

Nitrogen uptake by size-fractionated plankton in permanently well-mixed temperate coastal waters

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Nitrogen uptake by net- (15–200 μm), nano- (1–15 μm) and picoplankton (<1 μm) was measured over seasonal cycles at two stations with different patterns of biological and chemical cycles in the Morlaix Bay (western English Channel). Though assimilable dissolved N nutrient pool at both stations was nitrate-dominated, characteristics of biomass and N uptake by netplankton differed from conventional patterns in two respects. In the first, biomass (26–30%) and N uptake (36–43%) were less important than those of nanoplankton. In the second, the netplankton did not show any marked preference for nitrate over ammonium (nitrate to ammonium uptake ratios of 0.98 and 1.08). In contrast, nanoplankton had a preference for ammonium over nitrate (ammonium to nitrate uptake ratios of 2 and 1.2). N uptake by picoplankton was only 8% of total N uptake at both stations and was supported mainly by regenerated N (66% ammonium and 17% urea), with nitrate uptake detectable in only one instance and nitrite uptake in none. Substrate-dependent uptake of ammonium in all fractions and a higher ammonium uptake in the nanoplankton fraction in summer at both stations when ambient ammonium concentrations were high indicated that while nitrate may satisfy a part of N requirements, availability of ammonium and its flux through nanoplankton determine the magnitude of total N uptake in these waters. Most of the N uptake in picoplankton appears to be autotrophic, suggesting that a substantial part of heterotrophic uptake, if any, could be localized in the fractions >1 μm , and mediated by free-living and particle-bound bacteria.

INTRODUCTION

The composition and size structure of the autotrophic assemblages are major determinants of the quality (new or regenerated) and quantity of primary production in a given ecosystem [see (Wilkerson *et al.*, 2000)]. The size distribution, in turn, depends on nutrient availability and preferences (Stolte *et al.*, 1994). As far as two decades back, Malone (Malone, 1980) showed that eutrophic areas tend to support relatively large phytoplankton standing stocks that are dominated by addition of large size classes, of the size range 20–200 μm . Conversely, oligotrophic areas tend to have large biomass of smaller size classes, nano- (2–20 μm) and picoplankton (<2 μm). Since then, it has quite often been shown from

laboratory studies and in most natural populations that smaller size fractions have a higher ‘preference’ for ammonium over nitrate than the large fraction (Dortch, 1990). The commonly accepted trend, in the context of nitrogenous nutrients, is that the larger cells become dominant in high nitrate waters whereas smaller cells tend to become important in low nitrogen waters (Chisholm, 1992). The spatial differences also find expression in temporal scales: N uptake patterns could vary substantially between seasons, depending on whether new or regenerated N is dominant in the ambient waters (Riegman *et al.*, 1998; Riegman and Noordeloos, 1998).

The permanently well-mixed waters of the English Channel are characterized by different patterns of

seasonal changes of biological and chemical properties as a function of water column depth (Wafar *et al.*, 1983; Birrien *et al.*, 1991; L'Helguen *et al.*, 1996; Maguer *et al.*, 1996). Besides, in spite of being nitrate-dominated, ammonium uptake in these waters on annual and seasonal scales tends to be as important as nitrate uptake (L'Helguen *et al.*, 1996; Maguer *et al.*, 1996). These led us to speculate that N uptake by different size groups in these waters could have patterns different from those known from elsewhere (Dortch, 1990; Chisholm, 1992). The objective of this paper is to demonstrate, by measuring N uptake by three size fractions over an annual cycle at two stations with different hydrographic properties in the Morlaix Bay, that it is indeed the case. Unlike in most studies with fractionated populations, however, we measured uptake of all four nutrients (nitrate, ammonium, urea and nitrite) that have been commonly accepted as N sources for phytoplankton.

METHOD

Measurements of N uptake by size-fractionated plankton populations were made during 22 field trips to Astan (Figure 1) between January and December 1988 and 15 field trips to Duons (Figure 1) between March 1992 and March 1993. Samples were obtained at monthly intervals during autumn and winter and at shorter intervals during spring and summer. Astan is a deep-water station ($Z \sim 50$ m) with the water column depth greater than euphotic zone depth at any time of the year. Duons is a shallow water station ($Z \sim 20$ m) where the entire water column comes within the euphotic zone during spring–summer. The biological and chemical cycles tend to their seasonal maxima/minima during summer

at Astan and spring at Duons (L'Helguen *et al.*, 1996; Maguer *et al.*, 1996).

In each of the collections, typical hydrographic (temperature), chemical (nitrate, ammonium, nitrite and urea) and biological (Chl *a* and particulate organic nitrogen) parameters were measured. Temperature was recorded with Richter and Weiss reversing thermometers. Nitrate, nitrite and urea were measured in a Technicon Auto-Analyzer II following the methods given by Tréguer and Le Corre (Tréguer and Le Corre, 1975) and Aminot and Kérouel (Aminot and Kérouel, 1982). Ammonium concentrations were measured manually by the Indophenol blue method (Koroleff, 1970). Analytical precisions for the measurements of nitrate, nitrite, urea and ammonium were respectively ± 0.1 , ± 0.01 , ± 0.01 and $\pm 0.05 \mu\text{mol N L}^{-1}$. Chl *a* was measured fluorometrically in a Turner Designs fluorometer with a precision of $\pm 0.05 \mu\text{g}$. Organic N concentrations of the particulate matter retained on pre-ignited (400°C , 4 h) Whatman GF/F filter pads were measured in a Perkin–Elmer model 240 Elemental analyzer with a precision of $\pm 0.1 \mu\text{mol N}$, with bipyridine as a standard.

Samples for size-fractionated N uptake were obtained from 50% light depth and serially filtered through 200 and 15 μm mesh bolting silk and 1 μm Nucleopore filter to obtain fractions $<200 \mu\text{m}$, $<15 \mu\text{m}$ and $<1 \mu\text{m}$. Inoculations with ^{15}N ($\text{Na}^{15}\text{NO}_3$, $\text{Na}^{15}\text{NO}_2$, $^{15}\text{NH}_4\text{Cl}$ and $\text{CO}^{15}\text{NH}_2$) at 95–97.5 atom % excess) were made at $\sim 10\%$ of the ambient concentrations of the N nutrients. Samples added with ^{15}N were incubated in 2.5 L polycarbonate bottles on deck, with the 50% irradiance simulated with calibrated nickel screens. The incubations were usually done for 2–3 h before the local noon. Since the results at Astan showed that picoplankton

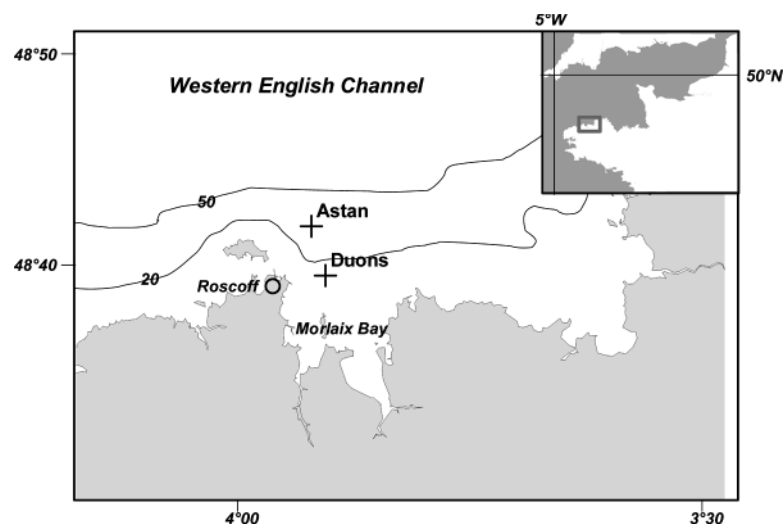


Fig. 1. Location of the study sites in the Morlaix Bay.

took up little of new N and only a small amount of urea (see below), only ammonium uptake by this fraction was measured at Duons.

The $^{15}\text{N}:^{14}\text{N}$ isotope ratio of particulate matter was determined by emission spectrometry in a GS1 Optical spectrometer (SOPRA, France). Absolute uptake rates (in $\text{nmol N L}^{-1} \text{ h}^{-1}$) of nitrate, nitrite and urea were calculated with the equation of Dugdale and Wilkerson (Dugdale and Wilkerson, 1986) where PON concentration at the beginning of the incubation is used. Ammonium uptake rates were calculated using either the equation of Glibert *et al.* (Glibert *et al.*, 1982a) or of Laws (Laws, 1984) depending upon whether there were measurable changes in the concentrations of ammonium during the incubations or not.

Consistent with our earlier paper (Le Corre *et al.*, 1996) we use the term 'netplankton' for the 15–200 μm fraction, 'nanoplankton' for the 1–15 μm fraction and 'picoplankton' for the $<1 \mu\text{m}$ fraction. Uptake rates, Chl *a* and PON were calculated by difference for the first two fractions

and directly for the last. Model II regressions were used in all statistical analyses. Ratios between variables (e.g. $\text{PON:Chl } a$) were computed from the slopes of the regressions.

RESULTS

N nutrients, Chl *a* and PON

Nitrate was the major N nutrient available at both stations (Figure 2) with winter concentrations ($>10 \mu\text{mol N L}^{-1}$) 20 or more times greater than those of the other three N nutrients. Only in spring (Duons) or summer (Astan) did nitrate concentrations decrease to $<1 \mu\text{mol N L}^{-1}$ and were similar to the concentrations of other N nutrients.

Seasonal changes of Chl *a* in all the three fractions at Astan were characterized by broad summer maxima (Figure 3). The nanoplankton fraction accounted for $\sim 59\%$ of the total Chl *a*, followed by net- (29%) and

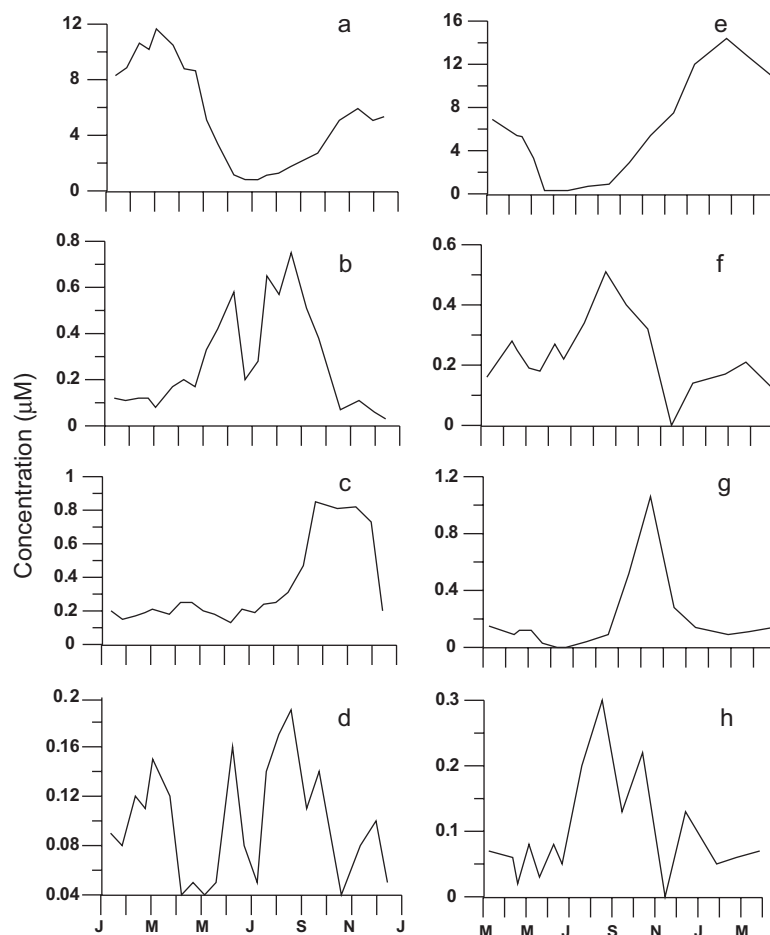


Fig. 2. Seasonal changes of the concentrations of nitrate, ammonium, nitrite and urea at Astan (a–d) and at Duons (e–h).

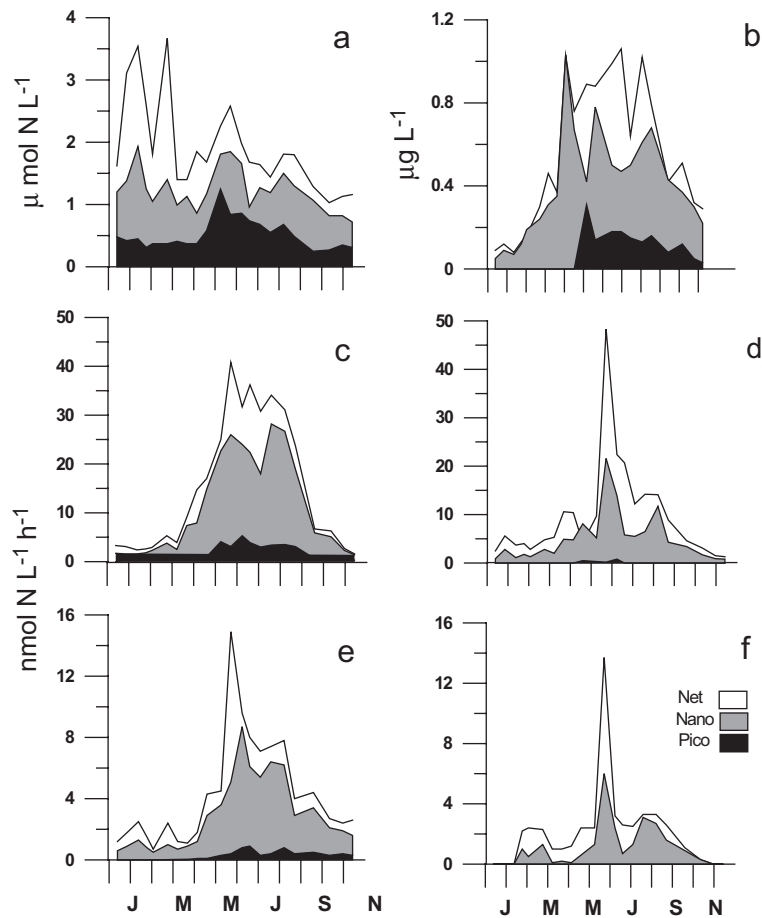


Fig. 3. Seasonal changes of (a) particulate organic nitrogen and (b) Chl *a* concentrations of net-, nano- and picoplankton and uptake rates of (c) ammonium, (d) nitrate, (e) urea and (f) nitrite in the three fractions at Astan.

pico- (12%) fractions (Table I). In the latter group, the Chl *a* concentrations rose above detection limits only from late spring onwards. The expected similarity in changes of PON with Chl *a* can be seen in the summer maximum tending to a decrease subsequently (Figure 3). The high winter–spring PON concentrations are probably related to transport with river waters (Wafar *et al.*, 1983). When these data were excluded, relationships between PON and Chl *a* were significant in the case of nano- and picoplankton fractions ($r = 0.82$ and 0.87 , $n = 12$ and 11 , $P < 0.01$), with PON:Chl *a* ratios respectively of 0.82 and $3.45 \mu\text{mol N} (\mu\text{g Chl } a)^{-1}$.

Seasonal changes of Chl *a* at Duons are notable for the spring peak (Figure 4) and the decreased importance of nanoplankton biomass (48%) (Table II). Surprisingly, this decrease was compensated by an increase in picoplankton biomass (22%) rather than in netplankton (30%). Unlike at Astan, Chl *a* was present in measurable concentrations in the picoplankton fraction throughout the year (Figure 4). The relationships between changes

of PON and Chl *a* were significant only in the case of nano- and picoplankton ($r = 0.64$ and 0.84 , $n = 13$ and 11 , $P < 0.01$), with PON:Chl *a* ratios [0.97 and $2.62 \mu\text{mol N} (\mu\text{g Chl } a)^{-1}$] more or less of the same order as at Astan.

N uptake

Astan

As can be expected of well-mixed temperate waters (Wafar *et al.*, 1983), N uptake rates by the three size fractions varied through an order of magnitude or more between winter and summer (Figure 3). The pattern of utilization of regenerated and new N forms, however, differed, within the seasonal cycle: uptake of ammonium and urea tended to a broad summer maximum whereas those of nitrate and nitrite were characterized by single summer peaks.

Nanoplankton were responsible for a major proportion of N uptake (Table I), with their importance

Table I: Seasonal and annual averages* of nitrogen uptake rates ($\text{nmol N L}^{-1} \text{h}^{-1}$) and Chl *a* ($\mu\text{g L}^{-1}$) of fractionated phytoplankton at Astan

	Fraction	ρNO_3	ρNH_4	ρUrea	ρNO_2	Total N uptake	Chl <i>a</i>
Winter (Dec–Feb)	M	2.11	1.13	0.83	0.68	4.75	0.025
	N	1.78	1.86	0.85	0.47	4.96	0.127
	P	0	0.27	0	0	0.27	0
Spring (Mar–May)	M	8.13	4.86	2.23	2.23	17.45	0.166
	N	6.43	11.83	2.25	1.38	21.89	0.518
	P	0.33	1.71	0.225	0	2.265	0.225
Summer (Jun–Aug)	M	7.30	8.20	1.40	0.95	17.85	0.348
	N	8.00	19.30	5.35	2.00	34.65	0.392
	P	3.70	0.80	0.60	0	5.1	0.160
Autumn (Sep–Nov)	M	0.92	0.66	0.77	0.20	2.55	0.076
	N	1.70	3.20	1.90	0.60	7.4	0.260
	P	0	0.48	0.38	0	0.86	0.700
Annual	M	4.96	4.00	1.35	1.46	11.77	0.164
	N	4.72	9.59	2.65	1.42	18.38	0.327
	P	0.45	1.71	0.43	0	2.59	0.07

M, netplankton; N, nanoplankton; P, picoplankton. *Uptake rates and concentrations temporally integrated for the season or year and expressed per h.

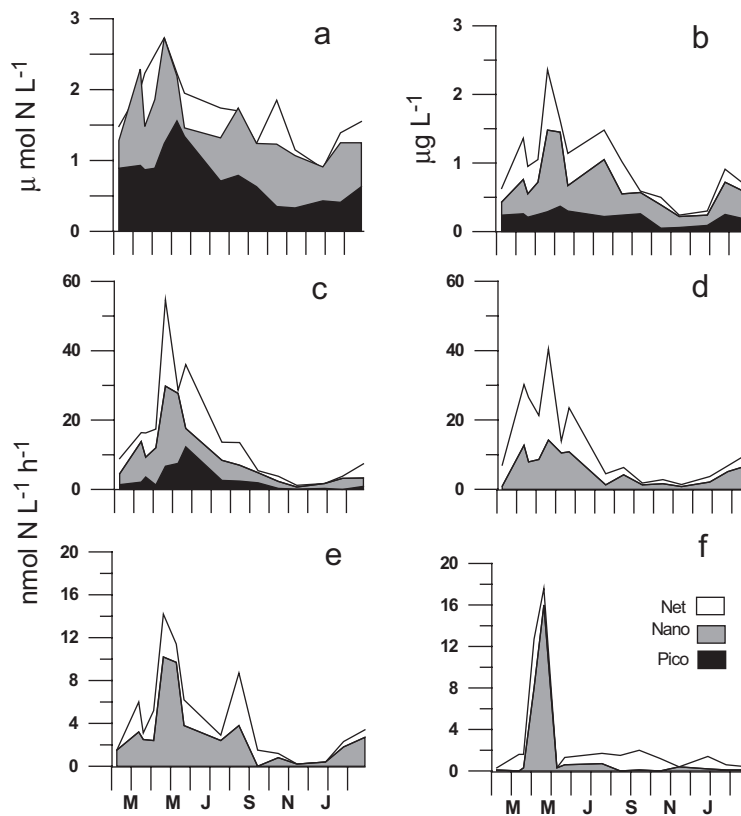


Fig. 4. Seasonal changes of (a) particulate organic nitrogen and (b) Chl *a* concentrations of net-, nano- and picoplankton and uptake rates of (c) ammonium, (d) nitrate, (e) urea and (f) nitrite in the three fractions at Duons.

Table II: Seasonal and annual averages* of nitrogen uptake rates (nmol N L⁻¹ h⁻¹) and Chl *a* (µg L⁻¹) of fractionated phytoplankton at Duons

	Fraction	ρNO ₃	ρNH ₄	ρUrea	ρNO ₂	Total N uptake	Chl <i>a</i>
Winter	M	3.22	2.28	0.30	0.55	6.35	0.14
	N	3.78	2.52	1.60	0.13	8.03	0.30
	P	–	0.65	–	–	0.65	0.19
Spring	M	12.60	8.46	1.88	1.30	24.24	0.39
	N	9.080	10.28	4.50	3.18	27.04	0.57
	P	–	4.48	–	–	4.48	0.26
Summer	M	4.28	6.20	1.90	0.64	13.02	0.31
	N	5.36	7.18	3.90	0.32	16.76	0.52
	P	–	4.96	–	–	4.96	0.23
Autumn	M	1.10	2.28	1.70	1.15	6.23	0.16
	N	2.00	2.53	1.20	0.13	5.86	0.28
	P	–	1.18	–	–	1.18	0.15
Annual	M	6.98	5.16	1.42	1.11	14.67	0.28
	N	5.56	6.41	2.84	1.68	16.49	0.44
	P	–	2.71	–	–	2.71	0.20

M, netplankton; N, nanoplankton; P, picoplankton. *Uptake rates and concentrations temporally integrated for the season or year and expressed per h.

remaining more or less the same throughout the year (50–70%). Most of the N assimilated by nanoplankton was in the form of ammonium (annual average: 9.6 nmol N L⁻¹ h⁻¹), even though ammonium formed only ~10% (range <1 to 30%) of the ambient assimilable N. The particularly strong preference for ammonium is also supported by the annual average ammonium uptake rate that was twice as high as that of nitrate, a substrate-dependent pattern of uptake ($r = 0.82$, $n = 22$, $P < 0.01$), and an average ammonium assimilation ratio [34 nmol N (µg Chl *a*)⁻¹ h⁻¹; $r = 0.63$, $n = 20$, $P < 0.01$] that was ~5–6 times higher than those of the other three nutrients [4.8–6.5 nmol N (µg Chl *a*)⁻¹ h⁻¹]. The strong autotrophic component of N uptake in this fraction can also be seen in the relationships of uptake rates of other three forms of N with Chl *a* that were statistically significant ($r = 0.42$ – 0.59 ; $n = 20$, $P < 0.05$). However, the intercepts of these regressions were not statistically different from zero, except in the case of nitrate (1.54 ± 1.3 nmol N L⁻¹ h⁻¹) suggesting that up to 30% of the nitrate taken up in the nanoplankton fraction could involve heterotrophic organisms.

Netplankton were responsible for an average of 36% of annual N uptake with relatively higher proportions in winter–spring (42–48%) than in summer–autumn (23–31%) (Table I). On an annual scale, the netplankton had no marked preference for nitrate or ammonium [uptake averages of 42 and 34% and assimilation ratios of 8.2 and 13.7 nmol N (µg Chl *a*)⁻¹ h⁻¹ respectively].

On a seasonal scale, however, they took up twice as much nitrate in winter and spring than ammonium. The increased assimilation of ammonium in summer–autumn could be a result of increased availability of ammonium in ambient N (from <1% in winter to >30% in summer) but could still be substrate-regulated ($r = 0.73$, $n = 21$, $P < 0.01$). Uptake of all the four nutrients correlated well with Chl *a* concentrations ($r = 0.5$ – 0.78 , $n = 17$, $P < 0.05$). However, the assimilation ratios [1.3–13.7 nmol N (µg Chl *a*)⁻¹ h⁻¹], except in the case of nitrate [8.2 nmol N (µg Chl *a*)⁻¹ h⁻¹], were much lower than those calculated for nanoplankton. Intercepts of the regressions in the case of nitrate (2.6 ± 1.5 nmol N L⁻¹ h⁻¹), nitrite (0.5 ± 0.3 nmol N L⁻¹ h⁻¹) and urea (0.7 ± 0.2 nmol N L⁻¹ h⁻¹) were significantly different from zero, suggesting an important component of heterotrophy in this fraction.

Uptake by picoplankton constituted only 8% of the annual N uptake, with >82% of it supported by regenerated N (ammonium 66% and urea 17%) (Table I). The assimilation ratios for ammonium and urea uptake rates were 17.5 and 2.8 nmol N (µg Chl *a*)⁻¹ h⁻¹ respectively. All of the urea uptake and ~70% of the ammonium uptake occurred when Chl *a* was detectable in this fraction. This, along with the significant correlations between ammonium and urea uptake rates and Chl *a* ($r = 0.92$ and 0.67 , $n = 20$ and 21 , $P < 0.01$) suggests a strong autotrophic component in this fraction. No measurable uptake of nitrite or nitrate (except in summer,

not exceeding $4 \text{ nmol N L}^{-1} \text{ h}^{-1}$) could be demonstrated in this fraction (Table I).

Duons

The pattern of a spring maximum of production described earlier at this station (Maguer *et al.*, 1996) could be seen in the N nutrition of all the three size fractions (Figure 4). Besides this, the characteristics of N utilization by the fractions differed in several respects from those at Astan.

Unlike at Astan, the nanoplankton were less important in total N uptake, with an average contribution of <50% that did not vary much seasonally (Table II). In addition, the fact that at no time of the year did ammonium uptake by nanoplankton exceed nitrate uptake by >30% and the fact that the difference between assimilation ratios for ammonium [$18.8 \text{ nmol N } (\mu\text{g Chl } a)^{-1} \text{ h}^{-1}$; $r = 0.91$, $n = 14$, $P < 0.01$] and the other three nutrients [$7.32\text{--}11.4 \text{ nmol N } (\mu\text{g Chl } a)^{-1} \text{ h}^{-1}$] was smaller compared with Astan, suggests that the nanoplankton at this station had a relatively lesser preference for ammonium. Among all the four nutrients, only the uptake of ammonium was substrate-dependent ($r = 0.68$, $n = 11$, $P < 0.01$).

Netplankton were responsible for a much higher proportion of N uptake (43%) than at Astan. The closeness of the assimilation ratios for nitrate and ammonium in this fraction [22.1 and $22.9 \text{ nmol N } (\mu\text{g Chl } a)^{-1} \text{ h}^{-1}$] suggests that the netplankton, like the nanoplankton, did not have any preference for ammonium over nitrate. Seasonally, nitrate was more important for netplankton nutrition in winter–spring and ammonium, in summer–autumn (Table II), evidently as a result of increased availability of ammonium (from <2% in winter–spring to >30% in summer–autumn) in ambient N.

The proportion of N flux through picoplankton (8%) was of the same order as at Astan. Ammonium uptake in this fraction was substrate-dependent ($r = 0.72$, $n = 11$, $P < 0.01$). The highly significant relation between ammonium uptake and Chl *a* ($r = 0.78$, $n = 13$, $P < 0.01$) suggests a strong autotrophic component. Surprisingly, the assimilation ratio for ammonium in this fraction [$23.8 \text{ nmol N } (\mu\text{g Chl } a)^{-1} \text{ h}^{-1}$] was as high as in the other two fractions.

DISCUSSION

A large number of measurements of chlorophyll and of carbon assimilation by size-fractionated plankton have shown that netplankton tend to dominate in well-mixed high nitrate waters, while nanoplankton prevail in stratified, low nitrate waters [see references in (Wilkerson *et al.*, 2000)]. Direct measurements of N uptake by

size-fractionated plankton in several studies tend to support this. For example, in Narragansett Bay, phytoplankton of $>10 \mu\text{m}$ accounted for 80% of Chl *a* and total N uptake in winter–spring when ambient N concentrations were in excess of $5 \mu\text{mol N L}^{-1}$ whereas cells of $<10 \mu\text{m}$ dominated Chl *a* and N uptake in summer when concentrations were $<1 \mu\text{mol N L}^{-1}$ (Furnas, 1983). Chang *et al.* (Chang *et al.*, 1992) observed that after an upwelling event in the inshore waters off Westland, New Zealand, netplankton accounted for >56% of the total N uptake whereas in offshore waters, their share in uptake was <25%. A comparative study of N assimilation in inshore, shelf and oceanic waters of the southern Benguela upwelling system (Probyn, 1985) showed that the proportion of N assimilated by netplankton decreased from inshore to oceanic waters.

The nitrate-dominant nature of these waters (Wafar *et al.*, 1983) where seasonal lowest concentrations of nitrate are several times higher than those of ammonium and there is a lack of a substrate-limitation of nitrate uptake, as shown by a comparison of trace and saturated uptake rates, at any time of the year (L'Helguen *et al.*, 1996) led us to expect a dominance of netplankton in biomass and N uptake. However, this was not so and the netplankton at both stations were less important on an annual scale for phytoplankton biomass and N uptake (Tables I and II). At Astan, the importance expected of the netplankton was evident only in winter–spring, with their N uptake accounting for 42–48% of the total N uptake and with nitrate forming 44–47% of the N substrate used by them. The pattern of seasonal changes of N uptake by netplankton at Duons was similar to that at Astan, except that the average contribution of netplankton to total N uptake was relatively higher.

In the few studies where uptake of new and regenerated N by fractionated plankton was measured on spatial and temporal scales, it has been demonstrated that when nitrate was abundant, the netplankton took up more nitrate than ammonium. Off the west coast of South Africa, in the inshore and shelf waters, larger cells assimilated more nitrate than ammonium and urea (Probyn, 1985). Similarly, the netplankton from waters off Westland, New Zealand, took up twice as much nitrate than ammonium in offshore waters and this increased to about six times in inshore waters where nitrate concentrations were $>1 \mu\text{mol N L}^{-1}$ (Chang *et al.*, 1989). In the North Sea, when nitrate concentrations were high during spring, the netplankton had a pronounced preference for nitrate (Riegman *et al.*, 1998), accounting for ~80% of the total N uptake. In the coastal waters influenced by transport from Mississippi River, high input of nitrate in spring favoured the blooms of chain-forming diatoms (Bode and Dortch, 1996). It has been suggested that

a strong diffusion limitation for ammonium in the larger cells would necessitate them to be dependent more on alternative N sources, especially on nitrate when it is available in high concentrations (Stolte *et al.*, 1994).

A greater flux of nitrate, which would be consistent with the above results and the ambient concentrations of nitrate that were an order or more of magnitude greater than those of ammonium, was not evident in the netplankton fraction of the Morlaix Bay. Instead, the rates of nitrate uptake in this fraction were no more different than those of ammonium throughout the year (Figure 5a and b), with annual average nitrate to ammonium uptake ratios (0.98 at Astan, $r = 0.86$, $n = 21$, $P < 0.01$; 1.08 at Duons, $r = 0.74$, $n = 15$, $P < 0.01$) that were close to unity. It is this inability to take advantage of high nitrate concentrations, which could perhaps be related to the specific hydrographic regime prevailing in the Morlaix Bay, that could be the reason for the low importance of netplankton in N uptake. It has been shown earlier (Wafar *et al.*, 1983) that because of the continual mixing the algal cells in these waters get entrained below the euphotic zone and this could prevent a rapid growth of phytoplankton, resulting in a seasonal pattern of productivity that is similar to that of incident light. Under these conditions, even though nitrate is abundant, the netplankton could still be

constrained by the relatively low levels of mean light energy available to them, since uptake of new N, unlike the regenerated N, is a light-dependent process (MacIsaac and Dugdale, 1972). Instances when nitrate uptake rates in netplankton were substantially higher than those of ammonium uptake were in June at Astan [nitrate uptake = 265 nmol N ($\mu\text{g Chl } a$)⁻¹ h⁻¹; ammonium uptake = 148 nmol N ($\mu\text{g Chl } a$)⁻¹ h⁻¹] and in April–May at Duons [nitrate uptake = 30–47 nmol N ($\mu\text{g Chl } a$)⁻¹ h⁻¹; ammonium uptake = 4–28 nmol N ($\mu\text{g Chl } a$)⁻¹ h⁻¹]. The former coincided with a transient stratification of the water column when the phytoplankton cells were retained within the euphotic zone for a longer time, and the latter, with the seasonal increase in surface-incident radiation. Both these situations were conducive to a better utilization of light energy and hence an enhanced nitrate uptake in netplankton.

Indirect evidence for the control of light on N uptake by netplankton can be seen in the higher proportions of Chl *a* and N uptake in the netplankton fraction at Duons than at Astan (Tables I and II). It is likely that the shallow water column at Duons retains the cells within the euphotic zone for longer periods than at Astan and this light-adaptation may have enabled the netplankton to take up more N. Figure 6 shows a comparison of the column uptake profiles of ammonium and nitrate at

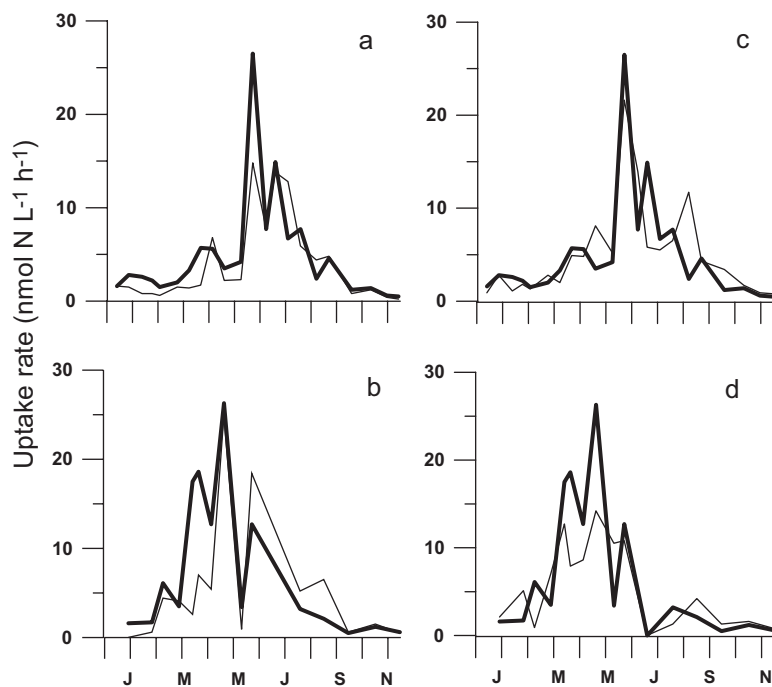


Fig. 5. Seasonal changes of nitrate (solid line) and ammonium (thin line) uptake by netplankton at (a) Astan and (b) Duons, and nitrate uptake by netplankton (solid line) and nanoplankton (thin line) at (c) Astan and (d) Duons.

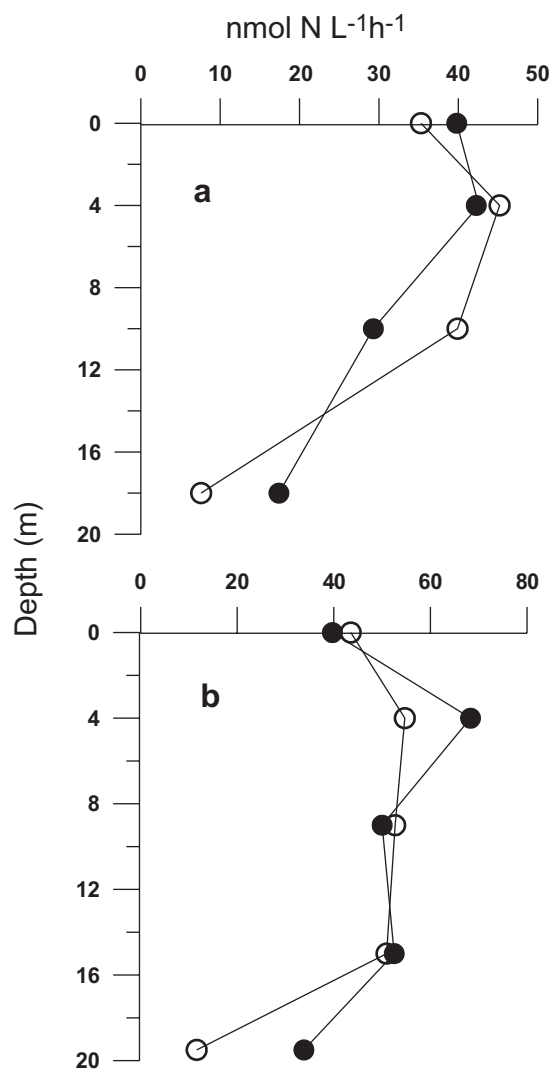


Fig. 6. Profiles of ammonium (closed circles) and nitrate (open circles) uptake rates at (a) Astan and (b) Duons during May.

Duons and Astan for the second fortnight of May. With both nutrients, uptake rate at any given light depth was higher at Duons than at Astan. As these samples were not fractionated, these results, understandably, cannot be taken as representing exclusively netplankton but the fact that the average Chl *a* content in netplankton fractions at Duons was higher than at Astan at any time would suggest a greater proportion of netplankton uptake in the total N uptake in these profiles.

The decreased ability of netplankton at Astan to utilize N could also have been compounded by a silicon limitation. The cyclic changes of nitrate and ammonium uptake by netplankton at this station had peaks in late March, mid April, late June and mid July (Figure 3).

Each of these coincided or was preceded by a Chl *a* maximum that, in turn, was related to a distinct species succession, from *Plagiogramma* through *Thalassiosira*, *Chaetoceros* and *Rhizosolenia* (L'Helguen, 1991). The collapse of successive blooms, leading to low biomass in between, could thus have been an added cause of the marked reduction in N uptake in netplankton. The seasonal succession of diatoms at this station, from thick-walled diatoms to thin-walled ones, has earlier been shown to be related to changes in the concentrations of ambient silicon (Wafar *et al.*, 1983). While the seasonal increase in ambient light and temperature in spring–summer could be expected to play a significant role in enhancing N uptake rates by netplankton, species succession, induced by silicon limitation, could thus have had a marked effect on N uptake by netplankton.

Dominance of nitrate as a N source did not seem to have favoured an enhanced uptake by nanoplankton either. On annual and seasonal scales, the nanoplankton at Astan and Duons used new N only in quantities similar to those used by netplankton, with no substantial differences in the new N utilization efficiency (Figure 5c and d, Tables I and II). It was only with the regenerated forms of N that the differences became marked, especially at Astan, with more than twice the uptake by nanoplankton than by netplankton. This is consistent with the general trend of a preference for ammonium by most flagellates (Dortch, 1990). The high uptake of regenerated N is prominent in spring–summer in the case of ammonium and in summer–autumn in the case of urea. The affinity of nanoplankton to regenerated forms of N thus determined the magnitude of N production in these waters. Such a preference is not unexpected, since it has often been demonstrated that ammonium utilization by nanoplankton is quantitatively more important than nitrate or urea and that at times all of the ammonium uptake was related to the nanoplankton fraction (Glibert *et al.*, 1982b; Furnas, 1983; Probyn, 1985).

Differences in total uptake of ammonium in the nanoplankton fraction between Astan ($41.7 \mu\text{mol N L}^{-1} \text{y}^{-1}$) and Duons ($22.1 \mu\text{mol N L}^{-1} \text{y}^{-1}$) are striking. As the uptake of ammonium in the nanoplankton fraction was highly substrate-dependent at both stations, the relatively higher flux at Astan than at Duons could only be related to the absolute ambient ammonium concentrations, which had a seasonal maximum at Astan ($0.75 \mu\text{mol N L}^{-1}$) that was higher than at Duons ($0.51 \mu\text{mol N L}^{-1}$). Shaded portions on Figure 7 show the period when the ambient ammonium concentrations at Astan exceeded the maximum at Duons and the corresponding excess in ammonium uptake by nanoplankton at Astan. This excess, between June and

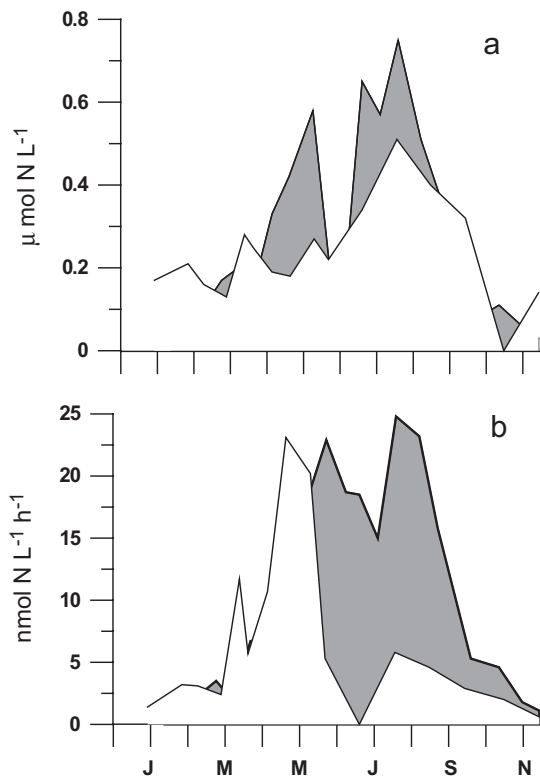


Fig. 7. Comparison of (a) ambient concentrations and (b) uptake of ammonium in the nanoplankton fraction between Astan and Duons. The shaded portions show the periods when these were in excess at Astan.

August, was $\sim 20 \mu\text{mol N L}^{-1}$, equivalent to $\sim 50\%$ of the annual ammonium flux in the nanoplankton fraction. The rates at which ammonium is made available for assimilation, therefore, determine not only the magnitude of N productivity in the Morlaix Bay but also the differences in it between the stations. In this context, it is of interest to note that the annual regeneration of ammonium by microheterotrophs at Astan [$1342 \text{ mmol N m}^{-2}$ (L’Helguen, 1991)] was $\sim 30\%$ higher than at Duons [$1048 \text{ mmol N m}^{-2}$ (Maguer *et al.*, 1996)] and that ammonium uptake and regeneration at both sites were closely coupled.

Nitrate–ammonium interactions in phytoplankton N nutrition are complex, among which repression of nitrate uptake in the presence of ammonium (Flynn *et al.*, 1997) and the decrease in such repression at high nitrate concentrations and low temperatures (Flynn, 1999) are ecologically important. The observed pattern of changes of nitrate-to-ammonium uptake ratios in net- and nanoplankton at both stations (Figure 8) reflect these: both the fractions assimilated more nitrate in winter when water temperature was at seasonal minimum and ammonium concentrations were low but progressively less later in the seasonal cycle when concentrations of ambient ammonium increased, even though nitrate concentrations were still twice as high as those of ammonium. Nonetheless, the effect of size could also be discerned to some extent: the consistently higher nitrate-to-ammonium uptake ratios in netplankton (0.8–2.8 at Astan and

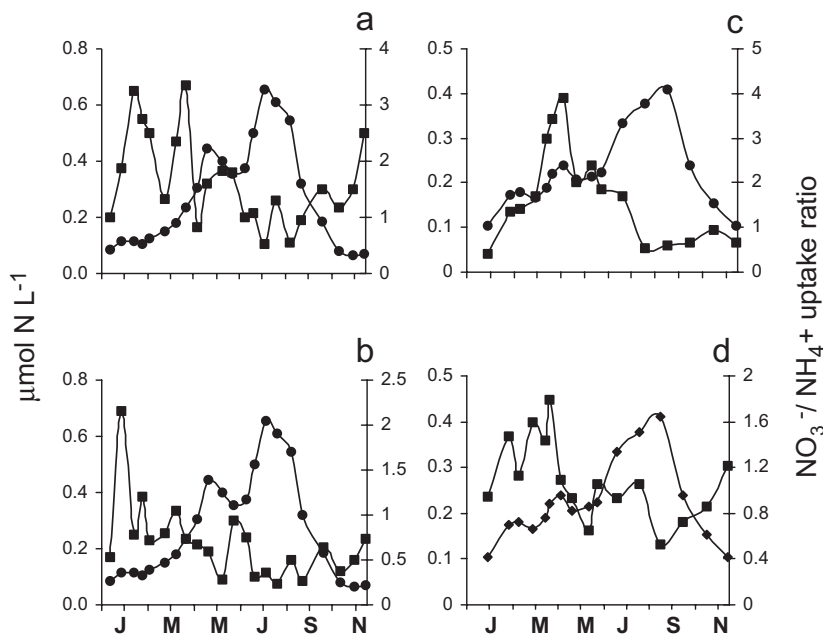


Fig. 8. Seasonal changes of nitrate-to-ammonium uptake ratios (squares) of netplankton at (a) Astan and (c) Duons and of nanoplankton at (b) Astan and (d) Duons along with those of ambient ammonium concentrations (closed circles).

0.4–3.9 at Duons) than in nanoplankton (0.3–1.4 at Astan and 0.5–1.8 at Duons) and the greater flux of ammonium through nanoplankton in summer at both stations are pointers to this. The pattern of N nutrition is thus size-independent when concentrations of nitrate are high, those of ammonium low, and temperature is low, and becomes ammonium- and to some extent size-dependent when the opposite conditions prevail.

The low proportion of N assimilation in the picoplankton fraction (8%) agrees with the general trend of decrease in their importance from oceanic to nearshore waters (Probyn, 1985; Probyn *et al.*, 1990). So also is the preference for reduced forms of N, since the picoplankton, as a group, are known to have a strong preference for reduced N (Probyn, 1985; Harrison and Wood, 1988; Chang *et al.*, 1989; Probyn *et al.*, 1990). Unlike the net- and nanoplankton, however, the picoplankton, except in one instance, did not take up new N. This contrasts with the observations (Probyn and Painting, 1985; Harrison and Wood, 1988) that on occasions picoplankton could also use predominantly nitrate. It would appear that while the affinity of picoplankton to reduced forms is universal, preference for new N is not, even when the ecosystem is nitrate-dominated, as in the present case.

It has often been suggested that a substantial fraction of ammonium assimilation in the marine microbial populations is associated with non-photosynthetic organisms (Laws *et al.*, 1985) and in the picoplankton size range it is largely accounted for by heterotrophic bacteria (Wheeler and Kirchman, 1986). The statistically significant correlations between N uptake and Chl *a* in the picoplankton at both stations suggests, however, a dominance of autotrophs. This is not unexpected since contribution of picoplankton to photosynthetic biomass (Platt and Li, 1986) and N assimilation (Probyn *et al.*, 1990) is generally non-negligible, and often substantial. It is more likely that the organisms responsible for a heterotrophic uptake of N are found in the nano- and netplankton fractions, probably as particle-bound bacteria. Size-fractionated counts of free-living bacteria at Astan have shown that ~25% of them could be in the nanoplankton fraction (Le Corre *et al.*, 1996) and the abundance of particle-bound bacteria could be as important as that of the free-living ones (L'Helguen, 1991).

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