Kinetic characteristics of the N-uptake regime at the Iberian Margin during upwelling situations

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Introduction

Our investigations during the ongoing OMEX phase I project have underlined the importance of vertical transport of nitrate in sustaining primary production at the ocean margin of the North-eastern Atlantic. Overall nitrate uptake rates (new production) paralleled carbon fixation rates and *f*-ratio followed the well-known function of nitrate (Elskens *et al*., 1999, Joint *et al*., 1998). However, these studies emphasised a leading role for ammonium in regulating the removal of nitrate (Elskens *et al*., 1997). Ammonium started to accumulate in the water column in late spring and during this period, both remineralisation data and turnover times of ammonium supported that the generation of ammonium and possibly other reduced nitrogen substrates meet the daily nitrogen requirement of the phytoplankton. The mechanism of supply and the kinetics of utilisation of dissolved inorganic nitrogen are believed to play a critical role in determining the productivity, size structure and species succession of phytoplankton communities of the world's ocean (Harrison *et al*., 1996). Therefore, the work described here is an attempt to investigate more systematically the kinetics of nitrate and ammonium uptake and the inhibitory effects of ammonium on nitrate uptake with natural plankton assemblages in waters seasonally regulated by upwelling events. Results are presented for the last two OMEX cruises aboard RV *Belgica*.

Methods

Two cruises were conducted at the Iberian Margin aboard RV *Belgica*. The first one was from 21/06 to 29/06/1997 and the second from 27/06 to 07/07/1998.

Surface water was collected at sunrise at 10-m depth with 10-litres Niskin bottles during the 2 cruises. During the second cruise, water was also collected at the bottom of the photic layer. Sampled stations are presented in Figure 1.

Nutrient concentrations were determined aboard. Nitrate + nitrite analyses were performed with a Technicon Autoanalyser by the VUB in 1997 and by X. Salgado in 1998. Ammonium and urea were manually analysed on board according to Koroleff (1969) and Goeyens *et al.* (1998), respectively.

Suspended matter for particulate organic carbon (POC) and particulate nitrogen (PN) determinations was collected on precombusted (450 °C) Whatman GF/F filters, dried at 60 °C and stored in polystyrene Petri dishes until analysis in the home lab. Measurements were carried out with a Carlo Erba NA 1500 CN Analyser, after carbonate elimination in HCl saturated vapour.

Experiments for the determination of nitrogen uptake rate were started by addition of labelled (^{15}N, 99%) nitrate, ammonium or urea into 0.7-l polycarbonate incubation bottles. Tracer additions resulted in increases of 0.1 µM for nitrate and 0.05 µM for ammonium and urea, respectively. All incubations were carried out during 6 hours in an incubator thermostated with running seawater and illuminated with an incident radiation of 200 µE m^{-2} s^{-1}. PN, collected on Whatman GF/F filters after incubation, was converted to dinitrogen by a modified Dumas combustion technique and ^{15}N abundance was assayed by emission spectrometry using Jasco NIA-1 and N-151 ^{15}N analysers (Fiedler and Proksch, 1975). Rates of uptake were calculated from the equations of Dugdale and Goering (1967).

For both cruises, Michaelis-Menten kinetics were established for ammonium and nitrate uptake by measuring nitrate uptake rates at increasing nitrate concentrations (+0.7, +2, +4, +8 µM) and ammonium uptake rates at increasing ammonium concentrations (+0.2, +0.4, +0.8, +1.7 µM). At 2 occasions, this was also done for urea uptake rates at increasing urea concentration (+0.2, +0.4, +0.8, +1.7 µM).

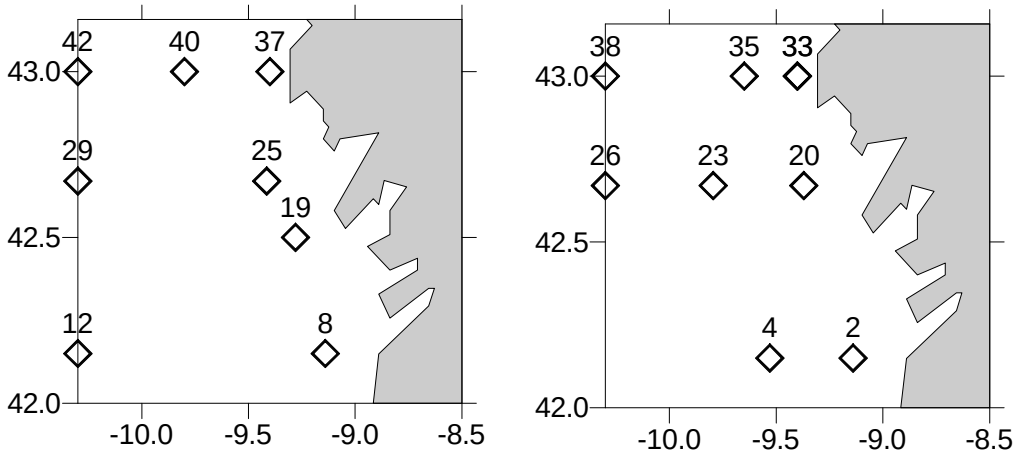

Figure 1: Location and number of the sampling stations in the Iberian Margin for the cruise of 1997 (left) and 1998 (right).

Kinetic parameters, maximum specific uptake rate (ν_{max}) and half-saturation constant (K_s), of the phytoplankton community are determined by fitting the results to the following equation using the computer program Origin (Microcal):

$$n = n_{MAX} \times \frac{C}{C + K_S}$$

with ν_{max}, specific uptake rate and C, substrate concentration (ammonium, nitrate or urea).

The inhibition kinetics of nitrate uptake by ammonium was also determined by measuring nitrate uptake rates at increasing ammonium concentration (+ 0.2, + 0.4, + 0.8, + 1.7 µM). Kinetic inhibition parameters, maximum inhibition (I_{max}) and half inhibition constant (K_i), were determined by fitting the results to the following equation using the computer program Origin (Microcal):

$$n = n_0 \times \left(1 - I_{\max} \times \frac{C}{C + K_I}\right)$$

with ν, specific nitrate uptake rate; ν_0, initial specific nitrate uptake rate (for ammonium concentration = 0) and C, ammonium concentration.

Results and discussion

End June 1997, the situation in the Iberian margin shows no surface upwelling except at one station located at Cape Finisterre, as indicated by the higher nitrate concentration (Figure 2).
The phytoplankton community displayed similar ν_{max} values for ammonium and nitrate uptakes (0.01 to 0.03 h^{-1}) and had a high affinity for both substrates (K_s < 1 µM) (Table 1). No kinetics was established for the uptake of urea during that time. With its high I_{max} and low K_i, the nitrate uptake of the phytoplankton community is also very sensitive to ammonium (Table 1).
At the same time period, the area was characterised by very low nitrate concentrations (< 0.05 µM) and higher ammonium and urea concentrations (> 0.1 µM) (Figure 2).
The resulting N-uptake regime of phytoplankton is that nitrate uptake is limited by the very low nitrate concentrations and higher ammonium concentrations. In these conditions, nitrate uptake is dominated by ammonium and urea uptakes. All *f*-ratio values are below 0.5 which is characteristic of regenerated N uptake conditions (Figure 5).
Only at one station located at the Cape Finisterre, nitrate concentration is higher showing the beginning of an upwelling event. At this station phytoplankton, taking advantage of its high affinity for that substrates, increases its uptake rate and the *f*-ratio increased (Figures 2 and 5).

	1997			1998		
NO_3^- uptake	station	ν_{max} (h^{-1})	K_s (µM)	station	ν_{max} (h^{-1})	K_s (µM)
	19	0.031	1.13	4	0.018	1.49
	25	0.027	0.87	26	0.016	6.85
	29	0.012	0.48	33	0.025	0.44
	40	0.014	0.25	35	0.046	5.63
	42	0.009	0.32			
NH_4^+ uptake	station	ν_{max} (h^{-1})	K_s (µM)	station	ν_{max} (h^{-1})	K_s (µM)
	12	0.025	1.05	4	0.017	0.13
	19	0.029	0.39	23	0.019	0.28
	25	0.017	0.21	26	0.011	0.06
	29	0.016	0.08	33	0.019	0.21
	40	0.031	0.59	35	0.03	0.22
	42	0.015	0.13			
Urea Uptake	station	ν_{max} (h^{-1})	K_s (µM)	station	ν_{max} (h^{-1})	K_s (µM)
				2	0.024	0.80
				38	0.018	0.26
Inhibition of NO_3^- uptake by NH_4^+	station	I_{max} (%)	K_i (µM)	station	I_{max} (%)	K_i (µM)
	12	90	0.04	2	62	0.62
	19	99	0.03	4	70	0.7
	25	98	0.79	20	100	0.37
	29	98	0.02	23	91	0.01
	40	100	0.06	26	91	0.00
	42	98	0.07	33	100	1.46
				35	87	0.17
				38	95	0.03

Table 1: Kinetic parameters of nitrate, ammonium and urea uptake and of the inhibition of nitrate uptake by ammonium determined during the two cruises as explained in "Methods". ν_{max}: maximum uptake rate, K_s: half-saturation constant, I_{max}: maximum inhibition, K_i: half-inhibition constant.

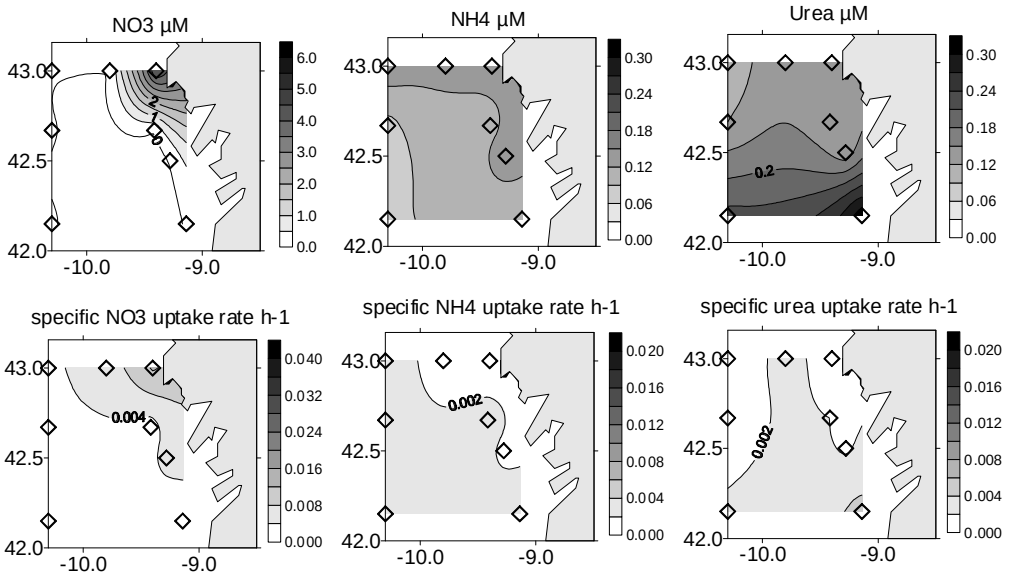

Figure 2: Contour plots of the spatial distribution of nitrate (NO_3), ammonium (NH_4) and Urea concentration and specific uptake rates. Surface waters, Iberian Margin 1997. Diamonds show the station locations.

Begin July 98, the situation evolved towards an extension to the south of the upwelling area starting at Cape Finisterre. Upwelling is also more pronounced at the bottom of the photic layer than in surface. Indeed, we see high nitrate concentration extending along the coast more to the south at the bottom of the photic layer than in the surface layer (Figures 3 and 4).

The phytoplankton community displayed slightly higher ν_{max} for nitrate uptake (0.02 to 0.05 h^{-1}) than in 1997 but had generally a lower affinity for this substrate (K_s > 0.5 µM) (Table 1). This is probably related to the fact that this community is associated to a water mass that is richer in nitrate. There is indeed no need to have a high affinity when concentrations are high.

On the contrary, ammonium uptake showed the same ν_{max} and K_s characteristics as in June 97 and the nitrate uptake was still very sensitive to ammonium (Figure 1).

In the upwelling area, at the bottom of the photic layer, nitrate concentrations are higher than in surface showing that water masses moving upwards in the water column are progressively depleted (Figures 3 and 4). Indeed, one can imagine that the phytoplankton present in the nitrate-rich upwelling water mass progressively receives more light, as that water mass is moving upwards. Taking advantage of the high nitrate concentration available and the increasing light, this phytoplankton community starts to grow, assimilating nitrate.

In the surface waters, nitrate concentrations are still high (>2 µM) while ammonium and urea concentrations are more limited (<0.1 µM) (Figure 3). Accordingly, and in spite of the poor affinity of phytoplankton for nitrate, nitrate uptake was dominant in this area, resulting in *f*-ratio higher than 0.5 (Figure 5).

Outside of this upwelling zone, in the extreme south of the area and to the open sea, the regeneration regime, as observed end June 1997, was still prevailing (Figures 3 and 5).

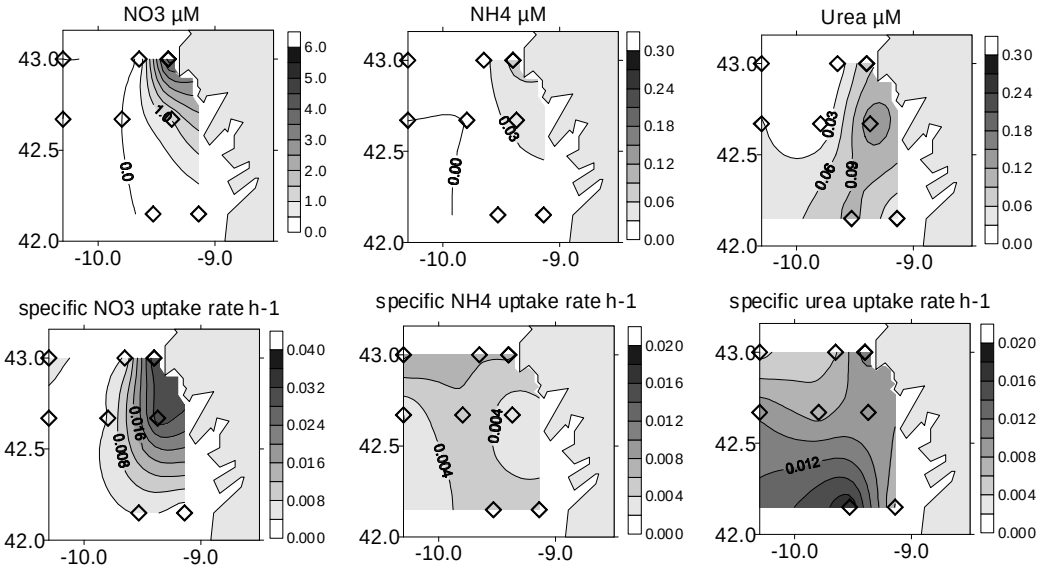

Figure 3: Contour plots of the spatial distribution of nitrate (NO_3), ammonium (NH_4) and Urea concentration and specific uptake rates. Surface waters, Iberian Margin 1998. Diamonds show the station locations.

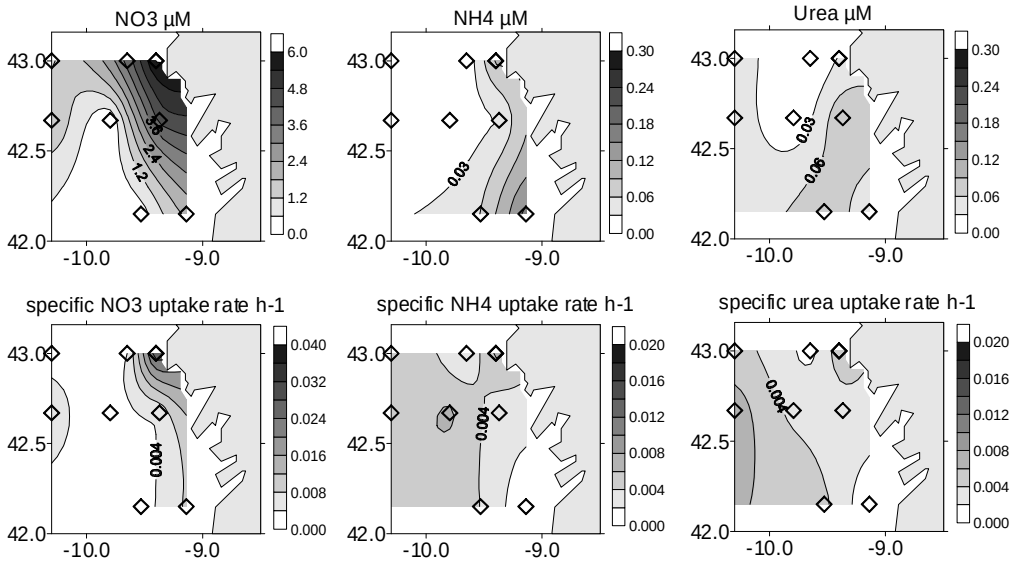

Figure 4: Contour plots of the spatial distribution of nitrate (NO_3), ammonium (NH_4) and Urea concentration and specific uptake rates. Bottom of the photic layer, Iberian Margin 1998. Diamonds show the station locations.

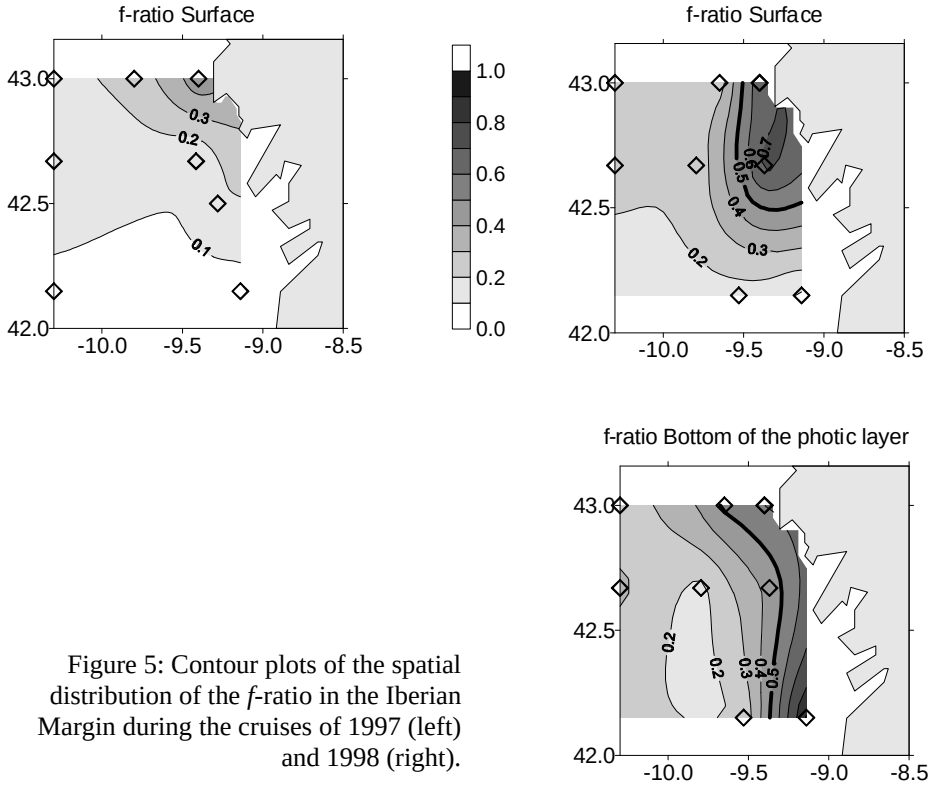

Figure 5: Contour plots of the spatial distribution of the *f*-ratio in the Iberian Margin during the cruises of 1997 (left) and 1998 (right).

Conclusions

With these 2 cruises conducted in the Iberian Margin end June 1997 and begin July 1998, we illustrate the transition between 2 contrasting N-uptake regimes characteristic of a non-upwelling situation and an upwelling situation. Conclusions are summarised in the following Table 2:

	End June 97	Begin July 98	
	No Upwelling	Upwelling area	No upwelling
Affinity for NO_3	High	Low	Low
Affinity for NH_4	High	High	High
Sensitivity of NO3 uptake to NH_4	High	High	High
NH_4 concentration	> 0.1 µM	< 0.1 µM	< 0.1 µM
Urea concentration	> 0.1 µM	< 0.1 µM	< 0.1 µM
NO_3 concentration	> 0.05 µM	> 2 µM	< 0.5 µM
N-uptake regime	Regenerated	New	Regenerated

Table 2: Summary of the major conclusions concerning the relation between uptake kinetics, N concentrations and N-uptake regime in the Iberian margin.

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