Progress in Oceanography 92–95 (2012) 43–49

Contents lists available at ScienceDirect

Progress in Oceanography

journal homepage: www.elsevier.com/locate/pocean

# Potential contribution of planktonic components to ammonium cycling in the coastal area off central-southern Chile during non-upwelling conditions

Veronica Molina [a,*], Carmen E. Morales [a], Laura Farías [a], Marcela Cornejo [a], Michelle Graco [b], Yoanna Eissler [c], Luis A. Cuevas [a]

[a] Departamento de Oceanografía y Centro de Investigación Oceanográfica en el Pacífico Sur-oriental (FONDAP-COPAS), Universidad de Concepción, Casilla 160-C, Concepción, Chile
[b] Dirección de Investigaciones Oceanográficas, Instituto del Mar del Perú (IMARPE), Esquina Gamarra y General Valle S/N Chucuito Callao, Peru
[c] Centro de Investigación y Gestión de Recursos Naturales, Departamento de Química y Bioquímica, Facultad de Ciencias, Universidad de Valparaíso, Gran Bretaña 1111, Playa Ancha, Valparaíso, Chile

ARTICLE INFO

*Article history:*
Available online 18 July 2011

ABSTRACT

The potential contributions of different microbial components (<20 μm) and metabolisms to ammonium cycling were assessed during non-upwelling conditions in a coastal area off Concepción (~36.5°S). Assays with specific inhibitors to estimate rates of ammonium consumption and production, and carbon assimilation associated with photolithotrophic and chemolithoautotrophic (nitrification) metabolisms in the water column were performed. Despite low water column concentrations of ammonium in wintertime, intense ammonium transformations were registered. Prokaryotes (or bacterioplankton) contributed most to ammonium generation rates over the entire water column; these rates increased with depth (0.4–3.1 μM $d^{-1}$). In surface waters (10 m depth), aerobic ammonium oxidation (potentially by Bacteria and Archaea) was the dominant consumption process (average 0.7 μM $d^{-1}$) whereas in the subsurface layer (20 and 50 m depth), unexpectedly, eukaryotes accounted for most of its consumption (average 2.1 μM $d^{-1}$). Nitrification oxidized an important proportion of the ammonium in both layers (from 25% to 100%) and provided regenerated nitrate. The integrated water column rates of chemosynthesis (0.005 g C $m^{-2}$ $d^{-1}$) represented a large proportion (51%) of the total dark carbon fixation during the non-upwelling season when integrated rates of photosynthesis are relatively low (0.42 g C $m^{-2}$ $d^{-1}$) and microbial food webs dominate the transfer of carbon within this coastal system.



## 1. Introduction

In the oceans, ammonium is rapidly cycled in the water column, and usually does not accumulate as other nitrogen compounds do, such as nitrate in subsurface waters. Ammonium cycling is carried out by organisms from different domains of life (Bacteria, Archaea and Eukarya) and with diverse metabolisms.

Ammonium in the water column is mainly produced by heterotrophic prokaryotes via the remineralization of organic matter, but also by heterotrophic eukaryotes via excretion (ammonium regeneration). It is consumed by eukaryotes (mainly photolithoautotrophic) and prokaryotes (mainly heterotrophic) via assimilative (ammonium uptake) (Lipschultz et al., 1990; Varela et al., 2003) and dissimilative pathways (chemolithoautotrophy), such as aerobic ammonium oxidation by Bacteria and the recently discovered Archaea (Könneke et al., 2005), and anaerobic ammonium oxidation (anammox) by Bacteria (Thamdrup et al., 2006).

All the above mentioned pathways occur above the continental shelf off Concepcion, an area subjected to strong seasonality caused by wind driven upwelling, defining two periods; the well-known upwelling-favorable period (austral spring/summertime, 57% of the year) and the less studied non-upwelling period (wintertime) (Sobarzo et al., 2007). Both periods differ substantially in terms of the planktonic community structure and functional metabolisms involved in nitrogen cycling. During summertime, large cells and diatoms dominate primary productivity (<10 g C $m^2$ $d^{-1}$) within the system, generating a large input of fresh organic matter towards the bottom (Daneri et al., 2000; Montecino et al., 2004; Gonzalez et al., 2007; Anabalón et al., 2007; Farías et al., 2009). In turn, this results in the development of noteworthy oxygen-deficiency in the water column, and a shift in biological communities towards the anaerobic metabolisms of nitrogen cycling (e.g., denitrification and anammox; Cornejo et al., 2006, 2007; Farías et al., 2009; Galan et al., this volume).

During wintertime, smaller cells and non-siliceous phytoplankton, i.e., phototrophic nanoflagellates (eukaryotes) and cyanobacteria (prokaryotes) (Böttjer and Morales, 2007; Morales et al., 2007) dominate and drive an increase in grazing by ciliates and heterotrophic dinoflagellates. Primary productivity is relatively

* Corresponding author. Present address: Biologia Marina, Facultad de Ecologia y Recursos Naturales, Universidad Andres Bello, Santiago, Chile. Tel.: +56 2 661 8243; fax: +56 2 661 8245.
*E-mail address:* vemolina@udec.cl (V. Molina).

0079-6611/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pocean.2011.07.006

lower (<0.5 g C $m^2$ $d^{-1}$) during wintertime. This latter condition, together with higher oxygenation of most of the shelf waters, might favor the dominance of alternative autotrophic metabolisms, such as chemosynthesis based on ammonium nitrification processes. During non-upwelling periods, ammonia oxidizing Archaea display high abundances (10,000–110,000 *amoA* copies $mL^{-1}$) in the system (Molina et al., 2010). Ammonia oxidizing Bacteria and Archaea can contribute significantly (20–76%) to dark inorganic carbon fixation in the system during the upwelling period (Farías et al., 2009); but the extent of total nitrifying activity and its chemosynthetic rates during the non-upwelling period is unknown. In addition, recent findings evidenced the importance of chemolithoautotrophic prokaryotes in fueling microbial food webs (Glaubitz et al., 2009). In effect, the microbial food web becomes more important in terms of carbon transfer during wintertime (Böttjer and Morales, 2005, 2007; Vargas et al., 2007), and thus ammonium cycling is expected to be higher compared with summertime when high rates of organic matter sedimentation occurs.

Little else is known about the contribution of the various planktonic components to ammonium transformations in the water column and the dominant pathways of nitrogen regeneration during the non-upwelling period. We expect that during this period, when the microbial food web is dominant, tighter coupling between ammonium production and consumption processes is more likely to occur. Moreover, nitrification is expected to contribute an additional source of nitrate to meet the demands of photolithoautotrophic plankton during this period, this may lead to competition for the use of ammonium. This study explores these aspects and includes an evaluation of ammonium cycling processes (production and aerobic ammonium oxidation) and chemolithoautotrophic activities related to nitrification (ammonium and nitrite oxidation), as well as of photolithoautotrophy, under non-upwelling conditions.

## 2. Material and methods

The study area off Concepcion, central Chile (Station 18, 36°30′S, 73°08′W; 90 m depth) was visited during August 2003 on board the R/V Kay Kay. Since 2002, this station has been sampled on a monthly basis, as part of the time series supported by the COPAS center (http://www.copas.cl/).

Hydrographic data (temperature, salinity and dissolved oxygen) were obtained with a CTD-Oxygenprobe (Seabird 23B Electronics, Bellevue, USA; accuracy at levels under 2% of saturation: ~2 μM oxygen). Water samples for the experiments and chemical analyses (ammonium, nitrate and nitrite) were collected using Niskin bottles (10 L) attached to a rosette sampler. $NH_4^+$ samples (40 mL, triplicate) were collected without filtration and the fluorometric reaction was immediately started on board (Turner Designs 10-AU fluorometer), following the method proposed by Holmes et al. (1999). This method provides highly sensitive (3 nM) and reproducible results for low ammonium concentrations (⩽5% standard error). Nitrite and nitrate samples (250 mL for the water column profiles and 20 mL for the experiments) were filtered (0.45 μm, Millipore filter) and stored frozen until analyses using standard colorimetric techniques (Parsons et al., 1984). The variability of nitrite and nitrate concentrations associated with each independent analysis was ⩽10%. In addition, discrete water samples were collected for estimating the abundances of the dominant pico- and nano-plankton components. Because of the time of year and the method used to collect samples, it is unlikely that larger planktonic components (e.g. diatoms chains, copepods, and other microplanktonic organisms) were of significant importance in terms of the experimental incubations and, therefore, were not included in the abundance assessments. Samples of heterotrophic (HNF) and autotrophic (also mixotrophic) nanoflagellates (ANF) were fixed immediately with glutaraldehyde (2% final concentration) and stored in the dark at 4 °C until analyses. Subsamples of HNF and ANF (20 mL) were filtered onto 0.8 μm black Millipore filters and stained with DAPI (10 μg $mL^{-1}$ final concentration) (Porter and Feig, 1980). Thereafter, the filters were mounted on glass slides and stored frozen (−20 °C) until later examination. Particle identification and counting was performed under up to 1600× magnification using a Zeiss epifluorescence microscope (Model Axioskop 2 plus, equipped with a blue and UV light filter set). Samples were analyzed in duplicate and 50 fields were randomly selected for counting. ANF were distinguished from the HNF by their autofluorescence. Picoplankton abundances, including bacterioplankton, cyanobacteria and eukaryotes, were estimated via flow cytometry. For this, samples (1.35 mL) of water were fixed with glutaraldehyde (0.1% final concentration) and stored at -80 °C until analysis. Subsamples (150 μL) were analyzed with a FACSCalibur flow cytometer equipped with an ion–argon laser (488 nm of 15 mW, Becton Dickinson). Among the photolithoautotrophs, coccoid cyanobacteria (*Synechococcus*) cells were identified by their size (light scatter) and autofluorescence in the orange wavelength; in the case of eukaryotes their scattering and fluorescence signals were used. The abundance of non-autofluorescent picoplankton (bacterioplankton) was estimated from separate samples previously stained with SYBR-Green I (10,000×; Molecular Probes) (Marie et al., 1997).

### 2.1. Experimental assessments of ammonium production-consumption and nitrification

Discrete seawater samples from three depths (10, 20 and 50 m) were collected to determine the rates of ammonium production and consumption following a previous study carried out off northern Chile (Molina et al., 2005). Seawater was collected at sunset and stored in the dark with running surface seawater until its arrival at the laboratory (~5 h). Thereafter, seawater from each depth was distributed into five sets of glass bottles (1 L, Duran Schott), each set in triplicate. One set was amended with allylthiourea (ATU), an ammonium oxidation inhibitor targeting autotrophic ammonium oxidizing Bacteria, at a concentration of 0.09 mM (Bianchi et al., 1997). A second set was amended with chlorate, a nitrite oxidation inhibitor targeting autotrophic nitrite oxidizing Bacteria, at a concentration of 10 mM (Bianchi et al., 1997). A third set was treated with cycloheximide (CX), an inhibitor of eukaryotic protein synthesis, at a concentration of 100 mg $L^{-1}$ (Wheeler and Kirchman, 1986). A fourth set was amended with a combination of both inhibitors (ATU + CX) in order to compare the effect of CX in ammonium oxidation; and a fifth set was used as a control (no treatment). Concentrations of dissolved inorganic N (DIN = ammonium, nitrate and nitrite) were measured in each bottle at the beginning of the experiment and then after seven hours of dark incubation in a temperature-controlled room (~11 °C). In order to evaluate the effect of CX on nanoflagellate abundance, initial and final times were also sampled in one of the CX and control bottles taken from 50 m depth; the data showed no significant changes (<30% difference) in the CX treatment (data not shown).

The DIN rate of change over time (μM $d^{-1}$) was calculated as follows:

$$R_{DIN} = (\text{Final DIN concentration} - \text{Initial DIN concentration}) \times \text{time}^{-1}$$

The rate of uncertainty (standard error) was calculated using the propagation error, which was estimated from the standard deviations of the averages. Positive $R_{DIN}$ values were indicative of

DIN production, whereas negative values indicated DIN consumption. The use of inhibitors implies that ammonium production and consumption represent "net" and "potential" rates since the experimental approach includes both production and consumption processes, and the estimates reflect the potentially dominant contribution of one process over the other, depending on the treatment used (Molina et al., 2005).

### 2.2. Assessment of the main planktonic contributors to ammonium cycling

Ammonium production and consumption rates were calculated from the differences between the average rates of the corresponding treatments in which one or two planktonic contributors were inhibited (Molina et al., 2005). Since ATU inhibits mostly the ammonium monooxygenase enzyme (AMO) of aerobic ammonia oxidizing Bacteria (Ginestet et al., 1998) and no clear influence of ATU over ammonia oxidizing Archaea has been demonstrated (Santoro et al., 2010), the experimental setup allowed us to distinguish between the process attributable to eukaryotes, prokaryotes and aerobic ammonium oxidizers (Bacteria). The contribution of heterotrophic prokaryotes to ammonium production is denoted by a positive (+) ammonium rate in the CX treatments (CX or ATU + CX). Prokaryotic contribution to ammonium consumption is explained by a negative (−) ammonium rate in the CX treatments. Eukaryotes are expected to influence ammonium production when a positive or zero production rate appears in the control and a negative rate in the CX treatment. Also, eukaryotes should influence ammonium consumption if a negative ammonium rate appears in the control and a positive or less negative ammonium rate in the CX treatment. On the other hand, in the control (no ammonium addition), aerobic ammonium oxidation activity is calculated from the difference between the ammonium net rate of change in the ATU treatment versus the control (when ammonium oxidation is inhibited, a higher ammonium production is expected in the ATU treatment). Under ammonium enrichment through the addition of CX (~0.1 μM; Wheeler and Kirchman, 1986), aerobic ammonium oxidation activity should increase.

The contribution of aerobic nitrite oxidation depends on the coupling between the activities of the nitrifying communities. ATU inhibits aerobic ammonium oxidation but not nitrite oxidation; however, if the two process are tightly coupled and if ATU stops nitrite production by aerobic ammonium oxidation, then nitrite would disappear in association with nitrite oxidation (if no other process, such as nitrite reduction, is acting). Therefore, nitrite oxidation rate can be estimated from the direct nitrite disappearance rate in the ATU treatment (Bianchi et al., 1997). In addition, if chlorate effectively inhibits nitrite oxidation and tight coupling between nitrification processes exists, then nitrite will accumulate in the chlorate treatment at a rate equivalent to that at which nitrite disappears in the ATU treatment. The contribution of each process was deemed valid when the expected response of the inhibitor was observed and when differences $\geqslant$30% were found between the averages ($n = 3$) of different treatments, coinciding with significant $t$-test probability analyses ($p \leqslant 0.05$).

### 2.3. Experiments to assess dark carbon fixation rates associated with ammonium and nitrite oxidation

Dark carbon fixation experiments were done with water from the same depth levels as above (10, 20 and 50 m) but were incubated in situ for 12 h. Immediately after sampling, the water was distributed among three polycarbonate (125 mL) bottles (wrapped with Al-foil and black nylon bags), leaving a headspace of <5% of the total bottle volume. The sampling time was approximately 7:00 a.m. (before sunrise). The bottles were amended with ATU and chlorate in triplicate and three others were left as controls. All the bottles and a "standard sample" received 10 μL of a 2 mCi $mL^{-1}$ $NaH_{14}CO_2$ stock solution (20 μCi final activities). After incubation, all samples were fixed with formaldehyde (final concentration 2%) and stored at 4 °C. In the lab, 50 mL were filtered onto 0.2 μm Nucleopore membrane filters and frozen until analysis. After the standard sample received $NaH^{14}CO_2$, a 50-μL aliquot was taken and stored frozen with an equivalent buffer addition (pH 10 NaOH) in a scintillation vial until analysis. Prior to activity determinations, the filters were fumed overnight with HCl gas in order to remove the $NaH^{14}CO_2$ fixed in $CaCO_3$. The filters were then transferred into 10 mL of scintillation cocktail (Ecoscint) in appropriate scintillation vials. The carbon activity in the samples (dpm) was measured with a Packard 1600 TR (Tri-Carb) liquid scintillation analyzer.

The apparent carbon fixation rate contributed by ammonium and nitrite oxidation was estimated following Joye et al. (1999), as the difference between the average dpm obtained in the control and ATU and chlorate treatments, respectively. The carbon fixation rate (μM C $d^{-1}$) by aerobic ammonium and nitrite oxidizers was calculated as follows:

$RC_{\text{fixation ammonium or nitrite oxidizers}}$ = [(dpm Control − dpmATU/dpm available) × dissolved inorganic carbon concentration)] × $time^{-1}$. The $RC_{\text{fixation ammonium or nitrite oxidizers}}$ was only estimated when the expected response of the inhibitor was observed and differences $\geqslant$30% were found between the averages ($n = 3$) of the different treatments, coinciding with significant $t$-test probability analyses ($p \leqslant 0.05$). A dissolved inorganic carbon (DIC) concentration of 25,000 μg C $L^{-1}$ was used for this estimation, assuming a standard value for comparative purposes (Molina and Farías, 2009).

### 2.4. Experimental assessment of photosynthetic primary production rates

Carbon fixation experiments to evaluate photosynthetic size-fractionated (0.2–5 and >5 μm) primary production were carried out with water collected from 5, 10 and 20 m depth. Immediately after sampling, the water was distributed between transparent ($n = 3$) and dark ($n = 3$) sets of polycarbonate bottles (125 mL), leaving a headspace of <5% of the total bottle volume. The samples were handled and analyzed as mentioned in the previous section on the contribution of nitrification to carbon fixation, except that 50 mL samples were filtered onto 5 and 0.2-μm pore-size polycarbonate membrane filters (Nuclepore; 25 mm diameter). Total photosynthetic carbon fixation (μg C $L^{-1}$ $d^{-1}$) was determined following Strickland and Parsons (1972). $RC_{\text{fixation primary producers}}$ = [(dpm transparent bottle − dpm dark bottle) × DIC concentration (25,000) × discrimination factor (1.05)] dpm $available^{-1}$ × $volume^{-1}$ × $time^{-1}$.

## 3. Results

### 3.1. Water column biogeochemical characteristics

Hydrographic conditions found in this study (Fig. 1) are similar to those observed during wintertime, characterized by the dominance of well-oxygenated, low-salinity Subantartic Waters (SAAW, Sobarzo et al., 2007). A shallow mixed layer (~10 m; ~11.9 °C) with low salinities (<33.5) and high dissolved oxygen (150–200 μM) was found. Below this layer, temperature decreased smoothly with depth and a sharp halocline was located between 10 and 25 m depth. Oxygen concentration below the subsurface maximum at 15 m (214 μM) decreased constantly with depth reaching less than 50 μM below 50 m depth.

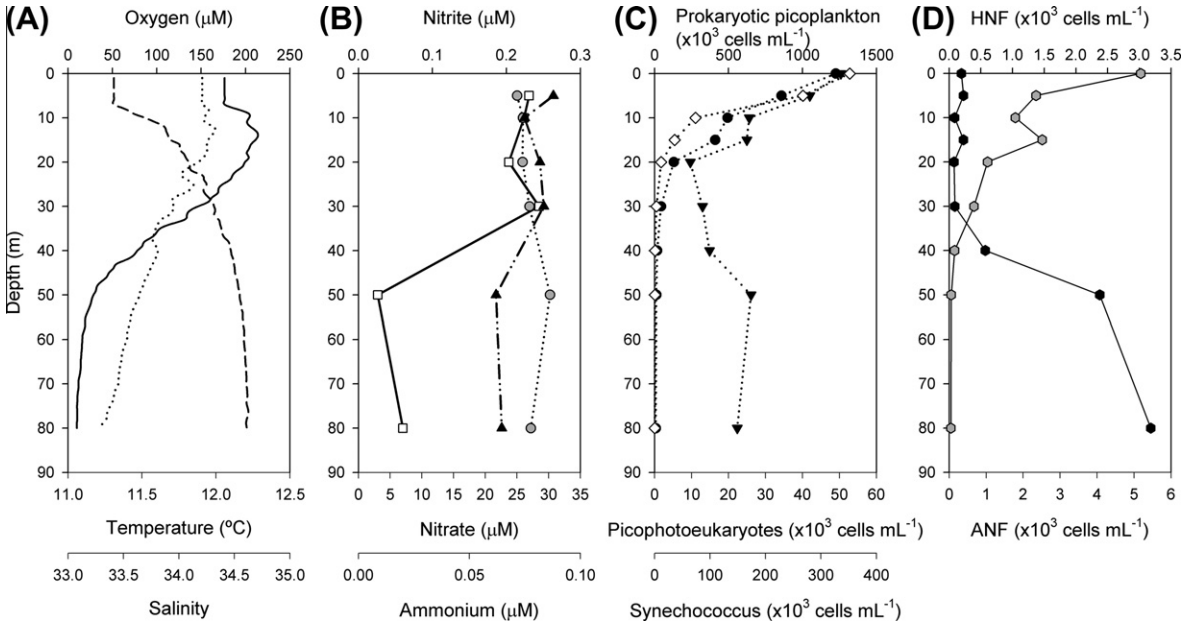


**Fig. 1.** Vertical distribution of physical and chemical variables and the abundance of planktonic microorganisms in the water column at the fixed sampling station (August 2003). (A) vertical profiles of oxygen (solid line), temperature (dotted line) and salinity (long dash line), (B) vertical profiles of nitrite (white square), nitrate (gray circle) and ammonium (black triangle), (C) vertical distribution of non-autofluorescent picoplankton (black triangle), photolithoautothophic eukaryotes (black circle) and *Synechococcus* (white diamond), (D) vertical distribution of heterotrophic nanoflagellates HNF (black circle) and autotrophic nanoflagellates ANF (gray circle).

Nitrate concentration was relatively high and was distributed uniformly in the water column. Slightly lower concentrations were located at the surface (<24 μM) compared with the subsurface waters (>28 μM). Nitrite and ammonium concentrations were higher in the upper 30 m (~0.2 and 0.08 μM, respectively) and decreased with depth.

The maximum abundances of non-fluorescent prokaryotes ($1258 \times 10^3$ cells mL$^{-1}$), *Synechococcus* spp. ($351 \times 10^3$), and photolithoautothophic pico-eukaryotes ($49 \times 10^3$) were located in the top 10 m. The latter two decreased sharply below this depth whereas non-fluorescent prokaryotes presented a secondary maximum ($652 \times 10^3$ cells mL$^{-1}$) at 50 m. The HNF abundance was lower within the upper 30 m and increased with depth, with maxima at 50 and 80 m (range: 0.07–3.18 $\times 10^3$ cells mL$^{-1}$). The ANF abundance presented the opposite trend, with higher abundances in the surface layer (range: 0.04–5.18 $\times 10^3$ cells mL$^{-1}$). In general, the abundances of non-fluorescent prokaryotic assemblages were within the range of those reported previously for Station 18 (Böttjer and Morales, 2007).

**Table 1**
Net rates of change in nutrient concentrations over time (μM d$^{-1}$) during the incubation experiments using different inhibitors and a control (no inhibitors), in samples of water from three different depths.

| Nutrient | Treatment | 10 m | | 20 m | | 50 m | |
|---|---|---|---|---|---|---|---|
| | | Rate | SE | Rate | SE | Rate | SE |
| Ammonium | Control | −0.21 | 0.05 | −0.12 | 0.03 | −0.22 | 0.08 |
| | ATU | 0.51 | 0.22 | 0.29 | 0.12 | 0.33 | 0.07 |
| | CX | 0.28 | 0.28 | 2.21 | 0.07 | 2.67 | 0.46 |
| | CX + ATU | 0.42 | 0.36 | 1.71 | 0.04 | 3.13 | 0.69 |
| | CHL | −0.19 | 0.05 | 0.16 | 0.10 | 0.61 | 0.05 |
| Nitrite | Control | −0.05 | 0.06 | −0.03 | 0.05 | −0.01 | 0.01 |
| | ATU | −0.07 | 0.05 | −0.01 | 0.04 | −0.03 | 0.00 |
| | CX | 0.03 | 0.04 | −0.21 | 0.04 | −0.08 | 0.02 |
| | CX + ATU | −0.01 | 0.07 | −0.13 | 0.03 | −0.06 | 0.02 |
| | CHL | 0.07 | 0.01 | −0.01 | 0.01 | −0.04 | 0.01 |

CX = cycloheximide, ATU = allylthiourea, CX + ATU = COMBINATION of both inhibitors.
CHL = chlorate.
SE = standard Error (see Section 2).

### 3.2. Ammonium cycling rates

Rates of change for ammonium in the control showed a dominance of net consumption at all depths ranging from −0.12 to −0.21 μM d$^{-1}$ (Table 1). Ammonium net production was observed in the treatments with different inhibitors. The highest ammonium production rates was observed in the CX treatments (CX and CX + ATU, Table 1), implying that prokaryotes were the main contributors to ammonium production, through organic matter remineralization, throughout the entire water column (Fig. 2A). Ammonium production by prokaryotes averaged 1.76 μM d$^{-1}$ (*SD* = 0.36) and was higher (>1 μM d$^{-1}$) at the subsurface (20 and 50 m) than in the surface layer (Fig. 2A).

A slightly higher ammonium accumulation was observed at 10 and 50 m in the CX + ATU treatment compared with the CX treatment (difference <40%), signaling the influence of ammonia oxidizing communities in the removal of ammonium (~0.10–0.46 μM d$^{-1}$) in these experiments. The ammonium build-up in the ATU treatment, as compared with the control (difference >100%), indicates that aerobic ammonium oxidizing communities were active throughout the water column, removing ammonium at an average rate of 0.56 μM d$^{-1}$ (*SD* = 0.15) (Fig. 2A). In addition, the differences between ATU and CX + ATU treatments (prokaryotes remineralization) indicates that the eukaryotic community contributed an average ammonium consumption of 1.38 μM d$^{-1}$ (*SD* = 0.41), suggesting that most of the contribution to ammonium consumption in the subsurface layer was due to this group (Fig. 2A).

### 3.3. Nitrite cycling

Rates of change for nitrite in the control also showed a dominance of net consumption at all depths, being an order of magnitude less than net ammonium cycling, ranging from −0.01 to −0.05 μM d$^{-1}$ (Table 1). Nitrite accumulation in the chlorate

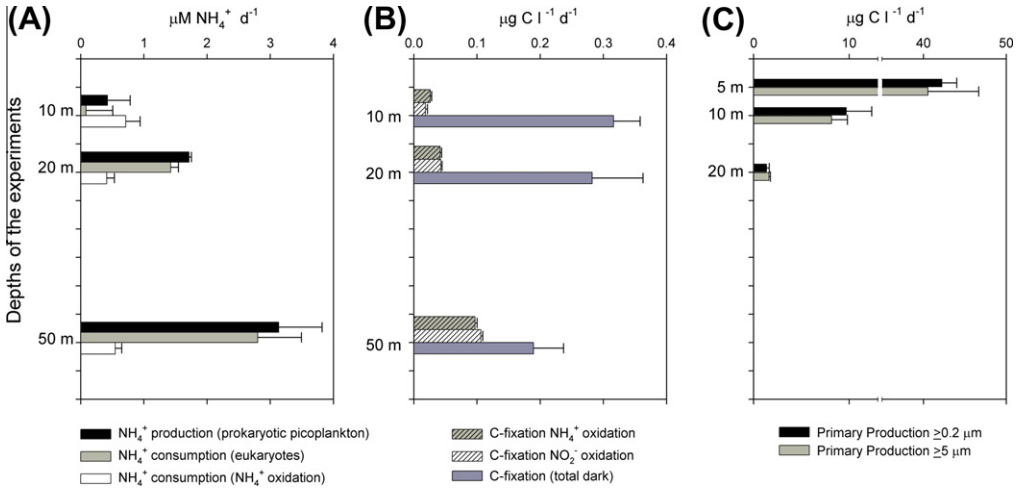


**Fig. 2.** (A) Net ammonium production and consumption rates (A), carbon fixation in nitrifying communities (B), and (C) fractionated, photosynthetic primary production experiments carried out at the fixed sampling station (August 2003) and at three different depths in the water column.

(CHL) treatment was only observed in the surface layer (ammonium oxidation rate coupled to nitrite oxidation). Within the surface layer, high rates of nitrite consumption were observed in the ATU and CX + ATU treatments compared with the control and CX, respectively (Table 1). These results indicate that aerobic ammonium oxidation was participating in nitrite cycling (i.e. nitrite oxidation rate coupled to ammonium oxidation) (Table 1).

### *3.4. Photosynthetic primary production and nitrifiers chemolithoautotrophic activities*

Photolithoautotrophic production rates ranged between 1.36 and 42.32 μg C $L^{-1}$ $d^{-1}$ and was mainly associated with the >5 μm size fraction (Fig. 2C). The 0.2–5 μm size fraction represented a minor contribution to the photosynthetic production rate (average 1.6 μg C $L^{-1}$ $d^{-1}$ (*SD* = 0.1) (Fig. 2C). An integrated value of 0.42 g C $m^{-2}$ $d^{-1}$ was obtained for the euphotic zone (upper 20 m depth). Dark carbon fixation rates (0.18–0.31 μg C $L^{-1}$ $d^{-1}$) were detected in all the samples (Fig. 2B); and an integrated value of 0.01 g C $m^{-2}$ $d^{-1}$ was estimated for the 10 and 50 m depth layer. ATU-sensitive aerobic ammonium oxidizers and CHL-sensitive nitrite oxidizers presented similar carbon fixation rates, ranging from 0.018 to 0.106 μg C $L^{-1}$ $d^{-1}$ and increasing with depth (Fig. 2B). Considering the sum of both oxidizing processes (10–50 m depth), the integrated rate of total chemolithoautotrophic production was 0.005 g C $m^{-2}$ $d^{-1}$.

## 4. Discussion

### *4.1. Ammonium cycling main contributors*

In the study area, ammonium production in the water column was mainly carried out by prokaryotes (0.42–3.13 μM $d^{-1}$) and at magnitudes comparable with estimates obtained in other coastal areas using the N isotope approach, e.g., <0.29–6.5 μM $d^{-1}$ (Glibert, 1982; Glibert et al., 1982; Wheeler and Kirchman, 1986; Metzler et al., 2000). The same inhibitor approach was used in northern Chile, and similar prokaryote ammonium production rates (1.33 μM $d^{-1}$) were found in the oxycline layer (Molina et al., 2005). In contrast, in the coastal area off Concepcion eukaryotes contributed less to total ammonium production, both in the oxic and the oxygen-deficient waters (50 m depth). Prokaryote ammonium production rates showed a noteworthy increase with depth in the water column (Fig. 2A). The differences in rates between 20 and 50 m depth coincide with an increase in prokaryote abundance at the latter depth (Fig. 1C). However, comparable prokaryote abundance was found between 10 and 20 m depth, but at 10 m depth the lowest ammonium net production rates were detected. This was explained by differences in the composition of the planktonic assemblages in the surface layer where high ammonium uptake was expected in the surface layer due to the presence of photolithoautotrophic communities such as cyanobacteria and eukaryotes (Fig. 1). Ammonium assimilation processes by cyanobacteria, such as *Synechococcus* spp., are carried out even during dark incubations (Alaoui et al., 2001), as found in this study. At the same time, a significant proportion of the ammonium can be taken up by heterotrophic prokaryotes, as reported in other marine systems (Lipschultz et al., 1990; Fernández and Raimbault, 2007).

Apparent aerobic ammonium oxidation was the dominant ammonium consumption process in the surface layer. However, in subsurface waters, aerobic ammonium oxidation represented only up to 25% of the total ammonium produced (Fig. 2A). The aerobic ammonium oxidation rates obtained in this study (0.41–0.71 μM $d^{-1}$) were within the wide range of those previously reported (0.001–4.3 μM $d^{-1}$) using ATU, N isotope and carbon fixation approaches (Hashimoto et al., 1983; Ward, 1987; Ward et al., 1989a; Ward and Zafiriou, 1988; Feliatra and Bianchi, 1993; Dore and Karl, 1996; Bianchi et al., 1997, 1999; Sutka et al., 2004). The rates here are similar to those previously reported by us within the oxycline and suboxic waters off northern Chile, 0.16–1.00 μM $d^{-1}$ (Molina et al., 2005; Molina and Farías, 2009).

The higher aerobic ammonium oxidizing potential by ATU-sensitive ammonia oxidizing microorganisms (including both Bacteria and Archaea) in surface layers is unexpected since photoinhibition was reported for this group of microorganisms (Olson, 1981; Guerrero and Jones, 1996). However, nitrification is active in the surface layers of marine systems (Ward et al., 1989b; Wankel et al., 2007), accounting for a significant portion of "regenerated" primary production in the oceanic euphotic zone (Yool et al., 2007).

In contrast to other coastal areas where prokaryotes account for a large part of the ammonium uptake (Wheeler and Kirchman, 1986; Le Corre et al., 1996; Tungaraza et al., 2003), the ammonium produced by prokaryotes was primarily taken up by eukaryotes within the subsurface layer (20–50 m). The maximum in eukaryotic ammonium consumption coincided with the HNF abundance peak at 50 m depth. Ammonium consumption is known to occur in planktonic heterotrophic–mixotrophic eukaryotes (Flynn and

Mitra, 2009), as well as in fungi and other groups of heterotrophs such as yeast (Javelle et al., 2003; Boeckstaens et al., 2007).

### 4.2. Carbon fixation

The dark carbon fixation rates obtained in this study (0.18–0.31 μg C $L^{-1}$ $d^{-1}$) were low compared to those reported in suboxic zones with sulfide and restricted water circulation conditions, i.e., the Cariaco basin and the Black Sea (3.6–30 μg C $L^{-1}$ $d^{-1}$), (Jφrgensen et al., 1991; Taylor et al., 2001). These rates are, however, typical of wintertime conditions in this area (0.20–0.46 μg C $L^{-1}$ $d^{-1}$), contrasting with the higher rates registered during the summertime (up to 84.9 μg C $L^{-1}$ $d^{-1}$). The latter is apparently associated with elevated concentrations and a larger variety of electron donors in the water column, which are available for diverse chemoautotrophic communities, e.g., methane and sulfide oxidizers (Farías et al., 2009).

Carbon fixation rates associated with the metabolisms of aerobic ammonia and nitrite oxidizers in this study (0.01–0.11 μg C $L^{-1}$ $d^{-1}$) were also within the lower range of those previously reported for other coastal marine systems and oxygen minimum zones, i.e., 0.01–4.6 μg C $L^{-1}$ $d^{-1}$ (Ward et al., 1989a; Feliatra and Bianchi, 1993; Bianchi et al., 1997). However, ATU sensitive dark carbon fixation rates were consistent with rates found using a similar approach off northern Chile (~0.15 μg C $L^{-1}$ $d^{-1}$; Molina and Farías, 2009), and using $^{13}C$ off Concepcion (0.03–0.99 μg C $L^{-1}$ $d^{-1}$; Farías et al., 2009). Despite our relatively low estimates of integrated carbon fixation rates by nitrifying assemblages, this process represented 51% of the total dark carbon fixation. On the other hand, the trend of increasing carbon fixation rates with depth by apparent nitrification with depth is comparable to the ammonium production rates by prokaryotes (Fig. 2). This suggests that both N-oxidizers ultimately depend on the ammonium regenerated via prokaryotic remineralization.

Primary production estimates in this study (0.42 g C $m^{-2}$ $d^{-1}$) are within the range of those previously reported during wintertime in the same area and using the same $^{14}C$ approach, i.e., 0.15–0.63 g C $m^{-2}$ $d^{-1}$ (Montecino et al., 2004); they are slightly lower than those obtained with the oxygen method, i.e., 0.5–0.9 g C $m^{-2}$ $d^{-1}$ (Daneri et al., 2000). The smaller fraction (0.2–5 μm) contributed only a low proportion (~8%) of the total primary production via photosynthesis. This finding agrees with previous reports from the study area that indicate a low contribution of prokaryotes to the total phytoplankton biomass (<10% of chlorophyll-*a*) year round (Vargas et al., 2007) and a dominance of the nanophytoplankton fraction (<20 μm) contribution to chlorophyll-*a* during the non-upwelling period (Böttjer and Morales, 2005, 2007; Vargas et al., 2007).

In this study, dark carbon fixation contribute to the carbon flux in the microbial food web, mainly below the euphotic zone, and may channel it towards the HNF community, which displayed maximum abundances deeper in the water column (50–80 m). HNF can consume >100% of the daily prokaryote production in this ecosystem (Cuevas et al., 2004; Vargas et al., 2007).

In summary, our results suggest that, under winter non-upwelling conditions in the coastal region off central-southern Chile, prokaryotes potentially play a dominant role in the production of ammonium, increasing in importance with depth in the water column. The ammonium regenerated was largely coupled to nitrification in the surface layer, whereas most of the ammonium produced at the subsurface was mainly consumed by eukaryotes. Nitrifying assemblages represent a potential contribution of carbon to the heterotrophic eukaryotic community found in the deeper, oxygen-deficient waters. Overall, during non-upwelling conditions, intense ammonium cycling is carried out by microbial assemblages in the coastal area off Concepcion.

## Acknowledgements

We acknowledge the crew of the R/V Kay Kay for their help during the cruise. We thank Dr. R. Escribano for running the COPAS time series off Concepción and the COPAS Kay-Kay time series staff. Financial assistance was provided by the COPAS Center (Project No. 150100007). V.M. was supported by a graduate fellowship from Fundación Andes, the MECESUP UCO0002 Project and by the Marine Genomics postdoctoral fellowship (PBCT RUE 004).

## References

Alaoui, S.E., Diez, J., Humanes, L., Toribio, F., Partensky, F., García-Fernández, J.M., 2001. In vivo regulation of glutamina synthetase activity in the marine chlorophyll *b*-containing cyanobacterium *Prochlorococcus* sp. Strain PCC 9511 (oxyphotobacteria). Applied and Environmental Microbiology 67, 2202–2207.

Anabalón, V., Morales, C.E., Escribano, R., Varas, M.A., 2007. The contribution of nano- and micro-planktonic assemblages in the surface layer (0-30 m) under different hydrographic conditions in the upwelling area off Concepción, central Chile. Progress in Oceanography 75, 396–414.

Bianchi, M., Feliatra, F., Tréguer, P., Vincendeau, M.A., Morvan, J., 1997. Nitrification rates, ammonium and nitrate distribution in upper layers of the water column and in sediments of the Indian sector of the Southern Ocean. Deep-sea Research Part I – Oceanographic Research Papers 44, 1017–1032.

Bianchi, M., Fosset, C., Conan, P., 1999. Nitrification rates in the NW Mediterranean Sea. Aquatic Microbial Ecology 17, 267–270.

Boeckstaens, M., André, B., Marini, A.-M., 2007. The yeast ammonium transport protein Mep2 and its positive regulator, the Npr1 kinase, play an important role in normal and pseudohyphal growth on various nitrogen media through retrieval of excreted ammonium. Molecular Microbiology 64, 534–546.

Böttjer, D., Morales, C.E., 2005. Microzooplankton grazing in a coastal embayment off Concepción, Chile (36°S) during non-upwelling conditions. Journal of Plankton Research 27, 383–391.

Böttjer, D., Morales, C.E., 2007. Nanoplanktonic assemblages in the upwelling area off Concepción (36°S), central Chile: abundance, biomass, and grazing potential during the annual cycle. Progress in Oceanography 75, 415–434.

Cornejo, M., Farías, L., Paulmier, A., 2006. Temporal variability in $N_2O$ water content and its air–sea exchange in an upwelling area off central Chile (36°S). Marine Chemistry 101, 85–94.

Cornejo, M., Farías, L., Gallegos, M., 2007. Seasonal variability in $N_2O$ levels and air–sea $N_2O$ fluxes over the continental shelf waters off central Chile (36°S). Progress in Oceanography 75, 383–395.

Cuevas, L.A., Daneri, G., Jacob, B., Montero, P., 2004. Microbial abundance and activity in the seasonal upwelling area off Concepción (36°S), a comparison of upwelling and non-upwelling conditions. Deep-sea Research Part II – Topical Studies in Oceanography 51, 2427–2440.

Daneri, G., Dellarossa, V., Quinones, R., Jacob, B., Montero, P., Ulloa, O., 2000. Primary production and community respiration in the Humboldt Current System off Chile and associated oceanic areas. Marine Ecology – Progress Series 197, 41–49.

Dore, J.E., Karl, D.M., 1996. Nitrification in the euphotic zone as a source for nitrite, nitrate, and nitrous oxide at Station ALOHA. Limnology and Oceanography 41, 1619–1628.

Farías, L., Fernández, C., Faúndez, J., Cornejo, M., Alcaman, M.E., 2009. Chemolithoautotrophic production mediating the cycling of the greenhouses gases $N_2O$ and $CH_4$ in an upwelling ecosystem. Biogeosciences Discussions 6, 6205–6247.

Feliatra, F., Bianchi, M., 1993. Rates of nitrification and carbon uptake in the Rhône River Plume (Northwestern Mediterranean Sea). Microbial Ecology 26, 21–28.

Fernández, C., Raimbault, P., 2007. Nitrogen regeneration in the NE Atlantic Ocean and its impact on seasonal new, regenerated and export production. Marine Ecology – Progress Series 337, 79–92.

Flynn, K.J., Mitra, A., 2009. Building the "perfect beast": modelling mixotrophic plankton. Journal of Plankton Research 31, 965–992.

Galan, A., Molina, V., Belmar, L., Ulloa, O., this volume.

Ginestet, P., Audic, J.-M., Urbain, V., Block, J.-C., 1998. Estimation of nitrifying bacterial activities by measuring oxygen uptake in the presence of the metabolic inhibitors allylthiourea and azide. Applied and Environmental Microbiology 64, 2266–2268.

Glaubitz, S., Lueders, T., Abraham, W.-R., Jost, G., Jurgens, K., Labrenz, M., 2009. $^{13}C$-isotope analyses reveal that chemolithoautotrophic Gamma- and Epsilonproteobacteria feed a microbial food web in a pelagic redoxcline of the central Baltic Sea. Environmental Microbiology 11, 326–337.

Glibert, P.M., 1982. Regional studies of daily seasonal and size fractionation variability in ammonium remineralization. Marine Biology 70, 209–222.

Glibert, P.M., Lipschultz, F., McCarthy, J.J., Altabet, M.A., 1982. Isotope dilution models of uptake and remineralization of ammonium by marine plankton. Limnology and Oceanography 27, 639–650.

Gonzalez, H.E., Menschel, E., Aparicio, C., Barria, C., 2007. Spatial and temporal variability of microplankton and detritus, and their export to the shelf sediments in the upwelling area off Concepción, Chile (~36°S), during 2002–2005. Progress in Oceanography 75, 435–451.

Guerrero, M.A., Jones, R.D., 1996. Photoinhibition of marine nitrifying bacteria I. Wavelength-dependent response. Marine Ecology – Progress Series 141, 183–192.

Hashimoto, L.K., Kaplan, W.A., Wofsy, S.C., McElroy, M.B., 1983. Transformation of fixed nitrogen and $N_2O$ in the Cariaco Trench. Deep-sea Research Part I – Oceanographic Research Papers 30, 575–590.

Holmes, R.M., Aminot, A., Kerouel, R., Hooker, B.A., Peterson, B.J., 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 56, 1801–1808.

Javelle, A., Morel, M., Rodríguez-Pastrana, B.R., Botton, B., André, B., Marini, A.-M., Brun, A., Chalot, M., 2003. Molecular characterization, function and regulation of ammonium transporters (Amt) and ammonium metabolizing enzymes (GS, NADP-GDH) in the ectomycorrhizal fungus *Hebeloma cylindrosporum*. Molecular Microbiology 47, 411–430.

Joye, S.B., Connell, T.L., Miller, L.G., Oremland, R.S., Jellison, R.S., 1999. Oxidation of ammonia and methane in an alkaline, saline lake. Limnology and Oceanography 44, 178–188.

Jϕrgensen, B.B., Fossing, H., Wirsen, C.O., Janasch, H.W., 1991. Sulfide oxidation in the anoxic Black Sea chemocline. Deep-sea Research Part I – Oceanographic Research Papers I 38, 1083–1103.

Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., Stahl, D.A., 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437, 543–546.

Le Corre, P., Wafar, N., Helguen, S.L., Maguer, J.F., 1996. Ammonium assimilation and regeneration by size-fractionated plankton in permanently well mixed temperate waters. Journal of Plankton Research 18, 355–370.

Lipschultz, F., Wofsy, S.C., Ward, B.B., Codispoti, L.A., Friedrich, G., Elkins, J.W., 1990. Bacterial transformations of inorganic nitrogen in the oxygen-deficient waters of the eastern tropical south-pacific ocean. Deep-sea Research Part I – Oceanographic Research Papers I 37, 1513–1541.

Marie, D., Partensky, F., Jacquet, S., Vaulot, D., 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Applied and Environmental Microbiology 63, 186–193.

Metzler, P., Glibert, P.M., Gaeta, S.A., Ludlam, J.M., 2000. Contrasting effects of substrate and grazer manipulations on picoplankton in oceanic and coastal waters off Brazil. Journal of Plankton Research 22, 77–90.

Molina, V., Farías, L., 2009. Aerobic $NH_4^+$ oxidation in the oxycline and oxygen minimum zone of the eastern tropical South Pacific off northern Chile (20°S). Deep-sea Research Part II – Topical Studies in Oceanography 56, 1032–1041.

Molina, V., Farías, L., Eissler, Y., Cuevas, L.A., Morales, C.E., Escribano, R., 2005. Ammonium cycling under a strong oxygen gradient associated with the Oxygen Minimum Zone off northern Chile (23°S). Marine Ecology – Progress Series 288, 35–43.

Molina, V., Belmar, L., Ulloa, O., 2010. High diversity of ammonia-oxidizing archaea in permanent and seasonal oxygen-deficient waters of the eastern South Pacific. Environmental Microbiology 12, 2450–2465.

Montecino, V., Astoreca, R., Alarcón, G., Retamal, L., Pizarro, G., 2004. Bio-optical characteristics and primary productivity during upwelling and non-upwelling conditions in a highly productive coastal ecosystem off central Chile (36°S). Deep-sea Research Part I – Oceanographic Research Papers I 51, 2413–2426.

Morales, C.E., González, H.E., Hormazabal, S.E., Yuras, G., Letelier, J., Castro, L.R., 2007. The distribution of chlorophyll-*a* and dominant planktonic components in the coastal transition zone off Concepción, central Chile, during different oceanographic conditions. Progress in Oceanography 75, 452–469.

Olson, R.J., 1981. Differential photoinhibition of marine nitrifying bacteria: a possible mechanism for the formation of the primary nitrite maximum. Journal of Marine Research 39, 227–238.

Parsons, T., Maita, Y., Lalli, C., 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.

Porter, K.G., Feig, Y.S., 1980. The use of DAPI for identifying and counting aquatic microflora. Limnology and Oceanography 25, 943–948.

Santoro, A.E., Casciotti, K.L., Francis, C.A., 2010. Activity, abundance and diversity of nitrifying archaea and bacteria in the central California Current. Environmental Microbiology 12, 1989–2006.

Sobarzo, M., Bravo, L., Donoso, D., Garcés-Vargas, J., Schneider, W., 2007. Coastal upwelling and seasonal cycles that influence the water column over the continental shelf off central Chile. Progress in Oceanography 75, 363–382.

Strickland, J.D.H., Parsons, T.R., 1972. A practical handbook of sea-water analysis. Bulletin Fisheries Research Board of Canada 167, 1–310.

Sutka, R.L., Ostrom, N.E., Ostrom, P.H., Phanikumar, M.S., 2004. Stable nitrogen isotope dynamics of dissolved nitrate in a transect from the North Pacific Subtropical Gyre to the Eastern Tropical North Pacific. Geochimica et Cosmochimica Acta 68, 517–527.

Taylor, G.T., Iabichella, M., Tung-Yuan, H., Scranton, M.I., Thunell, R.C., Muller-Karger, F., Varela, R., 2001. Chemoautotrophy in the redox transition zone of the Cariaco Basin: a significant midwater source of organic carbon production. Limnology and Oceanography 46, 148–163.

Thamdrup, B., Dalsgaard, T., Jensen, M.M., Ulloa, O., Farias, L., Escribano, R., 2006. Anaerobic ammonium oxidation in the oxygen-deficient waters off northern Chile. Limnology and Oceanography 51, 2145–2156.

Tungaraza, C., Brion, N., Rousseau, V., Baeyens, W., Goeyens, L., 2003. Influence of bacterial activities on nitrogen uptake rates determined by the application of antibiotics. Oceanologia 45, 473–489.

Varela, M.M., Barquero, S., Bode, A., Fernández, E., González, N., Teira, E., Varela, M., 2003. Microplanktonic regeneration of ammonium and dissolved organic nitrogen in the upwelling area of the NW of Spain: relationships with dissolved organic carbon production and phytoplankton size-structure. Journal of Plankton Research 25, 719–736.

Vargas, C.A., Martínez, R.A., Cuevas, L.A., Pavez, M.A., Cartes, C., González, H.E., Escribano, R., Daneri, G., 2007. The relative importance of microbial and classical food webs in a highly productive coastal upwelling area. Limnology and Oceanography 52, 1495–1510.

Wankel, S.D., Kendall, C., Pennington, J.T., Chavez, F.P., Paytan, A., 2007. Nitrification in the euphotic zone as evidenced by nitrate dual isotopic composition: observations from Monterey Bay, California. Global Biogeochemical Cycles 21, 7.

Ward, B.B., 1987. Nitrogen transformations in the Southern California Bight. Deep-sea Research Part I – Oceanographic Research Papers I 34, 785–805.

Ward, B.B., Zafiriou, O.C., 1988. Nitrification and nitric oxide in the oxygen minimum of the eastern tropical North Pacific. Deep-sea Research Part I – Oceanographic Research Papers I 35, 1127–1142.

Ward, B.B., Glover, H.E., Lipschultz, F., 1989a. Chemoautotrophic activity and nitrification in the oxygen minimum zone off Peru. Deep-sea Research Part I – Oceanographic Research Papers I 36, 1031–1051.

Ward, B.B., Kilpatrick, K.A., Renger, E., Eppley, R.W., 1989b. Biological nitrogen cycling in the nitracline. Limnology and Oceanography 34, 493–513.

Wheeler, P.A., Kirchman, D.L., 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. Limnology and Oceanography 31, 998–1009.

Yool, A., Martin, A.P., Fernández, C., Clark, D.R., 2007. The significance of nitrification for oceanic new production. Nature 447, 999–1002.