Nitrogen uptake by size-fractionated phytoplankton populations in the southern Benguela upwelling system

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ABSTRACT: Uptake of ^{15}N-labelled ammonium, nitrate and urea by picoplankton (< 1 μm), nanoplankton (< 10 μm) and the whole phytoplankton community was investigated in oceanic, shelf and inshore waters in the southern Benguela upwelling system during December 1983. Picoplankton and nanoplankton chlorophyll a accounted for 2 to 49 % and 13 to 99 % respectively, of the whole community chlorophyll a. Picoplankton were relatively more active in oceanic as opposed to coastal waters. Nitrogen uptake by picoplankton was 27 % of that by the intact phytoplankton community in oceanic waters compared to ca 10 % for the coastal regions. Similarly, turnover rates of particulate nitrogen in the < 1 μm size class were faster in oceanic than shelf or inshore waters. Relative preference indices showed a consistent preference for ammonium, to a lesser extent for urea, compared to nitrate by all 3 size classes. Nevertheless, nitrate was quantitatively the most important nitrogen source for the nutrition of the intact community over the shelf, supplying 71 % of the total nitrogen assimilated. The proportion of nitrate assimilated was directly related to algal size class at the shelf and inshore stations with picoplankton and nanoplankton production being based largely on regenerated nitrogen (ammonium and urea). In oceanic waters, production in all size classes was supported principally be regenerated nitrogen. Results are discussed with reference to the effect of qualitative and quantitative aspects of nitrogen availability on phytoplankton community structure.

INTRODUCTION

Rate measurements on size-fractionated natural phytoplankton assemblages have emphasized carbon fixation by the netplankton and nanoplankton size categories (reviewed by Malone 1980), with relatively few studies that address aspects of nitrogen assimilation by these size classes. Results of the few studies that have been attempted do not agree about the relationship between algal size and qualitative differences in nitrogen utilization. Netplankton blooms often develop in response to a large input of nitrate into the euphotic zone through upwelling (Malone 1980). The highest specific ammonium uptake rates by phytoplankton in Vineyard Sound have been shown to be almost exclusively in the nanoplankton (< 10 μm) size class (Glibert at al. 1982a). Seasonal shifts were evident so that during a winter diatom bloom period 30 to 90 % of ammonium uptake was due to netplankton (< 35 μm) activity (Glibert et al. 1982a). Nitrate uptake was not measured in this study. In contrast, Furnas

(1983) in his seasonal study in the nearby Narragansett Bay has shown that the whole phytoplankton community and nanoplankton assemblages utilize different nitrogen resources in a similar manner throughout the year with ammonium generally being quantitatively more important than either nitrate or urea. Other studies have been carried out on urea utilization and remineralization by size-fractionated plankton populations (Herbland 1976, Savidge & Hutley 1977); these experiments employed ^{14}C labelled urea as a tracer and consequently do not indicate the fate of urea-nitrogen.

Evidence is accumulating to suggest that bacterial-sized organisms play an important photo-autotrophic role in the pelagic environment. The ubiquitous and usually abundant presence of both cyanobacteria (Johnson & Sieburth 1979, Li et al. 1983) and eucaryotic algae (Johnson & Sieburth 1982) in this size class has been demonstrated in offshore and inshore waters. Organisms in this size range (< 1 μm) contribute between 20 and 80 % of the total primary production

(Gieskes et al. 1979, Joint & Pomroy 1983, Li et al. 1983). At present the nitrogen nutrition of natural assemblages of these important photo-autotrophs is poorly understood.

Detailed analyses of rate processes in natural populations of phytoplankton at sub-community levels of organization can provide valuable insight into the structure and functioning of the pelagic food web as well as the development and decay of phytoplankton blooms. The purpose of this study is to investigate the utilization of nitrogenous nutrients by broad size categories in the phytoplankton community of oceanic, continental shelf and close inshore waters, in the southern Benguela upwelling system. This study was undertaken to assess the spatial variation in nitrogen assimilation for this region and does not encompass any seasonal or shorter time scale variability.

METHODS

Sampling. Nitrogen uptake experiments were performed during December 1983 aboard RV 'Africana' off the west coast of South Africa. Stations were located over the 1000 m depth contour, the continental shelf, as
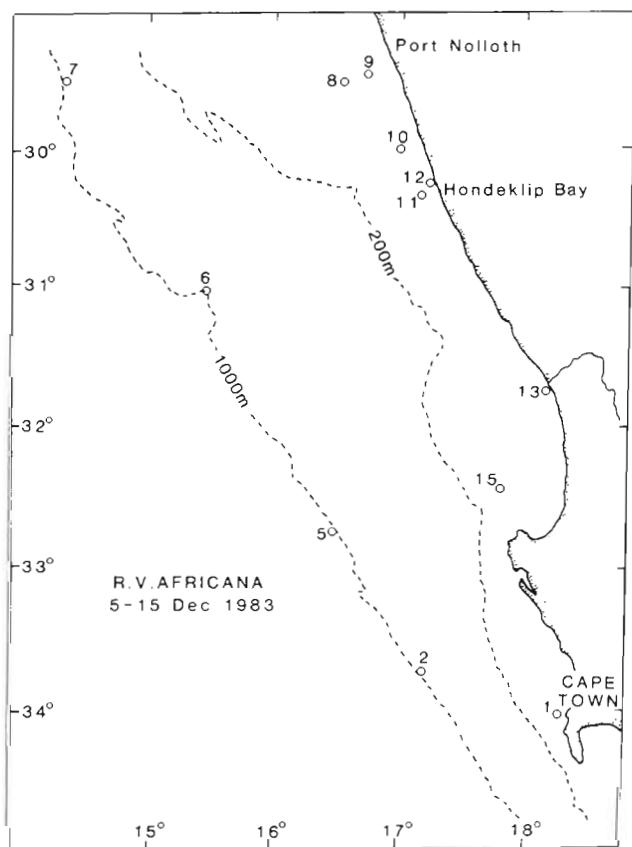

Fig. 1. Sampling area showing station numbers and 200 and 1000 m isobaths

delineated by the 200 m depth contour, and close inshore (Fig. 1). Surface water was sampled in the morning using a rosette sampler or plastic bucket and combined in a 30 l polyethylene carboy. Exactly 2 l of water was prescreened through either a 212 µm or 10 µm nylon mesh into glass incubation bottles. The < 1 µm fraction, the picoplankton (Sieburth et al. 1978) was obtained by gentle vacuum filtration through 1 µm Nuclepore filters. The < 10 µm fraction, (nanoplankton including picoplankton) will be termed the nanoplankton fraction unless otherwise stated, to enable comparison with other studies which have not differentiated between these size classes. The < 212 µm fraction is assumed to be representative of the intact phytoplankton community.

Analyses. Dissolved inorganic nitrogen concentrations were determined manually immediately after sampling and isotope addition. Nitrate was measured following the cadmium reduction method of Nydahl (1976) and ammonium according to Grasshoff (1976) scaled down to 5 ml samples. Samples for urea determinations were frozen immediately and analysed in the laboratory according to the diacetyl monoxime procedure (Grasshoff 1976).

Particulate matter was collected on glass fibre filters (Whatman GF/F) and frozen for subsequent chlorophyll *a* (Jeffrey & Humphrey 1975) corrected for phaeopigments (Strickland & Parsons 1972), and for particulate organic carbon and nitrogen (Heraeus CHN Rapid).

Bacterial and phytoplankton counts. Samples for counting were preserved with 1 % Lugol's iodine. Microbial populations were counted by epifluorescence microscopy, using acridine orange (Hobbie et al. 1977). Iodine was removed by addition of dilute sodium thiosulphate immediately prior to counting (Joint & Pomroy 1983). Bacterial biomass was calculated from the cell volumes using a specific gravity of 1.1 g cm^{-3} (Doetsch & Cook 1973) and the generally used ratio of carbon/wet biomass of 0.1 (Troitsky & Sorokin 1967). Phytoplankton (excluding the picoplankton) were counted using an inverted microscope. No distinction was made between autotrophic and heterotrophic forms in the nanoflagellate counts.

Nitrogen uptake measurements. Nitrogen uptake rates were determined according to Dugdale & Goering (1967). This method under-estimates ammonium and possibly urea uptake under conditions of active regeneration within the incubation bottle (Glibert et al. 1982b, Laws 1984). To 2 l of prescreened surface water were added either Na^{15}NO$_3$ (99.6 at %), ^{15}NH$_4$ Cl (99.7 at %) or CO (^{15}NH$_2$)$_2$ (99.1 at %) and the samples incubated for 4 to 6 h in unshaded simulated *in situ* deck boxes cooled by flowing surface seawater. Long-term incubations can be criticized on the basis of the

often observed non-linear nutrient uptake in laboratory and field experiments (Goldman et al. 1981). The high ambient nutrient concentrations encountered at most stations during this study, however, would reduce the possibility of a transient enhancement in uptake due to isotope addition or a reduction in uptake as a result of nutrient depletion. The quantity of ^{15}N isotope added depended on the initial nutrient concentration when prior analysis had been performed and amounted to 0.05 to 0.10 mg-at ammonium-^{15}N m^{-3}, 0.10 to 0.20 mg-at urea-^{15}N m^{-3}, and 0.10 to 2.50 mg-at nitrate-^{15}N m^{-3}. Enrichments at Stations 5 and 15 were 100 % for urea. Higher than trace additions were also made for urea at Station 8, ammonium at Station 10, and nitrate at Station 6.

Uptake experiments were terminated by filtration of the particulate matter onto glass-fibre filters (Whatman GF/F). Filters were stored frozen and analysed for ^{15}N content by emission spectrometry after a Kjeldahl-Rittenberg oxidation proceedure (Fiedler & Proksch 1975). With the system in operation at our laboratories on optimum of 1.43 μmole of ammonium is required for each ^{15}N determination. A reagent blank was prepared by measuring the dilution of ^{15}N-urea standards during sample preparation. A blank value of 1.44 μmole nitrogen for each sample was used to correct measured ^{15}N enrichments. Corrected Kjeldahl nitrogen and total nitrogen values obtained from CHN analysis agreed closely.

A relative preference index (RPI) was calculated for each nutrient to compare utilization relative to availability. For example, for ammonium (McCarthy et al. 1977):

$$\text{RPI NH}_4 = \frac{\varrho \text{ NH}_4}{\varrho \text{ NH}_4 + \varrho \text{ NO}_3 + \varrho \text{ urea}} \bigg/ \frac{[\text{NH}_4]}{[\text{NH}_4] + [\text{NO}_3] + [\text{urea}]} \quad (1)$$

where ϱ NH$_4$, ϱ NO$_3$ and ϱ urea = absolute uptake rates for each nutrient; [NH$_4$], [NO$_3$] and [urea] = their ambient concentrations.

Particulate nitrogen doubling times were calculated from the combined specific uptake rates (h^{-1}) assuming urea was taken up only in the light (12 h) and that dark ammonium and nitrate uptake were 25 and 10 % of the light rate, respectively (Dugdale & Goering 1967, unpubl. own results).

RESULTS

Physical and chemical characteristics

The stations have been separated into 3 regions depending on their physical and chemical characteristics:

(1) Shallow inshore (Stations 1, 12 and 13). Stations 1 and 12 are located at 2 active upwelling sites off the Cape Peninsula and off Hondeklip Bay (Nelson & Hutchings 1983). These stations were typically well mixed with cold, nitrate-rich water at the surface (Table 1). Station 13, located near the mouth of the Olifants River, is included in this group on the basis of temperature and water depth.

(2) Oceanic offshore (Stations 2, 5 and 6). All these stations were characterised by warm, low nitrate, high salinity surface water (Table 1).

Table 1. Environmental data for the stations sampled showing nitrogenous nutrient concentrations, station depth, surface water temperature and salinity

Station	Ammonium	Substrate Nitrate	Urea	Station depth	Temp	Salinity
		(mg-at N m^{-3})		(m)	(°C)	(%)
Inshore						
1	0.75	20.38	3.13	+ +	10.08	34.81
12	0.40	24.35	0.32	29	10.22	34.88
13	0.06	7.91	0.22	37	11.50	34.90
Shelf						
8	0.11	1.90	0.21	144	16.57	34.98
9	0.22	1.05	0.59	135	15.31	34.94
10	0.11	0.89	0.46	127	16.32	34.92
11	0.24	1.45	0.71	120	13.28	34.92
15	0.92	6.84	0	135	12.70	34.90
Oceanic						
2	0.56	0.92	0.83	1045	20.68	35.23
5	0.44	0.08	0	1050	18.73	35.22
6	0.32	0.02	0.34	1030	18.95	35.29
+ + Not measured						

Table 2. Abundances of the numerically important diatom and dinoflagellate species. Nanoflagellate (< 10 μm) counts include both heterotrophic and autotrophic forms. Bacterial carbon and tintinnid counts are also shown

	Inshore			Shelf					Oceanic		
	1	12	13	8	9	10	11	15	2	5	6
Diatoms ($\times\ 10^6\ m^{-3}$)											
Nitzschia delicatissima	0.85	1.10	15.90	16.40	33.50	3.20	3.89	8.90	11.12	1.59	
N. seriata	0.03			0.10				121.20	0.25	0.11	0.05
Chaetoceros affinis		3.80	4.60				559.40	7.30	0.25		
C. compressus		2.50				6.94	3419.30	17.80		0.23	
C. sp. (small)					21.60						
Thallasiosira sp.	0.03	27.90	3.20				3.80				
Skeletonema costatum	0.72							8.60			
Leptocylindricus danicus			30.20					3.20			
Asterionella sp.	0.06			2.00	0.10			614.30			
Rhizosolenia alata									0.32		
R. setigera									0.25	0.14	
R. delicatula											6.47
Other diatom species		0.80	2.70		0.70	0.50	0.80	1.40	0.41		0.08
Dinoflagellates ($\times\ 10^6\ m^{-3}$)											
Protoperidium spp.		0.20	6.50	0.20	0.10	0.80	6.20				0.99
Gymnodinium spp.									0.09		
G. sp. (colourless)	0.12		1.90			18.90			0.65	0.40	0.90
Ceratium spp.				0.10					0.09	0.11	0.12
Gonyaulax digitalis		3.00									
Prorocentrum mucum					0.10						
Scripsiella sp.			57.00					0.05			
Other dinoflagellate species		0.09	5.10	0.20			2.70	11.20		0.13	
Nanoflagellates ($\times\ 10^8\ m^{-3}$)	2.66	11.72	38.95	3.13	7.71	0.24	31.51	33.20	1.36	1.36	5.46
Tintinnids ($\times\ 10^6\ m^{-3}$)	0.72		63.70	2.02	3.16	1.20	11.30	1.70	0.45		0.26
Bacteria (mg C m^{-3})	9.34	13.29	41.55	52.64	78.80	31.25	61.43	58.82	12.21	17.89	24.72

(3) Continental shelf (Stations 8, 9, 10, 11 and 15). These stations were intermediate in temperature and salinity and located between the 100 and 200 m depth contour.

Phytoplankton and bacterial abundances

Phytoplankton counts and bacterial carbon are shown in Table 2. Both diatom numbers and bacterial carbon were higher in coastal than in offshore waters. Similarly, the highest nanoflagellate counts (including both autotrophic and heterotrophic forms) were recorded in the inshore and shelf waters. The < 10 μm size class was made up largely of these nanoflagellates.

Particulate matter

A summary of the various biomass parameters is given in Table 3. The oceanic stations were characteristically low in chlorophyll a (chll a) and particulate organic matter (POM). Highest chll a and POM values were recorded over the continental shelf. At all but Station 11 more than 50 % of the POM was < 10 μm. A significant fraction (11 to 100 %) of the chll a was also recovered in this nanoplankton size class. At inshore Station 1 and oceanic Station 5 some 40 to 50 % of the chll a was < 1 μm. There was a good correlation between particulate nitrogen and chll a concentrations for the different size classes (Fig. 2). The slope of the regression for the < 212 μm size class (b = 1.16) being lower than the < 10 μm (b = 1.64) and < 1 μm (b = 1.73) size classes indicates the intact community is chll a enriched compared to the smaller size fractions.

Nitrogen assimilation rates

Ammonium concentrations were monitored routinely before and after each uptake experiment. Initial concentrations for a particular sample were generally higher for the < 212 μm size class than either the < 10 μm or < 1 μm size class suggesting stimulation of microzooplankton excretion after passing through the 212 μm screen and confinement in the incubation bottle. Ammonium concentration changes during the incubations indicate that the surface water picoplankton are net mineralisers while the intact community (< 212 μm) was generally ammonium consuming (Fig. 3).

Table 3. Concentrations of chlorophyll a (chll a), particulate organic nitrogen and carbon (PON and POC), and carbon/nitrogen ratios (C/N) for the 3 size fractions

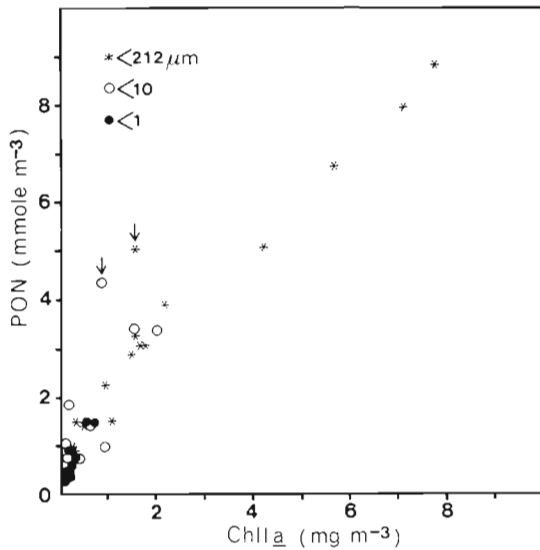| | Chll a | | | PON | | | POC | | | C/N | | |
| | $< 212\,\mu m$ | $< 10\,\mu m$ | $< 1\,\mu m$ | $< 212\,\mu m$ | $< 10\,\mu m$ | $< 1\,\mu m$ | $< 212\,\mu m$ | $< 10\,\mu m$ | $< 1\,\mu m$ | $<212\,\mu m$ | $< 10\,\mu m$ | $< 1\,\mu m$ |
	(mg m^{-3})	(%)		(mmole N m^{-3})	(%)		(mmole C m^{-3})	(%)				
Inshore												
1	0.45	89	49	1.45	53	24	12.25	53	23	7.00	7.09	6.80
12	1.47	11	8	2.93	63	17	19.75	69	25	5.78	6.31	8.36
13	1.50	99	21	3.36	98	25	22.5	90	24	5.28	5.75	5.65
Shelf												
8	2.14	88	27	4.36	90	34	27.75	88	29	5.34	5.45	4.57
9	5.56	–	–	6.79	–	–	57.50	–	–	7.26	–	–
10	1.45	–	–	5.14	–	–	42.83	–	–	7.13	–	–
11	7.88	26	9	8.93	38	17	62.08	37	18	5.96	5.87	6.52
15	7.08	13	2	8.00	59	12	51.08	56	11	5.47	5.24	5.07
Oceanic												
2	0.57	13	5	0.93	54	30	6.75	61	41	6.23	7.14	8.38
5	0.18	70	42	0.96	78	41	6.67	89	48	5.93	6.81	7.00
6	0.33	30	8	1.42	75	33	11.08	69	26	5.65	5.13	5.23

Fig. 2. Relationship between particulate organic nitrogen (PON) and chlorophyll a for the 3 size fractions. Regression equations were: $< 1\,\mu m$, PON = 0.301 + 1.734 chll a (r = 0.90); $< 10\,\mu m$, PON = 0.651 + 1.643 chll a (r = 0.93); $< 212\,\mu m$, PON = 0.995 + 1.164 chll a (r = 0.99). Points marked with arrows were excluded from regression analyses

Fig. 3. Measured changes in ammonium concentrations (ΔNH_4) over the time course of incubations (normalized to 4 h) for the 3 size classes

Fig. 4. Relative preference indices for ammonium, nitrate and urea (RPI's) as a function of the total nitrogenous nutrient concentration (ΣN). Nitrate RPI's are also plotted against ammonium concentration

A relative preference index (RPI) for each nutrient was calculated from the uptake rates for the 3 size fractions (Fig. 4). No relationship between size class and RPI was apparent in these data. The RPI's for ammonium were, with one exception, always in excess of unity, indicating a marked preference for this nutrient for all size fractions. Nitrate on the other hand was discriminated against at most stations, as indicated by the RPI's of less than unity. The RPI for urea was variable and as with ammonium tended to be positively related to the total nitrogenous nutrient
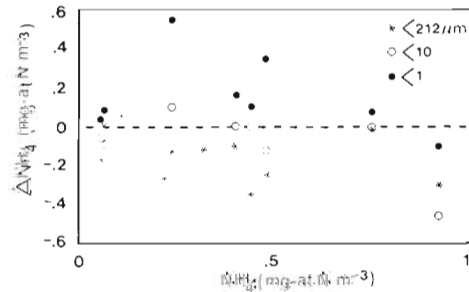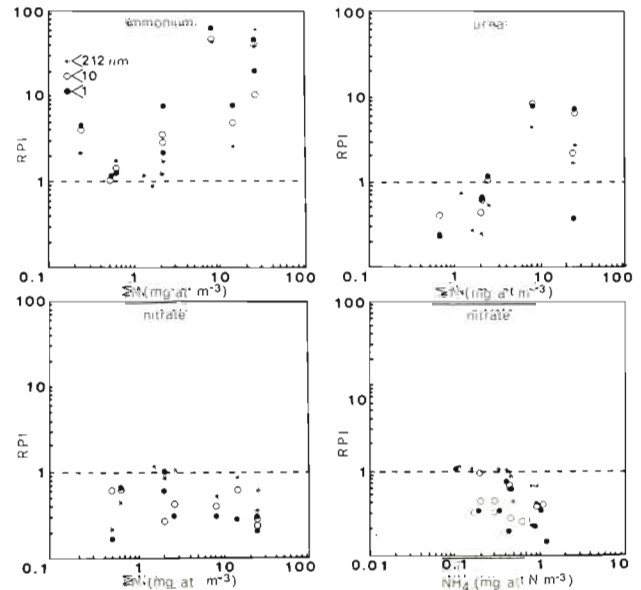
concentration (Fig. 4). Nitrate RPI's, though independent of total dissolved nitrogen concentration, tended towards unity and above with diminishing ammonium concentrations (Fig. 4).

Ammonium accounted for most of the nitrogen assimilated for all 3 size fractions at the oceanic stations (Fig. 5). The proportion of ammonium assimilated
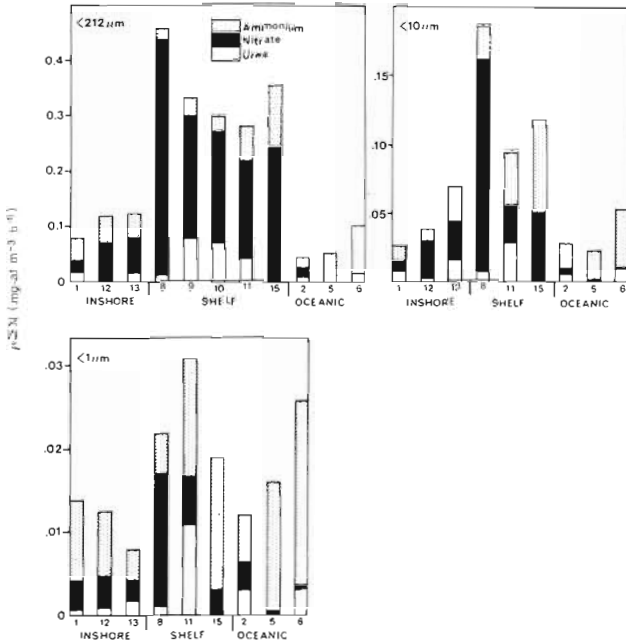

Fig. 5. Uptake rates for ammonium, nitrate and urea (ϱΣN) for the 3 size classes in inshore, shelf and oceanic regions

increased when the larger size fractions were screened out at the shelf and inshore stations. The result being that, in all stations except Station 8, ammonium supplied the most nitrogen for growth of the picoplankton. Urea assimilation was variable but important at some stations especially for the picoplankton and nanoplankton. The proportion of total nitrogen assimilated as nitrate was directly related to increasing size for each of the shelf and inshore stations (Fig. 5).

Table 4 shows that nitrate formed the major portion of the nitrogen taken up by the intact community at the shelf and inshore stations. Ammonium and urea were

Table 4. Mean proportion of the total nitrogen assimilated as either ammonium, nitrate or urea for the intact phytoplankton community (< 212 µm)

Substrate		Region	
	Inshore	Shelf (%)	Oceanic
Nitrate	48.0	71.2	13.3
Ammonium	38.5	15.1	73.7
Urea	13.5	13.7	13.1

quantitatively the most important for phytoplankton nutrition at the oceanic stations. The picoplankton and nanoplankton size classes generally had longer particulate nitrogen doubling times than the whole community (Fig. 6). Mean doubling times for the intact
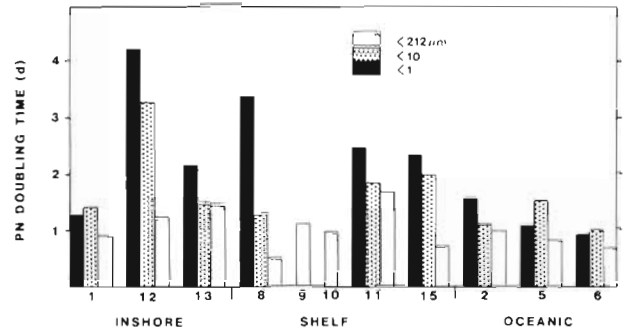

Fig. 6. Particulate nitrogen doubling times for the 3 size classes in inshore, shelf and oceanic regions

community were similar for the oceanic, shelf and inshore regions being 0.83 d, 1.00 d and 1.19 d, respectively. Mean doubling times for the < 1 µm size class were shorter in the oceanic environment (1.19 d) compared to the shelf (2.76 d) and inshore (2.75 d) waters. This trend was less obvious for the < 10 µm fraction.

As one would expect from the doubling times, the picoplankton were relatively more active in nitrogen assimilation at the oceanic stations than coastal stations (Table 5). The contribution of the 1 to 10 µm size

Table 5. Mean proportion of the total nitrogen assimilated by the picoplankton (< 1 µm), nanoplankton (< 10 µm), and the netplankton (10 to 212 µm) in inshore, shelf and oceanic regions

Size class			Region	
		Inshore	Shelf (%)	Oceanic
Picoplankton	(< 1 µm)	11.1	7.1	26.9
Nanoplankton	(1–10 µm)	29.6	28.8	24.5
	(< 10 µm)	40.7	35.8	51.4
Netplankton	(10–212 µm)	59.3	64.5	48.6

class (i.e. picoplankton exclusive) to total nitrogen assimilation was similar for the 3 regions.

The effect of substrate concentration on uptake rate was determined at three oceanic stations. The half-saturation constants (k_m) and maximum uptake velocities (V_{max}) are shown in Table 6. The results indicate that ammonium is the most efficiently assimilated nitrogen resource at both low and high ambient concentrations.

Table 6. Kinetic parameters for ammonium, nitrate and urea uptake for the intact phytoplankton community at 3 oceanic stations. Michaelis-Menten parameters, K_m (half-saturation constant) and V_{max} (maximal uptake velocity) were estimated from Hanes linear transformation

Station	Substrate	Correlation coefficient	K_m (mg-at N m^{-3})	V_{max} (h^{-1})	V_{max}/K_m
5	Ammonium	0.99	0.10	0.0098	0.0980
7	Urea	0.99	0.17	0.0041	0.0241
6	Nitrate	0.98	0.93	0.0082	0.0088

DISCUSSION

Biomass

The proportion of chll a recovered in the surface water picoplankton fraction (2 to 50 % of total chll a) is lower than the 25 to 90 % depth integrated estimate of Li et al. (1983) for the eastern tropical Pacific Ocean. The higher picoplankton chll a estimates in the latter study probably indicates the greater importance of picoplankton as primary producers in tropical than temperate regions. In tropical waters picoplankton can account for 20 to 80 % of the total inorganic carbon fixation (Li et al. 1983, Platt et al. 1983) while in the Celtic Sea they contribute 20 to 30 % of the total primary production (Joint & Pomroy 1983). Li et al. (1983) found that the picoplankton contribute a greater proportion to photosynthesis near the bottom of the euphotic zone in tropical waters than near the surface; this was not the case in the Celtic Sea study (Joint & Pomroy 1983).

The large amount of chll a in the < 10 μm and < 1 μm size classes at Station 1 indicate that newly upwelled water can be seeded by very small photoautotrophic cells, presumably mainly flagellates. Olivieri (1983) has shown that the mean cell size of 2 of the dominant diatom species increases as upwelled water matures in this upwelling area. This situation contrasts with Hondeklip Bay (Station 12) where newly upwelled water was dominated by larger diatom species, notably Thallassiosira sp.

Total nitrogen assimilation

One trend that did emerge from the uptake data and was not evident in the biomass data, was that the picoplankton and nanoplankton (inclusive of the picoplankton) were relatively more active nitrogen assimilators at the oceanic stations than over the shelf or inshore (Table 5). This finding is in line with the generalizations of Malone (1980) regarding the importance of netplankton and nanoplankton photosynthesis in such areas. The relatively long doubling times for the < 1 μm and < 10 μm size classes compared to the whole community at all stations is in agreement with the results of Furnas (1983) for particulate nitrogen doubling times in the < 10 μm fraction. Direct measurements of mean microflagellate and non-motile ultraplankton biomass changes in diffusion chambers confirm that they turn over at a slower rate than the community as a whole (Furnas 1982). These findings appear to be at variance with the general trend of an increase in half-saturation constant for nitrogen uptake with an increase in cell size (Eppley et al. 1969). However, one might only expect such a relationship between cell size and nitrogen uptake in a low nutrient environmental, with concentrations at or below the K_m values. Under nutrient saturated conditions large cells might grow more rapidly than small cells assuming they have a faster maximum growth rate (Parsons & Takahashi 1973). On a less speculative note, it does appear from the regressions in Fig. 2 that the intact community (< 212 μm) is chll a enriched compared to the < 10 μm and < 1 μm fractions. In demonstrating the importance of netplankton nitrogen assimilation, these findings might explain the shorter doubling times of the whole community.

Assimilation of individual nitrogen sources

The combined data for the relative preference indices (RPI's) for the 3 nutrients (Fig. 4) is in good agreement with previous studies (McCarthy et al. 1977, Glibert et al. 1982a) with ammonium generally the preferred substrate and nitrate usually rejected. Nitrate may be preferred under conditions of low (< 1 μM) ammonium concentration (Furnas 1983). A negative relationship between ammonium concentration and nitrate RPI was evident in the present study though nitrate was not taken up preferentially even at ammonium concentrations approaching 0.1 μM. (Fig. 4). Michaelis-Menten kinetics calculated for oceanic waters reveal that the efficiency of nitrogen uptake, measured as V_{max}/K_m (Healey 1980) follows the pattern of RPI's: ammonium > urea > nitrate.

Relative preference indices provide a useful phy-

siological measure but are not necessarily indicative of the routing of nitrogen into a cell on a quantitative basis. Ammonium and urea were generally quantitatively the more important nitrogen resource for the nutrition of the picoplankton and nanoplankton. Glibert et al. (1982a) similarly demonstrated that ammonium uptake was almost exclusively limited to the < 10 μm size class. Over the shelf and inshore areas nitrate uptake by netplankton dominated, with *Nitzschia* and *Chaetoceros* the important genera. Since these were the productive regions, the overall result was that nitrogen productivity for the intact community increased with the proportion of nitrate assimilated (Fig. 7). A similar relationship has been demonstrated

Fig. 7. Proportion of nitrate uptake ('new' production) as a function of total nitrogen uptake ($\rho\Sigma N$) for the 3 size classes. Curve fitted by eye for the < 212 μm fraction

for the depth-integrated proportion of nitrate assimilation versus primary production (Eppley et al. 1979). This relationship quantifies the importance of nitrate supply (advection or diffusion) to the primary production of an area not influenced by terrestrial runoff or benthic input of ammonium. The high rate of primary production characteristic of upwelling regions is apparently achieved only when the nitrate ration ('new' production) is in excess of 50 % of the total nitrogen assimilated (Eppley et al. 1979, Yoder et al. 1983). This trend is progressively dampened with the exclusion of the netplankton and nanoplankton through differential screening (Fig. 7) and again illustrates the importance of the reduced nitrogen sources, ammonium and urea, to the nutrition of the picoplankton and the nanoplankton.

In a steady-state environment the advective and diffusive flux of nitrate should balance the loss or sinking

rate i.e. 'new'/total production provides an index of the input to the aphotic water column and sediments (Eppley & Peterson 1979, Eppley 1981). Harrison et al. (1983) have demonstrated a good agreement between sedimentation rates calculated in this manner and from sediment trap measurements in the Middle Atlantic Bight shelf. The mean ratio of 'new'/total production of 71 % measured at the shelf in this study is in close agreement with the depth-integrated value of 67 % for the Middle Atlantic Bight (Harrison et al. 1983). The southern Benguela shelf region can be treated as a steady-state flow system, with an inshore flow of nutrient rich water into the euphotic layer due to upwelling and a flow of nutrient impoverished water out of the layer due to sinking down the thermocline and northward drift (Andrews & Hutchings 1980). The situation is, however, complicated by the intermittent nature of upwelling and patchy plankton distribution especially in the Cape Peninsula system (Hutchings 1981, Shannon et al. 1983).

The open ocean is probably reasonably close to a steady-state over suitably long temporal and spatial scales (Goldman et al. 1979). Thus the small ratio of 'new'/total production at the oceanic stations indicates little loss of primary production from the surface waters (ca 13 %) with most production being supported by regenerated nitrogen. This estimate of the flux of primary production from the euphotic zone is in good agreement with the values reported by Eppley & Peterson (1979) for other offshore oceanic regions. Eppley (1981) suggests that the ratio of 'regenerated'/'new' production provides a measure fo the number of times nitrogen cycles before being exported from the upper water column. Experimental values for the southern Benguela system of < 1 for coastal waters and >> 1 for oceanic stations (Fig. 8) agree well with the generalizations of Eppley (1981).

The above discussion regarding the importance of 'new' production to the shelf and oceanic surface waters is biased because of the inherent error of not taking isotope dilution into account in measurements
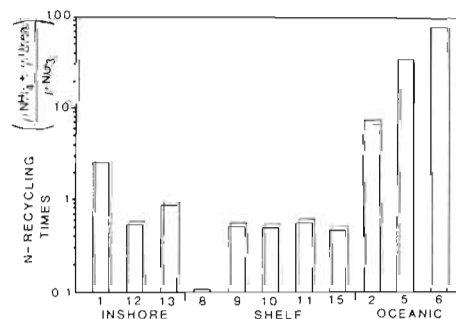

Fig. 8. Nitrogen recycling times in surface waters for inshore, shelf and oceanic regions; calculated from the ratio of 'regenerated' to 'new' production

of ammonium uptake. Glibert et al. (1982b) have shown that under a variety of field conditions ammonium uptake is typically underestimated by a factor of ca 2 when a constant ^{15}N enrichment is assumed. Using the same correction factor with the present data reduces 'new' production for the intact community to 65 % over the shelf and 11 % in oceanic waters. Accounting for ammonium isotope dilution in the < 1 μm and < 10 μm size fractions would strengthen the claim for a dominant role of 'regenerated' nitrogen in the nutrition of picoplankton and nanoplankton.

It is most likely that urea is supplied to the euphotic zone through bacterial degradation and animal excretion (McCarthy 1980). The relative contribution of urea to nitrogen assimilation was characteristically highly variable (range 1 to 35 %) and was somewhat lower than found in other studies (McCarthy 1972, Kristiansen 1983). In a coastal lagoon off Long Island, urea on average provided 50 % of the nitrogen utilized, possibly a result of assimilation by the large numbers of small (1 to 4 μm) chlorophyceae and cyanophyeae in this area (Kaufman et al. 1983). There was no clear correlation between ϱ urea/total nitrogen assimilation and < 10 μm flagellate or bacterial counts in the present study.

In conclusion, this study suggests that in the inshore and shelf waters influenced by upwelling, the picoplankton do not play as significant a role in nitrogen uptake as in the oceanic waters. The importance of this size class to nitrogen cycling in the surface waters both inshore and offshore is probably in ammonium regeneration (Harrison 1978, Glibert 1982, Harrison et al. 1983). In the oceanic environment the reduced nitrogen sources of ammonium and urea are quantitatively the most important for the nutrition of all size classes within the phytoplankton community.

This study supports the hypothesis that netplankton productivity is largely nitrate controlled, whereas nanoplankton productivity is regulated by regenerated nitrogen (Malone 1980, Glibert et al. 1982a). This generalization can be extended to include the picoplankton along with the nanoplankton. It should be remembered that the present study gives no indication of possible seasonal or shorter time-scale trends in the pattern of nitrogen assimilation which, in a pulsed system such as the southern Benguela, are likely to be appreciable. Expanding on these findings one would predict that moderate shifts in the strength and direction of the prevailing wind field over the upwelling season would result in measurable changes in the size structure of the phytoplankton community. The proportion of nanoplankton and picoplankton is likely to increase with a reduction in upwelling intensity and hence nitrate supply. Results of an upwelling monitoring cruise off the Cape Peninsula during March 1983,

an anomalous warm-event year, indicated a predominance of small flagellates over diatoms (Hutchings et al. 1984) providing indirect support for this hypothesis. The smaller mean size of the primary producers under these conditions might necessitate additional intermediaries in the transfer of carbon and nitrogen from phytoplankton to zooplankton, with possible detrimental affects on the overall efficiency of the food chain leading to pelagic fish.

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