Nanoheterotrophs grazing on bacteria and cyanobacteria in oxic and suboxic waters in coastal upwelling areas off northern Chile

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The vertical distribution and abundance of microbial assemblages and the grazing of nanoheterotrophs upon prokaryotes in oxic and suboxic waters were examined in two coastal upwelling areas off northern Chile where a shallow Oxygen Minimum Zone (OMZ) is characteristic. Prokaryotic prey included bacterioplankton and cyanobacteria (Synechococcus); both displayed a bimodal distribution, with abundance maxima above and within the upper OMZ. Flagellates numerically dominated the nanoplankton and were mostly concentrated in the oxic layer. Mean ingestion rates of cyanobacteria by nanoflagellates (vacuole content method) ranged from 0.2 to 1.1 cells flagellate–1 h–1 and mean consumption rates (34–160 cells mL–1 h–1) were four times higher in the oxic layer. With the selective inhibitors technique, specific grazing rates on bacteria were low (<0.1 h–1) and consumption did not control bacterial production in the surface layer but did so in the suboxic layer (accounting for >100% of bacterial production). With the same method, the specific grazing rate on cyanobacteria ranged between zero and 0.23 h–1 with no clear differences between oxygen conditions; prey growth and production were always higher than the grazing pressure (accounting for <17% of cyanobacterial production). The impact of grazing by nanoheterotrophs in regulating the production of prokaryotes in oxic and suboxic waters in this region is discussed.

INTRODUCTION

The main factors controlling the abundance and production of picoplanktonic assemblages in the upper layers of the oceans include, in the case of bacterioplankton (mainly focusing on heterotrophic bacteria; Sherr and Sherr, 2000), temperature, substrate availability, viral lysis and grazing (Billen et al., 1990; Pedrós-Alió et al., 2000; Thingstad, 2000) coupled with other factors that influence bacterial growth efficiency (del Giorgio and Cole, 2000). Among the autotrophic picoplankton, the cyanobacteria Synechococcus and Prochlorococcus are often important contributors to phytoplankton abundance and photosynthetic production in the upper layer of coastal and oceanic waters, while pico-eukaryotes are more important in terms of biomass (DuRand et al., 2001; Shalapyonok et al., 2001; Worden et al., 2004). The factors controlling the abundance and production of autotrophic picoplankton are, however, poorly understood (Zubkov et al., 1998; Worden and Binder, 2003). Protozoan grazing, mainly by heterotrophic nanoflagellates (HNF) and small ciliates, can exert an important control on heterotrophic bacteria (e.g. Pedrós-Alió et al., 2000) but its impact upon autotrophic pico-prokaryotes (e.g. Landry et al., 1995; Dolan and Simek, 1999; Christaki et al., 2002) and pico-eukaryotes (Liu et al., 2002; Christaki et al., 2005) has received far less attention. In addition, the way in which low oxygen concentrations in the water column (a condition receiving increasing attention in the oceans; e.g. Grantham et al.,
2004; Service, 2004) affect the distribution of microorganisms in general has been documented in only a few studies in the marine environment (e.g. Detmer et al., 1993; Dennett et al., 1999; Park and Cho, 2002; Gowing et al., 2003).

In the Humboldt Current System (HCS) where coastal upwelling is one of the most conspicuous processes in the water column, an important proportion of the high primary production (Marin et al., 1993; Daneri et al., 2000) can be channeled through the bacteria (Troncoso et al., 2003) or directly through HNF (Böttjer and Morales, 2005) suggesting a significant contribution of the microbial web in the trophodynamics of the HCS coastal system (Cuevas et al., 2004). Off northern Chile (18–24°S), coastal upwelling is moderate (upwelling favorable winds <6 m s⁻¹), persistent through the year, with a relatively narrow area of influence (20–40 km), except at specific sites where a plume extends further offshore; complete mixing is restricted to the most inshore area (Blanco et al., 2001; Morales et al., 2001). A permanent and shallow oxygen minimum zone (OMZ) is a characteristic of this region; a strong oxycline, away from the upwelling centre and closely related to the seasonal thermocline (and sometimes also to a shallow salinity minimum), separates well-oxygenated waters at the surface (<25 m layer) from suboxic waters (<1 mL O₂ L⁻¹) at depths between 25 and 100 m, depending on the distance from the coast and the temporal variability in the distribution of the Equatorial Subsurface Waters (Morales et al., 1999).

In the waters off northern Chile, two fluorescence maxima usually appear—a primary fluorescence maximum (PFM) in the surface oxic waters dominated by large phytoplankton (González et al., 1998) and a secondary fluorescence maximum (SFM) at the upper boundary of the OMZ dominated by heterotrophic bacteria and cyanobacteria, including Synechococcus and Prochlorococcus (Molina et al., 2005). The OMZ in this region presents a barrier to the vertical distribution and migration of most mesozooplanktonic populations (Morales et al., 1996, 1999; Escrúbano et al., 2000) and understanding its impact upon the structure and dynamics of microbial assemblages remains a challenge. Since, here, prokaryotes are an important component of the SFM in the upper OMZ, and since the shallow OMZ could constitute a barrier for the vertical distribution of nano- and micro-heterotrophs, the trophic interactions in surface oxic and suboxic waters are expected to be different. The aim of this study was to assess the role of heterotrophic nanoplanckton grazings upon prokaryote, picoplanktonic assemblages under surface oxic and subsurface suboxic conditions in two coastal upwelling areas off northern Chile (~20 and 23°S).

### METHODS

#### Field sites

Two sites were intensively sampled off northern Chile: (i) a fixed station off Mejillones (22°51′S–70°33′W; ~28 km from the coast) during December 2002 on board RV Paritabaar and (ii) a fixed station off Iquique (20°06′S–70°19′W; ~20 km from the coast) during March 2004 on board RV Carlos Porter. The vertical distributions (0–100 m) of water column characteristics (temperature, salinity, fluorescence and dissolved oxygen) were determined at least twice during the sampling using a CTD (SeaBird 25) equipped with oxygen and fluorescence sensors. Oxygen concentrations from the CTD sensor and measured by the Winkler method were highly correlated (Spearman test) off Mejillones (r = 0.8; P = 0.004; Molina et al., 2005) and off Iquique (r = 0.9; P = 0.01).

#### Microbial distribution and abundance

Seawater samples for bacteria, cyanobacteria, autotrophic and heterotrophic nanoplanckton were collected at 10–12 depths within the top 100 m. These samples were taken directly from Niskin bottles into duplicate sterile tubes (50 mL capacity), preserved with buffered formaldehyde (2% final concentration), then stored in the dark and refrigerated. In the sampling off Mejillones, all the microorganisms were analysed by epifluorescence microscopy. Samples were stained with DAPI (4', 6-diamidino-2-phenylindole; 0.01% final concentration) and concentrated by filtration as follows: bacteria and cyanobacteria (2 mL sample) onto 0.2-μm pore and nanoplanckton (20 mL sample) onto 0.8 μm pore, black polycarbonate filters (Porter and Feig, 1980). Cell counts were made using a Zeiss Axioscope Plus 2 epifluorescence microscope equipped with multiple light filters (UV, blue and green) at ×1600 magnification. A minimum of 500 (bacteria), 100 (cyanobacteria), and 50 (autotrophic and heterotrophic nanoplanckton) cells were counted whenever possible but some of the samples had too few cells; coefficients of variation (CV) of duplicate samples were <20% for the prokaryote prey. In the sampling off Iquique, the prokaryotic cells were analysed by flow cytometry (Marie et al., 2000), on the basis that the enumeration is comparable with epifluorescence microscopy (e.g. Ducklow, 2000). Samples of bacteria and cyanobacteria were fixed with fresh para-formaldehyde (0.1% final concentration) and counted using a Becton Dickinson FACScanlibur flow cytometer (flow rate: 28–32 μL min⁻¹; >10 000 events counted). Yellow-green fluorescence from the nucleic acid stain SYBR-I Green (530 nm) was used in the estimation of
heterotrophic bacterial abundance. Forward scatter (FSC), side scatter (SSC), orange fluorescence from phycoerythrin (585 nm) and red fluorescence from chlorophyll (>650 nm) were measured after 488 nm laser excitation and used as an estimate of cyanobacterial abundance. In this study, only the abundance of *Synechococcus* in the samples was considered to make these estimates comparable to those obtained with the epifluorescence microscope since the latter is thought to underestimate *Prochlorococcus* abundance (Partensky et al., 1999). CVs of duplicate samples were <4% for bacteria and <10% for *Synechococcus*. A comparison between microscopic and flow-cytometer estimates on data obtained during these studies (linear regression) revealed a satisfactory agreement (linear regression) for both bacterial ($r^2=0.63$, $P<0.01$) and cyanobacterial ($r^2=0.66$, $P<0.05$) counts.

**Grazing rates by nanoheterotrophs**

Two different approaches were selected for the estimation of grazing by the heterotrophic nanoplanктon on prokaryotes, on the basis of minimal disturbance of the organisms and the maintenance of the *in situ* oxygen conditions: (i) the food vacuolar content (FVC) method (e.g. Dolan and Simek, 1998; Christaki et al., 2002) for estimating the ingestion of cyanobacteria and (ii) the selective inhibitor technique (SIT) (e.g. Fuhrman and McManus, 1984; Campbell and Carpenter, 1986; Sherr et al., 1986) for measuring eukaryotic grazing on prokaryotic prey (bacteria and cyanobacteria).

**FVC**

Intensive temporal sampling of the prokaryotic prey and HNF predators was made at selected depths, representing oxic and suboxic conditions. Off Mejillones, samples were obtained during a 28-h cycle (between 6 and 7 December 2002) with a 2- to 3-h frequency, while off Iquique, samples derived from a 69-h cycle (between 21 and 24 March 2004) with a 6-h sampling interval. These samples (100 mL) were treated as described in Dolan and Simek (Dolan and Simek, 1999), that is, preserved in buffered formaldehyde (2% final concentration), kept in darkness and refrigerated; these authors tested various fixatives and concluded that buffered formalin induced less vacuole content evacuation. Subsequently, these samples were stained with DAPI and concentrated as above. All the analyses of FVC for cyanobacterial prey were made by epifluorescence microscopy (duplicates) so that only *Synechococcus* abundance was determined. Ingestion rates were obtained by multiplying the FVC value (cyanobacteria predator$^{-1}$) by a mean digestion rate constant of 1.1% min$^{-1}$ (Dolan and Simek, 1998), with no correction for temperature (Christaki et al., 2002). Consumption rates were obtained from the product of the abundance of predators and their ingestion rates.

**SIT**

Samples for the incubations were obtained from two layers representing the oxic-PFM and the suboxic-SFM layers, during day or night. For all experiments, water was gently transferred directly from Niskin bottles to large containers through a 200-μm mesh to remove larger planktonic organisms. This water was then distributed in acid-washed, 500 mL polycarbonate bottles using silicon tubing and avoiding air bubbles. In the case of samples from low oxygen conditions, the whole of the Niskin bottle was surrounded by a sealed bag containing an atmosphere of nitrogen. Cycloheximide (100 mg L$^{-1}$) was used as a eukaryotic inhibitor in three experimental bottles while three other bottles had no inhibitor (controls). Incubations lasted for 4 (Mejillones) or 6 h (Iquique) and were kept in running surface seawater in Mejillones ($\sim$16°C) and by placing an *in situ* array in Iquique, at the same depths from which the experimental samples were taken. In all experiments, a 1-h acclimation time to the inhibitors was allowed before taken the initial samples. Triplicates samples for prey and predator abundances were taken from each bottle at the beginning and end of the incubations; enumeration was carried out with epifluorescence microscopy for the Mejillones samples (3 incubation experiments) and flow cytometry for the Iquique samples (4 incubation experiments). The instantaneous or specific gross growth coefficient ($k$) and the net or apparent ($\mu$) growth coefficient of the prey in the experimental and control bottles were obtained from the changes in prey abundance during the incubation time, the difference between the two representing the instantaneous or specific grazing or mortality coefficient ($g$). Growth and grazing rates or coefficients were calculated from the following equations based on an exponential model of population growth (Campbell and Carpenter, 1986; Weisse, 1989; Liu et al., 1995):

$$k = (1/t) \times \ln(C_t/C_0) \text{ treatment with inhibitor}$$  
$$\mu = (1/t) \times \ln(C_t/C_0) \text{ treatment without inhibitor}$$  
$$g = k - \mu$$

where $C_t$ and $C_0$ are concentrations of prey at the end and the beginning of the incubation; $t$ = incubation time. These parameters are equivalent to those used in the dilution method for estimating growth of prey and grazing by nano- and micro-heterotrophs (Landry et al., 1995; Moisig and Goecke, 2003).

These specific rate values were used to derive daily carbon production (with the $k$ values) and consumption
rates (with the g values) (e.g. Campbell and Carpenter, 1986; Weisse, 1989; Liu et al., 1995), based on the following conversion factors: 20 fg C cell⁻¹ for heterotrophic bacteria (Lee and Fuhrman, 1987) and 100 fg C cell⁻¹ for Synechococcus populations from upwelling areas (Zubkov et al., 1998; Worden et al., 2004).

RESULTS

Water column structure and the vertical distribution of microorganisms

In the area off Mejillones (Fig. 1a and b), the base of the thermocline was located at 25–35 m depth during this study; temperature ranged between ~16.5°C at the surface and 13°C at 100 m depth. Salinity was relatively constant (34.7–34.9), except for a narrow (<10 m) and shallow (20–30 m) salinity minimum (<34.7) in one of the samplings. Dissolved oxygen concentration changed from ~4.0 at the surface to ≤0.1 mL O₂ L⁻¹ below 40 m, separated by a strong oxic line. The fluorescence displayed a well-defined surface PFM (0–15 m) while small SFM peaks appeared in the 30–50 m depth range. The pico-prokaryotic assemblages (Fig. 1c and d) were composed of bacteria (0.40–4.20 × 10⁶ cells mL⁻¹) and cyanobacteria (Synechococcus; 0.03–2.40 × 10⁵ cells mL⁻¹). Both groups presented bimodal distributions, with maximum abundances in the top 15 m layer and secondary peaks at 50–60 m depth, just below the oxic line and in the vicinity of the SFM; bacteria also appeared in significant numbers (>1 × 10⁶ cells mL⁻¹) well into the OMZ (Fig. 1c and d). The nanoplankton (Fig. 1e and f) was dominated by flagellates (0.03–1.67 × 10⁷ cells mL⁻¹), with higher abundances in the surface layer. Autotrophic nanoflagellates (ANF) assemblages were higher (0.01–1.22 × 10⁵ cells mL⁻¹) than the heterotrophic flagellates (HNF; 0.01–0.44 × 10⁵ cells mL⁻¹) but appeared to contribute little to the SFM when compared with the fluorescence profiles (Fig. 1a and b).

In the area off Iquique (Fig. 2a and b), the base of the thermocline appeared at 15–30 m depth; temperature ranged from ~20°C at the surface to 13°C at 100 m depth. Salinity variation was small (34.7–34.9) in one of the sampling intervals. Dissolved oxygen concentration changed from ~6 mL O₂ L⁻¹ at the surface to <0.1 mL O₂ L⁻¹ below 30–40 m depth, with a strong oxic line in between. The fluorescence profile presented a well-defined subsurface PFM (10–15 m) while the smaller SFM was more dispersed (30–80 m). The pico-prokaryotic assemblages (Fig. 2c and d) included heterotrophic bacteria (0.01–2.34 × 10⁶ cells mL⁻¹) and cyanobacteria (Synechococcus; 0.007–2.34 × 10⁵ cells mL⁻¹); only the bacteria displayed a bimodal distribution, with maximum values in the oxic and suboxic layers whereas Synechococcus abundances were higher in the oxic layer in association with a less defined SFP. The nanoplankton (Fig. 2e and f) was dominated by flagellates (0.10–3.84 × 10⁶ cells mL⁻¹) with ANF in higher abundances (0.00–3.43 × 10⁵ cells mL⁻¹) than the HNF (0.13–0.49 × 10⁵ cells mL⁻¹) but they also contributed little to the SFM when compared with the fluorescence profiles (Fig. 2a and b).

Diel variations in the abundance of prokaryotic prey and nanoheterotrophs, and their food vacuolar content, in the oxic and suboxic layers

The data on the abundance of microorganisms over diel cycles at the two study areas was grouped into three main periods: morning (6:00–14:00 hours), afternoon (14:00–22:00 hours) and night (22:00–06:00 hours); sunset during the austral summer at this latitude occurs at around 20:00 hours (all times local). A general analysis of the different CTD profiles obtained during the sampling periods (data not shown) suggested that the hydrographic conditions remained relatively stable during the period; consequently, it was assumed that the physical processes were not determining the changes in the abundances of prey and predators. In terms of the mean abundances and standard deviations for each of these periods, no evidence of a consistent pattern of diel changes in the abundances of the prokaryote prey and their nano-grazers was detected at the study sites though there was a relatively high dispersion of the values around the mean values (Table I).

In the surface oxic layer (10 m) off Mejillones, the mean abundances of bacteria during the three periods were similar. The cyanobacterial abundance (Synechococcus) displayed a lower mean value during the afternoon while the HNF abundance was higher during the morning. At the subsurface suboxic level (30 m), most of the mean abundance values were lower (range: –26 to –80%) than in the overlying water during each period (Table I), as seen in the vertical profiles (Fig. 1c–f). There was no evidence of diel variation in the abundances of bacteria and HNF, while the cyanobacteria displayed a tendency of lower mean values during the morning. Off Iquique, at the oxic level (5–10 m), bacterial abundances displayed diel variability, with lower values during the afternoon period; the cyanobacteria and HNF did not show diel variability. At the suboxic level (40–60 m), the abundances were again lower (range: –29 to –96%) than in the oxic layer (Table I), as seen in the vertical profiles (Fig. 2c–f). Diel variations were displayed in all three groups, bacteria being most abundant during the morning hours and cyanobacteria
during the afternoon period, while the HNF were less abundant during the nighttime.

The mean FVC values of HNF feeding on the cyanobacteria *Synechococcus* (Table I) at the Mejillones site were similar during the diel cycle and between the two levels, oxic and suboxic; the total values ranged between zero and 2.10 cyanobacteria HNF⁻¹. The FVC values at the Iquique site attained lower maximum values than at
Mejillones and they were higher in the surface layer (0.27–0.85 cyanobacteria HNF⁻¹) than in the suboxic layer (0.02–0.44 cyanobacteria HNF⁻¹), with no large variation during the diel cycle.

**Grazing on prokaryotes by HNF at oxic and suboxic levels**

Owing to the lack of a clear diel variation in the abundance of prokaryotic cells and FVC of HNF, mean
Table I: Variation in the abundance of prokaryotic prey (BA, bacteria and CyaB, cyanobacteria), heterotrophic nanoflagellate (HNF) predators and of the HNF food vacuolar content (FVC) during intensive sampling of surface oxic (PFM, primary fluorescence peak) and subsurface suboxic (SFM, secondary fluorescence peak) layers off northern Chile (20–23°S).

<table>
<thead>
<tr>
<th>Place, period</th>
<th>Surface oxic, PFM</th>
<th>Subsurface suboxic, SFM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BA 10^6 cells mL^-1</td>
<td>CyaB 10^5 cells mL^-1</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Mejillones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning</td>
<td>2.71</td>
<td>1.16(7)</td>
</tr>
<tr>
<td>Afternoon</td>
<td>2.67</td>
<td>0.92(4)</td>
</tr>
<tr>
<td>Night</td>
<td>2.62</td>
<td>0.42(4)</td>
</tr>
<tr>
<td>Iquique</td>
<td>1.66</td>
<td>0.13(2)</td>
</tr>
<tr>
<td>Morning</td>
<td>0.63</td>
<td>0.26(3)</td>
</tr>
<tr>
<td>Afternoon</td>
<td>2.45</td>
<td>1.80(3)</td>
</tr>
<tr>
<td>Night</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD, standard deviation; n, numbers of samplings.
values FVC values for the whole data set were obtained to calculate cyanobacterial ingestion with the FVC method (Table II). The FVC-derived mean ingestion rates off Mejillones were similar for the oxic and suboxic waters (∼1.0 cyanobacteria HNF⁻¹ h⁻¹) but the mean consumption rates were four times higher in oxic waters due to higher abundances of HNF, though with high variability around the mean values. Off Iquique, the mean ingestion rates in suboxic waters were about half as those in the oxic layer (∼0.2 and 0.4 cells HNF⁻¹ h⁻¹, respectively). Mean consumption rates were, as off Mejillones, four times higher in the oxic level as compared with the suboxic layer; these rates were very similar at the two study sites due to the differences in FVC and HNF abundances.

Estimates of specific grazing and growth coefficients with the SIT method are shown in Table III. Off Mejillones, the grazing values on bacteria were negligible (<0.1 h⁻¹) in the surface oxic layer (15 m) and bacterial growth did occur. In the suboxic layer (40–50 m), grazing equaled the growth of the bacteria. Grazing by HNF on cyanobacteria (Synechococcus) occurred at similar rates

\[ \text{Table II: Ingestion and consumption rates by heterotrophic nanoflagellates (HNF) feeding on cyanobacteria (CyaB) in oxic and suboxic layers in two areas off northern Chile (20–23°S)} \]

<table>
<thead>
<tr>
<th>Area</th>
<th>Depth (m)</th>
<th>Layer</th>
<th>CyaB 10⁶ cells mL⁻¹</th>
<th>HNF 10³ cells mL⁻¹</th>
<th>FVC cells HNF⁻¹</th>
<th>Ingestion cells HNF⁻¹ h⁻¹</th>
<th>Consumption cells mL⁻¹ h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mejillones</td>
<td>10</td>
<td>Surface oxic, PFM</td>
<td>2.11 ± 1.20 (15)</td>
<td>0.14 ± 0.13 (14)</td>
<td>1.65 ± 0.25 (14)</td>
<td>1.09 ± 0.16 (14)</td>
<td>160.5 ± 144.3 (14)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Suboxic, SFM</td>
<td>1.00 ± 1.34 (15)</td>
<td>0.04 ± 0.05 (14)</td>
<td>1.41 ± 0.28 (14)</td>
<td>0.93 ± 0.19 (14)</td>
<td>43.1 ± 47.3 (14)</td>
</tr>
<tr>
<td>Iquique</td>
<td>5–10</td>
<td>Surface oxic, PFM</td>
<td>2.08 ± 0.81 (7)</td>
<td>0.41 ± 0.10 (6)</td>
<td>0.57 ± 0.20 (6)</td>
<td>0.38 ± 0.13 (6)</td>
<td>160.2 ± 76.2 (6)</td>
</tr>
<tr>
<td></td>
<td>40–60</td>
<td>Suboxic, SFM</td>
<td>0.30 ± 0.16 (6)</td>
<td>0.21 ± 0.05 (7)</td>
<td>0.26 ± 0.10 (7)</td>
<td>0.17 ± 0.09 (7)</td>
<td>33.9 ± 21.2 (7)</td>
</tr>
</tbody>
</table>

SD, standard deviation; n, numbers of samplings. Estimates of ingestion and consumption are based on the mean abundances of prey and predator and food vacuolar content (FVC) using the data in Table I. A digestion rate factor of 1.1% cell content min⁻¹ (Dolan and Simek, 1998) was used in the calculations of ingestion. Other abbreviations as in Table I.

\[ \text{Table III: Specific grazing rates (g; h⁻¹) by heterotrophic nanoflagellates (HNF) feeding on bacteria (BA) and cyanobacteria (CyaB) and specific prey growth rates (k; h⁻¹), using the selective inhibitor technique (triplicate samples at the beginning and end of the incubations), and estimates of production (P) rates (µg CL⁻¹ d⁻¹) by prokaryotes and consumption (C) rates (µg CL⁻¹ d⁻¹) by HNF feeding on BA and CyaB in oxic and suboxic conditions off Mejillones and Iquique, northern Chile} \]

<table>
<thead>
<tr>
<th>Depth</th>
<th>Layer</th>
<th>Abundance BA 10⁶ cells mL⁻¹</th>
<th>CyaB 10⁶ cells mL⁻¹</th>
<th>HNF 10³ cells mL⁻¹</th>
<th>BA k g</th>
<th>CyaB k g</th>
<th>P µg CL⁻¹ d⁻¹</th>
<th>C µg CL⁻¹ d⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mejillones</td>
<td>15 Oxic</td>
<td>1.26 ± 0.10</td>
<td>0.35 ± 0.26</td>
<td>0.12 ± 0.13</td>
<td>0.03</td>
<td>0.00</td>
<td>18.72</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>40 Suboxic</td>
<td>0.44 ± 0.03</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.09</td>
<td>0.07</td>
<td>0.07</td>
<td>13.44</td>
<td>13.44</td>
</tr>
<tr>
<td></td>
<td>50 Suboxic</td>
<td>0.56 ± 0.20</td>
<td>0.14 ± 0.02</td>
<td>0.05 ± 0.09</td>
<td>-</td>
<td>-</td>
<td>2.69</td>
<td>0.13</td>
</tr>
<tr>
<td>Iquique</td>
<td>10 Oxic</td>
<td>1.87 ± 0.11</td>
<td>1.34 ± 0.10</td>
<td>1.34 ± 0.04</td>
<td>0.00</td>
<td>0.00</td>
<td>20.60</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>20 Oxic</td>
<td>0.83 ± 0.06</td>
<td>0.20 ± 0.01</td>
<td>0.40 ± 0.03</td>
<td>0.00</td>
<td>0.00</td>
<td>0.29</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>50 Suboxic</td>
<td>0.55 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>0.37 ± 0.04</td>
<td>0.00</td>
<td>0.00</td>
<td>0.08</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>70 Suboxic</td>
<td>1.39 ± 0.04</td>
<td>0.02 ± 0.00</td>
<td>0.32 ± 0.02</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Initial experimental mean abundances (n = 6) of BA, CyaB and HNF (<10⁶, 10⁶ and 10³ cells mL⁻¹, respectively) at the selected depths (m) are also shown (mean ± standard deviation). Incubation off Mejillones: 15 and 50 m—daytime, 40 m—nighttime; incubations off Iquique: 10 and 50 m—daytime, 20 and 70 m—nighttime. Other abbreviations as in Table I.
(<0.2 h⁻¹) in the oxic and the shallower suboxic layer (40 m) but was lower (<0.1 h⁻¹) in the deeper suboxic layer (50 m). Prey growth was balanced with grazing at the surface level, was slightly higher in the shallower suboxic layer, and was four times higher in the deeper suboxic layer.

Off Iquique, the estimates of specific grazing by HNF and of specific growth by prokaryotes with SIT were, in general, much lower than off Mejillones (Table III). Grazing rates on bacteria were negligible in oxic and suboxic conditions. Only HNF grazing on cyanobacteria (Synechococcus) in the surface layer was similar to the results obtained in Mejillones at the deepest layer (50 m). These differences in grazing between the two areas also reflect the differences in the ingestion rates of cyanobacteria observed with the FVC method (Table II). Ingestion of Prochlorococcus by HNF was not detectable with this method though they appeared in significant numbers (maximum of 0.8 x 10⁵ cells mL⁻¹) in the suboxic layer (50 m water incubations) (data not shown).

Estimates of carbon prey production and carbon consumption (or grazing pressure) by HNF were obtained using the resulting g and k rate values together with cell carbon data (see Methods). At both study sites (Table III), HNF control of bacterial production was not detectable in the surface layer but did so in the suboxic waters (>100% grazing impact on bacterial production). In the case of Synechococcus, prey production was always higher than the HNF grazing pressure (<17% consumption of cyanobacterial production), independent of site and oxygen conditions. By applying the same carbon conversion factors to the estimates of HNF consumption of cyanobacteria obtained with the FVC method (Table II), carbon consumption rates range between 0.08 and 0.4 μg C L⁻¹ d⁻¹, being in the lower range of those estimated with the SIT technique.

**DISCUSSION**

This study is one of the few attempts that have been made to analyse both the abundances of microorganisms and grazing under oxic and suboxic conditions in the water column of marine areas characterized by a relatively shallow OMZ or hypoxic environments (Dettmer et al., 1993; Dennett et al., 1999; Park and Cho, 2002). Part of the limitation in estimating grazing derives from the potential oxygenation by manipulation of the samples; minimal manipulation is thus desirable. Also, the duration of the incubations needs to be curtailed due to the potential effects of reducing even further the oxygen concentration by respiration processes. Here, two grazing methods were considered as the most suitable, the FVC method for HNF grazing on Synechococcus and the SIT for HNF grazing on bacteria and cyanobacteria.

**HNF grazing on cyanobacteria using FVC**

This method has the advantage of being relatively simple for estimating in situ ingestion rates but a value for the digestion rate (a constant) has to be applied since this is a difficult variable to assess directly for natural assemblages (Christaki et al., 2002). It has not been frequently used perhaps due to the restrictions associated with assessing the natural fluorescence of prey in the vacuoles. Further, only one digestion rate constant is available in the literature (1.1% content min⁻¹ at 22°C) to obtain an ingestion rate (Dolan and Simek, 1998). No temperature correction has been made in the present study for the 16–20°C in the oxic layer and 13–14°C in the suboxic layer assuming that digestion rates are relatively constant and unaffected by the physiological state of the heterotrophic flagellate (Dolan and Simek, 1998). In this sense, our estimates are relative but other related studies, with ingestion rate being estimated from FVC under different temperature regimes, have also used this digestion constant (Christaki et al., 2002). Another potential problem with the FVC is the evacuation of particles with the addition of a fixative but, in the present case, the fixative with the lowest impact (Dolan and Simek, 1999) was chosen. The main aspect here, however, is the fact that our analysis only allowed the detection of Synechococcus ingestion whereas Prochlorococcus is a more important component in the suboxic zone (Molina et al., 2005). In this sense, HNF grazing on cyanobacteria assemblages as a whole has been underestimated in this study.

HNF ingestion on Synechococcus, using the FVC method, and including the two study areas and two oxygen conditions, ranged from 0.2 to 1.1 cells HNF⁻¹ h⁻¹ (Table II). This range is in the upper level and varied within a narrower range than that reported in the literature with the same method (0.0005–2.7 cells HNF⁻¹ h⁻¹), the estimates obtained under a variety of environmental and experimental conditions (Dolan and Simek, 1999; Christaki et al., 2001, 2002). In considering only field data previously reported (Christaki et al., 2002), this range is narrower (0.004–0.35 cells HNF⁻¹ h⁻¹), with our field values being comparatively higher. Part of the explanation for this may be associated with higher cyanobacterial prey concentrations in our FVC field observations (mean values of 0.1–2.7 x 10⁵ cells mL⁻¹; Table I), compared with the wider range reported by the above authors when using the same method (0.02–5 x 10⁵ cells mL⁻¹) and the much narrower range for their field observations (0.002–0.55 x 10⁵ cells mL⁻¹). Also, a functional relationship between ingestion rates and food concentration
appears to apply to the feeding of HNF on *Synechococcus* (Christaki et al., 2002, Fig. 5); part of the ingestion and concentration values in our study are, however, outside the upper range of that relationship (Table II).

**HNF grazing on prokaryotes using SIT**

The SIT technique is relatively simple in terms of handling for the experimental estimation of specific grazing rates. However, it only applies for grazing on prokaryotes since the metabolic inhibitors commonly used cause the suppression of either eukaryotic cells (e.g. cycloheximide, colchicine) or prokaryotic cells [a greater specificity of inhibitors; e.g. penicillin, streptomycin, and chloramphenicol for bacteria; kanamycin for cyanobacteria; plus other types of inhibitors (Molina et al., 2005)]. An assumption of this method is that the use of inhibitors in the experiments does not affect organisms other than those for which the inhibitor is intended, as proved in some studies performing a test for this (Fuhrman and McManus, 1984; Caron et al., 1991). This method, as in the case of the FVC method, has not been frequently used for estimating bacterivory under natural conditions (Fuhrman and McManus, 1984; Sherr et al., 1986; Weisse, 1989; Caron et al., 1991) but has provided results which are directly comparable to those obtained by other independent methods, such as the dilution technique (Campbell and Carpenter, 1986) and the fluorescently labeled bacteria method (Liu et al., 1995).

The SIT technique provides a total estimate of community grazing, both of micro-grazers (20–200 μm) and nano-grazers (2–20 μm), on prokaryote prey. Our analyses only considered the impact of HNF since the microheterotrophs (e.g. dinoflagellates, ciliates) presented very low abundances (data not shown) and we have assumed that their grazing impact in the small incubation volumes (0.5 L) was minimal. Moreover, low abundance estimates for ciliates and heterotrophic dinoflagellates have been previously obtained off Mejillones (Vargas and González, 2004), though published data are scarce for the region off northern Chile. On the other hand, HNF are considered to be the main consumers of the picoplankton size range (Weisse, 1993), a conclusion shown to apply in the Mejillones area (Vargas and González, 2004).

HNF instantaneous grazing rates (g) in the present study reached up to 0.07 h⁻¹ for bacteria and 0.23 h⁻¹ for cyanobacteria (Table III), both maxima obtained off Mejillones. In this study, the HNF abundances were inversely related to the g values (Table III) suggesting potential food limitation at the Iquique site but not at the Mejillones site. Other results on g rates obtained with the SIT technique include those of Liu et al. (Liu et al., 1995), which range between 0.04 and 0.50 d⁻¹ for *Prochlorococcus* and between 0.09 and 0.73 d⁻¹ for *Synechococcus*; Campbell and Carpenter (Campbell and Carpenter, 1986) give values between 0.33 and 0.79 d⁻¹ for *Synechococcus*. For grazing on bacteria, Weisse (Weisse, 1989) reports g values of 0.01–0.1 h⁻¹ and mean daily rates of 1 d⁻¹; he also obtained similarly high growth values for the prey. Concerning cyanobacterial growth rates (k), maximum *in situ* rates of 1.4 and 2.1 d⁻¹ for *Prochlorococcus* and *Synechococcus* have been reported but up to 4 d⁻¹ during short term intervals (Furnas and Crosbie, 1999). Literature reviews on g values in the case of cyanobacteria as prey have been made, including different methods (Christaki et al., 1999, 2002); the range of values reported is wide but the highest mean values so far reported are between 2.1 (HNF *Pseudobodo* sp.) and 4.4 d⁻¹ (mixed flagellate culture). Our maximum estimates then, if converted to daily values, are in the upper part of the literature range; part of this can be explained by the short time incubations used in our case.

According to the literature, the results obtained in short (<6 h) versus long incubations (>20 h) are not clear (Caron et al., 1991; Liu et al., 1995) or long incubations yield lower grazing estimates due to a decrease in prey abundance (Fuhrman and McManus, 1984). Moreover, eukaryote consumption by other eukaryotes and nutrient changes influenced by preys and predators during the incubation can be minimized with short incubation times.

**Potential production of prokaryotes in oxic and suboxic waters off northern Chile**

Estimates of primary and bacterial production in the HCS off northern Chile are scarce (Daneri et al., 2000; Troncoso et al., 2003; Escribano et al., 2004) and are mostly referred to total, integrated production, without distinction between the communities living under oxic or suboxic conditions. This is of particular relevance in the region off northern Chile and Peru since the OMZ impinges on the euphotic layer, except probably, under El Niño conditions (Morales et al., 1999). Moreover, it is only recently that it has been found that the composition of the plankton in the primary and secondary fluorescence peaks are different, being dominated by eukaryotes in the surface oxic layer but with significant numbers of *Synechococcus*, and by prokaryotes, mainly *Prochlorococcus*, in the suboxic layer (O. Ulloa, U. Concepción, unpublished data).

The estimates of instantaneous growth rates (k) from the SIT experiments, and from other types of grazing experiments providing k values (e.g. dilution method), can be used as measures of the *in situ* carbon production rates (e.g. Campbell and Carpenter, 1986; Landry et al.,
2003; Moigis and Goecke, 2003). During the present study, no simultaneous measurements of fractionated primary production with the traditional methods (\(^{14}\)C or O\(_2\) production) nor of bacterial production were carried out. Therefore, our derived estimates can only be compared in general terms with other data for the same region. Bacterial production in our study ranged between 0.1 and 19 \(\mu\)g C \(\text{L}^{-1} \text{d}^{-1}\), the lower values being obtained at 50 m depth, well into the OMZ (Table III). These values are comparable to those obtained off Antofagasta (mean: 0.4 g C \(\text{m}^{-2} \text{d}^{-1}\), equivalent to 6.35 \(\mu\)g C \(\text{L}^{-1} \text{d}^{-1}\)) during January 1997 (austral summer and pre-El Niño condition), using (methyl-\(^{3}\)H)-thymidine and applying the same carbon conversion factor as in here (González et al., 1998). For the same data set off Antofagasta but combining oceanic and costal stations, Iriarte and others (Iriarte et al., 2000) reported a mean value of 15.8 \(\mu\)g C \(\text{L}^{-1} \text{d}^{-1}\) (range: 1.9–60.5 \(\mu\)g C \(\text{L}^{-1} \text{d}^{-1}\)) and lower values during July 1997 (austral winter); the depth at which these values apply is not specified. Also, for the same data set, and combining coastal and oceanic as well as summer and winter data, Troncoso and others (Troncoso et al., 2003, Fig. 6) reported bacterial production values ranging from 0 to 300 \(\mu\)g C \(\text{L}^{-1} \text{d}^{-1}\), up to 5 times higher in magnitude than the maximum values reported in Iriarte and others (Iriarte et al., 2000).

In the present study, the estimates of carbon primary production contributed by the picoplankton, specifically by the cyanobacteria, range between zero and 20.6 \(\mu\)g C \(\text{L}^{-1} \text{d}^{-1}\), being highest in the surface waters at both sites (Table III). There are only a few studies of fractionated primary production in the HCS off northern Chile to compare with and they only include surface or photic layer (1% light) data. One of them, referred to above (Iriarte et al., 2000), using the \(^{14}\)C method, found that the picoplankton and nanoplanckton size fractions can make a significant contribution (60–80%) to the total production and biomass in the upwelling area off Antofagasta. Their primary production estimate for the picoplankton size fraction in the coastal zone, for the same season as in this study (austral summer) and assuming a daily rate based on a 12 h light period, was \(\sim 24\)
\(\mu\)g C \(\text{L}^{-1} \text{d}^{-1}\) (range: 12–36 \(\mu\)g C \(\text{L}^{-1} \text{d}^{-1}\)). More recently (austral summer 2001) and also using the \(^{14}\)C method in Mejillones Bay, González and others (González et al., 2004) reported values on fractionated primary production. Again, calculating a daily rate based on a 12 h light period, a value of 15 \(\mu\)g C \(\text{L}^{-1} \text{d}^{-1}\) was registered by them in the photic layer, representing only 7% of the total primary production. In general, then, our estimated values for the surface layer agree with those obtained previously in the same coastal region. No doubt, many more investigations are needed before the levels of primary and bacterial production in this region can be characterized and the factors controlling them identified. More importantly, very little is known about the contribution of the different taxonomic and size assemblages, as well as those of the oxic and suboxic communities, to the overall production of the system.

The role of HNF grazing in oxic and suboxic waters off northern Chile

Estimates of microzooplankton grazing in the study region are also scarce. Only recently, Vargas and González (Vargas and González, 2004) indirectly estimated protozoan ingestion rates (including HNF, ciliates and heterotrophic dinoflagellates) in the surface oxic layer (0–25 m) of an area in and around Mejillones Bay using the model proposed by Peters (Peters, 1994), based on the abundances and biomasses (as bio-volumes) of prey and predators, and temperature. These authors concluded that HNF were largely bacterivorous, with no data on cyanobacteria consumption being included; total HNF consumption rates, estimated for a similar period as in this study, ranged between 3 and 16 \(\mu\)g C \(\text{L}^{-1} \text{d}^{-1}\). These values are well within the range found during the present study but we only detected grazing in the suboxic layer whereas at the surface layer it was zero (Table III). Our results also show that HNF grazing on Synechococcus, both in oxic and suboxic waters during the austral summer, is not large and can be disregarded as an important cause of mortality during the summer period. Whether this is also the case with Prochlorococcus as prey, which can attain significant numbers in the suboxic layer, and other types of picoplanktonic autotrophs present though in lower abundances in the study region (O. Ulloa, U. de Concepción, unpublished data), remains to be assessed.

Few other estimates of HNF grazing under oxic and suboxic conditions are available. In the eutrophic Masan Bay (Korea), HNF consumption rates on bacteria, measured using the fluorescent labeled bacteria technique, ranged between 0.1 and 32.2 \(\times 10^7\) bacteria \(\text{L}^{-1} \text{h}^{-1}\), without significant differences between the surface oxic and hypoxic layers (Park and Cho, 2002). In the Baltic Sea, estimates of HNF grazing rates on bacteria and cyanobacteria under oxic and anoxic conditions obtained using the dilution technique revealed considerable grazing rates on Synechococcus (1.2–1.3 \(\text{d}^{-1}\)) and bacteria (0.4–0.7 \(\text{d}^{-1}\)) in anoxic waters, together with similar growth rates (0.9–1.3 and 0.4–0.9 \(\text{d}^{-1}\), respectively); the growth rates were also similar in the different layers but grazing was higher (\(\sim 50\%\)) in the anoxic level (Detmer et al., 1993). The grazing impacts estimated by these authors ranged between 50 and 180% of prey production.
In general, the present study provides only the very first evidence of the significance of HNF grazing on prokaryotic prey in the coastal upwelling region off northern Chile, including the surface oxic layer and the suboxic layer immediately below the shallow oxycline. Whether HNF grazing is a key factor controlling the production of bacteria or cyanobacteria requires further methodological comparisons, the development of new approaches and the perspective of multidisciplinary research on the carbon and nitrogen fluxes in this region.

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