Contrasting effects of substrate and grazer manipulations on picoplankton in oceanic and coastal waters off Brazil

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Abstract. In two contrasting regions off the coast of Brazil, picoplankton (<1 µm) responses to removal of larger grazers and to the additions of glucose and amino acids were determined. Effects of glucose and amino acid additions (1 µM) on particulate nitrogen and chlorophyll a concentrations, and on rates of NH₄⁺ uptake and regeneration, were observed after 5 h pre-incubation. In the oceanic waters, removal of the >1 µm fraction had no significant effect on the chlorophyll a of the picoplankton after 5 h. However, the addition of glucose stimulated both uptake and regeneration by a mean of 27%, and the addition of arginine led to significant decreases in the rates of NH₄⁺ uptake and regeneration. In contrast, in the coastal waters, significant increases in chlorophyll and particulate nitrogen concentrations were found after 5 h incubation in both the amended samples and in the controls, and mean rates of NH₄⁺ uptake and regeneration were affected to a lesser degree by the additions of either glucose or amino acids than in the oceanic waters. The oceanic responses were suggestive of carbon limitation of heterotrophic bacteria. In the coastal region, on the other hand, the supply of organic carbon and nitrogen was likely to have been sufficient to meet the nutritional requirements of the heterotrophic bacteria and cyanobacteria. Grazing by larger organisms on the picoplankton appeared to play a more significant role in the nitrogen cycle in the coastal waters than in the oceanic waters.

Introduction

Marine picoplankton, consisting of organisms <1 µm (Li et al., 1983), have been shown to be abundant all over the world (Stockner and Antia, 1986; Le Boutieller et al., 1992; Fogg 1995). This size class includes prokaryotic and eukaryotic autotrophs and heterotrophs, with the primary contributors to this size class being cyanobacteria and heterotrophic bacteria (Selmer et al., 1993). Of the prokaryotes, Synechococcus and Prochlorococcus have been found to be the predominant components in this small fraction, although their relative contribution depends on location and depth, among other factors (Chisholm et al., 1988; Campbell and Vaulot, 1993; Liu et al., 1995; Moore et al., 1995; Furnas, 1999). The picoplanktonic autotrophs have been shown to contribute up to 90% of total chlorophyll a (Chl a) concentration, and between 20 and 80% of total primary production, in oligotrophic waters (Li et al., 1983; Takahashi and Bienfang, 1983; Probyn, 1985; Teixeira and Gaeta, 1991; Jiao and Wang, 1994). These small organisms also play an important role in the nitrogen cycle in marine environments due to their rapid cycling of nutrient elements and energy (Fogg, 1995).

Heterotrophic bacteria can be a major sink for organic carbon and nitrogen. Much of their variation can be explained by variations in primary production (Cole et al., 1988). They can assimilate a wide variety of organic and inorganic compounds for growth, including dissolved free amino acids (Wheeler and Kirchman, 1986; Coffin, 1989; Kirchman et al., 1989; Keil and Kirchman, 1991;
Jørgensen et al., 1993, 1994), dissolved combined amino acids (Coffin, 1989; Tupas and Koike, 1990; Jørgensen et al., 1993, 1994), nucleic acids (Jørgensen et al., 1993), and NH$_4^+$ (Kirchman et al., 1989; Tupas and Koike, 1990). Uptake of NH$_4^+$ by heterotrophic bacteria has been shown to be controlled by the supply of dissolved organic carbon as well as by the supply of dissolved organic nitrogen (Kirchman et al., 1990; Keil and Kirchman, 1991). Kirchman et al. observed that glucose additions stimulated NH$_4^+$ depletion in 0.8 µm fractionated water in the Subarctic Pacific (Kirchman et al., 1990). Suttle et al., based on observations of increased NH$_4^+$ uptake by bacteria with decreasing NH$_4^+$ additions, suggested that bacteria out-compete phytoplankton for NH$_4^+$ at low concentrations (Suttle et al., 1990). Depending on the nutritional status of the bacteria, and the nutritional quality of the available substrates, bacteria may either consume or release NH$_4^+$ (Goldman et al., 1978; Goldman and Dennett, 1991; Jørgensen et al., 1994).

Within a natural population, it is highly likely that some bacteria may be actively consuming NH$_4^+$ while others are releasing it (Tupas and Koike, 1990). Picoplankton biomass and production, both autotrophic and heterotrophic, may also be tightly regulated by grazers (Fuhrman and McManus, 1984; Wikner and Hagström, 1988; Kirchman, 1990; Hadas et al., 1998). Grazers may not only regulate biomass through direct predation, but the process of grazing may also enhance the supply of dissolved organic and inorganic substrates (Sherr et al., 1988; Weisse and Scheffel-Möser, 1991; Miller et al., 1997; Glibert, 1998). Thus, while grazing results in a reduction in biomass, the process of grazing results in production of substrates that may stimulate production. The extent to which substrate supply and/or grazing regulate picoplankton biomass or production may depend on subtle shifts in the relative availability of substrates, the nutritional status of the component organisms, the number of trophic interactions, as well as environmental parameters such as light and temperature (Glibert, 1998).

The purpose of this study was twofold. First, we aimed to examine the contribution of the picoplankton biomass relative to the larger plankton in two contrasting regions off the coast of Brazil. Second, we sought to determine the relative effects of the removal of grazers >1.0 µm, and the addition of glucose and amino acids on biomass and rates of NH$_4^+$ uptake and regeneration in this fraction at these contrasting sites. By altering both the trophic interactions by removal of larger grazers and the resource supply in terms of additions of organic carbon and organic nitrogen, we hoped to tease apart the more subtle differences in the microbial fractions of these regions. We hypothesized that if populations were tightly grazer controlled, then removal of the grazers would impact biomass regardless of substrate amendments. Alternatively, we hypothesized that if the populations were nutritionally limited, organic substrate additions would impact the rates of NH$_4^+$ regeneration and uptake without affecting biomass on the time scales of these experiments.

**Method**

Experiments on the effects of grazer removal and organic additions on the <1 µm fraction were conducted in the South Atlantic off Brazil in March 1994 and...
December 1994 (Figure 1). The March samples were collected during a cruise of the project entitled 'Ocean Circulation in the Western Region of the South Atlantic' (COROAS—Circulação Oceânica da Região Oeste do Atlântico Sul) aboard the R/V ‘Prof. W. Besnard’, while those from December were collected from daily excursions from the University of São Paulo research station at Ubatuba, aboard R/B ‘Albacora’. All experiments were conducted with subsurface samples (50% surface incident irradiance), and were repeated twice in March and four times in December.

Samples were collected with 10 l Niskin or Go-Flo bottles (General Oceanics Inc., FL) and filtered through a 20 µm screen before being dispensed into 20 l polyethylene containers. Filtration of subsamples onto Whatman GF/F filters (2 layers for <1 µm fraction and 1 layer for <20 and <200 µm fractions) was performed immediately after collection of sample and after pre-incubation (for each treatment) to determine the concentration of Chl \( \text{a} \) and particulate nitrogen (PN). Filters for Chl \( \text{a} \) determination were stored at \(-20^\circ\text{C}\) with dehydrated silica gel and Chl \( \text{a} \) concentrations were measured by fluorescence on an Aminco-Bowman fluorometer (SLM Instruments Inc., IL) after extraction in 5 ml of acetone (90%) at \(-4^\circ\text{C}\) for 12 h (Yentsch and Menzel, 1963). Filters for determination of PN were also stored at \(-20^\circ\text{C}\) and dried at 50°C before being analyzed on a Control Equipment CHN analyzer (Control Equipment Corporation, MA). Filtrates were frozen for later analysis of \( \text{NH}_4^+ \) concentration following the method described by Aminot and Chaussepied (Aminot and Chaussepied, 1983).

![Fig. 1. Map showing location of oceanographic stations occupied in March 1994 and the coastal station (*) located between Rio de Janeiro and Santos occupied in December 1995. Picoplankton experiments were conducted at Stations 14 and 26.](https://academic.oup.com/plankt/article-abstract/22/1/77/1549632)
Filtration of the sample for picoplankton experiments was initiated within 15–30 min of sample collection. Samples were filtered through 1.0 µm Nuclepore polycarbonate filters under a vacuum of less than 50 mmHg. The fractionated sample (<1.0 µm) was dispensed into six, 2 l polycarbonate bottles. To two of the incubation bottles, 1.0 µM of glucose was added, to the second pair of bottles 1.0 µM of a commercial amino acid mixture (Pierce 20089: coastal samples) or arginine (oceanic samples) was added, and the third pair of bottles was used as a control without any substrate addition. The bottles were incubated for 5 h under simulated in situ conditions. Light intensity of sampling depth was simulated with neutral density screening. Temperature was maintained by on-deck circulating surface water baths on the March cruise, while in December, bottles were attached to a float and incubated in surface water in neutral density screen bags. The labor-intensive nature of these experiments precluded replication of each experiment on each day. However, each experiment was replicated 2–4 times in each region over the course of the studies at the contrasting sites.

After 5 h, one of each pair of treatment bottles was filtered for the analysis of the concentration of Chl a and of particulate nitrogen (PN) and carbon (PC), as described above. The other pair of bottles was used for the determination of the rates of 15NH4+ uptake. Trace concentrations of 99% enriched 15N preparations of NH4+ were added. Additions were <10% of ambient or 0.03 µg atom N l–1 when NH4+ ambient concentrations were below detection limits. Samples were incubated for an additional hour at the same in situ simulated conditions of the pre-incubation. Incubations were terminated by filtration onto two layers of Whatman GF/F filters (pre-combusted at 500°C for 1 h) to increase retention of bacteria (Glibert et al., 1995). Both the filter and filtrate were collected and frozen at –20°C. The 15N atom % enrichments of the filters were determined by mass spectrometry as described by Glibert and Capone (Glibert and Capone, 1993). Samples were ground with copper oxide (Baker #1820–05; pre-combusted at 600°C for 3 h), placed with wire copper metal (Alpha Resources) in Pyrex glass tubes (also pre-combusted), evacuated, sealed and combusted at 550°C for 2.5 h.

Specific and absolute uptake rates [as V (h–1) and ρ (µg at N l–1 h–1), respectively] of NH4+ were calculated according to Glibert and Capone (Glibert and Capone, 1993). Although samples were originally collected for the determination of NH4+ regeneration by isotope dilution [e.g. (Glibert et al., 1982)], approximately 50% of the filtrates for isotopic analysis in the isotope dilution experiments were contaminated by 14N–NH4+. This contamination appeared to be associated with the use of foil-lined caps that were used on the vials for storing the distillate prior to mass spectrometry. Inasmuch as the contamination could not be accurately estimated, all rates of NH4+ regeneration were thus calculated using the following formula:

\[ D = \frac{(C_f - C_i)}{t} + \rho \]

where \( D \) is the NH4+ regeneration rate (µg atom N l–1 h–1), \( C_f \) is the final ammonium concentration, \( C_i \) is the initial ammonium concentration, \( t \) is the incubation duration and \( \rho \) is the rate of NH4+ uptake (µg atom N l–1 h–1).
The measurements on the larger size fractions, <20 µm and <200 µm, included Chl a and PN, as described above. Rates of nitrogen uptake were determined for the <200 µm fraction and have been reported in Metzler et al. (Metzler et al., 1997).

Results

General water column structure

General water column structure, water mass dynamics and the patterns of nitrogen and carbon uptake of the plankton communities studies, have been presented in Metzler et al. (Metzler et al., 1997). Stations along transect 3 and station 30 had strong thermal gradients, resulting from the rising of South Atlantic Central Water over the continental shelf. Those stations further offshore (stations 6–26), on the other hand, were dominated by warm, high salinity Tropical Water (Metzler et al., 1997).

The coastal station, which was occupied over a period of 7 days, showed a variation between mixed and stratified conditions. The initial 2 days of sampling were during a period of well-mixed conditions; however, according to temperature and salinity data, a thermocline developed on the fourth day, resulting in stratification and pronounced effects on production (Metzler et al., 1997). Here, we distinguish these periods as pre- and post-stratification.

Oceanic experiments: ambient nitrogen and size fractionated biomass

In the oceanic waters, the surface-mixed layer was characterized by low phytoplankton biomass (Chl a ≤0.13 µg l⁻¹) and low nitrogen nutrient concentrations (NH₄⁺ <0.03 µg atom N l⁻¹; NO₃⁻ ≤0.74 µg atom N l⁻¹; urea <0.03 µg atom N l⁻¹ with the exception of one station; Table I). Concentrations of Chl a in the <1 µm fraction represented on average ~60% of the total Chl a (Table II), while ~90% of the PN was in this fraction (Table II).

Oceanic experiments: responses to glucose and amino acid additions

Incubation of the <1 µm fraction with glucose or arginine had no significant effect on Chl a concentrations (Figure 2A), and the net change in Chl a concentration in the controls and amended treatments were not significantly different (Figure 2C). However, increases in PN, relative to the control treatments, were observed upon addition of glucose (Figures 2B, D). Relative to the control treatments, these increases in PN averaged 52% and were significantly different (P < 0.01) from the responses in the control and arginine-treated samples, which decreased slightly in PN concentration (Figure 2D).

The changes in NH₄⁺ concentrations of the oceanic samples upon incubation with glucose or arginine were very small and were not significant (data not shown). The addition of glucose to oceanic samples resulted in an average increase of 27% in the rate of NH₄⁺ uptake and regeneration (Figure 3A, B). In contrast, the addition of arginine led to a significant decrease in the rates of NH₄⁺
uptake and regeneration, averaging 53%. The net differences in the effects of glucose and arginine were significant at $P < 0.01$.

**Coastal experiments: ambient nitrogen and size fractionated biomass**

At the coastal station, low NO$_3^-$ concentrations (0.08–0.26 µg atom N l$^{-1}$), and variable NH$_4^+$ concentrations (0.31–1.05 µg atom N l$^{-1}$) and urea concentrations (0.53–0.67 µg atom N l$^{-1}$; Table I), characterized the near-surface samples. In sharp contrast to the oceanic station samples, at the coastal site there was considerably more biomass in the planktonic fractions >1 µm (Table II). The <1 µm fraction represented only ~20% of the total Chl $a$ before stratification, and ~35% after stratification. The 1–20 µm fraction had the largest fraction of Chl $a$ overall. The distribution of PN among the three measured size classes was somewhat different from that of Chl $a$ (Table II). While most of the PN was in the size classes <20 µm, ~30% was in the <1 µm fraction prior to stratification and ~40% was in this fraction after stratification.

Fig. 2. Mean biomass changes as Chl $a$ and PN in the <1 µm fraction from the oceanic, coastal pre-stratification and coastal post-stratification samples in response to additions of substrates. Light gray bars represent the concentrations before incubation, clear bars represent the control samples, black bars represent the glucose treatments and the hatched bars represent the amino acid treatments. Panels A and B give concentrations within the sample treatments and Panels C and D give the net changes between the ambient concentration and the incubation treatments. Error bars represent the standard deviation of the mean.
Coastal experiments: responses to glucose and amino acid additions

In contrast to the oceanic stations, after 5 h of pre-incubation, a significant increase in Chl $a$ was observed in all treatments, including the controls (Figure 2A, C). The increase in Chl $a$ was, on average, 290% for samples collected pre-stratification and 65% for samples collected post-stratification. An increase in PN was also observed after incubation, but not as large an increase as with Chl $a$, on average, 53 and 44% increase for samples collected under pre- and post-stratification conditions, respectively (Figure 2B, D).

### Table I. Ambient nitrogen and Chl $a$ concentrations for the stations sampled in the two regions indicated

<table>
<thead>
<tr>
<th></th>
<th>Depth (m)</th>
<th>NH$_4^+$ (µg atom N l$^{-1}$)</th>
<th>NO$_3^-$ (µg atom N l$^{-1}$)</th>
<th>Urea (µg atom N l$^{-1}$)</th>
<th>Chl $a$ (µg l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oceanic</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>&lt;0.03</td>
<td>0.16</td>
<td>0.26</td>
<td>0.11</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>&lt;0.03</td>
<td>0.54</td>
<td>&lt;0.03</td>
<td>0.11</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>&lt;0.03</td>
<td>0.24</td>
<td>&lt;0.03</td>
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<td>12</td>
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<td>&lt;0.03</td>
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<tr>
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<td>0.24</td>
<td>n.d.</td>
<td>0.05</td>
</tr>
<tr>
<td>23</td>
<td>15</td>
<td>&lt;0.03</td>
<td>0.04</td>
<td>&lt;0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>25</td>
<td>12</td>
<td>&lt;0.03</td>
<td>0.35</td>
<td>&lt;0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>26</td>
<td>12</td>
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<td>0.74</td>
<td>n.d.</td>
<td>0.09</td>
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<tr>
<td>30</td>
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<td>0.55</td>
<td>n.d.</td>
<td>0.12</td>
</tr>
<tr>
<td>39</td>
<td>12</td>
<td>&lt;0.03</td>
<td>0.44</td>
<td>&lt;0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>61</td>
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<td>&lt;0.03</td>
<td>0.02</td>
<td>&lt;0.03</td>
<td>0.1</td>
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<tr>
<td>Coastal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-strat.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 December</td>
<td>4</td>
<td>0.62</td>
<td>0.15</td>
<td>0.67</td>
<td>0.32</td>
</tr>
<tr>
<td>2 December</td>
<td>4</td>
<td>0.68</td>
<td>0.16</td>
<td>0.53</td>
<td>0.18</td>
</tr>
<tr>
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<td>0.08</td>
<td>n.d.</td>
<td>0.18</td>
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<tr>
<td>4 December</td>
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<td>1.05</td>
<td>0.08</td>
<td>n.d.</td>
<td>0.18</td>
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<td>Post-strat.</td>
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<td>0.53</td>
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<tr>
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<td>0.11</td>
<td>n.d.</td>
<td>0.22</td>
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<tr>
<td>8 December</td>
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<td>0.63</td>
<td>0.26</td>
<td>n.d.</td>
<td>0.22</td>
</tr>
</tbody>
</table>

n.d., no data available.

### Table II. Means and standard deviations of biomass of the plankton in each size class measured for the two regions indicated off Brazil. Chl $a$ is given in units of µg l$^{-1}$ and PN is given in units of µg atom N l$^{-1}$

<table>
<thead>
<tr>
<th></th>
<th>&lt;1 µm</th>
<th>1–20 µm</th>
<th>20–200 µm</th>
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</thead>
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<td>Oceanic sites</td>
<td></td>
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<td></td>
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<tr>
<td>Chl $a$</td>
<td>0.06 ± 0.03</td>
<td>0.04 ± 0.03</td>
<td>0.01 ± 0.04</td>
</tr>
<tr>
<td>PN</td>
<td>0.59 ± 0.07</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Coastal sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chl $a$</td>
<td>0.06 ± 0.03</td>
<td>0.16 ± 0.07</td>
<td>0.01 ± 0.07</td>
</tr>
<tr>
<td>PN</td>
<td>0.62 ± 0.06</td>
<td>0.68 ± 0.28</td>
<td>0.37 ± 0.24</td>
</tr>
</tbody>
</table>

Coastal experiments: responses to glucose and amino acid additions

In contrast to the oceanic stations, after 5 h of pre-incubation, a significant increase in Chl $a$ was observed in all treatments, including the controls (Figure 2A, C). The increase in Chl $a$ was, on average, 290% for samples collected pre-stratification and 65% for samples collected post-stratification. An increase in PN was also observed after incubation, but not as large an increase as with Chl $a$, on average, 53 and 44% increase for samples collected under pre- and post-stratification conditions, respectively (Figure 2B, D).
At the coastal site, the NH$_4^+$ concentrations showed a great deal of variation during the study period but did not vary significantly with state of stratification (data not shown). During the pre-stratification period, glucose had virtually no effect on rates of NH$_4^+$ uptake or regeneration relative to control treatments, but amino acid additions led to modest decreases (Figure 3A, B). However, after stratification, it was the glucose-amended samples that had depressed rates of NH$_4^+$ uptake, while the amino acid-amended samples were unchanged relative to the controls (Figure 3A, B). Except for the depression in NH$_4^+$ upon glucose addition under pre-stratification conditions, substrate amendments had no significant effects ($P > 0.01$) on rates of NH$_4^+$ uptake and regeneration.

**Discussion**

The experiments conducted in this study were aimed at examining the relative effects of substrate additions and grazer removal on nitrogen metabolism and biomass in the Brazilian subtropical waters. Whereas we had anticipated differential responses between the control, glucose and amino acid-enriched treatments, we had not anticipated that these effects would vary significantly between regions. Nor had we expected the large change in biomass in the short interval of

![Fig. 3](https://academic.oup.com/plankt/article-abstract/22/1/77/1549632)
our incubations observed in the coastal samples. These responses are suggestive of different factors regulating the biomass and nutrition of the microbial biota in each region. Before considering the biological factors affecting these patterns, we first consider the experimental factors which differed between the oceanic and the coastal experiments.

**Experimental considerations**

There are two differences in the manner in which the offshore and inshore experiments were conducted, but we do not believe these differences alone explain these overall patterns. First, the oceanic stations were occupied in March and the inshore stations in December. While there may have been some effect due to the different time of year, both of these periods represented subtropical summer. Second, in the oceanic region, the amino acid addition used was solely arginine, while that in the coastal region was a mixture of several amino acids. We did not, unfortunately, have the opportunity to compare the responses of a single sample to both amino acid additions. While some amino acids may be taken up in preference to others, both amino acid additions provided both a carbon and nitrogen substrate for the picoplankton, and it was the relative responses of the amended treatments compared with the controls that were of interest.

**Trophic effects**

By fractionating the <1 µm sized particles in this experiment, we altered the natural trophic interactions in the microbial food web. We do not have data on community composition, but the biomass and nutrient data are indicative of different relationships at the contrasting sites.

Across all treatments (control, glucose, amino acids), the net changes in PN and Chl a during the incubation period were remarkably similar within regions (oceanic versus coastal), but not between regions (Figure 2). In the oceanic region, net increases in PN were noted, while net changes of Chl a or NH₄⁺ were barely measurable in any treatment. However, for the coastal site, significant increases in PN and Chl a were noted for all treatments, including the controls. The lack of net NH₄⁺ or Chl a response to removal of organisms >1 µm in the oceanic samples suggests that either there was a lack of tight grazer control, or that the dominant grazers themselves were also <1 µm. There is some evidence that a tightly coupled microbial food web may be present even in very small size classes [e.g. (Fuhrman and McManus, 1984; Wikner and Hagström, 1988)], and the role of viruses must also be considered in such a scenario (Proctor and Fuhrman, 1991; Suttle et al., 1991). The size distribution of the biomass (Table II) is consistent with little biomass in the >1 µm fraction in these waters. The lack of response may also suggest a sufficiently slow growth rate that within the 5 h incubation, a significant change would not have been observed.

Mean net increases in PN and Chl a at the coastal site were similar in the control and amended treatments (Figure 2). In this region of the Brazilian inner shelf,
nanoplanktonic (<20 µm) organisms have been previously found to comprise the largest fraction of the phytoplankton community (Susini, 1990; Metzler, 1991). Abe (Abe, 1993), studying the contribution of micro-, nano- and picophytoplankton to total biomass and productivity, found that nanoplankton comprised up to 53% of the total phytoplankton biomass (as Chl \(a\)), while picoplankton Chl \(a\) concentrations represented, on average, 8% of the total Chl \(a\) concentration in summer. Our size-fractionated Chl \(a\) distributions for summer 1994 (Table II) are generally consistent with the Abe findings (Abe, 1993). Our results are suggestive of significant ‘top-down’ control on the <1 µm fraction. Release from grazing pressure by pre-incubation likely permitted the biomass in this fraction to increase, even in the relatively short incubation period of our experiments.

Significant control of bacterial or picoplankton growth and production by grazing has been demonstrated by several studies. Indeed, Rassoulzadegan and Sheldon suggest that at least four predator-prey interactions may occur among organisms in a <10 µm fraction (Rassoulzadegan and Sheldon, 1986). Furthermore, Wikner and Hagström observed that increases in bacterioplankton biomass could be detected after 2–3 h when grazers were removed (Wikner and Hagström, 1988). Similar effects, but over longer periods of incubation, were found by Weisse and Scheffel-Möser (Weisse and Scheffel-Möser, 1991). Thus, the increases we noted within 5 h are certainly consistent with the notion of reduction in grazing pressure.

Changes in Chl \(a\) concentration may also be a result of photoadaptation without concomitant biomass increases. While we made every effort in our incubation to simulate the light level of sample collection, photoadaptive changes may have occurred in our samples. Increases in shading over the ambient surface irradiance from which these samples were collected would result in the synthesis of additional Chl \(a\) per cell. Regardless, photoadaptation would not explain the observed increases in PN.

Waterbury et al., in an extensive study of *Synechococcus*, has shown that for this picoplankter, there is a distinct diel pattern in the division of these cells, with maximum division occurring 4–5 h after the light period begins and continuing for the remainder of the light period (Waterbury et al., 1986). Hagström et al. also observed similar bursts in division of cyanobacteria in the Mediterranean Sea (Hagström et al., 1988). The periods of the day during which these division bursts appear to occur coincide with the time of day during which our samples were incubated. Based on changes in Chl \(a\), we calculate division rates of 2.5–2.8 divisions day\(^{-1}\) for the control samples prior to stratification and 0.8–1.6 after stratification. These rates are within the range reported for the cyanobacterial fraction by Waterbury et al. for temperate coastal waters (Waterbury et al., 1986), and by Bienfang and Takahashi, and Landry et al., for subtropical waters (Bienfang and Takahashi, 1983; Landry et al., 1984).

In mass balancing the net NH\(_4\)\(^+\) depletion with net PN production in the coastal experiments, there was insufficient NH\(_4\)\(^+\) consumed to support the increase in biomass. While ambient concentrations of NO\(_3\)\(^-\) were consistently <0.3 µg atom N l\(^{-1}\) (Table I), concentrations of urea tended to be slightly higher, from 0.5 to 0.6 µg atom N l\(^{-1}\), and represented ~50% of the ambient available nitrogen.
(Metzler et al., 1997). Thus, other sources of nitrogen were available to support the increased PN.

Substrate effects

Whereas the coastal sites showed consistent increases in biomass regardless of the treatment of stratification state, they differed with respect to the rates of NH$_4^+$ fluxes during the pre- and post-stratification periods. Glucose additions have previously been shown to stimulate bacterial productivity and/or bacterial abundance in estuarine and mesohaline waters (Chin-Leo and Benner, 1992; Shiah and Ducklow, 1994), leading to increased draw-down of NH$_4^+$ (Kirchman et al., 1990). Carbon substrate limitation in these samples is suggested. The depression of NH$_4^+$ uptake upon amino acid addition, as observed at two stations, lends further support to this notion. Amino acids are considered to be preferred by bacteria (Billen, 1984; Kirchman et al., 1989), and direct uptake of amino acids by bacteria has been shown to result in higher growth rates than direct uptake of NH$_4^+$ (Payne and Wiebe, 1978).

The effects and interactions of substrates may vary significantly depending on the time scale of the response, and interpretations regarding limitation of a particular substrate are not necessarily straightforward. Goldman and Dennett have noted that the efficiency with which NH$_4^+$ is utilized upon addition of glucose or amino acids depends on the relative concentration of the substrates and not their absolute concentration (Goldman and Dennett, 1991). Furthermore, the assimilation of nitrogen and bacteria depends on the internal cellular status of carbon cycling, and short-term depressions in the rate of assimilation of nitrogen may occur following glucose or amino acid additions if conditions lead to increases in respiratory demands (Turpin et al., 1988). Thus, during the post-stratification period, glucose appeared to have a negative effect on NH$_4^+$ uptake, but this effect was likely to have been transitory. Kirchman showed for the subarctic Pacific that addition of amino acids stimulated bacterial production and that the effect of amino acid additions was generally greater than the effect of additions of glucose plus NH$_4^+$ (Kirchman, 1990). Similar findings have been reported for other lakes (Jørgensen, 1987). Additionally, in a survey of factors influencing heterotrophic bacteria in fresh and marine waters, Gasol and Vaqué suggested that substrate limitation was typical of these organisms (Gasol and Vaqué, 1993).

In summary, these measurements on the picoplankton fraction in Brazilian oceanic and coastal regions suggest differing factors controlling biomass and NH$_4^+$ consumption and production. The coastal site showed evidence of ‘top-down’ control, with increases in Chl $a$ when the larger size fraction grazers were removed, regardless of substrate additions. However, the oceanic samples did not change appreciably in Chl $a$ and had modest increases in PN with glucose additions. The latter suggests that the bacterial fraction responded, but not the autotrophic fraction. In the coastal samples, where both Chl $a$ and PN increased, the response was more likely to have been by autotrophic picoplankton. These relationships suggest differing trophic pathways and nutritional status in these oceanic and coastal regions.
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