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Contribution of heterotrophic plankton to nitrogen regeneration in the upwelling ecosystem of A Coruña (NW Spain)

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The contribution of heterotrophic plankton to nitrogen (N) regeneration in the water column, and its significance for the requirements of phytoplankton, were studied at the seasonal scale in the coastal upwelling ecosystem of A Coruña (Galicia, NW Spain). During 1995–1997, monthly measurements were taken of hydrographic conditions, dissolved nutrients, and abundance and biomass of microplanktonic heterotrophs (bacteria, flagellates and ciliates), phytoplankton and mesozooplankton (>200 μm). Additionally, series of experiments were conducted to quantify N fluxes, including primary production (^{14}C method), phytoplankton uptake of nitrate, ammonium and urea (^{15}N-labelling techniques), microheterotrophic regeneration of ammonium, mesozooplankton grazing (chlorophyll gut-content method) and excretion of ammonium by mesozooplankton. Two N budgets were built for the average situations of high (>100 mg C m^{-2} h^{-1}) and low (<100 mg C m^{-2} h^{-1}) primary production. The results revealed that phytoplankton relied strongly on regenerated ammonium all year round (33 and 43% of total N uptake in high and low production situations, respectively). This demand for ammonium was closely matched by regeneration rates of microplankton (0.14–0.25 mmol N m^{-2} h^{-1}), whereas zooplankton contributed on average <10% to N regeneration. Likewise, zooplankton grazing had little direct control on phytoplanktonic biomass. The results obtained indicate that in the A Coruña upwelling system, N biomass of heterotrophic plankton is generally higher than phytoplankton N biomass. The high rates of N regeneration measured also suggest that a large proportion of the organic matter produced after an upwelling pulse is recycled in the water column through the microbial food web.

INTRODUCTION

In upwelling ecosystems, food webs were considered short and efficient, channelling a large portion of primary production into pelagic fish, and the accumulation of phytoplankton biomass regulated mainly by the increase in production fuelled by the new nutrients (Ryther, 1969; Codispoti, 1983; Minas *et al.*, 1986; Dugdale *et al.*, 1990; Dickson and Wheeler, 1995). Various factors have been studied as drivers of biomass loss in upwelling areas: offshore transport of coastal waters (Head *et al.*, 1996; Joint *et al.*, 2001) and sinking of cells, sometimes helped by the active downward transport of surface waters (Varela *et al.*, 1991), and biological losses due to grazing by zooplankton (Braun *et al.*, 1990; Varela *et al.*, 1991; Head *et al.*, 1996) and release of dissolved organic matter (Teira *et al.*, 2001).

The usage of nutrients following the relaxation of the upwelling pulse can be partly compensated by *in situ* nutrient regeneration (Bidigare, 1983; Codispoti, 1983; Probyn *et al.*, 1990). Earlier estimations suggested that zooplankton and fish supplied a large part of the nitrogen (N) required by primary production (Whitledge and Packard, 1971; Smith and Whitledge, 1977), and also that sedimentary regeneration might be a significant source of nutrients (Rowe *et al.*, 1977). However, later studies in the field indicated that most of the regenerated N is remineralized by the microplankton (Bidigare, 1983; Probyn, 1987; Probyn *et al.*, 1990). The inclusion of microbial food webs in upwelling ecosystems has added several trophic levels to the transfer of organic matter from phytoplankton to fish, challenging the earlier perceptions of simplicity in these systems (Moloney,

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1992). Simulation studies have shown that changes in the trophic structure of the upwelling can alter the relative importance of microbes and zooplankton in carbon (C) and N flows (Newell *et al.*, 1988; Moloney and Field, 1991; Moloney, 1992).

The efficiency of N regeneration by heterotrophs largely depends on the biochemical composition of their food (Bidigare, 1983; Glibert, 1993; Miller *et al.*, 1997), but also the interactions between zooplankton, phytoplankton and microbial organisms affect in a complex way the amount and the form of N released (Glibert, 1998). On one hand, zooplankton contribute to N removal directly by grazing phytoplankton and indirectly by eating microorganisms, which are the primary remineralizers of N (Batten *et al.*, 2001). On the other hand, zooplankton also contribute directly to N release by excretion of ammonium and urea, and sloppy feeding. Seasonal shifts in the relative effects of grazing and N regeneration by heterotrophic plankton were found in estuarine waters (Glibert *et al.*, 1991), and changes in shorter time scales were illustrated by mesocosm (Glibert, 1998) and microcosm experiments (Glibert *et al.*, 1992; Miller *et al.*, 1997). However, to date, there has been no experimental verification of the relative importance of zooplankton and microorganisms for the supply and removal of N in a coastal upwelling ecosystem in relation to the pulsating levels of primary production.

Earlier studies in coastal upwelling ecosystems of NW Spain suggested that most of the regenerated N could be provided by the microplankton (Bode and Varela, 1994), while there were controversial results about the importance of zooplankton grazing (Braun *et al.*, 1990; Varela *et al.*, 1991; Tenore *et al.*, 1995; Barquero *et al.*, 1998; Fileman and Burkill, 2001; Halvorsen *et al.*, 2001a; Bode *et al.*, 2003). Recently, C budgets constructed for the upper water column in the shelf both near the Ria de Vigo (Barbosa *et al.*, 2001; Halvorsen *et al.*, 2001b) and near A Coruña (Teira *et al.*, 2003) highlighted the predominant role of microbial processes in this upwelling area. With the aim of clarifying these important questions, the present study addresses the relative importance of the different groups of heterotrophic plankton as regenerators or consumers of N during phases of high and low primary production in the A Coruña upwelling system.

METHOD

From January 1995 to December 1997, approximately monthly measurements were taken at one station 80 m deep (43°25′N, 8°25′W) near the coast of A Coruña (Galicia, NW Spain) located in the area under the influence of the upwelling (Figure 1). Measurements included oceanographic conditions, plankton biomass and rates of primary production. Additionally, for various periods between January 1995 and December 1997, experiments were conducted to estimate zooplankton grazing, N uptake and N regeneration (Table I). Vertical profiles of temperature, salinity and density (σ_t) were recorded with a SBE-25 CTD with attached sensors for photosynthetically available irradiance (LiCor spherical sensor) and *in situ* fluorescence (SeaTech). Water samples were collected with Niskin bottles at 3–5 depth points within the euphotic zone (>1% of surface irradiance) and at 10-m intervals below. Dissolved nitrate and ammonium were determined with an autoanalyser (Technicon AA-II) using the procedures described in Grasshoff *et al.* (Grasshoff *et al.*, 1983). Urea concentration was measured by the urease method (McCarthy, 1970). Seston C and N were analysed in 0.5–2 L water samples filtered onto Whatman GF/F filters, using an elemental analyser (Perkin Elmer 2400 CHN).

The chlorophyll (Chl) *a* concentration was measured by fluorometry in acetone extracts of phytoplankton filtered onto Whatman GF/F filters (UNESCO, 1994). Phytoplankton biomass was estimated from Chl *a* concentrations by assuming conversion factors of 50 C:Chl *a* [weight; (Varela *et al.*, 1988)] and 6.6 C:N [molar; (Redfield *et al.*, 1963)]. Primary production rates were determined at five depths within the euphotic zone by the ^{14}C method (UNESCO, 1994). Triplicate samples from each depth were incubated for 2–3 h around noon in simulated *in situ* conditions of light and temperature (Bode *et al.*, 1994; Bode and Varela, 1998).

The abundance of bacteria and flagellates was determined by epifluorescence microscopy in glutaraldehyde-preserved samples (Porter and Feig, 1980). Water samples of 5–10 mL were stained with 4′,6-diamidino-2-phenylindole and filtered onto 0.2 μm, black polycarbonate membrane filters (25 mm; Poretics). The filters were mounted on microscope slides using low-fluorescence oil and stored frozen. Bacteria and flagellates were counted under an epifluorescence microscope (Olympus BH-2) using UV light. Flagellates were grouped in three size-classes using an eyepiece graticule: <2, 2–5 and 5–10 μm. Autotrophic flagellates were distinguished by their red autofluorescence under blue light. For the purpose of this study, only the abundance of heterotrophic flagellates is used. Ciliates were counted in Lugol-preserved samples under an inverted microscope and grouped in three size-classes: <30, 30–100 and ≥100 μm. At least 300 bacteria and 50–100 flagellates were counted in each sample. The abundance of each group and size-class was converted into C and N biomass using the factors given in Table II. For further calculations, heterotrophic flagellates and ciliates were considered as two whole

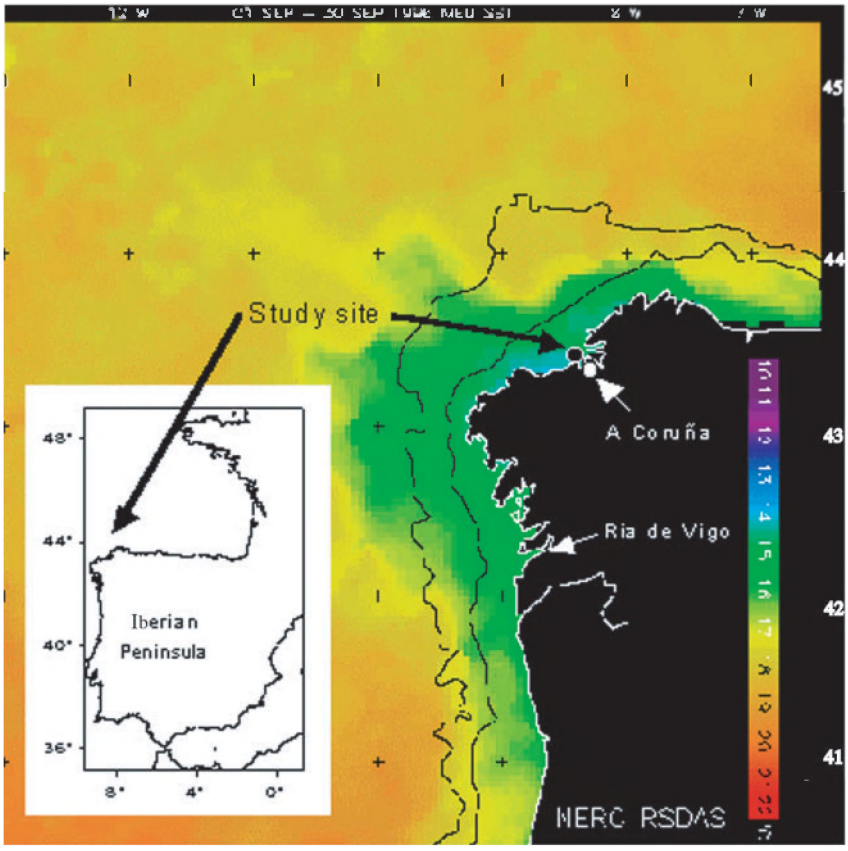

Fig. 1. Distribution of sea surface temperature in NW Spain with indication of the study site near A Coruña. The image was a composite of the best daily AVHRR satellite data received from 1 to 30 September 1996. The distribution of upwelling waters is shown by green and blue colours (<16°C). The image was processed at CCMS (Plymouth Marine Laboratory). The black lines indicate the contours of the 200 and 2000 m isobaths.

Table I: Variables measured and sampling periods

Variable	Period of measurement
Temperature, salinity, density (σ_t)	January 1995–December 1997
Dissolved nutrients, seston C and N	January 1995–December 1997
Chlorophyll *a* and primary production	January 1995–December 1997
Nitrate and ammonium uptake	March 1995–July 1996
Urea uptake	July 1996
Abundance of bacteria and flagellates	January 1995–December 1996
Ciliate abundance	January 1995–December 1997
Ammonium regeneration by microplankton	March 1995–July 1996
Mesozooplankton biomass	January 1995–December 1997
Size-fractionated copepod abundance	February 1996–June 1997
Size-fractionated copepod grazing	February 1996–June 1997
Size-fractionated copepod excretion	October 1996–September 1997

groups and their biomass expressed as the total sum of their size-classes' biomass.

Uptake rates of nitrate, ammonium and urea by phytoplankton were measured with ^{15}N-labelling techniques. Water was taken from two (urea) or five (nitrate and ammonium) depths within the euphotic zone. Duplicate water samples in 250 mL polycarbonate bottles were amended with ^{15}N-labelled substrates and incubated

Table II: Mean biovolumes (BV) and factors employed to convert abundance values to C and N biomass for microplanktonic groups

Group	BV (μm^3)	C:BV (fg C μm^{-3})	Cell C (ng C $cell^{-1}$)	C:N (molar)
Bacteria	4.1×10^{-2}	–	1.1×10^{-5}[a]	4.95[b]
Heterotrophic flagellates (<2 μm)	1.1	220[c]	2.6×10^{-4}	5.83[d]
Heterotrophic flagellates (2–5 μm)	5.6	220[c]	1.2×10^{-3}	5.83[d]
Heterotrophic flagellates (5–10 μm)	5.5×10^{1}	220[c]	1.2×10^{-2}	5.83[d]
Ciliates (<30 μm)	2.1×10^{3}	190[e]	4.0×10^{-1}	5.13[f]
Ciliates (30–100 μm)	7.2×10^{4}	190[e]	1.4×10^{1}	5.13[f]
Ciliates (≥100 μm)	2.1×10^{6}	190[e]	4.0×10^{2}	5.13[f]

[a](Iriberri *et al.*, 1990).
[b](Nagata, 1986).
[c](Borsheim and Bratbak, 1987).
[d](Fenchel and Blackburn, 1979).
[e](Putt and Stoecker, 1989).
[f](Verity and Langdom, 1984).

for 2–3 h around noon, in parallel to the ^{14}C incubations. Mean (± SE) experimental additions were 0.62 ± 0.04 μM [^{15}N]nitrate, 0.33 ± 0.03 μM [^{15}N]ammonium (n = 114) and 0.49 ± 0.10 μM [^{15}N]urea (n = 12). The ^{15}N incubations were terminated by filtration (Whatman GF/F) and the ^{15}N enrichment in the particulate material was analysed in an isotope-ratio mass spectrometer (Europa Scientific; Integra-N). Also, following the procedure of Glibert *et al.* (Glibert *et al.*, 1982), initial and final ^{15}N enrichment of the dissolved ammonium was determined in aliquots of the filtrate of every bottle to simultaneously correct the ammonium uptake estimation for isotope dilution and estimate regeneration rates of ammonium. The uptake rates of nitrate and urea were computed following the procedure of Dugdale and Goering (Dugdale and Goering, 1967) and those of ammonium following Glibert *et al.* (Glibert *et al.*, 1982). In those cases where direct computation of the ammonium regeneration rate was not possible because contamination was suspected, regeneration was estimated using the model of Kanda *et al.* (Kanda *et al.*, 1987). To avoid further manipulation, the water samples used for ^{15}N experiments were not pre-screened, but due to the small volume of the incubation bottles (250 mL), we can safely assume that the measured rates correspond to organisms smaller than 200 μm (microheterotrophs, mostly bacteria, flagellates and ciliates).

Mesozooplankton (>200 μm) dry weight was measured at all sampling dates on samples collected with oblique tows of a Juday–Bogorov net of 200 μm mesh between the surface and the bottom (Valdés *et al.*, 1991). Samples were collected onto Whatman GF/C glass fibre filters, dried at 60°C and weighed. Values of dry weight were converted into N biomass using the equations given in Bode *et al.* (Bode *et al.*, 1998a). In addition, abundance and biomass of copepods were determined in samples collected with total vertical hauls of Bongo-type nets of 200 μm mesh and screened in three size-classes of mesozooplankton (small, 200–500 μm; medium, 500–1000 μm; large, ≥1000 μm) using mesh filters. Individuals of potentially herbivorous and omnivorous species in each size-class were identified using a binocular microscope. Herbivorous grazing by each size-class of copepods was estimated following the Chl gut content method of Mackas and Bohrer (Mackas and Bohrer, 1976), as described by Barquero *et al.* (Barquero *et al.*, 1998), and the gut evacuation rates measured following the procedure described by Dagg and Wyman (Dagg and Wyman, 1983). For the gut evacuation experiments, aliquots of freshly collected copepods from the three size-classes were incubated in filtered sea water (Millipore 0.2 μm membrane). Details on the procedures and results of copepod grazing in these experiments can be found in Bode *et al.* (Bode *et al.*, 2003).

To measure net excretion rates of ammonium by mesozooplankton from the three size-classes, freshly collected zooplankton samples were placed in filtered sea water at simulated natural densities. Two 1-L polycarbonate bottles for each size-class were moored at 50 m for 24 h at the sampling station. Initial and final concentrations of ammonium were measured in each bottle, along with the N biomass of mesozooplankton.

Finally, all rates and stock values were converted into N using C:N ratios indicated in Table II and integrated in the euphotic layer (i.e. to the depth of 1% of surface irradiance, typically 30–40 m), except with the mesozooplankton values, which were integrated in the whole water column. The resulting values were used to compute

N budgets considering dissolved N sources, phytoplankton, total seston and heterotrophic plankton compartments. The latter were partitioned into mesozooplankton (including three size-classes of copepods) and microheterotrophs (bacteria, heterotrophic flagellates and ciliates). Fluxes included N uptake by phytoplankton, primary production, herbivorous grazing by copepods, and N excretion by mesozooplankton and by microheterotrophs. Since not all the rates were simultaneously determined during the study, the observations were grouped according to the value of water-column integrated primary production. In those cases when primary production was higher than a reference value of 100 mg C m^{-2} h^{-1}, the observations were classified as 'high production' (HP), and conversely as 'low production' (LP) when primary production was lower than the reference value. Previous studies in this region showed that phytoplankton blooms reached a mean primary production value of 87 mg C m^{-2} h^{-1} and Chl *a* concentrations of 60 mg C m^{-2} (Bode *et al.*, 1996).

RESULTS

Oceanographic conditions and dissolved N

The distribution of oceanographic variables and dissolved nitrate and ammonium (Figure 2) observed during 1995–1997 followed the seasonal dynamics described for the upwelling ecosystem of A Coruña in preceding studies (Valdés *et al.*, 1991; Casas *et al.*, 1997). Nearly complete vertical mixing of the water column was typical of winter, followed by progressive thermal stratification of the upper surface layer during spring and summer. In general, dissolved nitrate and ammonium were higher during mixing periods and lower during summer, when they reach minimum concentrations near the surface (Figure 2). However, from March to October, the upwelling introduced waters from the outer shelf into the subsurface layers (indicated by $\sigma_t > 26.9$ in Figure 2b), thus altering the stratification and increasing nutrient concentrations, particularly nitrate (Figure 2c). Such nitrate enrichment was higher during the summer. Ammonium concentrations were generally <1 μmol N L^{-1}, except on some sampling dates, probably due to resuspension of bottom sediments.

Interannual differences appeared because of the variability of the timing and intensity of upwelling events (Lavín *et al.*, 2000), which probably affected the amount of nitrate in the water column. For instance, most of the subsurface water in 1995 had lower temperature, higher density and higher nitrate concentration than subsurface water in 1996 and 1997. Considering the period March–July, the mean value of the upwelling index off Galicia in 1995, computed from the data in Lavín *et al.* (Lavín *et al.*, 2000), was 356 m^3 s^{-1} km^{-1}, while that of 1996 was 147 m^3 s^{-1} km^{-1}. Correspondingly, mean nitrate concentrations in the same period in 1995 were almost double the concentrations measured in 1996 (Table III).

Phytoplankton: primary production and N uptake

The distribution of phytoplankton biomass, indicated by Chl *a*, and production showed maximum values near the surface and coincident with the onset of surface stratification in spring and during summer upwelling pulses (Figure 3). These phytoplankton blooms are typical of those described for this ecosystem and were characteristically dominated by diatoms (Casas *et al.*, 1997, 1999; Barquero, 1999). It must be noted that both Chl *a* and primary production values for blooms in 1995 were higher than those for 1996 and 1997, following the described changes in water characteristics (particularly nitrate concentrations). In this way, average concentrations of particulate nitrogen (PN) during March–July 1995 were significantly higher than those measured for the same period in 1996 (Table III).

Nitrate uptake displayed a seasonal variation similar to that of primary production, with maximum values generally near the surface during blooms (Figure 4a). Weak blooms, like those produced during 1996, had maximum nitrate uptake rates near the lower limit of the euphotic layer (30–40 m), probably because of the lower nitrate concentration in this layer in 1996 compared to the concentration measured in 1995. In contrast to nitrate uptake, ammonium uptake did not show a clear seasonal pattern, although high values generally coincided with phytoplankton blooms (Figure 4b). Also, maximum values of ammonium uptake occurred at various depths of the water column, without a clear preference for surface or bottom layers. Nevertheless, nitrate and ammonium uptake values were significantly correlated to each other ($r = 0.723$, $n = 74$, $P < 0.001$) as well as to Chl ($r = 0.611$ for nitrate uptake and $r = 0.790$ for ammonium uptake, $n = 74$, $P < 0.001$) and PN concentrations ($r = 0.665$ for nitrate uptake and $r = 0.861$ for ammonium uptake, $n = 74$, $P < 0.001$). Ammonium uptake reached lower values in 1996 than in 1995, following the trend observed in PN and nitrate concentration values (Table III). However there were no significant differences between both years in mean values of nitrate uptake for the March–July period. The possible effect of differences in the experimental determinations of ^{15}N uptake between 1995 and 1996 was analysed by considering the amount of ^{15}N inoculated in the experimental bottles (% atom enrichment)

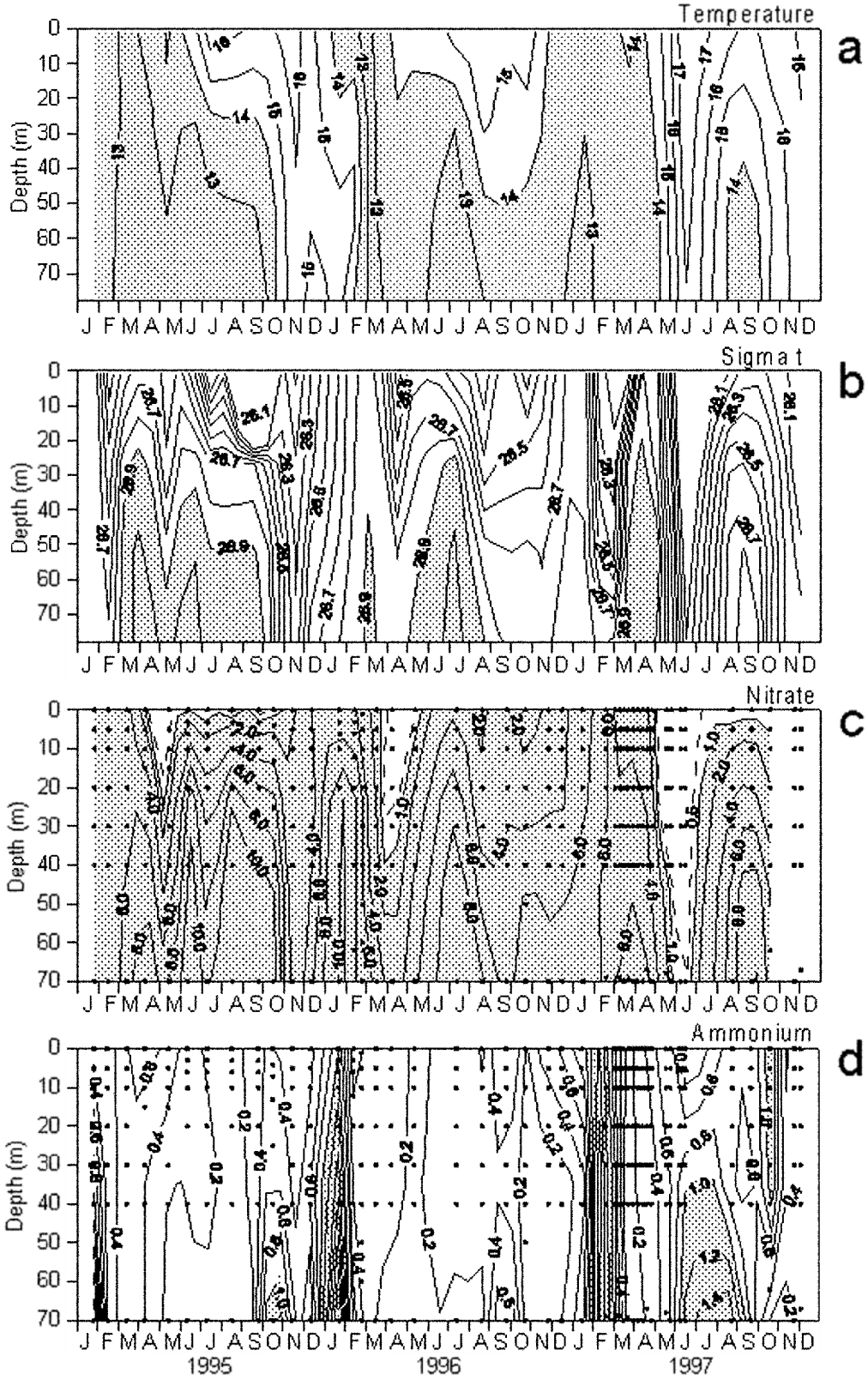

Fig. 2. Distribution of temperature (**a**, °C), density (**b**, σ_t), nitrate (**c**, μM) and ammonium concentrations (**d**, μM). Areas with temperature <14°C, σ_t >26.9, and nitrate and ammonium concentration >1 μM are shaded for descriptive purposes (see the text).

Table III: Mean (± SE) values of N uptake rates and variables employed in their computation for the periods March–July 1995 and March–July 1996

Variable	Units	1995			1996			*P*
		Mean	SE	*n*	Mean	SE	*n*	
Ammonium concentration	μM	0.81	0.04	50	0.67	0.09	62	0.000
Nitrate concentration	μM	2.75	0.36	50	1.65	0.01	62	0.000
PN	μM	1.34	0.15	50	0.74	0.03	62	0.001
% atom enrichment NH_4^+	%	27.8	1.4	50	32.9	3.1	62	0.756
% atom enrichment NO_3^-	%	33.1	2.5	50	19.8	2.2	62	0.000
^{15}N atom excess PN_{NH4}	%	1.7686	0.2079	50	0.3919	0.0400	62	0.000
^{15}N atom excess PN_{NO3}	%	1.4588	0.2500	50	0.4505	0.5970	62	0.005
ρNH_4^+	nmol L^{-1} h^{-1}	23.1	7.0	25	2.0	0.4	31	0.000
ρNO_3^-	nmol L^{-1} h^{-1}	19.4	6.0	25	12.4	5.3	31	0.459

Individual values were taken from each incubation bottle, except for uptake rates, which were computed using the average value of two replicate bottles. PN, particulate nitrogen; % atom enrichment, per cent increase in the dissolved N source caused by the added ^{15}N label; ^{15}N atom excess, percent excess over natural abundance in the atmosphere (0.3676%) of ^{15}N in the PN after the incubation. The significance of differences between means (*P*) was analysed by Mann–Whitney tests.

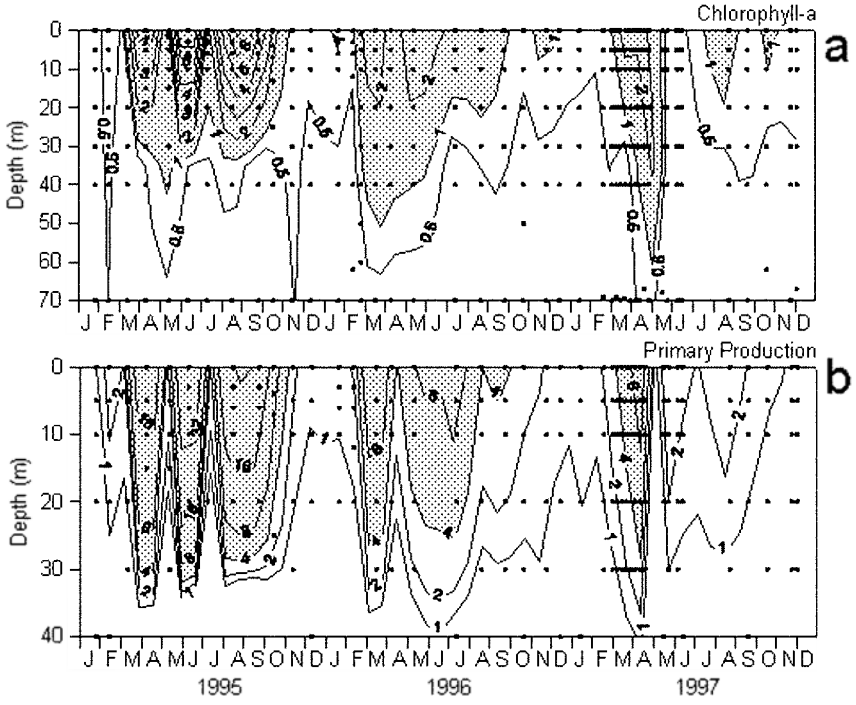

Fig. 3. Distribution of Chl *a* concentration (**a**, mg Chl *a* m^{-3}) and primary production (**b**, mg C m^{-3} h^{-1}). Areas with values >1 mg Chl *a* m^{-3} and >4 mg C m^{-3} h^{-1} are shaded for descriptive purposes (see the text).

and the measured isotopic enrichment in the particulate matter after the incubations (^{15}N atom excess). No significant differences were found in the initial enrichment of ammonium bottles, and even when the nitrate bottles in 1995 were initially more enriched than those in 1996, the final uptake rates did not reflect such differences (Table III). We concluded that the differences in ammonium uptake rates between 1995 and 1996 were primarily caused by upwelling conditions and plankton biomass rather than by experimental conditions.

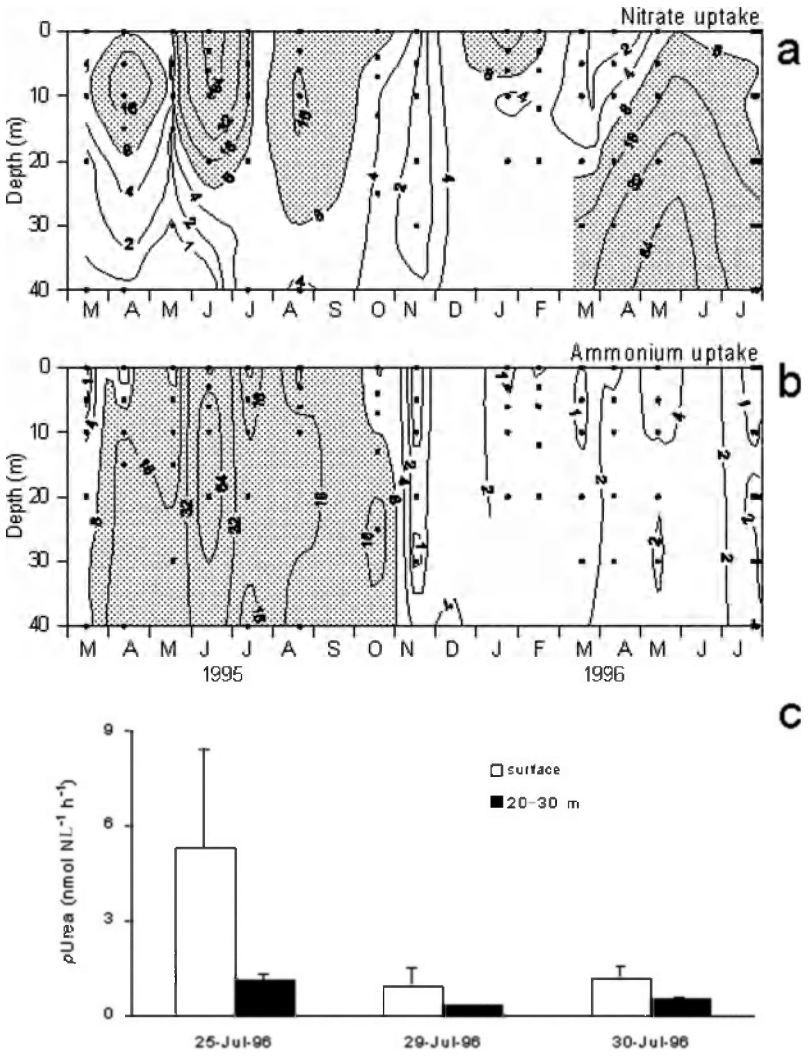

Fig. 4. Uptake rates of nitrate (**a**, nmol N L^{-1} h^{-1}), ammonium (**b**, nmol N L^{-1} h^{-1}) and urea (**c**, ρurea, nmol N L^{-1} h^{-1}). Areas where uptake was >8 nmol N L^{-1} h^{-1} are shaded for descriptive purposes (see the text). Urea uptake rates were averaged (± SE, $n = 2$) for the surface (white bars) and the layer of the subsurface Chl maximum (black bars).

Urea uptake was only determined on three sampling dates in July 1996 (Figure 4c), during the decline of a phytoplankton bloom induced by upwelling. Maximum values were higher than those of ammonium uptake, but lower than those of nitrate during the same sampling dates, and decreased towards the end of July 1996. Urea uptake rates were always higher at the surface than at the subsurface Chl maximum, the latter located between 20 and 30 m and approximately coincident with the depth of 10% of surface irradiance.

Microheterotrophs: bacteria, flagellates and ciliates

Bacteria had their maximum abundance in summer near the surface, generally after phytoplankton blooms (Figure 5a). More than 10^6 bacteria mL^{-1} were found during most of the year in 1995 and 1997, but only in a few cases during 1996. Maximum abundance values of heterotrophic flagellates were generally coincident with those of bacteria (Figure 5b). Ciliates dominated mainly during summer, generally near the surface (Figure 5c). Considering the March–July period, bacteria were more abundant in 1995 than in 1996 (mean ± SE values were 1.2 ± 0.1 and 0.8 ± 0.1 × 10^6 cells mL^{-1} for 1995 and 1996, respectively, Mann–Whitney test, $P < 0.05$), while heterotrophic flagellates followed a converse pattern (5.8 ± 0.9 and 22.1 × 10^3 cells mL^{-1} for 1995 and 1996, respectively, Mann–Whitney test, $P < 0.001$). No significant differences were found in the case of ciliates (Mann–Whitney test, $P > 0.05$).

Ammonium regeneration by microheterotrophs (Figure 6) was significantly correlated to ammonium uptake ($r = 0.985$, $n = 74$, $P < 0.001$), revealing the same

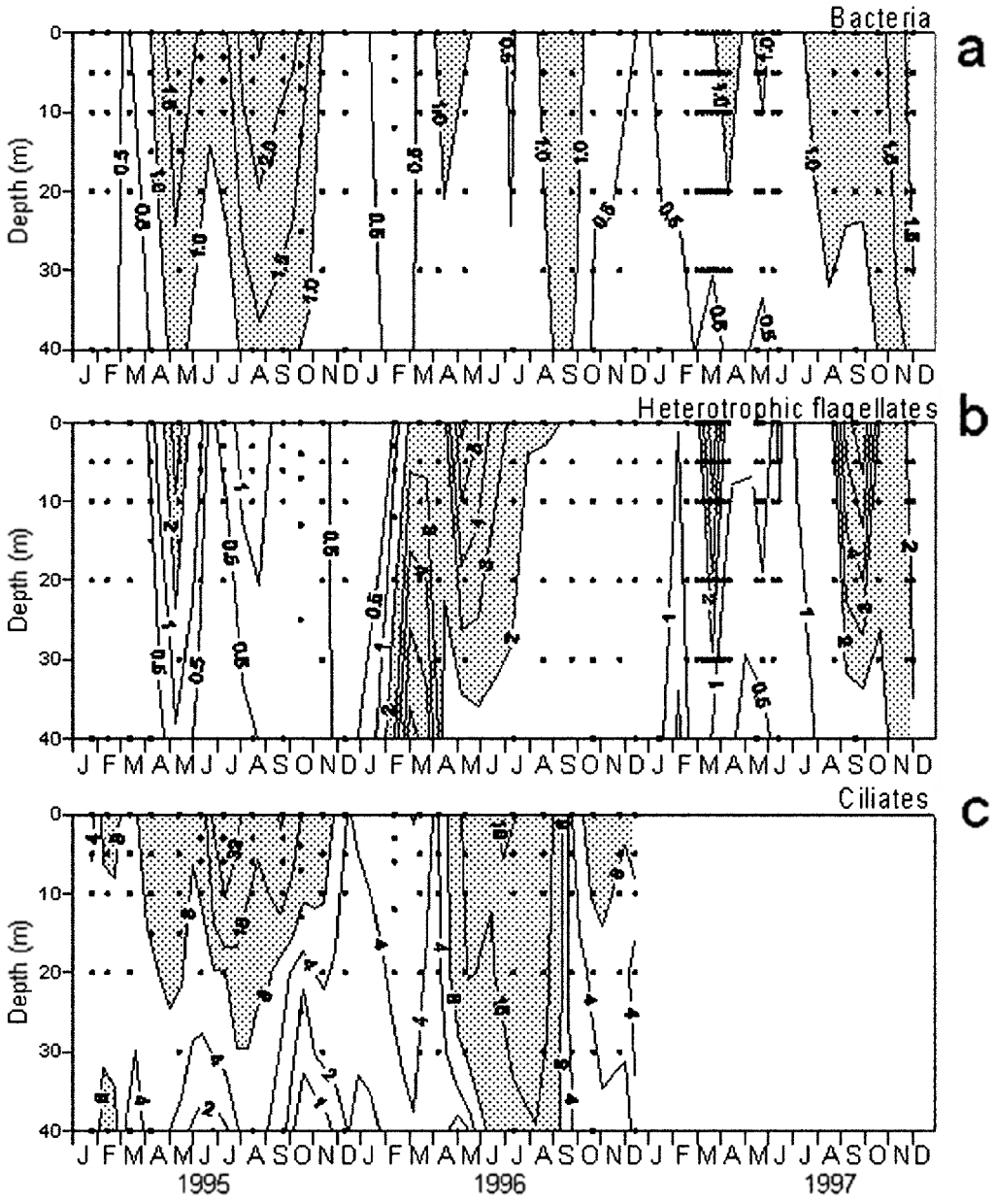

Fig. 5. Abundance of bacteria (**a**, $\times 10^6$ cells mL^{-1}), heterotrophic flagellates (**b**, $\times 10^4$ cells mL^{-1}) and ciliates (**c**, cells mL^{-1}). Abundances of all size-classes of flagellates and ciliates considered (see Method) were combined. Areas with $>10^6$ bacteria mL^{-1}, $>2 \times 10^4$ flagellates mL^{-1} and >8 ciliates mL^{-1} are shaded for descriptive purposes (see the text).

contrasting pattern with values of 1995 higher than those of 1996. In both years, maximum regeneration values generally occurred during phytoplankton blooms (Figure 3).

Mesozooplankton

Mesozooplankton biomass (Figure 7a) showed two annual maxima in spring and late summer, while minimum values were found in winter. In 1995, a marked increase was also observed in autumn. A description of the main species and taxonomic groups present in these samples was given by Barquero (Barquero, 1999). Abundance of copepods (Figure 7b) peaked generally during summer (small and medium size-classes) and autumn (large size-class). Large variations in abundance were observed during phytoplankton blooms (as those in March and July 1996), particularly in the small

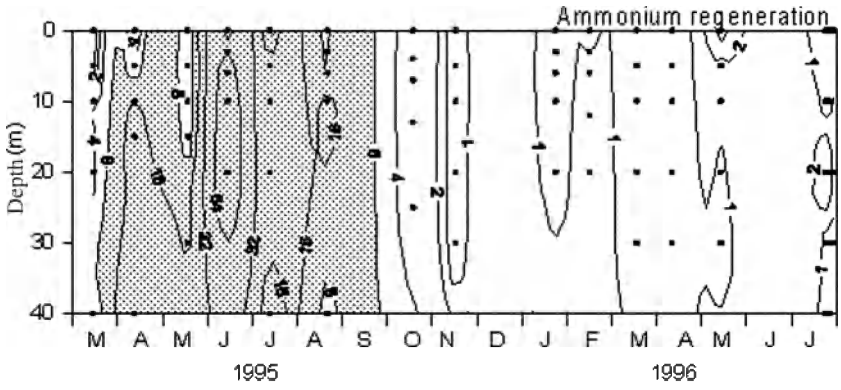

Fig. 6. Ammonium regeneration rates by microheterotrophs (nmol N L^{-1} h^{-1}).

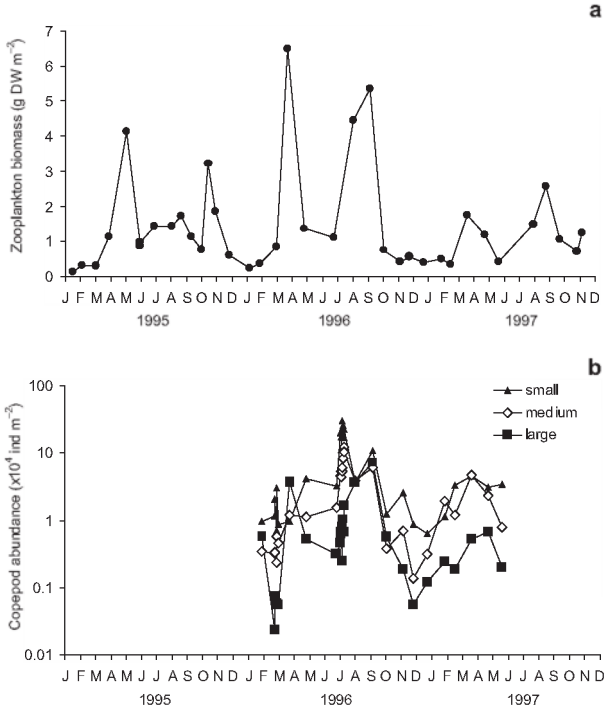

Fig. 7. Water-column integrated biomass of total mesozooplankton (**a**, mg dry weight m^{-2}) and abundance (**b**, $\times 10^4$ ind. m^{-2}) of three size-classes of copepods (small, 200–500 μm; medium, 500–1000 μm; large, 1000–2000 μm).

size-class. Nitrogen biomass values (not shown) displayed a distribution pattern similar to that of abundance. Maximum mesozooplankton abundance and biomass values often occurred after phytoplankton blooms.

Grazing rates by herbivorous copepods were very variable during the study (Figure 8a). The highest grazing rates were measured in summer 1996, although values for the largest size-class were also high in autumn. Secondary grazing maxima were also found in spring of both years. Grazing rates were not determined for most of summer 1997, and therefore there are no data for comparison with those of the previous year. Nevertheless, the differences in grazing rates of both spring and autumn between years suggest that interannual variations are of some importance, as they were originated mainly by the differences in copepod abundance (Figure 7b). Considering the whole study period, grazing was mainly due to medium and large size-classes, while the small size-class had a low contribution to total mesozooplankton grazing despite its high abundance (Bode *et al.*, 2003).

The measured ammonium excretion rates were higher in spring and late autumn (Figure 8b), with the medium and large size-classes contributing the most. Although excretion experiments were not carried out in July 1996, when the highest grazing rates were measured, it can be assumed that excretion would also reach high values at that time because both rates were significantly correlated ($r = 0.750$, $n = 21$, $P < 0.001$).

Nitrogen budgets

HP situations were generally related to high Chl *a* concentrations (sometimes exceeding 100 mg Chl *a* m^{-2}), as occurred with the spring and summer phytoplankton blooms (Figure 9). It must also be noted that maximum values of both integrated primary production and Chl *a* decreased from 1995 to 1997.

The average N budgets show that HP situations have higher nutrient concentrations (particularly nitrate), phytoplankton biomass, primary production, and biomass of microheterotrophs, but lower biomass of mesozooplankton, than LP situations (Figure 10). Phytoplankton, on average, constituted 99% of total seston during HP, while they were only 60% during LP. Also, average primary production and total N uptake in HP exceeded by 6- and 3-fold, respectively, the values measured in LP. Nitrate was the form of N generally preferred by

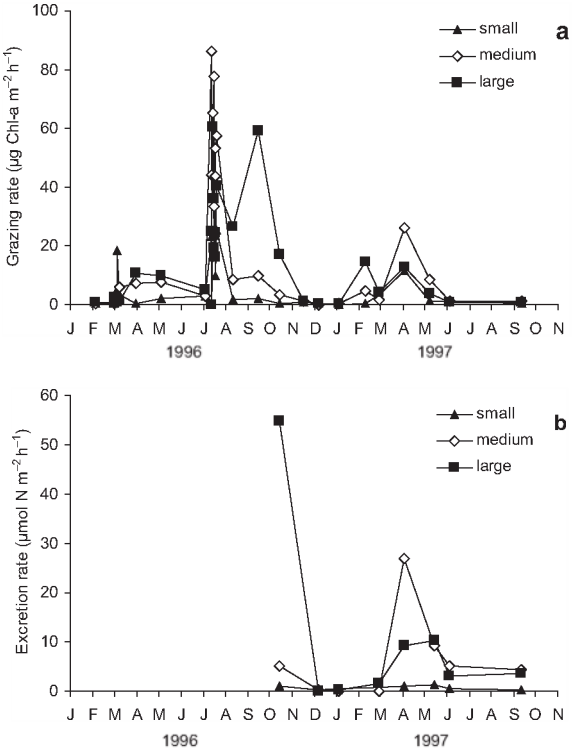

Fig. 8. Water-column integrated grazing rates (**a**, µg Chl *a* m^{-2} h^{-1}) by three size-classes of copepods (small, 200–500 µm; medium, 500–1000 µm; large, 1000–2000 µm) and ammonium regeneration by mesozooplankton (**b**, µmol N m^{-2} h^{-1}).

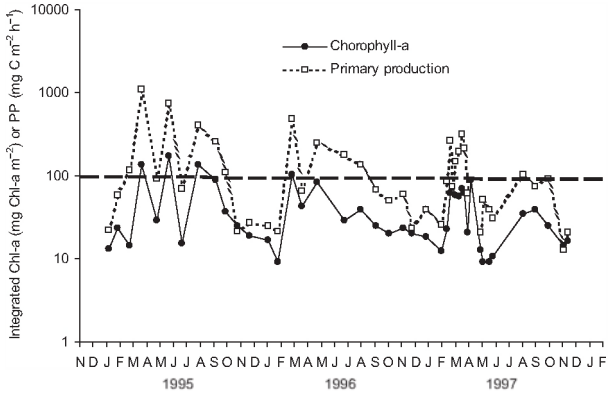

Fig. 9. Water-column integrated Chl *a* (mg Chl *a* m^{-2}) and primary production (mg C m^{-2} h^{-1}). The dashed line indicates the reference value of 100 mg C m^{-2} h^{-1} used to separate high and low production periods.

phytoplankton during HP, while in LP, ammonium and nitrate were taken up at equivalent low rates. In both situations, urea contributed <10% to total N uptake. However, the values of the *f* ratio [nitrate uptake/(nitrate + ammonium uptake)] calculated for each sampling date and averaged for HP and LP were very close in both situations (Table IV). Hourly rates of N uptake only accounted for a fraction of primary production in all situations (Figure 10) and uptake C:N ratios were generally much higher than those of seston (Table IV). These results suggest that N uptake and C fixation processes were uncoupled in both situations at the short time scale considered.

In this coastal ecosystem, grazing by copepods seems to represent only a minor loss of phytoplankton relative to primary production rates measured (on average 0.3–5.8% of primary production in HP and LP, respectively). Therefore, a large excess of primary production was available for export and support of the microplanktonic community, as the latter were also the main contributors to ammonium regeneration (on average 93 and 88% of total ammonium regeneration in HP and LP, respectively). Interestingly, biomass of microheterotrophs exceeded total mesozooplankton biomass from 3 to >10 times (even when the latter was integrated in the whole water column), revealing the ability of microheterotrophs to use the excess of organic matter produced during phytoplankton blooms.

DISCUSSION

In the last decade, an increasing number of studies describing the various components of the pelagic ecosystem off A Coruña have been published (Valdés *et al.*, 1991; Bode and Varela, 1994; Casas *et al.*, 1997, 1999; Bode *et al.*, 1998a), but while most of the information on C fluxes concentrated on primary production (Bode *et al.*, 1994; Bode and Varela, 1998), there was comparatively less information about C and N fluxes through heterotrophic plankton (Bode *et al.*, 2002; Valencia *et al.*, 2003). A preliminary estimation of the main C and N fluxes using available data suggested that microheterotrophs could provide a significant amount of N during upwelling relaxation periods (Bode and Varela, 1994). Recently, Teira *et al.* examined the coupling between the microbial community and the phytoplankton at short time scales through the release of dissolved organic carbon (DOC) (Teira *et al.*, 2002). In the present study, we provide new and experimental data that allow for an evaluation of the role of heterotrophic plankton as remineralizers of N in this ecosystem, including in the balance the biomass of components of the microbial system (bacteria, heterotrophic flagellates and ciliates) and zooplankton, and by measuring fluxes of N between these heterotrophic organisms and phytoplankton. These data are representative of the main changes in this ecosystem, mainly related to the seasonal and episodic occurrence of

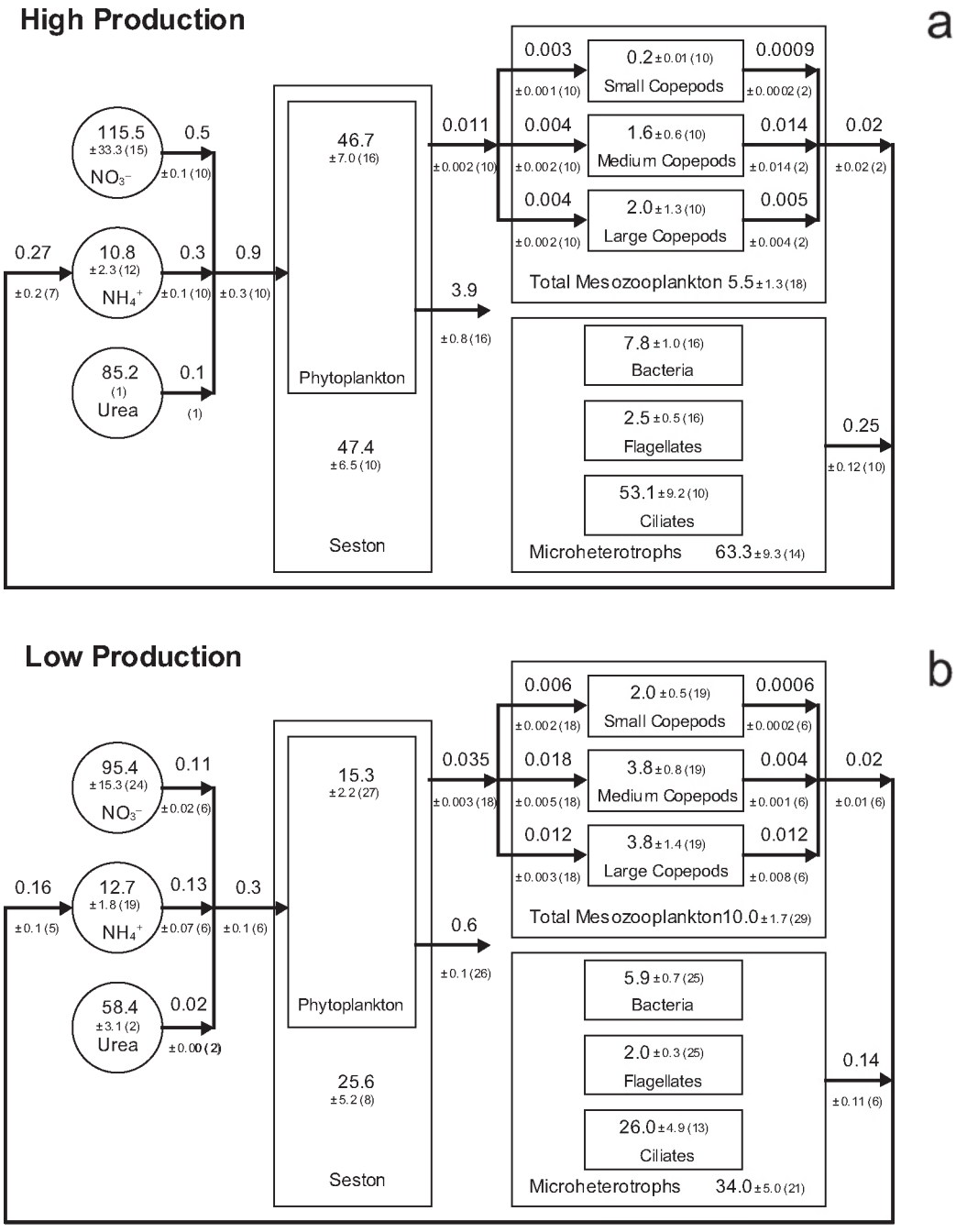

Fig. 10. Planktonic N budget computed for high (**a**) and low (**b**) primary production periods (as determined from Figure 9). Biomass stocks are in mmol N m^{-2} and fluxes in mmol N m^{-2} h^{-1}. Values (mean ± SE) were integrated in the euphotic layer, except for values of mesozooplankton, which were integrated in the whole water column. Primary production is represented by the arrow coming out of the phytoplankton compartment. The number of samples appears in parentheses.

upwelling pulses, as described previously (Valdés *et al.*, 1991; Casas *et al.*, 1997).

One first concern with ^{15}N-labelling techniques is the potential overestimation of N uptake by the experimental addition of ^{15}N-nutrients. Our measured rates, however, do not appear to be overestimated, as described for oligotrophic oceanic waters (Harrison *et al.*, 1996), even if most of our experimental additions of ^{15}N could not be considered trace additions, i.e. <10% of ambient concentration (Dugdale and Goering, 1967). For instance,

Table IV: Mean (± SE) values of the f ratio [nitrate uptake/(nitrate + ammonium uptake)] and molar C:N uptake and composition ratios for periods of high and low production (see the text)

Period	*f* ratio	Uptake C:N	Seston C:N
High production	0.7 ± 0.1 (10)	76.7 ± 17.1 (10)	7.6 ± 0.2 (10)
Low production	0.6 ± 0.1 (6)	25.9 ± 7.2 (6)	7.8 ± 0.2 (6)

Ratios were computed from euphotic zone integrated values. The number of samples is in parentheses.

considering the kinetic model of Harrison *et al.* (Harrison *et al.*, 1996) for the estimation of the effect of enhancement of uptake by the N additions, the average values in HP and LP situations (Figure 10) would change by <15% in the case of ammonium and by <1% for nitrate. Also, the differences between rates measured in the March–July period of 1995 and 1996 were not clearly related to variations in the experimental conditions (Table III). The ranges of values (0.07–128 nmol N L^{-1} h^{-1} for nitrate and 0.03–123 nmol N L^{-1} h^{-1} for ammonium) are within those published for other coastal upwelling ecosystems (Dickson and Wheeler, 1995), and mean values (13.17 and 10.31 nmol N L^{-1} h^{-1} for nitrate and ammonium, respectively) were close to those measured off Portugal during weak upwelling conditions (Slawyk *et al.*, 1997) and in nearby zones off the Ria de Vigo (Joint *et al.*, 2001; Alvarez-Salgado *et al.*, 2002). The measured urea uptake (0.3–5.3 nmol N L^{-1} h^{-1}), although measured only on a few dates, seems off the lower ranges published for upwelling environments (Kokkinakis and Wheeler, 1988; Dortch and Postel, 1989; Probyn *et al.*, 1990). Recently, for an offshore upwelling filament near the Ria de Vigo, Joint *et al.* reported water-column integrated uptake rates of urea in excess of those of nitrate and ammonium (Joint *et al.*, 2001). These rates were on average 2.5 times higher than those measured in our study. However, it could be premature to discuss further the urea uptake rates for A Coruña before more complete measurements are available.

One possible adaptation of the phytoplankton to the nutrient-rich environment of A Coruña would be a low sensitivity to average N additions. For instance, the calculated mean value of the Chl-specific nitrate uptake rate, 6.99 μmol N mg^{-1} Chl *a* h^{-1}, which is an indicator of the nitrate uptake activity per unit of biomass of the phytoplankton cells, is rather low compared to values reported for growing phytoplankton. As an example, Dickson and Wheeler measured up to 24 μmol N mg^{-1} Chl *a* h^{-1} during an upwelling pulse in the NE Pacific (Dickson and Wheeler, 1995). A likely explanation for this low biomass-relative nitrate uptake is that the frequent exposure to upwelling nutrients prevents N limitation within phytoplankton cells, which are less sensitive to uptake enhancement by N additions than cells living in other more oligotrophic environments (Harrison *et al.*, 1996).

Our results show a systematic excess of C production compared to N uptake (Table IV). This excess may be due to the different processes measured by the methods employed for primary production and N uptake determinations. On one hand, our experimental incubations of phytoplankton with inorganic ^{14}C for 2–3 h are likely to measure mostly gross production (Williams, 1993). Gross primary production (GPP) includes C incorporated into organic matter and later used for growth in biomass, respiration and losses as DOC. Respiration may be equivalent to 15% of incorporated C (Setchell and Packard, 1979). Recently, Teira *et al.* estimated a mean DOC release of 37% off A Coruña (Teira *et al.*, 2003). On the other hand, our measurements of N uptake, even when ammonium uptake rates were corrected for isotope dilution (Glibert *et al.*, 1982), did not take into account the release of recently incorporated ^{15}N as dissolved organic nitrogen (DON), which may be a significant loss during short incubations. In this regard, Bronk and Ward reported a mean DON release of 29% of total N uptake in coastal waters (Bronk and Ward, 2000). Therefore, according to the definitions of Slawyk *et al.* (Slawyk *et al.*, 1998, 2000), we measured net N uptake rates in our N experiments.

Considering longer (daily) time scales, the ratio between the rates of C and N incorporation into organic matter is likely to converge towards the biomass C:N ratio of phytoplankton, i.e. 6.6 by mole (Redfield *et al.*, 1963). For example, the C:N uptake ratio for an average summer day (using C and N uptake rates of 512 mg C m^{-2} h^{-1} and 728 μmol N m^{-2} h^{-1}, respectively; photoperiod = 10 h C respiration = 15% of GPP; DOC release = 37% of GPP; DON release = 29% of gross N uptake), assuming no reduction in inorganic N uptake during the night period (Glibert and Garside, 1992), would reduce from 77 to 17 when computed from hourly or daily rates, respectively. Still this daily value of the C:N uptake ratio was higher than the mean C:N value measured for seston (7.7), suggesting larger losses of C at daily time scales than those assumed and/or the use of additional sources of N by

phytoplankton. On one hand, Teira *et al.* showed that, on average, DOC release was sufficient to support bacterial C uptake off A Coruña at daily time scales (Teira *et al.*, 2003). However, for any particular sampling date, both processes were often uncoupled, leading to temporal accumulations of DOC or excess bacterial uptake. Also, DOC release was not a constant fraction of GPP (Teira *et al.*, 2003). On the other hand, one of the possible additional sources of N may be urea, as recent studies revealed that it could sustain phytoplankton requirements when both nitrate and ammonium are at low concentrations (Riegman and Noordeloos, 1998; Riegman *et al.*, 1998; Joint *et al.*, 2001). The likely sources of urea in coastal waters are bacterial breakdown of dissolved organic matter (Berman *et al.*, 1999) and mesozooplankton excretion (Conover and Gustavson, 1999). In our study, we measured concentrations of urea between 1.12 and 2.53 μM, exceeding those of ammonium and nitrate in surface waters, although uptake of urea seemed to contribute only to a small fraction of total N uptake. Further research is required to assess the significance of urea in this ecosystem.

As expected, new N, such as nitrate introduced by the upwelling, contributed significantly to phytoplankton nutrition in the studied ecosystem, even during phases of low productivity. In fact, nitrate was always at concentrations detectable with the conventional colorimetric method used (i.e. >0.05 μM). Furthermore, due to the existence of generally <1 μM ammonium, it is not likely that nitrate uptake rates were significantly inhibited by ammonium (Dortch, 1990). However, the relatively high rates of ammonium uptake in all situations indicate that regenerative processes contributed significantly to sustain primary production, since ammonium did not accumulate in the water and showed ambient concentrations generally lower than those of nitrate. In addition, even the low urea uptake rates caused a reduction in the relative importance of nitrate for primary production, as shown by the average situations illustrated in Figure 10. Such dependence on regenerated N confirms the results found in other upwelling areas (Bidigare, 1983; Probyn, 1987; Probyn *et al.*, 1990) and our earlier predictions (Bode and Varela, 1994). As a result, nitrate would account for roughly half of N uptake during an average HP period and only over one-third during an LP period if urea uptake rates were considered in the computation of *f* ratios, although mean values for each period computed using *f* ratios from individual sampling dates were >0.5 (Table IV). The apparent discrepancy between *f* ratio values computed from individual observations (as in Table IV) and average values given in Figure 10 can be explained by the different time scales implicit in each computation. On one hand, individual observations reflect instantaneous uptake rates, where nitrate is generally the preferred source given the dominance of diatoms and the frequent occurrence of upwelling pulses. The preference for nitrate is even higher in the case of individual depths. For instance, the maximum *f* value (0.98) was found in a sample taken at 30 m depth (near the nitracline) in July 1996. On the other hand, uptake values averaged through HP or LP situations (as values in Figure 10) tend to be closer to time-integrated rates, as extreme values are reduced, and consequently produce lower *f* ratio values. Interestingly, the dominance of nitrate uptake at short time scales in the study area is not paralleled by large export rates, at least when the latter are determined by *in situ* particle sedimentation (Bode *et al.*, 1998b; Teira *et al.*, 2003). However, recent studies in this upwelling region indicated that a significant fraction of new production is exported as dissolved organic matter (Alvarez-Salgado *et al.*, 2002; Teira *et al.*, 2003; Varela *et al.*, 2003).

Heterotrophic microplankton release most of the ammonium in the coast of A Coruña. Mesozooplankton, however, seem to be only a minor source of ammonium (on average, from 8 to 14% of ammonium regeneration during HP and LP, respectively). This result contrasts with the high capability of zooplankton for ammonium production in other ecosystems, where it can meet daily phytoplanktonic demands (Whitledge and Packard, 1971; Smith and Whitledge, 1977; Alcaráz *et al.*, 1994). Similarly, grazing by copepods consumes only a small fraction of phytoplanktonic biomass in all situations studied, which confirms earlier results obtained during phytoplankton blooms in the area (Barquero *et al.*, 1998). In spite of the small impact of grazing, mesozooplankton, and particularly copepods, represent a key mechanism of transport of phytoplankton-derived matter to the sediments through the package of algae in faecal pellets (Bode *et al.*, 1998b), and probably contributed to a large fraction of the production of DON by sloppy feeding, as was reported for other coastal ecosystems (Miller *et al.*, 1997; Hasegawa *et al.*, 2000).

In other estuarine, non-upwelling ecosystems, an alternative dominance of microplankton- and zooplankton-driven regeneration of ammonium has been observed, which depends on the time scale observed (Glibert *et al.*, 1991, 1992; Miller *et al.*, 1997; Glibert, 1998). As a precaution, we assessed the short-term changes in the zooplankton rates to estimate the ranges of variability of grazing and ammonium regeneration rates of these organisms. With this intention, we estimated the variability of the grazing rate during two 1-week periods of daily samplings, corresponding to HP and LP situations. The resultant coefficients of variation were 24 and 73% for HP and LP, respectively, which suggests a substantial daily variability of grazing,

and presumably also of excretion rates of N, particularly during LP periods. Even if we acknowledge some degree of variability in the relative contribution of zooplankton to N regeneration, we are confident that most of the seasonal maxima of activity were sampled during our study, which covered the main seasonal features already described for the coastal ecosystem near A Coruña (Casas *et al.*, 1997). Thus, our averaged estimations of grazing and ammonium excretion by the zooplankton are reasonable, and it is possible to conclude that the mesozooplankton have a very limited control over the phytoplankton in this coastal upwelling system. However, when taking into account energy demands, small copepods must fulfil their metabolic requirements by consuming significant amounts of microplankton (e.g. protozoans and other crustacean plankton), as found in studies near the Ria de Vigo (Batten *et al.*, 2001) and off A Coruña (Bode *et al.*, 2003). Even in the worst case (73% variability in the measured rates), the maximum expected zooplankton excretion rates would not exceed the excretion rates by the microplankton. Furthermore, the total biomass of the community of microheterotrophs (bacteria, flagellates and ciliates) always outweighed the mesozooplankton by 3- to 10-fold (LP–HP).

The importance of microplankton for the recycling of N available for the phytoplankton in A Coruña upwelling supports the growing evidence that food webs in upwelling ecosystems are more complex than previously thought, and that the heterotrophic microorganisms are the major agents of N regeneration (Bidigare, 1983; Probyn, 1987; Probyn *et al.*, 1990). The latter conclusion was also reached in a study of microplanktonic N regeneration in the central eastern north Atlantic (Gaul *et al.*, 1999). One additional indication of the importance of the microbial system is the large abundance and biomass of ciliates found, confirming previous observations (Figueiras and Pazos, 1991; Bode and Varela, 1994; Batten *et al.*, 2001). Given the pivotal role of ciliates in the microbial food web, as consumers of flagellates and bacteria and as prey for larger zooplankton (Glibert, 1998), a more detailed study of these organisms in the NW Spanish shelf is very much needed.

The present study demonstrates the primary role of the heterotrophic microplankton, namely bacteria, flagellates and ciliates, in the regeneration of N for the primary production in the upwelling ecosystem of A Coruña. The contribution of the microplankton is greater during the low productivity intervals between upwelling pulses, but even during blooms the microplankton still regenerate a significant fraction of the N needed by the growing phytoplankton, thus reducing the total amount of primary production that can be exported towards open-shelf waters. In contrast, the mesozooplankton have little direct impact on both the consumption and regeneration of N throughout the year. Finally, the N budgets proposed for typical high and low productivity situations suggest an overall predominance of heterotrophic organisms in this coastal upwelling ecosystem.

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