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Nitrate storage by phytoplankton in a coastal upwelling environment

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Abstract The storage of nitrate by phytoplankton cells during the early phases of upwelling was studied in coastal stations off northern Spain (southern Bay of Biscay) between 1990 and 1994. In this region, a persistent upwelling during summer is characterised by intermittent pulses of variable intensity, and increased nutrient concentrations in the surface layer. The main effect of an upwelling pulse on phytoplankton distribution is the shifting of the chlorophyll *a* and primary production maxima to near the surface. When the upwelling relaxes, thermal stratification of the water column occurs, and a distinct subsurface chlorophyll maximum develops below the production maximum. An accumulation of intracellular nitrate characterized the early phases of upwelling (mean = 2.73 μmol N m^{-3}), maximum concentrations being attained at depths where biomass and production values were moderate. In contrast, phytoplankton cells from non-upwelling situations contained significantly lower concentrations of intracellular nitrate (mean = 0.17 μmol N m^{-3}). The variations in the intracellular pool of nitrate may result from the differential allocation of resources within the cell as a result of variations in the energy available, since the uptake and assimilation of nitrate is a relatively expensive process involving several enzymatic systems. We hypothesize that nitrate storage by phytoplankton cells is characteristic of early phases of upwelling and is linked to patterns of carbon fixation. Average nitrogen budgets for upwelling and non-upwelling situations indicate that intracellular nitrate reserves are not responsible for maintaining high phytoplankton growth rates, since they only account for <2% of daily primary production during upwelling events.

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Introduction

Dissolved inorganic nitrogen is the nutrient that usually limits phytoplankton productivity in the sea, especially during periods of surface stratification (Ryther and Dunstan 1971; Codispoti 1983). Upwelling of deep waters into the upper layers of the ocean greatly enhances local productivity, mainly because of the input of new nitrogen (Dugdale and Goering 1967, Codispoti 1983). However, investigation of the relationship between new nitrogen and productivity in upwelling systems is complicated by the fact that most measurements of phytoplankton production in the field only detect the highest nitrate-uptake rates, or inorganic carbon-fixation rates after nitrate has almost been depleted from surface waters (Dugdale et al. 1990). In addition, in upwelling systems, productivity measured by carbon uptake does not correspond to productivity measurements estimated by nitrogen uptake (e.g. Dortch and Postel 1989). One of the reasons of the time-lag between nutrient-enrichment and enhanced phytoplankton productivity is that phytoplankton cells must first undergo a period of physiological adaptation to the changed ambient light and nutrient concentrations. The various processes leading to membrane transport, assimilation, and incorporation of external dissolved nitrogen into biochemical compounds inside the cell involve several enzymatic mechanisms (Syrett 1981; Falkowski 1983). These mechanisms need time to develop and adapt to the changes in ambient conditions. When upwelling relaxes and the supply of new nutrients decreases, phytoplankton

populations may survive by using regenerated forms of nitrogen such as ammonium and urea. In fact, a significant proportion of the nutrients used in primary production after an upwelling pulse may be supplied by regeneration of the phytoplankton biomass in the same area where upwelling occurs (Codispoti 1983).

The dynamic nature of upwelling systems means that the phytoplankton must be able to adapt to rapid changes in nutrient concentrations, and therefore possess mechanisms to buffer environmental variability. Transient storage of several nitrogen compounds has been described for cultures of marine microalgae, mostly diatoms (DeManche et al. 1979; Dortch 1982; Dortch et al. 1984; Raimbault and Mingazzini 1987; Martinez 1991; Marsot et al. 1992). Such storage allows further growth after external nitrogen concentrations have decreased to limiting concentrations. Dortch et al. (1985) hypothesized that the intracellular accumulation of nitrogen pools may buffer large variations in external nitrogen when this nutrient is supplied sporadically. The largest intracellular pools of unassimilated nitrogen are accumulated during periods when uptake rates exceed growth rates, in healthy, non-limited phytoplankton cells (Collos and Slawyk 1976; Dortch 1982; Dortch et al. 1984; Martinez 1991). Among the various compounds that can be analyzed (mainly nitrate, ammonium, amino-acids and proteins), nitrate has the advantage of being easily determined by standard analysis and of being more directly related to phytoplankton cells than other nitrogen compounds that may be also present in significant amounts in bacteria, microzooplankton, and detritus. Kirchman (1994) reviewed several studies of nitrate assimilation by marine bacteria, and concluded that nitrate uptake by these organisms in the plankton may be "safely ignored as a first approximation" compared to phytoplankton uptake-rates. The analysis of intracellular nitrate pools can be used as an index of nitrogen-sufficiency in the field. Some measurements made in coastal waters (Dortch et al. 1985) suggest that, despite low concentrations of dissolved inorganic nitrogen in surface waters, phytoplankton may avoid severe nitrogen limitation by utilizing internal pools of nitrogen.

The seasonal upwelling off the northern coast of Spain (Southern Bay of Biscay) affects coastal waters mainly during the thermal stratification period, from May to September (Botas et al. 1990). Upwelling is induced primarily by north-east winds, and surface-cooling is often connected with the stronger upwelling area of north-west Spain (Dickson and Hughes 1981). Nearshore waters intermittently affected by the upwelling exhibit higher phytoplankton biomass concentrations and production rates than stratified waters (Botas et al. 1990; Fernández and Bode 1991; Fernández et al. 1991). In some cases, high rates of primary production have been shown to be largely unsupported by the external nutrient concentrations (Botas et al. 1990), leading to the hypothesis that in situ nitrogen regeneration must constitute an important nitrogen source between upwelling pulses.

This paper studies the relationships between nitrate storage, the distribution of dissolved inorganic nitrogen, and phytoplankton biomass and productivity in upwelling and non-upwelling situations, using field measurements to evaluate the adaptation of the local phytoplankton populations to rapid changes in the supply of new nitrogen. The contribution of the accumulated nitrate to sustaining high primary-production rates in coastal waters is assessed by comparison with other inorganic nitrogen sources.

Materials and methods

Intracellular nitrate concentrations, together with temperature, salinity, inorganic nutrient concentrations, phytoplankton biomass and primary production were studied at 28 stations along the northern Spanish coast (43 30′ to 44 00′ N; 6 00′ to 7 00′ W), in some cases employing repeated sampling, during the summer cruises ASFLOR-I, ASTURIAS-793, and ASTURIAS-794. The selected stations covered a large range of shelf areas from the coast to the ocean, with a bathymetric range of 20 to 2000 m. Water samples were collected with 5-litre Niskin or 20-litre Van-Dohrn bottles. Temperature and salinity were measured either using reversible thermometers and an induction salinometer (ASFLOR-I cruise) or a CTD probe SeaBird SBE-2501 (ASTURIAS cruises). Inorganic nutrients were analysed using a Technicon AAII autoanalyser and the methods described by Grasshoff et al. (1983). The detection limit for nitrate was 0.05 mmol m^{-3}.

Intracellular nitrate concentrations were measured by the method described by Dortch (1982) and Dortch et al. (1984). Five litres of water were filtered through glass-fibre Whatman GF/F filters, which were then rinsed with nitrate-free filtered seawater and stored frozen. Nitrate was extracted by adding boiling deionized water to the filters, and the nitrate concentration in the extract was determined with the autoanalyser. Blank filters were analysed together with the samples and showed undetectable nitrate concentrations (<0.05 mmol m^{-3}). Chlorophyll *a* concentrations were determined by fluorometric analysis of acetonic extracts (Yentsch and Menzel 1963). Primary-production rates were determined by measuring the incorporation of $H^{14}CO_3^-$ by phytoplankton cells, as described by Fernández and Bode (1991). Incubations were performed on deck for 2 to 3 h, and were terminated by filtration through glass-fibre GF/F filters. In the absence of other measurements, daily primary-production rates were approximated by multiplying the hourly production rates by 12 h of effective light.

Nitrogen budgets for upwelling and non-upwelling situations were computed using average values and the procedure described by McCarthy and Carpenter (1983). The purpose was to compare the measured intracellular nitrate storage with other nitrogen pools and fluxes in the different situations, rather than to obtain accurate estimates of the nitrogen fluxes. The upward flux of inorganic nitrogen, J (mol N m^{-2} d^{-1}), was computed using the formula:

$$J = wS_N + K_z(dN{:}dz),$$

where wS_N describes vertical advection and K_z ($dN{:}dz$) the diffusive flux. The advective flux term w (m d^{-1}) for upwelling situations was calculated from direct observations of the vertical displacement of the pycnocline during several days at one station in the study area (Botas 1990). The value for non-upwelling situations was taken from Munk (1966). The eddy diffusivity coefficient K_z (m^2 d^{-1}) was computed from the temperature gradient through the thermocline using the empirical formula of Anderson (1978), as quoted by McCarthy and Carpenter (1983). The values of K_z obtained by this procedure were within the range of values estimated from vertical microstructure gradients (Table 1 of Denman and Gargett 1983). The gradient $dN{:}dz$ (mol N m^{-4}) and concentration S_N (mol N m^{-3}) of various forms of dissolved inorganic nitrogen were estimated from the average vertical profiles for upwelling and non-upwelling situations.

The nitrogen requirements of the phytoplankton were estimated using the measured carbon-fixation rates and a compositional C:N ratio of 7.59 mol C (mol N)$^{-1}$ (Ríos et al. 1987). This is likely to produce conservative estimates, given the large variations in C:N-uptake ratios measured in upwelling environments [6 to 24 mol C (mol N)$^{-1}$, Fisher et al. 1982]. Phytoplankton nitrogen was computed using a ratio of 50 g C (g chlorophyll *a*)$^{-1}$ and the C:N ratio cited above.

Results

Vertical distribution of phytoplankton and nitrogen

Clear differences in the vertical distribution of water-column characteristics were observed between stations affected by upwelling and those with stratified surface waters. The examples in Fig. 1 illustrate the presence of a sharp thermal gradient of ~7 C° between the surface and 40 m depth in the non-upwelling station, while in the upwelling station the difference in temperature was ~3 C°. Water of <15 °C was detected below 10 m in the upwelling station and below 30 m in the non-upwelling station. Inorganic nitrogen concentrations (especially nitrate) were higher in the upwelling station, and chlorophyll *a* concentrations in the surface layer showed a tenfold increase relative to the non-upwelling station. Primary production rates in the upper 15 m were also higher in the upwelling station. However, the most marked differences were in intracellular nitrate concentrations. Only the sample at 30 m depth in the non-upwelling station contained detectable nitrate (~1 μmol N m^{-3}). In contrast, all the samples from the upwelling station contained detectable concentrations of intracellular nitrate, with the maximum concentration (~9.5 μmol N m^{-3}) at 15 m depth coinciding with the production maximum.

All stations were classified as upwelling or non-upwelling conditions. Upwelling conditions had surface temperatures <17 °C and nitrate concentrations of >0.5 mmol m^{-3} in the upper 20 m. The average vertical profiles (Fig. 2) revealed lower surface temperature, higher salinity and higher nitrate concentrations for upwelling than for non-upwelling conditions. Table 1 indicates that these differences are statistically significant. During upwelling the surface layer is moderately mixed, since there is weak thermal and saline stratification in the upper 20 m. The average nitrate concentration at the surface in our upwelling conditions was 10 mmol m^{-3} lower than in the bottom waters. The mean temperature and salinity profiles in non-upwelling conditions displayed a higher degree of stratification than upwelling stations, but no marked thermo- or haloclines. This suggests that mixing of the upper layer occurred frequently; lack of sharp profiles could have

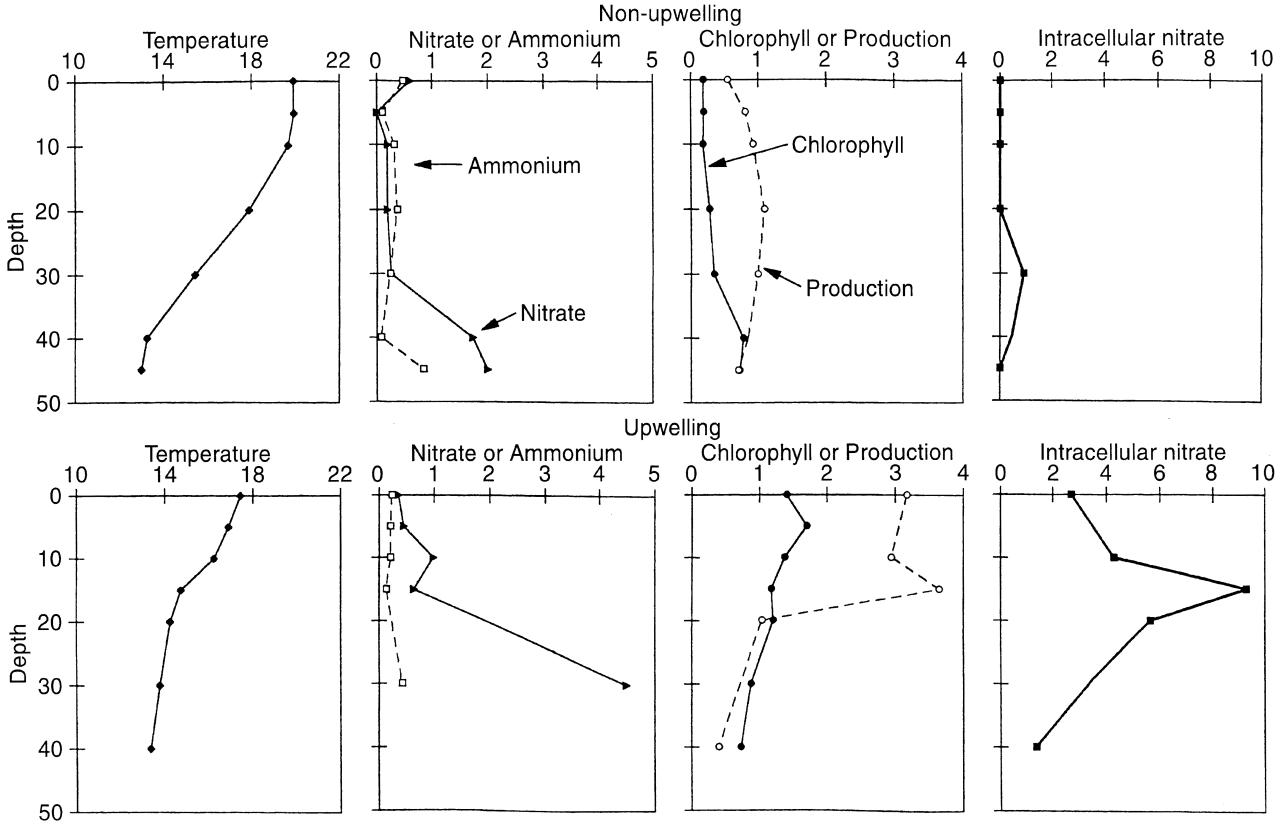

Fig. 1 Selected vertical profiles of temperature (°C), nitrate and ammonium concentrations (mmol m^{-3}), chlorophyll *a* (mg m^{-3}), primary production (mg C m^{-3} h^{-1}) and intracellular nitrate (μmol m^{-3}) in upwelling and non-upwelling stations

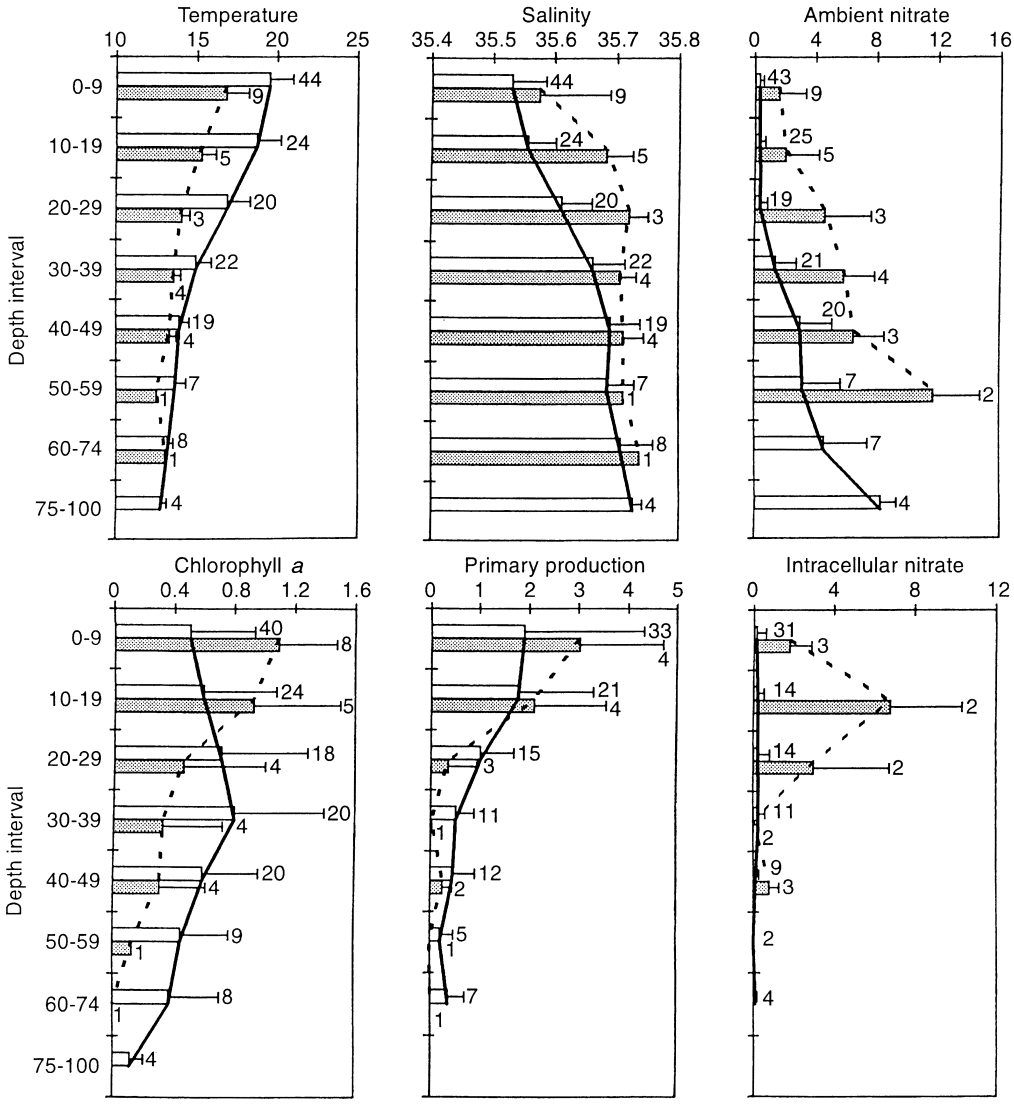

Fig. 2 Average (+ SEM) vertical profiles of temperature (°C), salinity (‰), ambient nitrate (mmol N m^{-3}), chlorophyll *a* (mg m^{-3}), primary production (mg C m^{-2} h^{-1}), and intracellular nitrate (µmol N m^{-3}) in upwelling (*shaded bars and dashed lines*), and non-upwelling (*open bars and continuous lines*) conditions. Values grouped in depth intervals (m) *Numbers beside bars* numbers of samples averaged)

arisen from past upwelling events or other mechanisms of coastal water-mixing, such as tides. The chlorophyll maximum in non-upwelling conditions was between 30 and 40 m, whereas that in upwelling conditions was at the surface. However, Table 1 shows that the differences between the average concentrations of these maxima were statistically non-significant. The profiles for primary production were similar in all cases, with maximum rates at the surface decreasing exponentially with depth. Most primary productivity took place in the upper 20 m in upwelling conditions, with an average profile similar to that for chlorophyll *a* (Fig. 2).

Only 35% of the samples collected from non-upwelling stations contained measurable amounts of intracellular nitrate. In contrast, all samples studied from upwelling stations contained measurable intracellular nitrate. The differences between average intracellular nitrate concentrations for each upwelling situation and the shape of the vertical profiles displayed in Fig. 2 were highly significant (Table 1). Most intracellular nitrate was present in upwelling situations, generally between 10 and 20 m depth. Intracellular nitrate concentrations were not correlated to either extracellular nitrogen, chlorophyll *a* or primary production (Pearson's *r*, $p > 0.05$).

Nitrogen budgets

The daily budgets calculated for nitrogen in the euphotic zone in upwelling and non-upwelling situations indicate that the external dissolved inorganic pools contained sufficient nitrogen to support the relatively high primary-production rates measured (Fig. 3). In both situations, ammonium concentrations were low but able to support the estimated uptake rates for 1 d (upwelling) or 2 d (non-upwelling). However, the main source of inorganic nitrogen may well have been nitrate, since the nitrogen stocks in the surface layer could support the observed phytoplankton production for a period of 6 to 10 d. Vertical advection constituted the main source of nitrate to the surface mixed-layer during an upwelling

Table 1 Results of ANOVA tests performed on variables in Fig. 2. Two qualitative factors were analysed in nested model: depth (in 10 m intervals from surface to 50 m) and upwelling (upwelling and non-upwelling conditions) (*SS* sum-of-squares; *df* degrees of freedom; *F* *F*-statistic; *p* probability of significance of *F*)

Variable, factor	SS	(*df*)	*F*	*p*
Temperature				
depth	623.06	(4)	99.00	0.000
upwelling	110.33	(1)	70.13	0.000
depth (upwelling)	19.05	(4)	3.03	0.020
residual variance	226.56	(144)		
Salinity				
depth	0.56	(4)	46.24	0.000
upwelling	0.09	(1)	29.14	0.000
depth (upwelling)	0.03	(4)	2.63	0.037
residual variance	0.43	(143)		
Ambient nitrate				
depth	187.14	(4)	29.44	0.000
upwelling	132.62	(1)	83.45	0.000
depth (upwelling)	36.32	(4)	5.71	0.000
residual variance	225.68	(142)		
Chlorophyll *a*				
depth	0.45	(4)	0.49	0.744
upwelling	0.18	(1)	0.79	0.375
depth (upwelling)	3.81	(4)	4.13	0.003
residual variance	31.56	(137)		
Primary production				
depth	46.32	(4)	4.28	0.003
upwelling	0.62	(1)	0.23	0.634
depth (upwelling)	5.65	(4)	0.52	0.720
residual variance	259.96	(96)		
Intracellular nitrate				
depth	8.68	(4)	4.02	0.005
upwelling	59.18	(1)	109.69	0.000
depth (upwelling)	38.52	(3)	23.80	0.000
residual variance	43.16	(80)		

pulse, whereas eddy diffusion supplied >99% of nitrate in non-upwelling conditions. Intracellular nitrate comprised but a small fraction of phytoplankton nitrogen (0.5% in upwelling, and 0.1% in non-upwelling conditions) and would support 1.5% of daily primary production measured during upwelling, and only 0.1% of primary-production in a non-upwelling situation. In the latter, phytoplankton biomass integrated throughout the water column was almost twice that in upwelling situations, but primary production was similar in both (Fig. 3). This resulted from the fact that the surface mixed-layer was twice as deep in non-upwelling conditions, and that the nitrogen supply was sufficient to sustain active phytoplankton growth in both cases. Nitrogen regeneration in the surface layer may be the main mechanism supplying primary-production requirements in non-upwelling conditions, since diffusion of nitrogen from the subsurface layer through the pycnocline accounts for only 10% of the nitrogen required for the measured daily primary-production rates.

Discussion

Nitrate storage and upwelling dynamics

The present study has shown that intracellular nitrate storage is a good indicator of recent upwelling along the northern Spanish coast, even when external concentrations of inorganic nitrogen are only slighty higher than in more stratified, non-upwelling conditions. This result confirms previous findings of sizeable nitrogen pools in areas where new nitrate inputs occur as sporadic pulses (Collos and Slawyk 1976; Dortch et al. 1985; Dortch and Postel 1989). Although accumulation of unassimilated nitrogen has been detected in laboratory cultures of

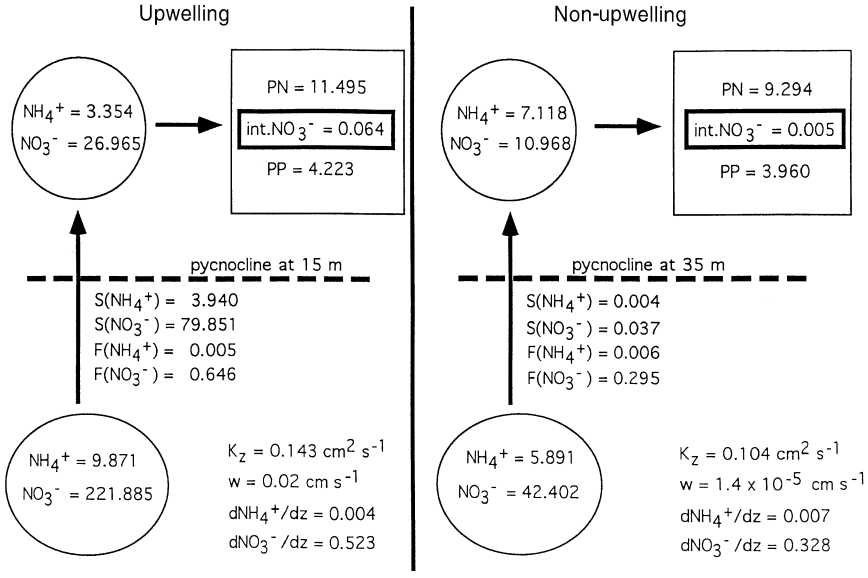

Fig. 3 Average nitrogen budgets for upwelling and non-upwelling conditions calculated for 60 m-depth coastal station. See "Materials and methods" for details of calculations [*PN* phytoplankton nitrogen; *PP* primary production, *int.*NO_3^- intracellular nitrate; K_z eddy diffusivity coefficient; *w* advective flux coefficient; *F*(*N*) diffusive flux; *S*(*N*) advective flux; *dN*/*dz* nitrogen gradient through pycnocline; *ringed data* dissolved nitrogen pools, *boxed data* particulate nitrogen, – all values in mmol N m^{-2})] Nitrogen fluxes, including PP, are in mmol N m^{-2} d^{-1}

species representative of the main phytoplankton groups, the size of the internal nitrogen pools accumulated by different species varies greatly (DeManche et al. 1979; Dortch 1982; Dortch et al. 1984; Raimbault and Mingazzini 1987; Martinez 1991; Marsot et al. 1992). Much variability related to adaptation of phytoplankton to nutrient conditions of cultures has also been reported (Dortch 1982; Martinez 1991). Most of such data were for diatoms, whose vacuoles can attain large sizes and fill most of the cytoplasm. Diatoms are often the dominant species when external nitrogen ("new nitrogen": Dugdale and Goering 1967) enters the euphotic zone (Malone 1980). Although in the present study the species composition was not assessed systematically in all samples, we presumed that diatoms constitute a significant part of the phytoplankton during the initial phases of upwelling, as in the nearby upwelling area of Galicia (Varela et al. 1991). In late phases of upwelling, and in stratified conditions, a mixture of varying proportions of diatoms, flagellates and dinoflagellates has been described for the study area (Botas et al. 1990; Fernandez and Bode 1994). In diatom cultures, nitrate accumulation can comprise >20% of the total cell nitrogen (DeManche et al. 1979; Raimbault and Mingazzini 1987), but more often values ranging from 0.1 to 4% have been found in the field (Dortch et al. 1985; Dortch and Postel 1989), which are similar to those recorded in the present study for both upwelling and non-upwelling situations. Our results comply with those of Martinez (1991), who found that intracellular nitrate storage was maximum when cells had been nitrogen-starved for a relatively short time previously. This suggests that phytoplankton populations in areas subject to frequent nitrate enrichment, such as the upwelling situations in the present study) are able to take up the nutrient immediately, with complete assimilation into organic matter occurring later.

The vertical distribution of the intracellular nitrate pool in upwelling conditions was quite different from that of biomass and primary production. Laboratory and field experiments have established that nitrate-uptake rates of phytoplankton are higher in the light than in the dark (Dortch 1982; Price et al. 1985; Dortch and Postel 1989; Glibert and Garside 1992), as observed for other plants (Hattori 1962; Beevers and Hageman 1972; Duke and Duke 1979, 1984; Maldonado and Aparicio 1987). In contrast to other nitrogen sources (e.g. ammonium), each step of the processes of nitrate uptake and assimilation requires specific enzymes to transport the molecule through the cell membrane, reduce it to nitrite and ammonium, and finally incorporate the nitrogen into carbon skeletons (Syrett 1981; Falkowski 1983). Even when merely the first step is considered, it is evident that some energy supply must be involved. This may explain why intracellular nitrate accumulates near the surface, where irradiance levels are higher. However, maximum rates of primary production often occur at depths shallower than the intracellular nitrate maximum. A possible explanation of the mismatch between these processes is that nitrate accumulation must balance two basic requirements: sufficient light to support the energy requirements of the enzymatic transport activity, and sufficient external nitrate concentrations to supply the enzymatic mechanism. The fact that nitrate uptake and carbon fixation compete for metabolic energy (Falkowsky and Stone 1975; Collos and Slawyk 1979) agrees well with this explanation. The observed maximum of intracellular nitrate may be at an optimal depth where both conditions are satisfied.

An alternative explanation is that nitrate does not accumulate in the surface layer because of its rapid incorporation into intracellular organic molecules. Harrison (1990), after examination of an extensive database of vertical phytoplankton profiles with definite subsurface chlorophyll and productivity maxima, concluded that the shallow primary-production maxima were fuelled mainly by nitrogen regenerated in situ. This means that reduced forms of nitrogen would be the main source for phytoplankton growth in the surface mixed-layer. While this may be the case for non-upwelling situations, nitrogen concentrations at the surface in the upwelling stations of the present study (Figs. 2, 3) suggest that there was sufficient external inorganic nitrogen (either nitrate or ammonium) to support the observed primary-production rates. Fernandez et al. (1991) described the preferential production of lipids and low molecular weight metabolites during the day by an active growing phytoplankton population in some of the samples used in the present study, while carbon incorporation (and probably nitrogen also) into proteins ocurred mainly during the night. Similar results were reported by Marañon et al. (1995) in the same upwelling area. Therefore, the second explanation seems less probable, but direct measurements of the uptake and storage of different nitrogen sources in this ecosystem will be necessary to test this.

The intracellular nitrate concentrations observed in this study may represent a transient pool of nitrogen that can be incorporated later into proteins and other nitrogen-rich organic molecules. Since all our samples were taken during the day, we do not know the extent of such intracellular storage at night. If night reallocation of carbon synthesized during the day is a general feature of the daily production cycle of phytoplankton (Cuhel et al. 1984; Fernández et al. 1991; Marañón et al. 1995), we would expect a decrease in the concentration of intracellular nitrate and an increase in the concentration of organic nitrogen at night. Although some field studies in non-upwelling areas found no apparent daily cycles in the size of intracellular nitrate and other nitrogen pools (Dortch et al. 1985), other studies in upwelling areas did record significant variations in nitrate storage during the day (Collos and Slawyk 1976). Similarly, a large uncoupling between nitrate uptake and dark assimilation was demonstrated in laboratory experiments with cultures (Raimbault and Mingazzini 1987; Marsot et al. 1992). Such uncoupling supports the hypothesis of the dominance of assimilative processes at night.

Nitrogen budgets

The average nitrogen budgets demonstrate that nitrate storage in cells would have but a small impact on the maintenance of elevated instantaneous growth rates. In the most favourable case (upwelling), such nitrogen reserves accounted for only 1.5% of daily primary-production. Many other sources of nitrogen were more instrumental in supplying this element. Eddy diffusion (in both upwelling and non-upwelling conditions) and physical advection (in upwelling conditions) may supply up to two orders of magnitude more nitrogen for primary production requirements than stocks of intracellular nitrate (Fig. 3). In the late phases of upwelling events in the Galician coast, Bode and Varela (1994) estimated that up to 50% of the nitrogen requirements of phytoplankton could be provided by in situ regeneration of organic matter by the existing populations of heterotrophic organisms. This fraction of regenerated production is expected to increase with the degree of surface stratification (Harrison 1990). Furthermore, there are other mechanisms that supply nitrogen to the euphotic layer that were not measured in the study area. One is the atmospheric deposition of nitrogen; this can represent a major source in some environments (Owens et al. 1992). Using data collected at coastal stations and during cruises through the North Atlantic Ocean, the atmospheric deposition of inorganic nitrogen (nitrate and ammonium) through rain and dry deposition range from 11.1 to 521.3 mmol N m^{-3} (Church et al. 1991; Spokes et al. 1993). Taking a mean value of 266 mmol N m^{-3} and rainfall of 35 litres m^{-2} (median value for the summer period along the northern Spanish coast; Instituto Geografico Nacional 1991), this would add 9.3 mmol N m^{-2} to the surface water. If mixing is complete, atmospherically-derived nitrogen could support the observed production rates for two days. Another source of nitrogen, in this case of local importance, could be river runoff. Some coastal stations visited during this study exhibit salinities near 34.3‰ and inorganic nitrogen concentrations of $\simeq$15 mmol N m^{-3}. Even when the low-salinity waters flow as a thin layer of a few centimetres thick at the surface, its nutrient-loading might be wind-mixed. Assuming a median value of 100 litres of continental water per square meter at stations near the coast for the summer period in the study area (Botas 1990), the nitrogen supplied by runoff could be three times the requirements for production. Additional nitrogen inputs, such as fixation of atmospheric nitrogen by cyanobacteria, are expected to be of minor importance (e.g. McCarthy and Carpenter 1983).

Nitrogen limitation does not appear to be widespread in coastal waters of northern Spain during the summer. Even in stratified, non-upwelling conditions, there are enough physical and biological mechanisms to supply the nitrogen required to sustain the measured primary-production rates. The accumulation of intracellular nitrate in upwelling situations may be a consequence of differential resource allocation within the phytoplankton cells during the day and in the early phases of upwelling, with the available energy being used to store nitrate and carbon-rich molecules.

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