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Bacteria-flagellate interactions in the microbial food web of the oligotrophic subtropical North Pacific

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ABSTRACT: The number and relative strengths of trophic linkages in the microbial community of the oligotrophic subtropical North Pacific were studied in 12 experiments from August 1998 to April 1999 at Stn ALOHA (100 km north of Oahu, Hawaii). Collected seawater was manipulated by sequential size-fractionation to truncate the food web at different organism sizes (1, 2, 5, 10 and 20 µm), and the response variable, net bacterial growth rate, was assessed from flow cytometric analyses of the changes in cell abundance (combined heterotrophic bacteria and *Prochlorococcus*) after 24 h incubations. The corresponding size structure of the protistan grazer assemblage was measured microscopically. With a coefficient of variability of 7% and a 2-fold range overall, total bacterial abundance displayed relatively low temporal variability. Despite the relative constancy of standing stock, however, microbial community interactions varied markedly among the experiments. For experiments conducted at higher levels of bacterial biomass, the bacteria showed little growth response to the removal of predators and may have been resource limited. In contrast, the growth response was highest when conditions were defined by relatively low bacterial biomass and high heterotrophic flagellate biomass. Trophic cascades were evident only at intermediate to high levels of bacterial biomass, and may appear in transitions between high and low levels of bacterial biomass. These results suggest that resources and predators oscillate in importance in regulating open-ocean microbial populations. In such oscillations, the indirect influences of a protistan predatory chain may determine the balance between resource limitation and strong predatory control.

KEY WORDS: Planktonic food web · Microbial loop · Flagellates · Bacteria · Top-down · Bottom-up · North Pacific Subtropical Gyre

INTRODUCTION

Although the microbial food web paradigm (Azam et al. 1983, Sherr & Sherr 1988) has greatly expanded our view of plankton community structure and ecology, we are still far from understanding the dynamics of microbial populations in the oceans and the factors that control them. Vast regions of the tropical oligotrophic oceans, for instance, are characterized by the dominance and relative constancy of the microbial community (e.g., Campbell et al. 1997). However, the relative importance of mechanisms that set and maintain the ambient levels of population abundance are not so clear. Previous studies have considered the problem largely from 2 perspectives: the oscillations of a simple predator-prey system (Fenchel 1982b, Tanaka et al. 1997) and the dichotomy between substrate limitation (bottom-up) and predator control (top-down) (e.g., Gasol & Vaqué 1993, Gasol 1994). These mechanisms are complicated, however, by the cascading influences of a multi-level predatory chain (Carpenter et al. 1985, McQueen et al. 1986, Wickham 1995, Pace et al. 1998, Calbet & Landry 1999). The temporal oscillations of bacteria and heterotrophic flagellates (Hflag) are not

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so easily interpreted if Hflag do not comprise a single guild of bacterivores. Moreover, temporal variations in the relative strengths of trophic linkages in a predator chain could provide subtle indirect influences that tip the balance between bottom-up and top-down controls.

In the present study, we investigated trophic coupling in the subtropical North Pacific using size-fractionation to truncate the food web at various predator sizes and measuring the response as net bacterial growth rate. Our main goals were to determine the number and relative strengths of trophic linkages within the microbial community and to assess if and how they contributed to regulatory processes. In a previous experiment with a similar design, we found that 5 to 20 µm Hflag exerted a strong positive influence on net bacterial growth rate by feeding on the primary bacterivores (2 to 5 µm Hflag) (Calbet & Landry 1999). Consequently, our *a priori* expectation was that this would be a general result for experiments conducted over the range of seasonal conditions, although the relative magnitudes of the positive and negative influences might vary to maintain more-or-less steady population abundance. In the absence of trophic cascade effects, 2 other possibilities were also anticipated. The lack of a significant net growth rate response to the release of grazing by size-fractionation would provide, for example, an indication of substrate-limited (bottom-up) regulation. On the other hand, a gradual and substantial increase in net growth with each component of the Hflag removed would be more consistent with grazer (top-down) regulation and simple predator-prey dynamics. As described below, our results show a surprising variability in growth rate responses, suggesting that each of these effects may have some regulatory function within the natural range of bacterial abundance.

MATERIALS AND METHODS

We conducted 12 experiments (Table 1) from August 1998 to April 1999 at Stn ALOHA (22.75° N, 158° W), approximately 100 km north of the island of Oahu, Hawaii (Karl & Lukas 1996). Seawater was collected from the mixed layer (ML, 4 experiments) and the deep chlorophyll maximum (DCM, 8 experiments) with Niskin bottles on a CTD rosette. The experimental protocol consisted of truncating the microbial community at varying consumer sizes by gravity filtration (30 to 40 mm Hg) of the water through 45 mm polycarbonate membranes of 1, 2, 5, 10 and 20 µm pore sizes. Four replicates were prepared for each size-fractionated treatment by gently pouring the filtrate into either 250 ml (1, 2 and 5 µm treatments) or 500 ml (10 and 20 µm treatments) polycarbonate bottles. Four additional 500 ml bottles were filled with unfiltered water as controls for the natural (unmanipulated) condition. The bottles were incubated for 24 h at surface seawater temperature in blue-tinted Plexiglas incubator boxes screened to light intensities comparable to those at the depth of water collection (Table 1).

Initial and final subsamples (2 ml) were preserved with paraformaldehyde (0.2% final concentration), frozen in liquid nitrogen and stored at −85°C for flow cytometric (FCM) analysis (Vaulot et al. 1989). The FCM subsamples were thawed in the laboratory and stained with Hoechst 33342 (1 µg ml^{-1}) about 1 h before analysis (Monger & Landry 1993). Population abundance of heterotrophic prokaryotes (Hbact) and *Prochlorococcus* spp. (PRO) were enumerated using a Coulter EPICS 753 flow cytometer equipped with two 5 W Argon lasers, an MSDS volume-control sampling and Cytomation CICERO software (Campbell et al. 1997). Although the cytometer had the sensitivity to distinguish auto- and heterotrophic cells in initial samples, the population boundaries became less clear after incubation, particularly at mixed-layer light levels. Consequently, we combined these comparably sized prokaryotes into 1 category for rate calculations. The combined 'bacteria' are effectively what would be visualized by standard epifluorescence microscopy, but counting precision was substantially improved using flow cytometry (coefficient of variation ~1 to 2% of mean values; Monger & Landry 1993). Initial estimates of bacterial biomass were determined from cell counts assuming carbon contents of 12 and 35 fg C $cell^{-1}$ for Hbact and PRO, respectively (Garrison et al. 2000).

Auto- and heterotrophic nanoflagellates were quantified from microscopical slides prepared with 50 ml water samples from the depths of experimental water collection. The samples were preserved with paraformaldehyde (0.4% final concentration) for 1 to 2 h (6°C, in the dark) before being stained with proflavin (12 µg ml^{-1} final concentration) immediately prior to filtration. The preparations were also stained with DAPI (8 µg ml^{-1} final concentration) for ~30 s in the final stages of filtering onto 0.6 µm pore-size black polycarbonate membrane filters. The filters were mounted on glass slides with type B immersion oil (Calbet & Landry 1999). Counting and sizing were facilitated with a color image-analysis system consisting of a Zeiss epifluorescence microscope with a ZVS 3-chip CCD video camera connected to a computer with Zeiss Image Pro Plus software. At least 300 individuals were enumerated and sized for each sample, and flagellate equivalent spherical diameter, calculated from mean cell diameter, was converted to cell carbon using a factor of 0.22 pg C $µm^{-3}$ (Børsheim & Bratbak 1987).

Statistical analyses of the experimental results included ANOVA, Tukey's test and multiple regression. Multiple regression equations were derived by first generating a global model including all possible interactions between factors and later suppressing those that were not significant (JMP 3.1 Statistics package).

RESULTS

Population abundance and variability

For all experiments, the combined abundance of PRO + Hbact displayed coefficients of variability (CV) of 6.6% for samples taken in the DCM and 7.4% for ML samples (Fig. 1). Both populations were more variable when considered separately. For Hbact, the CVs were 13% for DCM and 18% for ML samples. The highest Hbact abundances were found in September (ML) and March (DCM), and the lowest in February (ML) and August/September (DCM). Thus, from the limited samples available, the highs and lows of Hbact abundance in the ML and DCM appeared to be out of phase. The CVs for PRO were 37% for DCM and 21% for ML samples. Although the data are insufficient for defining the seasonal cycle, the late-winter/spring (February and April, ML; March, DCM) highs in PRO abundance and the late summer (August/September) lows were out of phase with Hbact in the ML and in phase with Hbact in the DCM.

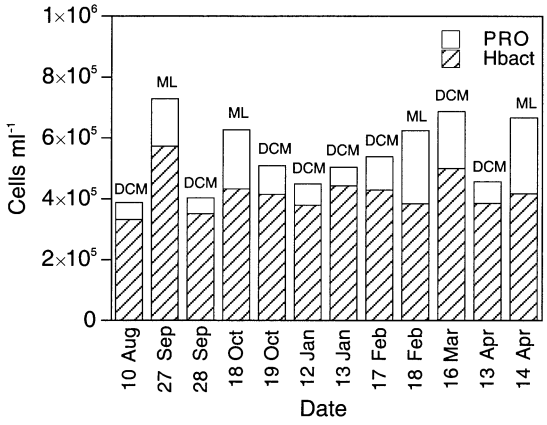

Fig. 1. Bacterial abundance (cells ml^{-1}) in the ambient environment at the start of each experiment. PRO = *Prochlorococcus* spp.; Hbact = Heterotrophic bacteria; ML= mixed layer; DCM = deep chlorophyll maximum

With CVs of 62% for DCM samples and 54% for ML samples, Hflag were more variable than bacterial populations (Fig. 2A). The highest Hflag densities were found during winter cruises (January, DCM; February, ML), the ML high corresponding to samples with the lowest Hbact abundance. The late summer low in Hflag abundance (August/September, DCM; September, ML) occurred during the time of lowest individual and combined populations of bacterial prey. When measured for experiments on the same cruises, Hflag were more abundant in ML samples than in the DCM

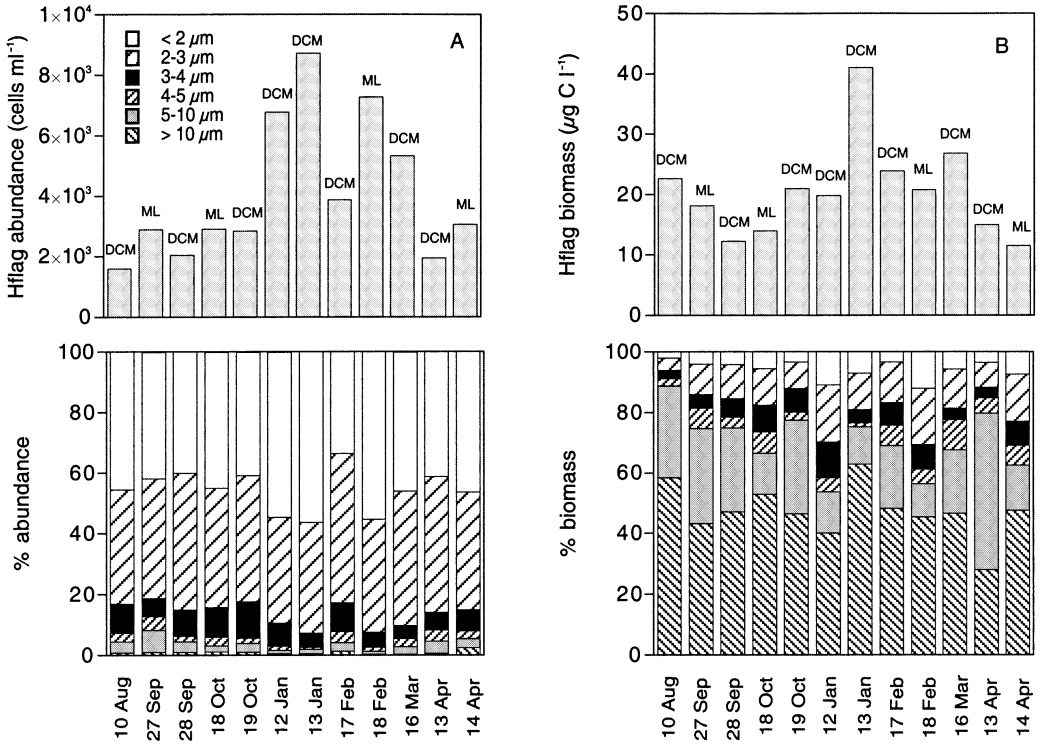

Fig. 2. (A) Abundance and (B) biomass of the different size-fractions of the heterotrophic flagellate community (Hflag) in the ambient environment at the start of each experiment. ML = mixed layer; DCM = deep chlorophyll maximum

Table 1. Initial conditions for size-fractionation experiments. Experiment number, date, depth of water collection for the experiments, and incubation temperature are shown. DCM = deep chlorophyll maximum; ML = mixed layer

Expt	Date	Depth (m)	Temp (°C)
1	10 Aug 1998	108 (DCM)	25.7
2	27 Sep 1998	25 (ML)	25.0
3	28 Sep 1998	130 (DCM)	25.0
4	18 Oct 1998	50 (ML)	24.5
5	19 Oct 1998	115 (DCM)	25.6
6	12 Jan 1999	100 (DCM)	23.5
7	13 Jan 1999	105 (DCM)	23.5
8	17 Feb 1999	125 (DCM)	23.5
9	18 Feb 1999	75 (ML)	23.2
10	16 Mar 1999	100 (DCM)	23.3
11	13 Apr 1999	125 (DCM)	23.6
12	14 Apr 1999	45 (ML)	23.5

by an average factor of 1.9 ± 0.9 SD. Cells <3 µm dominated the community in terms of abundance. However, larger cells accounted for most of the biovolume, and consequently dominated carbon estimates (Fig. 2b). Autotrophic flagellates (Aflag) generally comprised a lower portion of the nanoplankton community biomass than Hflag (Fig. 3A), except during the summer (Expt 1) when larger pigmented cells were at their seasonal maximum (Karl 1999, Scharek et al. 1999). Their CVs were also lower than those for Hflag (33% for DCM and 40% for ML samples).

Among the various experiments, the biomass ratio between bacteria and the likely consumers of bacteria, <5 µm Hflag, was quite variable (Fig. 4). For the 2 January experiments (Expts 6 and 7) <5 µm Hflag biomass exceeded bacteria. In February and March (Expts 8 to 10), Hflag biomass was about 70% of the standing biomass of bacteria. In the remainder of the experiments, however, bacterial biomass substantially exceeded Hflag, typically by a factor of 2 to 3.

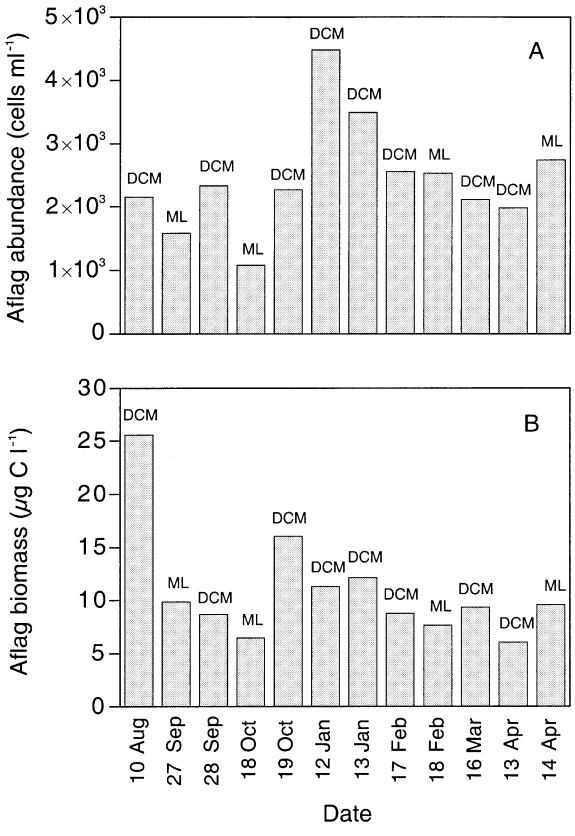

Fig. 3. (A) Abundance and (B) biomass of the autotrophic flagellate community (Aflag) in the ambient environment at the start of each experiment. ML = mixed layer; DCM = deep chlorophyll maximum

Food-web interactions

Fig. 5 shows the variations in net rates (d^{-1}) of bacterial growth for the different size-fractionations incubated in each experiment. The net growth of bacteria (Hbact + PRO) in unfiltered control treatments ranged from −0.12 to +0.16 d^{-1}, averaging +0.004 d^{-1} (SD = 0.084 d^{-1}). On the whole, therefore, the expected balance between bacterial growth and grazing losses was well demonstrated in samples containing the intact microbial community. In contrast, for most of the experimental manipulations, net growth was enhanced significantly ($p < 0.05$) in treatments filtered through the 1 µm membranes. The exceptions were Expts 1 and 6, for which the 2 µm treatments showed both significantly enhanced net growth compared to unfiltered water and the highest measured rates. In addition, bacterial growth in the <1 µm treatment was

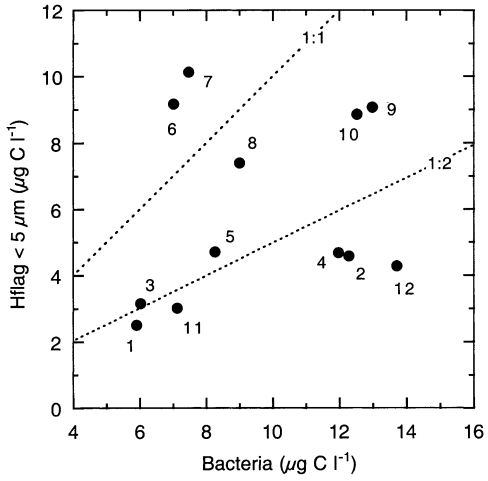

Fig. 4. Biomass relationship between bacteria and <5 µm heterotrophic flagellates (Hflag). Experiment number and lines 1:1 and 1:2 are also indicated

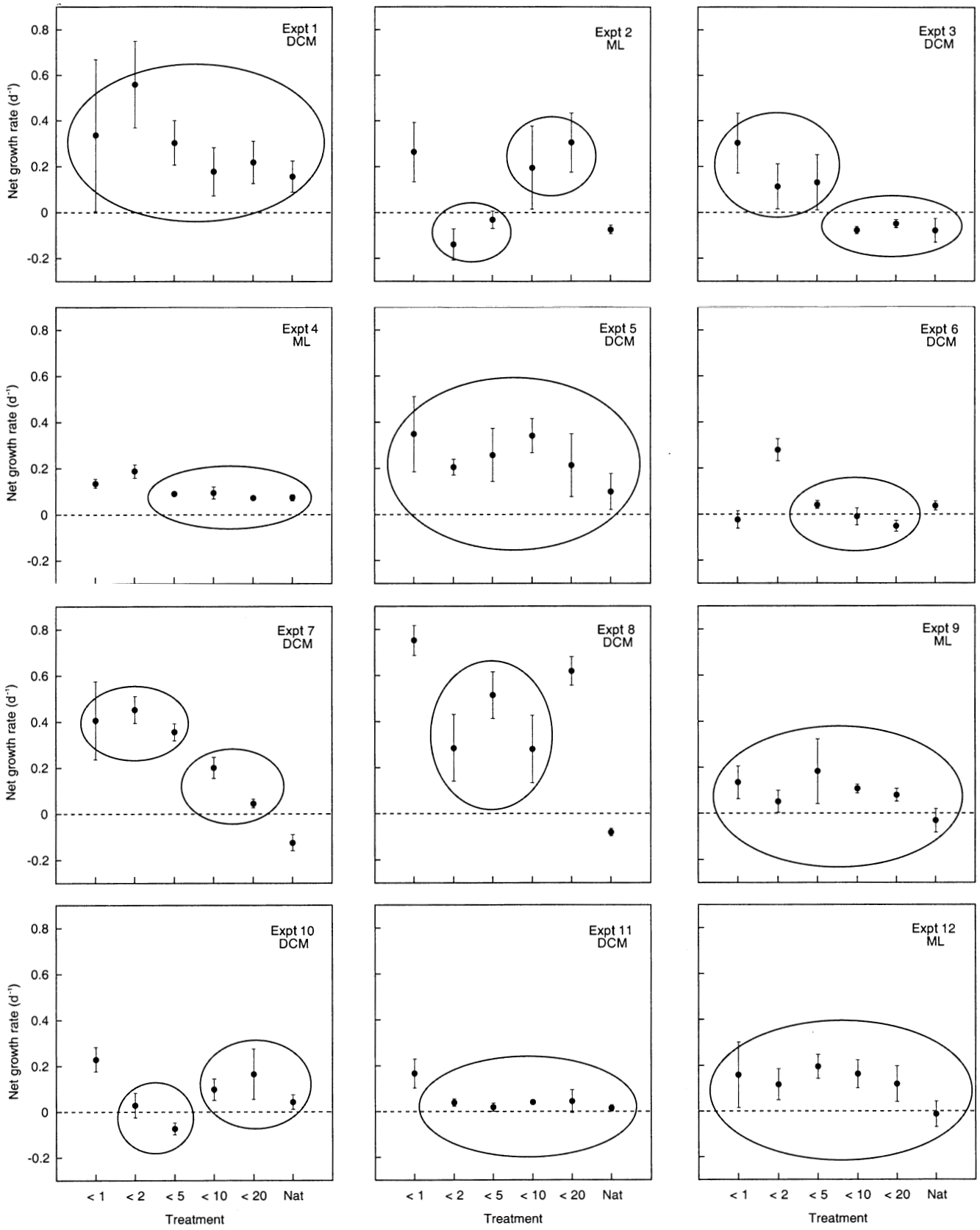

Fig. 5. Effects of removal of different size classes of consumers on the net growth rates of bacteria. Treatments are seawater samples filtered through 1 to 20 µm filters. Nat = natural growth rates from unfiltered controls. Vertical bars show standard deviation of 4 replicates. Circles group consecutive treatments that do not show significant differences (Tukey's test, $p < 0.01$). ML = mixed layer; DCM = deep chlorophyll maximum. Dashed line indicates growth = 0

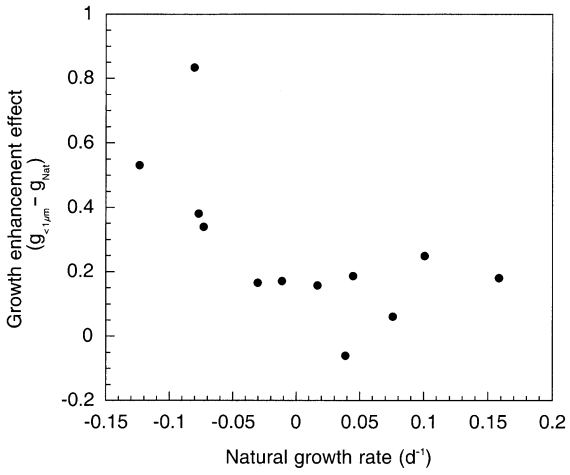

Fig. 6. Growth enhancement effect of the <1 µm treatment ($g_{<1\,\mu m} - g_{Nat}$) as a function of the net growth rate measured in the natural seawater control

only significantly lower than the control for Expt 6. Relative to the ambient seawater, prescreening of water through fine porosity filters (i.e., the <1 µm treatments; <2 µm for Expts 1 and 6) produced an average growth rate enhancement of 0.31 d^{-1} (SD = 0.21 d^{-1}). As shown in Fig. 6, the enhancement of bacterial growth rate in the <1 µm treatment only exceeded 0.3 d^{-1} in experiments where the net growth in the unfiltered control was negative. Presumably this reflected the occasions when protistan bacterivory was greatest.

The circles in Fig. 5 set off sequences in which the results were not significantly different (Tukey's test, $p < 0.01$) among adjacent size-fraction treatments. By this criterion, significant differences were not found in the treatment sequences for 4 of 12 experiments (Expts 1, 5, 9 and 12). For the experiments that showed such differences, the patterns were particularly strong for Expts 2, 3, 7, 8 and 10, with changes in net rate estimates up to 0.4 d^{-1} from one size-fraction to the next. Among the emergent patterns, Expts 1, 4, 6 and 7 showed modest increases to significantly (Expts 4 and 6) higher rates in the <2 µm filtrate, with net rates declining generally in samples passed through larger filters. Expt 3 conformed to the latter part of this pattern, but rate estimates for the <2 µm fraction were lower, though not significantly different, than the <1 µm treatment. Only for Expt 6 was the rate estimate for the unfiltered controls significantly higher than the estimate for the <20 µm treatment.

A second emergent pattern was evident in the results of Expts 2, 5 and 8 to 12. This pattern was characterized by highest net rates in the <1 µm size-fraction (significant for Expts 2, 8, 10 and 11), decreasing rates for intermediate size-fraction treatments, and modest to significant rate enhancement (significant for Expts 2, 8 and 10) in the <10 and/or <20 µm treatments. The alternating increases and decreases in net bacterial growth suggest a trophic cascade in which grazing impacts on bacteria are affected indirectly by removing successive layers of predators.

DISCUSSION

Population abundances and variability

Although our series of experiments was not sufficiently extensive to establish a definitive seasonal pattern, some variations in the abundance of bacteria and nanoplankton were evident (Figs. 1, 2 & 3). As expected from the relative constancy of bacteria in the open oceans, Hbact and *Prochlorococcus* displayed relatively low temporal variability. Both in terms of the variability of population estimates (CVs of 25 to 27%) and the ranges of densities determined over a decade of near-monthly sampling at Stn ALOHA (Landry & Kirchman unpubl.), the present estimates were representative of the study site. These prokaryotic components constitute a large fraction of the carbon biomass of the microbial community of these waters (Campbell et al. 1997). Thus, to a first approximation, total microbial biomass remains quite constant through the year even though daily turnover rates are substantial and likely dynamic.

In contrast to the bacterial assemblage, nano-sized flagellates displayed considerable variability in abundance among the different experiments. Since such organisms comprise a smaller fraction of the microbial community and are often overlooked in routine sampling of open-ocean ecosystems, their variations are not always considered in the general perception of relative constancy. Nonetheless, they may reflect meaningful oscillations in the functioning of the base of the food web.

Food web linkages

The main goals of our study were to identify the trophic linkages among microbial size fractions in an oligotrophic food web and to assess the temporal variability of these linkages. By truncating the grazer chain at predetermined sizes, we expected to find a trophic cascade effect marked by the alternation of positive and negative trends in bacterial net growth (Carpenter et al. 1985, McQueen et al. 1986, Wickham 1995, Pace et al. 1998, Calbet & Landry 1999). Given the apparent steady state of bacterial communities in oligotrophic systems, we further expected that the patterns of growth rate responses would be similar among

experiments conducted at different times and therefore indicative of a dominant regulatory mechanism.

Taken as a whole, our results provide some evidence for and some evidence against regulation of bacterial population growth by a protistan trophic cascade. The strongest cascade effects that we observed were in Expts 2, 8 and 10. The response patterns of Expts 5, 9 and 12 were similar but not significant according to our criteria. In Expts 1, 4 and 6, we observed a somewhat different and unexpected alternating pattern, with removal of the smallest category of Hflag reducing, rather than stimulating, net growth of bacteria. Lastly, the main pattern in Expts 3 and 7 was a general increase in bacterial growth with removal of successive size fractions. The variability of results achieved would lead us to believe that the regulatory mechanisms are more complex than we had hypothesized initially, perhaps involving an alternation among 2 or more modes of interaction.

By their very nature, well-defined cascades (e.g., Expts 2, 8 and 10) would imply narrow dietary preferences among small protistan consumers, with little sharing of prey between protists in adjacent size categories. Given physical limitations on prey capture and ingestion, it is easy to appreciate that sharp size cutoffs might exist on the large end of prey size spectra. For direct-interception feeding protists operating in a nutritionally dilute system, however, the arguments for sharp breaks in predation on smaller prey are less clear. Indeed, since ingestion probabilities are regulated by the physics and chemistry of encounter (Fenchel 1982a,b, Monger & Landry 1991) and since bacteria comprise a relatively large food resource in the open ocean (Fuhrman et al. 1989, Campbell et al. 1994), even modest-sized flagellates (5 to 10 µm) must randomly contact bacteria quite frequently. Ignoring the nutritional opportunity of such encounters simply because the potential also exists to consume slightly larger prey would seem to make little sense for such protists. Thus, even under the best of circumstances, some of the cascade effect of the predatory chain would likely be confounded by overlap in the size-structure of prey consumed. This might explain why the cascade pattern can sometimes appear quite subtle (Expts 5, 9 and 12).

The semi-gradual increases in bacterial net growth with size-fraction treatment in Expts 3 and 7 suggests that bacterivory may have been more broadly distributed within the protistan size spectrum in these experiments, compared to the others. Alternatively, the influence of >5 µm consumers may have been reduced, their sequential removal failing to elicit an initial positive then negative effect on bacterial growth. We see clear cascade influences of cells >10 µm in only 4 of the experiments (Expts 2, 6, 8 and 10). The more general emergent property of this study is the antagonistic effects of size class removal at the smallest end of the size range, particularly between the 1 and 2 µm filters. The directional changes are not the same in all experiments, but when the cascade effect of larger cells in the 2 to 5 µm fraction was negative, the subsequent removal of the 1 µm fraction was typically positive, and vice versa.

One way to examine the size relationships within this suite of experiments is by multiple regression analysis relating the 'growth enhancement effect', here defined as the difference in bacterial net growth rate in the 1 µm treatment and the unfiltered seawater control, to the size structure of protistan grazers. We assume that the effect measures something close to the mean specific growth rate of the bacterial assemblage caused by the removal of protists (Caron et al. 1999). Bacteria may have benefited indirectly from some DOM release during size-fractionation (Fuhrman & Bell 1985), but at the same time those filtered through the smallest pore sizes would have been cut off from production and remineralization feedbacks to larger auto- and heterotrophs (Ferrier-Pagès et al. 1998). In addition (discussed further below), growth rates in the smallest size-fraction treatment may have been less than optimal if some of the larger, faster growing bacteria were removed by size-fractionation, or if some of the smallest heterotrophic protists squeezed through the 1 µm pore. While it is difficult to assess the net effects of these potential experimental artifacts on the resulting rate estimates, on balance, they suggest that the 'growth enhancement effect' was a conservative measure of bacterial growth rate. The only growth-enhancing effect was the potential filtration release of DOM, which Fuhrman & Bell (1985) showed to be less problematic with decreasing filter pore size.

With these possible complications in mind, 88% of the variability in the measured growth enhancement effect (Y, d^{-1}) in the 12 experiments can be explained by 3 size components of the Hflag assemblage, the biomass (µg C l^{-1}) of <2, 2–3 and 3–5 µm heterotrophs. The resulting equation,

$$Y = 0.20 - 0.86\ [<2\ \mu m\ Hflag] + 0.71\ [2–3\ \mu m\ Hflag] - 0.30\ [3–5\ \mu m\ Hflag]$$

is significant at $p < 0.0002$ ($R^2 = 0.88$), and individual estimators are significant at $p < 0.0005$. Even though most of the heterotrophic protist biomass in our microscopical analyses resided in the >5 µm size fractions (Fig. 2B), taking larger grazers into account contributed no additional power to the regression analysis. This may be a consequence of the imprecision in biomass determinations because so few large cells were counted on individual slides. For possibly the same reason, including Aflag in the regression model also

had an insignificant effect on its ability to explain experimental variability.

According to the above equation, growth dynamics of bacteria in the subtropical North Pacific are significantly influenced by a 3-step predatory chain compressed within the <5 µm size fraction. Hflag of <2 µm size feed directly on bacteria, hence their negative interactive effect. Hflag of 2–3 µm in size positively influence net bacterial growth by predating on smaller flagellates, while 3–5 µm Hflag exert another negative influence by suppressing 2–3 µm flagellates. As noted previously, Calbet & Landry (1999) speculated from the results of a single size-fraction experiment that 2 to 5 µm Hflag were the likely principle predators of bacteria in the oligotrophic open ocean, and they, in turn, were consumed by 5 to 20 µm flagellates. Therefore, the present findings are in better agreement with the studies of Wright & Coffin (1984) and Wikner & Hagström (1988), which suggested smaller (1 to 3 µm) bacterivores.

Because we have arbitrarily chosen to group our size classes in whole µm integers and because cells may shrink somewhat when preserved (Stoecker et al. 1994), the implied sizes of organisms in this predatory chain are only approximate. In addition, direct comparison between the grazer size structure from microscopical analyses and the observed experimental effects of size-fractionated treatments is difficult due to the plasticity of living cells. Rassoulzadegan & Sheldon (1986) have argued, for instance, that some flagellates can pass filter pores half their cell width without damage. Accordingly, the apparent activity of grazers in the <1 µm treatments of some experiments must include cells enumerated microscopically in the <2 µm size class. Relatively subtle changes in the dimensions or plasticity of cells in the smallest size categories might explain why the growth response in the <1 µm fraction sometimes exceeded that in the <2 µm treatment, and sometimes not. While there is evidence that filtration through 1 µm filters usually eliminates grazing activity in open-ocean oligotrophic systems (Caron et al. 1999), even smaller pore sizes might be needed to remove all of the tiniest grazers. At the same time, decreasing filter pore size complicates the problem of maintaining the integrity of the bacterial assemblage. In the present experiments, for instance, an average of 7.9% (±3.3% SE) of the natural bacterial assemblage was retained on the 1 µm filters, most (78%) of which passed through the 2 µm filter. If these are larger bacterial cells or particle-associated bacteria that contribute disproportionately to community growth rate (e.g., Krambeck & Krambeck 1984, González et al. 1990, Gasol et al. 1995), one can conceivably lose more in terms of the net growth rate response of bacteria by using increasingly smaller pore sizes to extract the last possible grazers. In fact, this potential effect provides an alternate explanation for the net growth decrease in the <1 µm treatment (compared to <2 µm) in Expts 1, 4 and 7.

Regulation of the microbial community

The natural state of a system at or near equilibrium is to show negligible to low temporal change in its component populations. In the present study, the approximately 2-fold range in bacterial community abundance and biomass was consistent with long-term observations (Landry & Kirchman unpubl.), and net growth rates of the natural assemblage (–0.12 to 0.16 d^{-1}) differed little on average from steady-state expectations. However, the manner in which the relative constancy was maintained changed from experiment to experiment. In some (e.g., Expts 2, 3, 7 and 8), the balance appeared to be achieved by strong grazing pressure on fast growing bacteria (top-down), and in others (e.g., Expts 4, 9, 11 and 12) by weak grazing control of slower growing bacteria (bottom-up). According to the model proposed by Gasol (1994), our data fit close to the line of maximum attainable abundance of Hflag that a system can support with a given abundance of bacteria. Such strong bacteria-Hflag coupling is typical of extreme oligotrophic conditions and is consistent with the potential of bacterivorous nanoflagellates to regulate bacterial populations by predation. In contrast, a substantial deficiency in the relative abundances of Hflag and bacteria would imply predator control of bacterivorous flagellates, and therefore substrate regulation of bacterial abundance (Gasol 1994).

While the relationships between Hflag and bacterial biomass observed in our experiments (Fig. 4) do not completely resolve a pattern in regulatory processes (top-down vs bottom-up), they may offer some useful insights. For instance, when bacterial biomass was high (e.g., Expts 2, 4 9, 10 and 12), the bacteria generally showed little growth response to the removal of predators and may have been resource limited in most cases. Expt 2 stands out as atypical of this group. This experiment had the highest abundance of >5 µm Hflag of all experiments, which may explain the relatively large net growth swing when this size fraction was removed.

The highest net bacterial growth rates occurred in experiments in which the ratio of Hflag to bacterial biomass was initially high (Expts 7 and 8). Intuitively, one would expect bacterial growth rates to be elevated when standing stocks are low, particularly if predator levels are also relatively high. In Expt 6, however, and to a lesser extent Expts 1 and 7, the lower net growth rates in the <1 µm treatments compared to those in the

<2 µm samples may be complicated by size shifts. Mechanistically, higher grazing pressure may select for smaller bacterial cells (Andersson et al. 1986, González et al. 1990), and therefore smaller grazers capable of passing the 1 µm filters. Alternatively, these results could indicate specific circumstances in which grazer remineralization feedbacks contributed most growth to bacterial growth, hence, where their elimination had the most effect.

Trophic cascades were significant (Expts 2, 8 and 10) or suggestive (Expts 5, 9 and 12) at intermediate to high levels of bacterial biomass (Fig. 4). There was notably little indication of cascade shifts when bacterial biomass was low. We might speculate from these observations that heterotrophic protists may best organize themselves into a coupled predatory chain when the base of prokaryotic prey is relatively large. If so, the cascades may appear principally as transition phases between high (substrate limited) and low (grazer regulated) concentrations of bacterial biomass.

We had undertaken this study expecting to find relatively static response patterns, or perhaps subtle seasonal differences, in an oligotrophic open-ocean system defined by inherently low variability (McGowan & Walker 1979, Hayward et al. 1983, Venrick 1999). It is surprising, therefore, that the underlying microbial community interactions appear to be quite variable and perhaps indicative of regular oscillations between resource and predatory controls. Multiple levels of consumers indirectly affect the balance of resource and predator regulation, further complicating the mechanistic interpretations of these experiments. Future research needs to explore how such constraints relate to one another in a temporal context and combine to maintain microbial populations within relatively stable limits of variability.

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