibited or interfered with cell division. We noted many dividing cells during the first 12 h, which did not appear to translate into an increase in cell concentration, and the growth ''curve'' resembled a ''saw-tooth'' pattern in the presence of *Prochlorococcus* (Fig. 4). Unfortunately, no comparative data concerning the food value of *Prochlorococcus* for other bacteriovores exist.

Our experiments used ciliates grown on heterotrophic bacteria and thus, the grazers were not acclimatized to the experimental prey. It may be thought that this could have influenced our results, especially in the growth experiments; however, we believe that this was not the case. Regardless of previous exposure, *S. sulcatum* digests *Synechococcus* cells at the same rate (Dolan and Simek 1997) as the flagellate *Bodo saltans* (Dolan and Simek 1998). Given that digestion rates are insensitive to previous exposure to a prey item, there seems little reason to assume that growth rate should vary with previous exposure.

Field studies to date of growth and apparent grazing losses of autotrophic picoplankton are dominated by data on *Synechococcus,* with relatively little information on *Prochlorococcus* (Table 5). However, growth of both appear to be commonly in the range of one division per day with a corresponding grazing mortality of about 50% of the stock per day. Based simply on the clearance rates from our laboratory study, ciliates are probably less important as grazers on *Prochlorococcus* than on *Synechococcus.* The difference be-

Table 5. Growth (h^{-1}) and grazing rates (h^{-1}) of *Prochlorococcus* and *Synechococcus.*

Species	Growth of picoautotrophs (h^{-1})	Mortality of picoautotrophs (h^{-1})	Predator	Location	Technique	Source
Synechococcus spp.	0.01–0.04	0–0.0069	Diverse assemblage of micrograzers	Great Barrier Reef, Australia	Dilution technique (Landry and Hasset 1982)	Ayukai 1996
Synechococcus spp.	0.02–0.04	0.012–0.05	Diverse assemblage of micrograzers	NW Indian Ocean	Dilution technique	Burkill et al. 1993
Synechococcus spp.	0.063	0.01–0.034	Diverse assemblage of micrograzers	Warm core eddy Coastal station	Dilution technique + metabolic inhibitors	Campbell and Carpenter 1986
Prochlorococcus	0.004–0.02	ND†	ND	Sargasso Sea (surface layer)	^{14}C uptake	Goericke and Welshmeyer 1993
Synechococcus spp.	0.05–0.095 Mean: 0.095	0.008–0.016 Mean: 0.0125	Diverse assemblage of micrograzers	North Pacific	^{14}C-labelled *Synechococcus*	Iturriaga and Mitchell 1986
Synechococcus spp.	0.02–0.05	ND	ND	NW Atlantic	^{14}C uptake	Iturriaga and Marra 1988
Synechococcus spp.	0.1	0.04 0.06	Small flagellates Ciliates $>$ 10 μm	Oceanic surface waters, Japan	FDC frequencies of cell numbers	Kudoh et al. 1990
Synechococcus spp.	0.066	0.008	Diverse assemblage of micrograzers	Hawaii	Dilution technique	Landry et al. 1984
Prochlorococcus spp. *Synechococcus* spp.	0.028 0.016	ND	Diverse assemblage of micrograzers	Central Equatorial Pacific	Flow cytometry	Landry et al. 1996
Prochlorococcus spp. *Synechococcus* spp.	0.013–0.024 0.016–0.044	0.0016–0.02 0.0037–0.019	Diverse assemblage of micrograzers	Hawaii, coastal and oceanic station	Selective inhibitors of procaryotes	Liu et al. 1995
Prochlorococcus spp.	0.06*	Total mortality = growth	Diverse assemblage of micrograzers	Equatorial Pacific	Difference between observed and expected cell abundance	Liu et al. 1997
Prochlorococcus SS120 *Synechococcus* WH8103	0.02* 0.04*	ND	ND	Culture	Flow cytometry	Moore et al. 1995
Synechococcus spp.	0.0625	ND	ND		Cultures	Morris and Glover 1981
Prochlorococcus spp. *Synechococcus* spp.	0.01–0.038 0.0283	0.01–0.03 0.0279	Diverse assemblage of micrograzers	Red Sea	Measured as bulk Chla by flow cytometry in $<$20-, $<$10-, $<$3-, $<$2-μm size fractions	Reckermann and Veldhuis 1997
Prochlorococcus spp.	0.06*	ND	ND	Equatorial Pacific	Flow cytometry	Vaulot et al. 1995
Synechococcus spp.	0.02	0.01	Diverse assemblage of micrograzers	English Channel	^{14}C labelled *Synechococcus*	Xiuren and Vaulot 1992
Prochlorococcus SS120	0.002	0.004–0.056	*Strombidum sulcatum* (oligotrich)	Cultures	Flow cytometry	This study
Synechococcus WH8103	0.002	0.0011–0.016	*Uronema* sp. (scuticociliate)			

* Maximum growth.
† ND, not done.

comes even more apparent if one considers that often ciliates and *Synechococcus* are more abundant in surface waters than *Prochlorococcus.* Scuticociliates, such as *Uronema* sp., are generally more abundant at shallow depths (Dolan and Marrasé 1995), and recently, nanociliates (ciliates <20 μm in length) were found to be strongly correlated with zeaxanthin, a pigment associated with *Synechococcus,* over four diel cycles (Pérez et al. in press).

Unfortunately, little is known about the composition or abundance of the ciliate community in waters dominated by *Prochlorococcus.* However, the grazing losses experienced by *Prochlorococcus* are unlikely to be dominated by ciliates. The major consumers of *Prochlorococcus* are probably nanoflagellates, which, relative to ciliates, represent an additional trophic link between picoplankton primary producers and higher trophic levels. Thus, the pathway of *Prochlorococcus* carbon to higher trophic levels would involve consumption by nanoflagellates, followed by ciliate consumption of the nanoflagellates. This raises the possibility that carbon fixed by *Prochlorococcus* is more likely to be mineralized within the microbial food web than carbon fixed by *Synechococcus.* Our results suggest, then, that the different distributions of the two autotrophic picoplankters correspond with different roles in the microbial food web.

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