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NOTE

Grazing impact of two small heterotrophic flagellates on *Prochlorococcus* and *Synechococcus*

Laure Guillou[1,*], Stéphan Jacquet[1,], Marie-Josèphe Chrétiennot-Dinet[2], Daniel Vaulot[1]**

[1]Station Biologique de Roscoff, CNRS, INSU and Université Pierre et Marie Curie, Place Georges Teissier, BP 74, 29682 Roscoff Cedex, France
[2]Observatoire Océanologique de Banyuls, CNRS, INSU and Université Pierre et Marie Curie, BP 44, 66651 Banyuls-sur-mer, France

ABSTRACT: In open oceanic waters, phytoplankton biomass is dominated by organisms below 2 to 3 µm in size (pico- and small nanophytoplankton). The cell concentration of these populations is very stable in time and space as a consequence of nutrient limitation and strong grazing pressure. Although the identity of the organisms that directly graze on picoplankton is largely unknown, they are thought to be very small, i.e. <3 to 5 µm. Here, we analyze the grazing impact of 2 small flagellates, *Symbiomonas scintillans* and *Picophagus flagellatus*, upon 2 oceanic cyanobacteria, *Prochlorococcus* and *Synechococcus*. *S. scintillans* does not feed on the 2 cyanobacteria. In contrast, *P. flagellatus* appears as an active predator capable of drastically reducing prey concentrations. The flagellate displays a substantial division rate of the order of 2 doublings d^{-1} when fed on *Prochlorococcus* cells, but no significant growth is recorded when *Synechococcus* is used as prey. As the majority (>80%) of *P. flagellatus* cells can pass throughout a 2 µm filter, the impact of such tiny predators should be taken into consideration during field experiments that rely on size fractionation to separate grazers from prey.

KEY WORDS: Heterotrophic flagellates · Stramenopiles · Picoplankton · *Picophagus flagellatus* · *Symbiomonas scintillans* · *Prochlorococcus* · *Synechococcus* · Grazing

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In most oligotrophic and mesotrophic areas of the world oceans, primary production is dominated by cells of <2 to 3 µm (picophytoplankton) (Li et al. 1983, Platt et al. 1983). Picophytoplankton is composed of the 2 cyanobacteria genera *Prochlorococcus* (Chisholm et al. 1992) and *Synechococcus* (Waterbury et al. 1979) and a poorly defined assemblage of eukaryotic algae (Johnson & Sieburth 1982, Andersen et al. 1996). Despite growth rates of the order of 1 division d^{-1} (Liu et al. 1995, Vaulot et al. 1995), the abundance of these populations remains at nearly constant levels over time scales ranging from days to years (Campbell et al. 1997). *Prochlorococcus* and *Synechococcus* cells are too small to be consumed directly by mesozooplankton, including small copepods and cladocerans. The carbon sequestered as a result of photosynthesis moves to higher trophic levels via intermediate small grazers, mainly identified as flagellates (Sherr et al. 1986, Hagström et al. 1988). Size fractionation experiments have revealed that the first level of grazers is significantly smaller than 5 µm (typically below 2 to 3 µm in diameter) in coastal waters (Wikner & Hagström 1988), in the upper water column of the Arctic Ocean (Sherr et al. 1997), and in more oligotrophic ecosystems, such as the Mediterranean Sea (Zohary & Robarts 1992), the Sargasso Sea (Caron et al. 1999), and the Arabian Sea (Reckerman & Veldhuis 1997). Nevertheless, most of these tiny oceanic planktonic predators remain unidentified.

During an oceanographic cruise conducted in the equatorial Pacific Ocean (OLIPAC, November 1994), we made systematic isolations to investigate picoplanktonic diversity. Surprisingly, without any addition of organic matter, 2 different heterotrophic flagellates of very small size (<4 µm) were isolated from surface waters in the transition zone separating the oligotrophic gyre from the equatorial upwelling (Guillou et al. 1999a). Because very little is known about the potential grazing activity of such small flagellates on picoplanktonic populations, we conducted laboratory experiments to examine the consumption by *Picophagus flagellatus* and *Symbiomonas scintillans* of the 2 widespread very small marine cyanobacteria *Prochlorococcus* (0.6 µm) and *Synechococcus* (1.0 µm).

Present addresses:
*Institut de Ciències del Mar, CISC Passeig Maritim de la Barceloneta 37–45, 08003 Barcelona, Spain.
E-mail: laure.guillou@icm.csic.es
**Station INRA d'Hydrologie Lacustre, Laboratoire Dynamique et Evolution des Communautés Phytoplanctoniques, UMR CARRTEL, BP 511, 74203 Thonon Cedex, France

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Materials and methods. *Picophagus flagellatus* (Chrysophyceae, RCC 22, RCC = Roscoff Culture Collection, Station Biologique de Roscoff, France, see www.sb-roscoff.fr/Phyto/collect.html for more information) and *Symbiomonas scintillans* (Bicosoecida, RCC 24) were initially isolated in K medium (Keller et al. 1987) by serial dilution from equatorial Pacific waters (150° W, 11° 50′ S, 15 m depth). One month before the grazing experiments, cultures of *P. flagellatus* and *S. scintillans* were acclimated to the culture conditions of *Prochlorococcus* SS 120 (CCMP 1375, CCMP = Culture Center for Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME) and *Synechococcus* WH 8103 in PCR-S11 medium (Rippka et al. 2000), at 19°C with 15 µmol quanta m^{-2} s^{-1} of continuous blue light provided by cool-white fluorescent bulbs wrapped in a blue filter (Lee filter, Panavision, France). Thin sections were prepared as in Guillou et al. (1999a).

Long- and short-term experiments were conducted using the same controls and experiment flasks. Controls were prepared by mixing different solutions of 0.2 µm-filtered medium from *Prochlorococcus* (Pro) (C1), *Synechococcus* (Syn) (C2), and predator (C3) cultures: (1) 50 ml of Pro culture + 150 ml of C3; (2) 50 ml of Syn culture + 150 ml of C3; (3) Pro and Syn, 25 ml of each prey cultures + 150 ml of C3; (4) 150 ml of predator culture + 25 ml of both C1 and C2. The other flasks received 150 ml of predator culture and 50 ml of prey cultures ([5] Pro + predator; [6] Syn + predator; [7] Pro + Syn + predator). First, we performed long-term experiments (6 d) during which we analyzed the grazing behavior and growth response of a given diet, i.e. *Prochlorococcus* and *Synechococcus* using exponentially growing prey. Second, we determined grazing parameters (grazing, clearance and ingestion rates, and specific clearance, see below) from short-term experiments (10 h) to minimize changes in grazer concentration on the estimated parameter. The concentration of the prey (*Synechococcus* and *Prochlorococcus*) and *Picophagus flagellatus* were followed by sampling 1 ml every 2.5 h. Prey concentrations were adjusted to 2 and 7 × 10^5 cell ml^{-1} for *Synechococcus* and *Prochlorococcus* respectively, in order to mimic the highest concentrations found in oceanic waters. The concentration of *P. flagellatus* was adjusted to be 1 order of magnitude lower than that of the prey.

During these experiments, 1 ml was sampled from each treatment several times a day. Concentrations were determined in triplicates using flow cytometry (FCM, FACSort, Becton Dickinson, CA) after appropriate dilution using 0.2 µm-filtered seawater to avoid FCM coincidence. Because the cultures were non-axenic, we also monitored the concentration of heterotrophic bacteria. Heterotrophic bacteria and heterotrophic flagellates were counted after staining of intracellular nucleic acids by the SYBR Green I dye (Molecular Probes, Eugene, OR) according to Marie et al. (2000). When possible, the heterotrophic flagellates were also detected from the autofluorescence of their ingested prey. All parameters were normalized with 0.95 µm-calibrated beads. Data were collected as list mode files (i.e. parameter values were stored for each cell) and analyzed using the CYTOWIN software (Vaulot 1989; available at www.sb-roscoff.fr/Phyto/cyto.html). Growth and grazing parameters were calculated using the equations established by Frost (1972) and Heinbokel (1978) and revised by Marin and coworkers (1986), as summarized below:

Net growth rate, μ (d^{-1}), of the preys in the control flasks and of the predator in all flasks during long-term experiments was estimated as

$$\mu = \frac{\ln C(t_2) - \ln C(t_1)}{t_2 - t_1} \quad (1)$$

where $C(t)$ is the concentration (cell ml^{-1}) of the prey or of the predator at time t (d) and t_1 and t_2 correspond to the time at the beginning and the end of the experiment respectively.

Grazing rate, g (d^{-1}), was computed for the short-term experiment as

$$g = \frac{\ln C(t_1) - \ln C(t_2)}{t_2 - t_1} \quad (2)$$

where $C(t)$ is the concentration of the prey (cell ml^{-1}) mixed with the grazer (this equation assumes that the growth rate of the prey is close to zero) and $t_2 - t_1$ is the total duration of the experiment (10 h).

Clearance rate, F (ml $cell^{-1}$ h^{-1}), was computed for the short-term experiment as

$$F = \frac{g}{24 \times \bar{C}_g} \quad (3)$$

where $\bar{C}_g$ is the mean concentration of the grazer (cell ml^{-1}) during the experiment.

Ingestion rate, IR ([cells eaten] $flagellate^{-1}$ h^{-1}), was computed during the short-term experiment as

$$IR = F \times \bar{C}_p \quad (4)$$

where $\bar{C}_p$ is the mean concentration of the prey (cell ml^{-1}).

Specific clearance, SC (ml $cell^{-1}$ m^{-3} h^{-1}), was computed during the short-term experiment as

$$SC = \frac{F}{V_g} \quad (5)$$

where V_g is the biovolume of grazer (m^3).

The carbon conversion factors (B) used in this study were 250 fg C $cell^{-1}$ for *Synechococcus* (Kana & Glibert

1987), 50 fg C cell^{-1} for *Prochlorococcus* (Cailliau et al. 1996) and 100 fg C µm^{-3} for living *Picophagus flagellatus* (Bøsrsheim & Bratbak 1987). Because the previous size description of *P. flagellatus* was based upon fixed cells (Guillou et al. 1999a), we measured the size of living cells by optical microscopy (Olympus Fluoview) calibrated using a micrometer. *P. flagellatus* was found to have a mean width of 2.2 µm and a mean length of 3.2 µm. With a cell volume of 8.1 µm^3, as calculated from the volume of an ellipsoid, the carbon content of *P. flagellatus* was thus estimated to be 810 fg C cell^{-1}.

Carbon transfer efficiency (*CE*) was computed during the long-term experiment as

$$CE = \frac{(C_{g'} - C_g) \times B_g}{(C_p - C_{p'}) \times B_p} \quad (6)$$

where $C_{g'}$ is the final concentration of the grazer mixed with the prey, C_g the final concentration of the grazer in the control flasks (without prey), C_p the final prey concentration in the control flasks (without grazer), $C_{p'}$ the final concentration of the prey mixed with the grazer (cell ml^{-1}), and B_g and B_p the carbon biomass (fg C cell^{-1}) of the grazer and prey, respectively, estimated as detailed above.

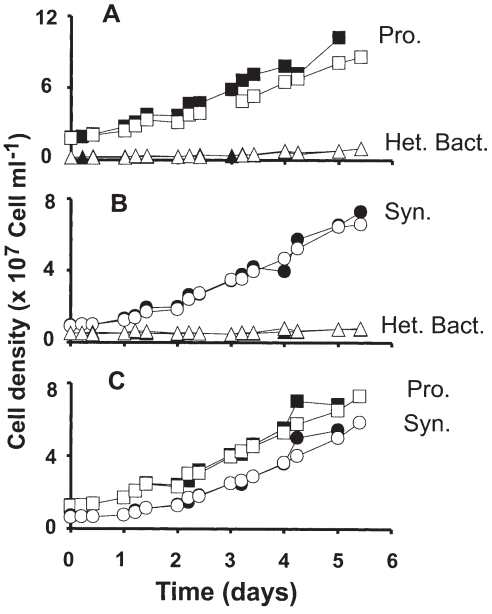

Fig. 1. Time-course changes in cell concentrations of *Prochlorococcus* (■, Pro), *Synechococcus* (●, Syn), and heterotrophic bacteria (▲, Het bact), during long-term experiments using *Symbiomonas scintillans*. Solid symbols: control cultures without heterotrophic flagellates; open symbols: autotrophic cultures mixed with the corresponding heterotrophic flagellates. (A–C) Changes in cell concentration of (A) *Prochloroccocus*, (B) *Synechococcus*, (C) mixed culture of *Prochlorococcus* + *Synechococcus* and (A,B) heterotrophic bacteria. Concentrations were determined in triplicates, counting error bars were always smaller than the data points

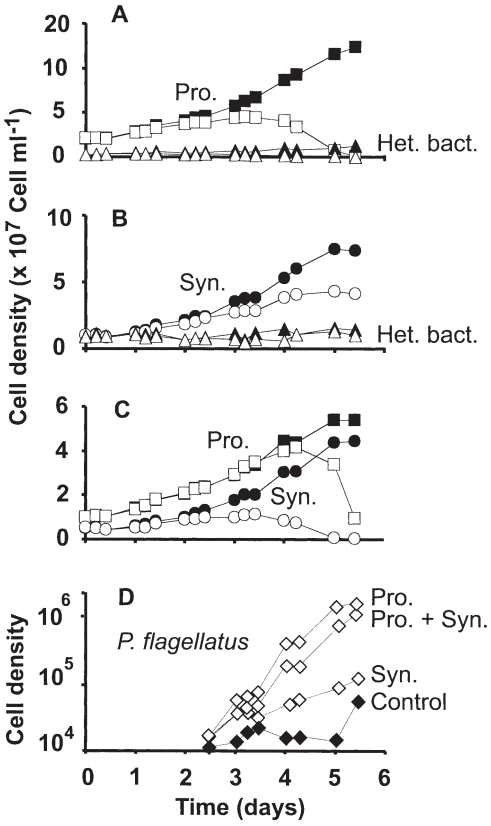

Fig. 2. Time-course changes in cell concentration of *Prochlorococcus* (■, Pro), *Synechococcus* (●, Syn), and heterotrophic bacteria (▲, Het bact), during long-term experiments using *Picophagus flagellatus* (◆). Solid symbols: control cultures without heterotrophic flagellates; open symbols: autotrophic cultures mixed with the corresponding heterotrophic flagellates. (A–C) Changes in the cell concentration of (A) *Prochloroccocus*, (B) *Synechococcus*, and (C) mixed culture of *Prochlorococcus* + *Synechococcus*. (D) Change in the cell concentration (log scale) of *P. flagellatus* alone or mixed with prey. Heterotrophic bacteria are also presented in flasks containing *Prochlorococcus* (A) and *Synechococcus* (B)

Results. No clear differences in cell numbers were observed between the control flasks and the different cultures mixed with *Symbiomonas scintillans* (Fig. 1). We were not able to count the flagellate by flow cytometry when it was mixed with autotrophic prey because of its relative low concentration and the overlapping of its signature by flow cytometry with those of heterotrophic bacteria.

In contrast, grazing of *Picophagus flagellatus* on *Prochlorococcus* and *Synechococcus* was clearly observed (Fig. 2) after 2 d of incubation. The predator cell number increased from 10^4 cells ml^{-1} initially to 10^6 cells ml^{-1} after 4 d. *P. flagellatus* fed on *Prochlorococcus* drastically reduced the prey population (the final *Prochlorococcus* concentration in the control flask was 1.2×10^8 cells ml^{-1} vs 5.8×10^5 cells ml^{-1} in the flask mixed with *P. flagellatus*, see Table 1). The maximal

Table 1. Long-term experiments (6 d). Growth rates and concentrations of *Picophagus flagellatus* feeding on *Prochlorococcus* (Pro), *Synechococcus* (Syn) and mixed cultures of *Prochlorococcus* and *Synechococcus* (Pro + Syn)

	Pro	Syn	Pro + Syn	
Picophagus flagellatus				
Maximum growth rate (d^{-1})	1.6	0.6	1.5	
Initial *P. flagellatus* concentration (cells ml^{-1}) ± SD	$10^4 \pm 2 \times 10^3$	$10^4 \pm 2 \times 10^3$	$10^4 \pm 2 \times 10^3$	
Final *P. flagellatus* concentration in flasks mixed with prey (cells ml^{-1}) ± SD	$1.7 \times 10^6 \pm 2 \times 10^5$	$10^5 \pm 8 \times 10^3$	$1.1 \times 10^6 \pm 3 \times 10^5$	
Increase in flagellate carbon (fg C ml^{-1}) in flasks mixed with prey	1.36×10^9	7.29×10^7	8.83×10^8	
Cyanobacteria prey				
Growth rate (d^{-1}) estimated from control flasks	0.3	0.3	0.24	0.38
Initial prey concentration (cells ml^{-1}) ± SD	$2 \times 10^7 \pm 5 \times 10^5$	$1 \times 10^7 \pm 1 \times 10^5$	$1 \times 10^7 \pm 8 \times 10^4$	$5 \times 10^6 \pm 6 \times 10^4$
Final prey concentration in control flasks (cells ml^{-1}) ± SD	$1.2 \times 10^8 \pm 2 \times 10^6$	$7.3 \times 10^7 \pm 3 \times 10^6$	$5.4 \times 10^7 \pm 7 \times 10^5$	$4.5 \times 10^7 \pm 1 \times 10^5$
Final prey concentration in flasks mixed with *P. flagellatus* (cells ml^{-1}) ± SD	$5.8 \times 10^5 \pm 6 \times 10^3$	$4 \times 10^7 \pm 6 \times 10^5$	$1 \times 10^6 \pm 5 \times 10^5$	$3 \times 10^5 \pm 6 \times 10^3$
Consumption of carbon (fg C ml^{-1}) in 6 d	5.9×10^9	8.2×10^9	2.6×10^9	1.1×10^{10}
Carbon transfer efficiency (%)	23	0.9	6	

growth rate of *P. flagellatus* feeding on *Prochlorococcus* was larger than 2 doublings d^{-1} (μ = 1.6 d^{-1}, Table 1), and the concentration of the predator reached a final density of 1.7×10^6 cells ml^{-1} (Table 1). The use of *Synechococcus* as a prey induced significantly less efficient prey removal (Fig. 2B) as well as a lower maximum growth rate (μ = 0.6 d^{-1}, Table 1) and a lower final concentration (10^5 cells ml^{-1}, Table 1) for *P. flagellatus*. When *P. flagellatus* was fed with the 2 photosynthetic prey given together, it grazed on them and drastically reduced both of them (Fig. 2C), although its growth rate and final cell numbers were lower than when it was fed on *Prochlorococcus* alone (Table 1). It is noteworthy that, at a similar prey concentration, *P. flagellatus* grazed first on *Synechococcus* rather than on *Prochlorococcus*. However, when *Prochorococcus* became about 3 times more concentrated than *Synechococcus*, it began to be eaten as well. In terms of carbon transfer efficiency, *Synechococcus* is a very poor food (less than 1% when offered alone). Comparatively, *Prochlorococcus* contributed to 23% of the carbon transfer efficiency (Table 1). At the end of the experiment, we observed that the side scatter (SSC) parameter of *P. flagellatus*, a proxy for cell size, was much higher when it was fed on *Synechococcus* (SSC = 2.2 relative to 0.95 µm beads) than on *Prochlorococcus* (SSC = 1.45) or on a mixture of the prey (SSC = 1.5). Microscopic observations also revealed that *P. flagellatus* lost its motility when fed on *Synechococcus* alone. Heterotrophic bacteria were also present, since the cultures were not axenic. At the beginning of the long-term experiment, their concentration was 10 times lower than *Prochlorococcus* and similar to that of the cyanobacteria in the other cases. However, in all experiments, they only represented a minor fraction of the consumed prey (Fig. 2A,B).

The concentration of *Picophagus flagellatus* did not change significantly during the short-term experiment (10 h) and remained around 2.5×10^4 cell ml^{-1} in all flasks. Under these conditions, a reduction in both *Prochlorococcus* and *Synechococcus* cells was evident after 2.5 h of incubation (Fig. 3). Clearance rates of *P. flagellatus* were estimated to be 2.3 nl $cell^{-1}$ h^{-1} for *Prochlorococcus* and 2.5 nl $cell^{-1}$ h^{-1} for *Synechococcus* (Table 2). When the 2 prey were mixed together, the clearance rate for *Synechococcus* was more than twice as high as that for *Prochlorococcus* (Table 2). The clearance rate for *Synechococcus* was also higher when it was mixed with *Prochlorococcus* (3.8 nl $cell^{-1}$ h^{-1}) than when it was offered alone (2.5 nl $cell^{-1}$ h^{-1}). Since in this study we used the highest prey concentration found in natural samples, our estimates of clearance rates are probably on the low side. Ingestion rate was higher with *Prochlorococcus* alone than with *Synechococcus* alone or mixed with *Prochlorococcus* (Table 2). Based on our calculations, an individual *P. flagellatus* ate an average of 1 *Prochlorococcus* h^{-1}. Based on electron microscopy sections, an individual *P. flagellatus* could indeed contain 1 or 2 entire or partly digested prey (Fig. 4).

Discussion. *Picophagus flagellatus* and *Symbiomonas scintillans* belong to the Stramenopiles (Guillou et al. 1999a). This lineage is known to include several active phagotrophic species such as *Paraphysomonas* spp. (Chrysophyceae) and *Cafeteria* spp. (Bicosoecida). A number of features suggested phagotrophy in

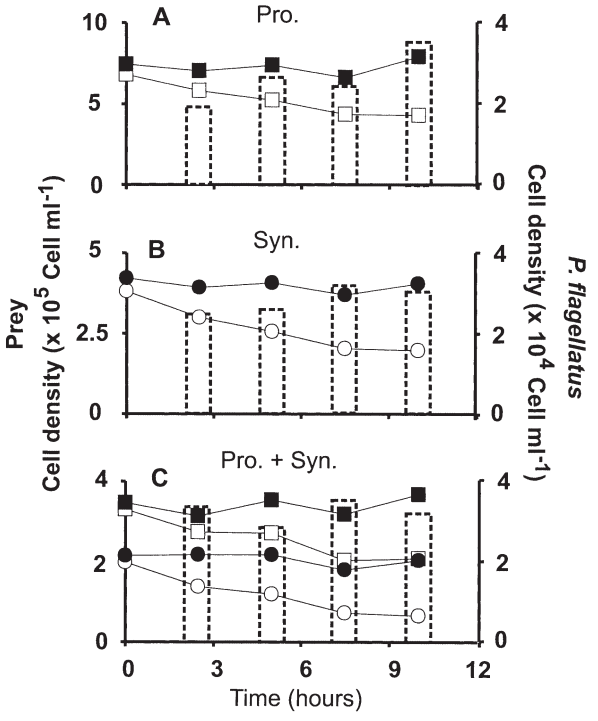

Fig. 3. Time-course changes in cell concentration of *Prochlorococcus* (■, Pro) and *Synechococcus* (●, Syn) during short-term experiments using *Picophagus flagellatus*. Prey (left scale): (A) *Prochlorococcus*, (B) *Synechococcus*, and (C) mixed culture of *Prochlorococcus* and *Synechococcus*. Bars (right scale) represent *P. flagellatus* concentration in the mixed culture

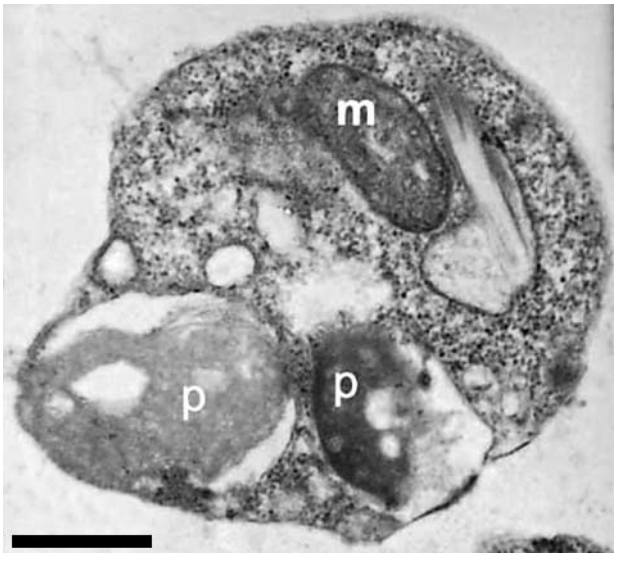

Fig. 4. Thin section of *Picophagus flagellatus* feeding with *Prochlorococcus* observed in transmission electron microscopy. A mitochondrion (m) and 2 food vesicle containing partially digested *Prochlorococcus* (p) are visible. Scale bar = 500 nm

P. flagellatus and *S. scintillans*. Indeed, both of them bear a hairy flagellum and a complex microtubule network (Guillou et al. 1999a), which are known to help in prey capture (Andersen & Wetherbee 1992). The long hairy flagellum of *P. flagellatus* is very spectacular in this context (over 6 times the cell diameter). Typically, half of the cellular volume of *P. flagellatus* is filled with large phagotrophic vesicles that sometimes contain recognizable bacteria, demonstrating that this flagellate feeds on free-living heterotrophic bacteria present in the cultures (as they are not axenic). Several micrographs also revealed the phagotrophic capacities of *S. scintillans* (see Fig. 1D from Guillou et al. 1999a). This latter species is characterized by the presence of several endosymbiotic bacteria located close to the nucleus (Guillou et al. 1999a). Although this species can apparently be phagotrophic (Guillou et al. 1999a), it does not appear to be an active predator of either *Synechococcus* or *Prochlorococcus* or of the free-living heterotrophic bacteria contained in our cultures. This suggests that *S. scintillans* may feed on other prey, such as smaller heterotrophic bacteria typically found in marine waters, but it may also feed by osmotrophy. In this context, the trophic role of *S. scintillans* endosymbiotic bacteria deserves further scrutiny.

In contrast, *Picophagus flagellatus* is an active predator of both *Prochlorococcus* and *Synechococcus*. At a similar prey concentration, *Synechococcus* is

Table 2. Short-term experiments (10 h). Growth and grazing parameters measured for *Picophagus flagellatus* feeding on *Prochlorococcus* (Pro), *Synechococcus* (Syn) and on mixed cultures of *Prochlorococcus* and *Synechococcus* (Pro + Syn)

Grazing parameters	Pro	Syn	Pro + Syn	
Prey growth rate (h^{-1}) determined in controls	0.0056	–0.0046	0.0058	–0.0057
Grazing rate (h^{-1})	0.052	0.061	0.045	0.107
Mean grazer concentration (cells ml^{-1}) ± SD	$2.3 \times 10^4 \pm 6 \times 10^3$	$2.4 \times 10^4 \pm 3 \times 10^3$	$2.8 \times 10^4 \pm 3 \times 10^3$	
Mean prey concentration (cells ml^{-1}) ± SD	$5.2 \times 10^5 \pm 1 \times 10^5$	$2.7 \times 10^5 \pm 8 \times 10^4$	$2.6 \times 10^5 \pm 5 \times 10^4$	$1.2 \times 10^5 \pm 5 \times 10^4$
Clearance rate (10^{-6} ml $cell^{-1}$ h^{-1})	2.3	2.5	1.6	3.8
Ingestion ([cells eaten] $flagellate^{-1}$ h^{-1})	1.2	0.7	0.4	0.4
Specific clearance (10^4 ml $cell^{-1}$ m^{-3} h^{-1})	28	30	19	47

grazed in preference to *Prochlorococcus* or heterotrophic bacteria. The clearance rates of *P. flagellatus* fall into the lower range reported for larger heterotrophic nano-flagellates both in cultures (mainly based on *Paraphysomonas* spp.) and in the field, i.e. 0.5 to 27 nl flagellate^{-1} h^{-1} for *Synechococcus* (Christoffersen 1994, Dolan & Šimek 1999) or 4 to 24 nl flagellate^{-1} h^{-1} for *Prochlorococcus* (Monger et al. 1999). However, it should be noted that our estimates are derived from experiments using very high prey concentrations. The specific clearance rate is higher for *Synechococcus* than for *Prochlorococcus*, and 10 to 100× higher than that calculated for ciliates (Christaki et al. 1999). However, *Synechococcus* is not efficiently assimilated, as is suggested by the very low carbon transfer efficiency we found. The *P. flagellatus* volume increases observed at the end of the long-term experiment could be due to the fact that *Synechococcus* cells are not digested. This poor digestibility of *Synechococcus* has been observed recently in appendicularians (Gorsky et al. 1999). Similarly, Caron et al. (1991) reported that protozoa displayed faster growth rates and higher cell yields on heterotrophic bacteria than on *Synechococcus*. In contrast, *Synechococcus* is efficiently consumed by some ciliates, such as *Strombidium sulcatum* (Christaki et al. 1999).

Using the particle-size model of Sheldon et al. (1972), Azam et al. (1983) suggested that in marine food chains the optimum size difference between a predator and its prey is about 1 order of magnitude in length (or 10^3 in volume) with a carbon transfer efficiency of 10% between each compartment. For microbial food webs, it is widely accepted that this predator:prey size ratio is smaller (Goldman & Caron 1985, Hansen et al. 1994). The current paradigm is that 0.4 to 1 µm bacteria are grazed by 5 µm nanoflagellates, themselves being grazed by 10 to 20 µm nanozooplankton or larger ciliates. If we consider a predator:prey size ratio of 3:1 and a 2 to 4 µm size for *Picophagus flagellatus*, this species can potentially graze all prey smaller than 1.3 µm in diameter. Of the cells of *P. flagellatus*, 87% pass through a 2 µm filter. Therefore, field experiments based upon size fractionation should take into account that the <2 to 3 µm fraction is not potentially free of efficient heterotrophic predators.

The existence of *Picophagus flagellatus* and *Symbiomonas scintillans* clearly demonstrates that the microbial food web may possess numerous additional steps and that the transfer efficiency of matter and energy to the higher trophic levels (i.e. to zooplankton such as copepods) is probably lower than previously hypothesized. In fact, the recent discovery of lineages based on the description of picoplanktonic species (Andersen et al. 1993, Guillou et al. 1999b) illustrates our very scant knowledge of the taxonomy of this size class. Cultured heterotrophic flagellates with sizes of less than 3 to 4 µm are restricted to date to the Stramenopile lineage. Recent molecular studies, based on the cloning and the sequencing of the small subunit ribosomal RNA gene, of the picoplankton fraction obtained from the euphotic zone in the equatorial Pacific Ocean (Moon-van der Staay et al. 2001) and from the deep aphotic zone in the Antarctic Polar Front (López-García et al. 2001) have provided evidence that cells passing through a 3 µm filter also contain 2 groups of previously unknown alveolates, which are very likely heterotrophic or parasitic given their close phylogenetic relatives. Clearly, this predator size class is much more taxonomically diverse than was previously thought. Their behavior and food preference are probably also very diverse, as exemplified by the difference in grazing behavior between *P. flagellatus* and *S. scintillans*. The next step will be to quantify their relative abundance in the field and to evaluate their role in carbon export towards the higher trophic levels. Detailed studies of the relationships between prey and predators are also necessary to better understand the structure and function of oceanic communities and to develop more accurate models of microbial food webs.

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