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SHORT COMMUNICATION

Predation on marine picoplankton populations examined with an 'add-in' approach

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Abstract. NW Mediterranean surface water was spiked with picoplankton prey (heterotrophic bacteria or cyanobacteria) or predators (bacterivorous microflagellates or ciliates) to investigate differential grazing pressure on picoplankton populations. Adding a particular prey type did not yield different growth patterns for heterotrophic bacteria and cyanobacteria, but gave either similar, positive, effects on both picoplankton types or similar negative effects. Natural populations of both predator types increased with additions of cyanobacteria, but not heterotrophic bacteria. Ciliate additions gave marked decreases in cyanobacteria. While individual groups of grazers may preferentially consume cyanobacteria, selective grazing is probably not responsible for the maintenance of apparently stable populations of different groups of picoplankters during the summer.

Picoplankton populations are a heterogeneous assemblage, dominated by prokaryotic osmotrophs and autotrophs, united by small cell size (0.2–2.0 μm). They are often important in primary and secondary production, especially in oligotrophic systems. For example, in the NW Mediterranean, surface layer populations of cyanobacteria and heterotrophic bacteria have been described as accounting for the majority of primary and secondary production, respectively (Hagström *et al.*, 1988).

Relative to larger algal cells, picoplankters are adept at absorbing nutrients, whether autotrophic or heterotrophic, because of their small cell size. If a nutrient salt is in sufficiently short supply, and given ample stocks of labile dissolved organic carbon to supply heterotrophic bacterioplankton, a single nutrient may limit both osmotrophic and autotrophic picoplankton (Thingstad and Rassoulzadegan, 1995). Thus, in a nutrient-poor, but dissolved organic carbon-rich system such as the NW Mediterranean (see Copin-Montegut and Avril, 1993), the importance of picoplankton may be coupled with a common limitation by phosphorus of both autotrophs and heterotrophs (Zweifel *et al.*, 1993; Dolan *et al.*, 1995).

In the NW Mediterranean, concentrations of heterotrophic bacteria and cyanobacteria in coastal waters are very stable, i.e. ± <10% over a scale of 1–4 weeks (Ferrier-Pages and Rassoulzadegan, 1994), despite the fact that heterotrophic and autotrophic picoplankton populations often grow at very different rates. For example, in early autumn, *Synechococcus* has a generation time of ≤24 h compared to heterotrophic bacteria doubling every 48 h (Hagström *et al.*, 1988). While the dominance of picoplankton in an oligotrophic system is not surprising, the question remains of what maintains the apparent stability of autotrophic and

heterotrophic picoplankton. The populations appear to grow at different rates, be competitors for the same limiting nutrient, yet show stable cell concentrations.

Different groups of picoplankton could be removed at different rates. Laboratory and some field data suggest that, in general, cyanobacteria are an inferior food for micro and nano grazers relative to heterotrophic bacteria, and may be discriminated against (Caron *et al.*, 1991). Nonetheless, some protozoa have been cultured on cyanobacteria (Dryden and Wright, 1987), including marine ciliates grown on *Synechococcus* (Johnson *et al.*, 1982). In natural populations of the NW Mediterranean, tintinnid ciliates, based on food vacuole contents, generally appeared to select against cyanobacteria (Bernard and Rassoulzadegan, 1993).

However, in the coastal NW Mediterranean, small aloricate ciliates dominate the ciliate community and have been reported as probably major consumers of picoplankton (Sherr *et al.*, 1989). Small marine aloricate ciliates may be similar to their freshwater counterparts. Among freshwater ciliates feeding on picoplankton, most appear to select for cyanobacteria relative to heterotrophic bacteria when measurements are made with fluorescently labelled cells, and the ciliates show clearance rates 2–3 times higher on cyanobacteria than heterotrophic bacteria (Simek *et al.*, 1995).

Experimental examination of picoplankton removal rates in natural populations poses very significant problems. Use of fluorescently labelled prey relies on the assumptions that only 'tracer' quantities, insufficient to change ingestion rates, have been used and that dyed prey are ingested at the same rate as natural prey; assumptions which are difficult to verify. Approaches such as size fractionation, metabolic inhibition or dilution all seek to eliminate or reduce grazers, but also eliminate or reduce feedback effects of grazers which may be important. For example, in weekly incubations of water filtered through 2.0 μm pore-size filters, both with and without grazer populations isolated in dialysis bags, growth of heterotrophic bacteria or cyanobacteria rarely occurred in the absence of a grazer population, and the apparent deleterious effects of removing grazers appeared to disproportionately affect cyanobacteria (Ferrier-Pages and Rassoulzadegan, 1994).

In this study, we examined an alternative approach to determine whether distinct picoplankton populations, heterotrophic bacteria and cyanobacteria, are removed at different rates. The basic idea was to add, rather than remove, components of natural communities to avoid disrupting feedback mechanisms. Cultured prey and predator populations were added to natural communities, increasing concentrations by a factor of 3–4, and short-term (15–30 h) changes in natural populations were monitored and compared to trends in controls to which nothing was added. The underlying hypothesis was that changes in growth or mortality rates of heterotrophic (or autotrophic) picoplankton would be evident if only one type of prey or predator was added. For example, if cyanobacteria were truly preferentially removed, their rate of disappearance or growth should be independent of the concentration of heterotrophic bacteria.

Two experiments were conducted; they began with a single surface water sample taken from Point B at the mouth of the Bay of Villefranche (43°41′10″N, 7°19′00′E) in the NW Mediterranean taken on 8 June or 15 July 1994. In

experiment 1, water was distributed into four 500 ml glass bottles and assigned one of four treatments: no additions (control); 10 ml added of a culture of heterotrophic bacteria (10^8 cells ml^{-1}); 10 ml of a culture of cyanobacteria, *Synechococcus* sp. (10^6 cells ml^{-1}); 10 ml of a culture of heterotrophic microflagellate, *Pseudobodo*-like (10^6 cells ml^{-1}). In experiment 2, water was distributed into 15 500 ml glass bottles and assigned either one of the four treatments given above or an additional treatment: 10 ml of a culture of an oligotrich ciliate, *Strombidium sulcatum* (10^2 cells ml^{-1}). Cultures of the microflagellate and ciliate were grown on bacteria. All micro-organisms were harvested as late log or early stationary phase cells. Culture protocols are presented in detail in Rivier *et al.* (1985) and Bernard and Rassoulzadegan (1990).

For both experiments, the bottles were placed in a running seawater incubator with a neutral density screen removing 70% of incident illumination. Samples were removed at time zero, and after 15 and/or 30 h of incubation. Ten millilitre samples were fixed with formalin for epifluorescence counts of picoplankton and heterotrophic microflagellates. For ciliate counts, in experiment 2, 100 ml were removed from a single replicate of each treatment and fixed with acid Lugol's for inverted microscope counts of ciliate microzooplankton. Average concentrations at time zero and after 30 h were compared for significant differences. In the results reported below and the discussion which follows, all changes represent significant differences, unless otherwise noted.

The results of the first experiment, in which treatments were not replicated, indicated that additions of either prey type affected both prey populations similarly (Table I). In the control bottle, heterotrophic bacteria increased by 68% and cyanobacteria remained unchanged. After an addition of heterotrophic bacteria, concentrations of heterotrophic bacteria and cyanobacteria both declined, and both populations increased with time following an addition of cyanobacteria. The population changes following increases in one or the other picoplankton group provided no clear evidence of selective grazing. Adding heterotrophic bacteria may have stimulated a grazer population, while adding cyanobacteria may have inhibited grazing on both prey types. However, heterotrophic microflagellates appeared to react differently to prey additions, increasing with the addition of cyanobacteria.

In the second experiment, heterotrophic bacteria increased and cyanobacteria decreased, both by ~25%, from t_0 to t_{30} in the control bottles (Table I, Figure 1). In the treatments with additions of either heterotrophic bacteria or cyanobacteria, concentrations of cyanobacteria remained unchanged, compared to the significant declines in controls, and heterotrophic bacteria increased by 25–50%, similar to controls. These results suggest that if selective grazing was maintaining the balance between the two picoplankton populations, the selectivity disappears when one population is present in excess.

In treatments which consisted of adding predators, similar increases in heterotrophic bacteria occurred in bottles with added ciliates or microflagellates (Table I, Figure 1). In contrast, cyanobacteria concentrations decreased 45% with ciliate additions and remained unchanged with heterotrophic flagellate additions. These results were interesting because they indicated that ciliates may selectively ingest cyanobacteria. It should be recalled that both of the predators added had been

Table I. Results from experiment I, 8 June 1994. Changes in the concentrations (cells ml^{-1}) from time zero (t_0) to 30 h (t_{30}) of picoplankton prey organisms, heterotrophic bacteria (Hetbac) and cyanobacteria (Cyanobac) and presumptive predators (heterotrophic microflagellates, Hflag) in natural water samples with and without the addition of cultured populations. The averages ± SD of triplicate counts are given. Differences between t_0 and t_{30} concentrations in per cent t_0 concentrations were calculated when the means of triplicate counts were significantly different. Note that additions of heterotrophic bacteria appeared to stimulate the removal of both heterotrophic bacteria and cyanobacteria, while additions of cyanobacteria yielded the largest per cent increases after 30 h for both groups. Heterotrophic microflagellates appeared to react positively to increases in concentrations of cyanobacteria in contrast to additions of heterotrophic bacteria

Group	Treatment	$[t_0]$	$[t_{30}]$	% difference
Hetbac	Control	$8.3 \times 10^5 \pm 0.30 \times 10^5$	$1.4 \times 10^6 \pm 0.20 \times 10^6$	168
	Add Hetbact	$1.1 \times 10^7 \pm 0.06 \times 10^7$	$5.8 \times 10^6 \pm 0.40 \times 10^6$	53
	Add Cyanobact	$8.6 \times 10^5 \pm 0.30 \times 10^5$	$1.6 \times 10^6 \pm 0.10 \times 10^6$	186
	Add Hflag	$9.9 \times 10^5 \pm 0.10 \times 10^5$	$1.2 \times 10^6 \pm 0.10 \times 10^6$	121
Cyanobac	Control	$1.8 \times 10^4 \pm 0.10 \times 10^4$	$2.1 \times 10^4 \pm 0.21 \times 10^4$	ns
	Add Hetbact	$2.0 \times 10^4 \pm 0.11 \times 10^4$	$1.8 \times 10^4 \pm 0.15 \times 10^4$	90
	Add Cyanobact	$2.2 \times 10^5 \pm 0.17 \times 10^5$	$2.6 \times 10^5 \pm 0.10 \times 10^4$	118
	Add Hflag	$1.9 \times 10^4 \pm 0.06 \times 10^4$	$1.5 \times 10^4 \pm 0.02 \times 10^4$	79
Hflag	Control	$2.9 \times 10^3 \pm 0.15 \times 10^3$	$3.7 \times 10^3 \pm 0.25 \times 10^3$	128
	Add Hetbact	$3.2 \times 10^3 \pm 0.06 \times 10^3$	$3.4 \times 10^3 \pm 0.21 \times 10^3$	ns
	Add Cyanobact	$3.3 \times 10^3 \pm 0.32 \times 10^3$	$4.6 \times 10^3 \pm 0.26 \times 10^3$	140

cultured on bacterial prey, using the same bacterial cultures as those used in the additions of heterotrophic bacteria. The differences in responses of natural communities of heterotrophic microflagellates and ciliates to additions of different picoplankton populations also suggested differences.

In control bottles and those with cyanobacteria added, microflagellates increased 60–100%, but did not change significantly in bottles to which heterotrophic bacteria were added (Figure 2), similar to the results of the first experiment. Ciliate counts, although based on only one replicate, showed a dramatic increase in concentration with the addition of cyanobacteria, compared to no changes in controls or treatments with heterotrophic bacteria added.

Despite some evidence of increased predator growth with additions of cyanobacteria compared to heterotrophic bacteria, the results of adding either type of picoplankter were similar in effect on both picoplankton populations. Such results argue against one type of picoplankter being subjected to highly selective removal by grazers. Thus, there is little evidence for a marked, differential grazing pressure on the two picoplankton types in the NW Mediterranean. This conclusion echoes that of Caron *et al.* (1991) concerning the NW Atlantic (Vineyard Sound, MA, USA) studied using eukaryote metabolic inhibitors to reduce grazing pressure.

However, in our experiments, different prey additions yielded distinct responses in the naturally occurring communities of microflagellates and ciliates. Natural populations of both microflagellates and ciliates increased with additions of cyanobacteria and reacted, at best, indifferently to additions of heterotrophic bacteria (Table I, Figure 2). Also, the treatment of adding ciliates gave a decrease in cyanobacterial concentrations and did not appear to affect abundance of heterotrophic bacteria (Figure 1E). Thus, there was some evidence that in the NW

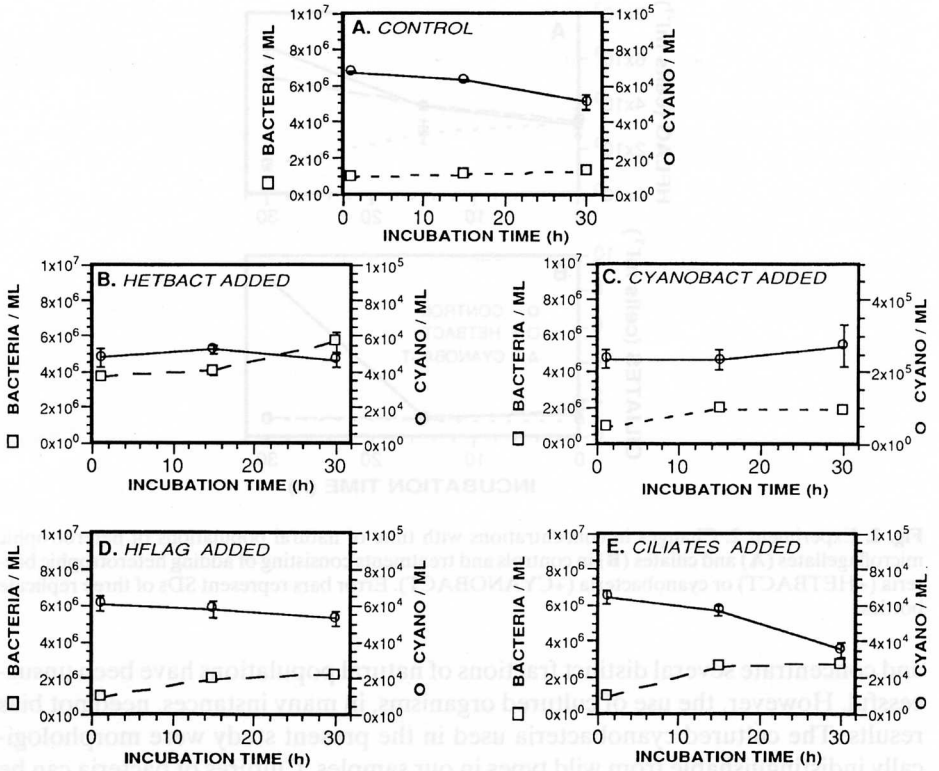

Fig. 1. Experiment 2. Changes in concentrations of heterotrophic bacteria (BACTERIA) and cyanobacteria (CYANO) with time in the control bottles (**A**) and those in which different microbial populations were added: heterotrophic bacteria (**B**), cyanobacteria (**C**), heterotrotrophic microflagellates (**D**) or oligotrichous ciliates (**E**). Error bars represent SDs of the three replicate bottles per treatment.

Mediterranean individual groups of grazers such as ciliates may feed selectively on cyanobacteria, as found by others for freshwater (e.g. Simek *et al.*, 1995). The selection may be size based as the average cyanobacterium is considerably larger than the average bacterium.

Overall, the results indicate that predation on the two types of picoplankton was not independent in our samples despite the fact that individual groups of predators may be selective feeders. Differential mortality, not due to grazing, between the heterotrophic bacteria and cyanobacteria could still explain apparently stable populations growing at different rates. Both heterotrophic bacteria and cyanobacteria are subjected to viral attacks (e.g. Suttle and Chan, 1994; Weinbauer and Peduzzi, 1995) and may have very different viral attack-related mortality rates.

The presented technique of adding rather subtracting components of the food web is promising, but not without drawbacks. For example, using the approach of a 'dose–response', adding a prey type in over a range of concentrations, prey switching by predators may be detectable. The major drawback is that the populations added should be identical to natural populations and in practice this is difficult to achieve. Our attempts to develop a reliable, relatively rapid, protocol to isolate

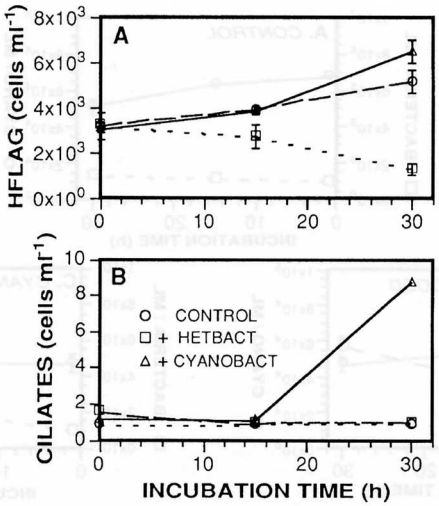

Fig. 2. Experiment 2. Changes in concentrations with time of natural populations of heterotrophic microflagellates (**A**) and ciliates (**B**) in controls and treatments consisting of adding heterotrophic bacteria (+HETBACT) or cyanobacteria (+CYANOBACT). Error bars represent SDs of three replicate bottles.

and concentrate several distinct fractions of natural populations have been unsuccessful. However, the use of cultured organisms, in many instances, need not bias results. The cultured cyanobacteria used in the present study were morphologically indistinguishable from wild types in our samples. Cultures of bacteria can be mixed, matched and manipulated to alter size or taxonomic composition. Some caution may be advisable in choosing a method to enumerate bacteria since recent studies have brought into question the DAPI procedure (Porter and Feig, 1980), considered a routine method (Turley, 1993). The DAPI procedure has been described as providing underestimates of total cell counts (Suzuki *et al.*, 1993) as well as overestimating numbers of living bacteria (Zweifel and Hagström, 1995).

Another possible concern in experiments using an 'add-in' approach is avoiding effects due, not to the addition of organisms, but to the solution in which the organisms were held. In the experiments described here, we tried to minimize such effects by adding small volumes (10 ml) of cultures, which were nearly exhausted (late log–early stationary), to relatively large volumes of seawater (500 ml). However, it may be desirable to specifically examine the effects of adding only the solution without the organisms.

An 'add-in' approach may prove especially valuable in elucidating trophic interactions within oligotrophic systems where predators are least likely to be unlimited by food and feedback effects are most likely to be important and could, in fact, be used to exaggerate them as a method of investigation. It should be noted that in systems in which predators or grazers are nearly saturated with available food, e.g. eutrophic waters, an 'add-in' approach is probably inappropriate. We do not propose the method as an all-purpose replacement methodology, but rather as a supplementary strategy.

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