Nitrogen uptake of phytoplankton assemblages under contrasting upwelling and downwelling conditions in the Ría de Vigo, NW Iberia

Sophie Seeyave[a,1], Trevor Probyn[b,2], Xosé Antón Álvarez-Salgado[c,3], Francisco G. Figueiras[c,4], Duncan Purdie[a,5], Eric D. Barton[c,6], Michael Lucas[d,7]

[a] National Oceanography Centre, Southampton, University of Southampton, Waterfront Campus, European Way, Southampton SO14 3ZH, UK.
[b] Marine and Coastal Management, Private Bag X2, Rogge Bay 8012, Cape Town, South Africa.
[c] CSIC, Instituto de Investigaciones Marinas (IIM/CSIC), Eduardo Cabello 6, 36208 Vigo, Spain.
[d] Zoology Department, University of Cape Town, Rondebosch 7701, South Africa.

[1] Present address: Plymouth Marine Laboratory, Prospect Place, Plymouth PL1 3DH, United Kingdom; ssve@pml.ac.uk; Tel. +44 (0)1752 633424; Fax. +44 (0)1752 633101.
[2] TrevorP@nda.agric.za
[3] xsalgado@iim.csic.es
[4] paco@iim.csic.es
[5] duncan.purdie@noc.soton.ac.uk
[6] e.d.barton@iim.csic.es
[7] milucas@uct.ac.za

Abstract

The Galician Rías, situated in the Iberian upwelling system, are regularly affected by blooms of toxic dinoflagellates, which pose serious threats to the local mussel farming industry. These tend to occur towards the end of summer, during the transition from upwelling to downwelling favourable seasons, when cold bottom shelf waters in the rías are replaced by warm surface shelf waters. Nitrate, ammonium and urea uptake rates were measured in the Ría de Vigo during a downwelling event in September 2006 and during an upwelling event in June 2007. In September the ría was well mixed, with a downwelling front observed towards the middle of the ría and relatively high nutrient concentrations (1.0-2.6 µmol L^{-1} nitrate; 1.0-5.6 µmol L^{-1} ammonium; 0.1-0.8 µmol L^{-1} phosphate; 2.0-9.0 µmol L^{-1} silicic acid) were present throughout the water column. Ammonium represented more than 80 % of the nitrogenous nutrients, and the phytoplankton assemblage was dominated by dinoflagellates and small flagellates. In June the water column was stratified, with nutrient-rich, upwelled water below the thermocline and warm, nutrient-depleted water in the surface. At this time, nitrate represented more than 80 % of the nitrogenous nutrients, and a mixed diatom assemblage was present. Primary phytoplankton production during both events was mainly sustained by regenerated nitrogen, with ammonium uptake rates of 0.035-0.063 µmol N L^{-1} h^{-1} in September and 0.078-0.188 µmol N L^{-1} h^{-1} in June. Although f-ratios were generally low (<0.2) in both June and September, a maximum of 0.61 was reached in June due to higher nitrate uptake (0.225 µmol N L^{-1} h^{-1}). Total nitrogen uptake was also higher during the upwelling event (0.153-0.366 in June and 0.053-0.096 µmol N L^{-1} h^{-1} in September). Nitrogen uptake kinetics demonstrated a strong preference for ammonium and urea over nitrate in June. This study underlined the importance of regenerated production (including organic nitrogen) in the Ría de Vigo in supporting both harmful algal bloom communities during the downwelling season, but also (to a lesser extent) diatom communities during stratified periods of weak to moderate upwelling.

Key words: harmful algal blooms, new production, phytoplankton ecology, regenerated production Ria de Vigo Spain, upwelling.

1. Introduction

Nitrogen is generally recognised as being the nutrient limiting primary production in coastal marine ecosystems (Dugdale, 1967; Ryther & Dunstan, 1971; Howarth & Marino, 2006). Furthermore, nitrogen inputs to coastal waters are increasingly thought to be implicated in the reported global increase in Harmful Algal Blooms (HABs) (Anderson et al., 2002), in particular due to the increase in dissolved organic nitrogen (Glibert et al., 2006). Nitrogen uptake measurements provide valuable information on the relative contributions of new and regenerated forms of nitrogen to primary production. Such measurements are particularly important for understanding the ecology of HABs, especially in upwelling systems, which are characterised by large fluctuations in nitrate (NO_3^-) concentrations. A number of nitrogen uptake measurements have been made in the California and Benguela upwelling systems (Dugdale et al., 1990; Probyn, 1992; Dugdale et al., 2006; Seeyave et al., 2009). In the Iberian upwelling system some previous measurements of nitrogen uptake and regeneration have been reported in shelf waters (Slawyk et al., 1997, Joint et al., 2001; Bode et al., 2004a,b; Bode et al., 2005) and a few reported in the rias (Bode et al., 2005; Varela et al., 2003). Published *f*–ratios have been calculated either from direct measurements using ^{15}N (but most of these have not included urea), or estimated from the NO_3^- flux into the euphotic zone caused by upwelling (Alvarez-Salgado et al., 2002), or based on satellite-derived primary production estimates (Aristegui et al., 2009). The relatively low seasonally-averaged ratios (0.20-0.33) derived from the latter study were attributed to low continental nutrient inputs, low nutrient concentrations in the source water, low average coastal winds and the importance of heterotrophy and therefore nutrient regeneration (Aristegui et al., 2006).

The Rías Baixas of Galicia are large coastal indentations situated on the north-west coast of the Iberian Peninsula, within the Iberian upwelling system (Figure 1). They are the largest producer of mussels worldwide, representing 40 % of European production and 15 % of world production, with a first sale value of 80 million US dollars (Labarta et al., 2004). The regular occurrence of HABs in the rías is therefore a major concern for the industry (Fraga, 1989), with total losses to the shellfish industry attributed to these toxic outbreaks estimated at 10-20 million euros per year (Hoagland & Scatasta, 2006).

Upwelling occurs from approximately March to September when northerly winds prevail, whereas the rest of the year is characterised by southerly winds and downwelling (Fraga, 1981). Short-term changes in wind direction generally drive upwelling/relaxation cycles of 1-2 weeks (Blanton et al., 1987), which in turn drive the subtidal circulation in the rías. During upwelling, positive estuarine circulation forces upwelled water from the shelf into the rías along the bottom while surface water flows out of the rías. During downwelling, surface water flowing into the rías converges with water flowing out and forms a downwelling front, with the outflow occurring at depth (Figueiras et al., 1994). During upwelling, the injection of nutrients into the rías stimulates phytoplankton growth and the resulting biomass is then exported out of the ría, where it may sink and become remineralised, and can later be re-injected into the rías along with the upwelled nutrients (Alvarez-Salgado et al., 1993). This “secondary remineralisation” allows the rías to support very high rates of primary production, particularly towards the end of the upwelling season (Alvarez-Salgado et al., 1997).

The abundance of diatoms is positively correlated to upwelling (Figueiras & Rios, 1993), and HABs tend to occur during downwelling events in late summer-early autumn (Fraga et al., 1988; Figueiras et al., 1994). The horizontal distribution of diatoms and dinoflagellates also reflects the intensity of upwelling or stratification along the rías, with diatoms dominating towards the interior, where upwelling is strongest, whereas dinoflagellates tend to occur in the outer, more stratified parts of the rías (Tilstone et al., 1994). The apparent increase in blooms of certain HAB species in the last 4 decades has been attributed to enhanced eutrophication of the rías as a result of increased sewage discharges, expansion of the mussel farms and increases in forest fires (Wyatt & Reguera, 1989), as well as a decrease in the duration and average intensity of the upwelling season (Alvarez-Salgado et al., 2008).

No consensus has yet been reached regarding the mechanisms leading to HAB development in the rías (Pitcher et al., 2010). Some studies have supported the hypothesis of advection of offshore populations into the rías (Fraga et al., 1993; Sordo et al., 2000), whereas others have suggested *in situ* HAB development (Fraga et al., 1990; Figueiras & Pazos, 1991a; Pazos et al., 1995; Figueiras et al., 1998). In any case, downwelling is thought to favour motile species such as *Gymnodinium catenatum*, which can maintain themselves in the surface layer (Fraga et al., 1988; Figueiras et al., 1994; Fermin et al., 1996). HABs can also develop during weak to moderate upwelling, which raises the nutricline without being sufficiently intense to mix the entire water column (Figueiras & Rios, 1993). In this situation, dinoflagellates can undertake diel vertical migrations that allow them to exploit the high nutrient concentrations at the nutricline during the night and photosynthesise during the day in the surface layer (Figueiras & Fraga, 1990; Fraga et al., 1992; Fraga et al., 1999). Using a box model, Rios et al. (1995) suggested that diatom growth was sustained by nitrate during the upwelling season, whereas autumn dinoflagellate populations relied on ammonium as their main source of nitrogen.

The aim of this study was to characterise the nitrogen nutrition of phytoplankton assemblages during upwelling and downwelling conditions in the Ría de Vigo, using the ^{15}N stable isotope tracer technique. These new measurements not only provide us with valuable information on the nutrient biogeochemistry of the ría, but also on the nitrogen sources that are utilised by HAB communities in these embayments.

2. Materials and methods

2.1. Sampling

Sampling was carried out on-board the *R/V Mytilus*, as part of the Galician programme CRIA (Circulation in a RIA). CRIA consisted of two parts, CRIA I targeting the downwelling, "HAB season" (26 to 30 September 2006) and CRIA II targeting the upwelling, "diatom" season (25 to 28 June 2007). Spatial surveys of temperature, salinity, chlorophyll-a (hereafter chl-a) fluorescence and turbidity were carried out using a lightweight towed undulating vehicle, MiniBAT FC60 (Ocean Scientific International Ltd.), fitted with an Applied Microsystems Ltd. (AML) Micro CTD, a Wet Labs WetStar fluorometer and a Campbell Scientific OBS 3 turbidity sensor.

Continuous measurements of temperature, salinity and chl-a fluorescence were also made on surface water collected underway (2.5 m depth). Continuous vertical profiles of these parameters were carried out at various stations along the ría (Figure 1) using a Seabird Electronics 911+ CTD system coupled with a Seatech fluorometer mounted on a sampling rosette fitted with 12-L Niskin bottles. Seawater samples were collected from 3-6 depths in acid-washed and Milli-Q rinsed 5- or 10-L carboys for routine chl-a and nutrient analyses among other parameters. These were stored in the dark until transported ashore (within <5 hours). At some stations water was only collected from the underway supply (2.5 m). Water for ^{15}N incubations and associated nitrate, ammonium and urea analyses was collected from ~3 m in both sampling periods and occasionally from the chl-a maximum (10-12 m) in 2007.

Wind data were obtained from the MeteoGalícia weather station (http://www.meteogalicia.es) on Islas Cíes in September 2006. In June 2007 the data were obtained from the Seawatch buoy off Cabo Silleiro that is maintained by Puertos del Estado (http://www.puertos.es/es/oceanografia_y_meteorologia/banco_de_datos/ viento.html). Locations of both weather stations are shown in Figure 1.

Figure 1

2.2. Nutrients and phytoplankton

Nutrient samples (nitrate NO_3^-, nitrite NO_2^-, ammonium NH_4^+, phosphate HPO_4^{2-} and silicic acid $Si(OH)_4$) were analysed within ~6 h of being collected in both years using an Alpkem autoanalyser following the method of Hansen & Grasshoff (1983) as modified by Mouriño & Fraga (1985) and Álvarez-Salgado et al. (1992). Ammonium was also measured using the fluorometric (o-Phthaldialdehyde, OPA) method of Holmes et al. (1999) for the samples that were incubated for NH_4^+ uptake determinations. After reagent addition, samples were incubated overnight in the dark and fluorescence was determined on a Turner Designs TD700 fluorometer. Urea was determined manually on fresh samples following the diacetylmonoxime thiosemicarbazide method of Mulvenna & Savidge (1992) adapted to room temperature using reaction times of 72-96 h (Goeyens et al., 1998) in 2006, but following the method of Grasshoff et al. (1999) in 2007. Precisions were <0.05 μmol N L^{-1} for all nutrients.

Chl-a concentrations were determined by fluorometry after filtering 100 mL of seawater through 25 mm GF/F filters (Welschmeyer, 1994). Samples for phytoplankton preserved in Lugol's iodine were settled overnight and counted under an inverted microscope and identified to species level, when possible, as previously described in Crespo et al. (2006).

2.3. Nitrogen uptake

For each incubation, water was decanted into two 0.5-L and one 1-L Nalgene polycarbonate bottles. The 0.5-L samples were inoculated with stock solutions of $K^{15}NO_3$ and urea $[CO(^{15}NH_2)_2]$ and the 1-L sample with $^{15}NH_4Cl$. All stock solutions had a concentration of 1 μmol N mL^{-1} and ^{15}N purities were 99.6, 99.1 and 99.7 % for $K^{15}NO_3$, $CO(^{15}NH_2)_2$ and $^{15}NH_4Cl$, respectively. The volume of ^{15}N spike in each case aimed to achieve a final concentration of approximately 10 % of the ambient nutrient concentration. However, at very low NO_3^- concentrations (<0.05 μmol L^{-1}), the aqueous enrichments were sometimes as high as 93 %. Therefore the correction for high spike addition of Eppley et al. (1977) was applied to some of the uptake rates (see below). Immediately after spiking the NH_4^+ sample, exactly 0.5 L was transferred to a separate 0.5-L polycarbonate bottle for incubation, while the remaining 0.5L was filtered through a 47-mm Whatman precombusted GF/F filter to measure time zero aqueous ^{15}N enrichment (R_0) in the filtrate. Subsamples were also taken from the filtrate for later analyses of ambient NO_3^-, NH_4^+ and urea.

Samples were incubated in a grey plastic box placed on-deck, maintained at *in situ* temperature by a flow of surface water. For subsurface samples, 50 % shading was provided by a nylon mesh. Incubations lasted for between 1h30 and 2h in 2006 and 2h30-3h in 2007 and took place between 10:00 and 14:00. Incubations were terminated by filtration onto pre-combusted GF/F filters, which were then rinsed with filtered seawater and dried at 60 ºC overnight. Filtration of $^{15}NO_3^-$ and ^{15}N-urea spiked samples was onto 25-mm Whatman GF/F filters, whereas the $^{15}NH_4^+$ spiked samples were filtered onto 47-mm Whatman GF/F filters using a different system that allowed clean collection of the filtrate for later isotopic dilution analyses. Aqueous enrichment at the start and end of the incubations was measured on filtrates from the start and end of each incubation. These were frozen for later recovery of aqueous NH_4 by diffusion onto ashed halved 25-mm

GF/F filters (Probyn 1987). Filters were processed and analysed in the same way as the ^{15}N uptake samples to determine the parameters R_0 and R_t in Equation 3 of Glibert et al. (1982).

Uptake rates were calculated from equations 1-3 of Dugdale & Wilkerson (1986) corrected for isotopic dilution of $^{15}NH_4^+$ by regenerated $^{14}NH_4$ according to Glibert et al. (1982) in September 2006, and for the NH_4^+ samples in June 2007. The equation of Eppley et al. (1977) was applied to all NO_3^- and urea measurements in June 2007 for consistency. Ammonium recycling was calculated from the Blackburn-Caperon model (Blackburn, 1979; Caperon et al., 1979) since the NH_4^+ concentration always changed during the incubation.

A nitrogen uptake kinetics experiment was carried out on 28 June 2007, on water collected from 2 m depth at station B3. Water collected from the CTD was decanted into eighteen 75-mL Sterilin Iwaki culture flasks. Six 75-mL samples were spiked with different volumes of 10 % enriched 1 mmol N L^{-1} NO_3^- solution, another 6 with 10 % enriched 1 mmol N L^{-1} NH_4^+ solution and the remaining 6 with 10 % enriched 2 mmol N L^{-1} urea solution to obtain final concentrations between 0.6 and 30 µmol N L^{-1} for NO_3^-, between 0.3 and 30 µmol N L^{-1} for NH_4^+ and between 0.2 and 60 µmol N L^{-1} for urea. The experiment was carried out in the same incubator as the standard uptake incubations and the incubation lasted 2h30. Incubations were terminated by filtration onto 25-mm precombusted GF/F filters and the filters were processed in the same way as for the standard uptake experiments. The PN-specific uptake rates were plotted against concentration of each nitrogen species and fitted to the Michaelis-Menten equation for uptake kinetics using SigmaPlot (Jandel Scientific) to derive the parameters K_m (half-saturation constant) and V_{max} (maximum uptake rate).

3. Results

3.1. Hydrographic setting

Figures 2 & 3

In September, southerly winds were predominant (Figure 2a) and the water column was relatively well mixed, as a consequence of downwelling (Figure 3b,c). The downwelling front, indicated by vertical temperature and salinity isolines, was observed in the vicinity of station B2 (Figure 3b,c, see also Romera-Castillo et al., 2011). By the end of the survey, the water column had warmed and salinity had dropped, and both horizontal and vertical gradients were weak (Figure 3e,f). In June, no water was upwelled during the 2 weeks prior to the survey, due to predominantly southerly winds. Winds switched to upwelling-favourable northerly flow during the 3 days preceding the survey, although with relatively weak components (< 4 m s^{-1}). Thus upwelling was not strong enough to mix the entire water column, and consequently the surface layer remained stratified. A thermocline was observed between 10 and 20 m (Figure 3h,i), showing positive estuarine circulation, with a warm, less saline surface layer flowing out of the ría (T = 18-20 °C, S = 33.1-34.9) and colder, more saline water (T = 13-15 °C, S = 35.4-35.9) flowing into the ría at depth. By 28 June the thermocline was uplifted to ~5-10 m following a pulse of upwelling, with surface temperatures of ~18 °C and salinities of 33.0-34.7 (Figure 3k,l).

3.2. Nutrients

Figure 4

In September, NO_3^- concentrations were relatively homogeneous throughout the water column, displaying an increase with depth of <1 µmol L^{-1} (Figure 4). Little horizontal variation was observed at the start of the survey, however at the end concentrations were up to 1.3 µmol L^{-1} higher at B5 relative to B2. Ammonium concentrations were highest at B2, with concentrations ranging from 1.1 to 4.7 µmol L^{-1} at the surface, whereas at B3 they ranged from 0.8 to 2.1 µmol L^{-1} and at B5 they remained ~0.9 µmol L^{-1}. Concentrations increased with depth, to maxima of 5.3, 5.5 and 4.3 µmol L^{-1} at B5, B3 and B2, respectively. Phosphate and $Si(OH)_4$ profiles were very similar to NH_4^+ profiles, displaying the same spatial and temporal variations (data not shown).

Figure 5

In June, all nutrient concentrations were very low at the surface at the start of the survey (<0.05 µmol L^{-1} NO_3^-, ≤0.1 µmol L^{-1} NH_4^+, Figure 5). Concentrations increased with depth to maximum values of 11.2 µmol L^{-1} NO_3^- and 3.6 µmol L^{-1} NH_4^+. By the end of the survey, concentrations had increased, consistent with a rising pycnocline caused by upwelling. Maximum surface concentrations increased to 5.5 µmol L^{-1} NO_3^- and 1.9 µmol L^{-1} NH_4^+.

3.3. Chl-a and phytoplankton community structure

Figure 6

Chl-a concentrations were relatively low in September 2006 (Figure 6a,b,c), particularly at the start of the survey (<5 µg L^{-1}). At this time there was little horizontal variation between stations B5 and B2. By 30 September chl-a had increased and showed a horizontal gradient, with maximum concentrations of 5.8, 7.1 and 8.1 µg L^{-1} at B5, B3 and B2, respectively (Figure 6). At the start of the survey, chl-a was relatively homogeneous throughout the water column, whereas on 30 Sept, chl-a concentrations had increased above initial values in the upper 15-30 m. In June 2007, a pronounced maximum developed with upwelling at ~10 m depth (Figure 6d,e,f). Maximum concentrations were 10.5, 15.8 and 6.5 µg L^{-1} at B5, B3 and B2, respectively on 25 June. On 28 June they had increased at B5 and B2, to 25.8 and 25.1 µg L^{-1}, respectively, whereas at B3 concentrations remained largely unchanged. Surface concentrations were not significantly different from those measured during the September survey, although concentrations in the sub-surface maximum at 10 m were significantly higher (Mann-Whitney U-test, $p < 0.05$).

Figure 7

In September, the phytoplankton community averaged over the top 10 m was numerically dominated by a mixture of dinoflagellates (up to 49 %) and small flagellates (up to 79 %), whereas the proportion of diatoms was <21 % (Figure 7a,b). Maximum concentrations were 0.14 x 10^6 for diatoms, 0.29 x 10^6 for dinoflagellates and 0.40 x 10^6 cells L^{-1} for small flagellates. The most abundant dinoflagellate species were *Cachonina niei*, *Ceratium fusus*, *Gymnodinium* spp. and *Prorocentrum* spp. (data not shown). Toxic species were present, but never numerically dominant. They were generally observed at the outer station B5 at the start of the survey then later appeared at the inner station B2. Maximum concentrations were 3.9 x 10^3 cells L^{-1} for *Dinophysis acuta,* 4.5 x 10^3 cells L^{-1} for *Dinophysis caudata* and 20.2 x 10^3 cells L^{-1} for *Gymnodinium catenatum* (data not shown).

In June, the phytoplankton community in the top 10 m at stations B5, B3 and B2 was dominated by diatoms, which represented 95 to 99 % of total phytoplankton cells, with concentrations as high as 9.5 x 10^6 cells L^{-1} (Figure 7c,d). Dinoflagellate concentrations only reached a maximum of 0.12 x 10^6 cells L^{-1}, representing up to 5 % of total phytoplankton cells, whereas small flagellates reached 0.04 x 10^6 cells L^{-1} (2 % of total cell concentration. The main diatom species were *Chaetoceros* spp., *Leptocylindrus* spp., *Nitzschia* cf. *americana* and *Skeletonema costatum*. *Pseudo-nitzschia* cf *delicatissima* and *P.* cf *seriata*, two groups potentially including toxic species, and the toxic dinoflagellate *Dinophysis acuminata* were also present. *P.* cf *seriata* was most abundant on 25 June (maximum 237.6 x 10^3 cells L^{-1} at B3), whereas *P.* cf *delicatissima* was most abundant on 28 June (maximum 72.8 x 10^3 cells L^{-1} at B5). *Dinophysis acuminata* was most abundant at B3, where it formed a sub-surface maximum at 10 m (15 x 10^3 cells L^{-1}) on 25 June (data not shown).

3.4. Nitrogen uptake

Table 1

In September, nitrogen was taken up predominantly in the form of NH_4^+ [52 to 74 % total ρ(N)], followed by urea (15 to 32 %), whereas ρ(NO_3^-) contributed <20 % (Table 1). Total ρ(N) showed little variation between stations and over time, except at B2 where it increased by 58 % between 26 and 29 September. Ammonium uptake ranged from 0.035 to 0.063 μmol N L^{-1} h^{-1}, ρ(urea) from 0.008 to 0.028 μmol N L^{-1} h^{-1} and ρ(NO_3^-) from 0.005 to 0.013 μmol N L^{-1} h^{-1}. Hourly-scaled *ƒ*-ratios were very low, ranging from 0.05 to 0.19. *ƒ*-ratios were lowest at the inner station B2 (<0.1), due to the very high contribution of ρ(NH_4^+), and increased seaward to values ≥0.1 at B3 and B5.

In June, NH_4^+ was also an important source of nitrogen in the surface, where it represented up to 89 % of total ρ(N), with uptake rates ranging from 0.078 to 0.188 μmol N L^{-1} h^{-1}. During stratified periods when surface NO_3^- was depleted, ρ(NO_3^-) was lower than ρ(NH_4^+) and often lower than ρ(urea). Surface NO_3^- uptake rates ranged from 0.001 to 0.043 μmol N L^{-1} h^{-1}, representing 8-26 % total ρ(N). Unfortunately, our sampling "missed" the upwelling pulse that occurred at the end of the survey, since this was localised around station B2, where there was no surface measurement of ρ(N). However, ρ(NO_3^-) was measured at 12 m at B2, following the upwelling pulse, and in this case it was 2-fold higher than ρ(NH_4^+), reaching 0.225 μmol N L^{-1} h^{-1}. Urea was also an important source of nitrogen, particularly at the surface at B3, with uptake rates reaching 0.161 μmol N L^{-1} h^{-1}. Highest total ρ(N) was measured at the central stations B3 and B2 (0.153-0.366 μmol N L^{-1} h^{-1}), whereas it was 0.153 and 0.158 μmol N L^{-1} h^{-1} at the outer and inner stations, respectively. Total ρ(N) was significantly higher (on average 4-fold) than in September (Student's t-test, $p < 0.0001$) and PN-specific uptake rates (V) were ~5-fold higher (data not shown). This increase in ρ(N) was significant for all 3 nitrogen sources (Mann-Whitney U-test, $p < 0.05$); it was 3-fold for ρ(NH_4^+), 7-fold for ρ(NO_3^-) and 6-fold for ρ(urea). *ƒ*-ratios were generally lower than expected for the upwelling season, as they were always <0.2 in the surface. The *ƒ*-ratio reached 0.61 at 12 m following the upwelling pulse on 28 June.

Figure 8

The nitrogen uptake kinetics experiment carried out on 28 June demonstrated a very strong preference for NH_4^+ relative to the other sources, with the maximum PN-specific uptake (V_m) being 5-fold higher than for urea and 13-fold higher than for NO_3^- (Table 2, Fig. 8). The half-saturation constant K_s displayed exactly the same differences between nitrogen sources, since K_s and V_m were positively correlated. Thus, the difference in the affinity constant ($\alpha = V_m/K_s$) was less pronounced between nitrogen sources, although it was still higher (40 %) for NH_4^+, indicating that this source was also preferred at limiting concentrations.

Ammonium regeneration rates [r(NH_4^+)] were highly variable, ranging from 0.034 to 0.451 µmol N L^{-1} h^{-1} in September and from 0.002 to 0.235 µmol N L^{-1} h^{-1} in June (Table 1). Although r(NH_4^+) was on average higher in September (0.161 ± 0.060 µmol N L^{-1} h^{-1}) relative to June (0.112 ± 0.032 µmol N L^{-1} h^{-1}), this difference was not statistically significant (Student's t-test, $p > 0.05$). There was no significant correlation between NH_4^+ uptake and regeneration. Regeneration rates were generally higher than uptake rates in the September survey, but mostly lower than or similar to uptake rates during the June survey.

Table 2

4. Discussion

The hydrographic conditions that prevailed in the Ría de Vigo in September 2006 and June 2007 were typical of the downwelling and upwelling seasons, respectively. The phytoplankton communities present during the two surveys were also fairly typical of the downwelling and upwelling seasons, whereby the downwelling community was dominated by a mixture of dinoflagellates and flagellates, and the upwelling community was dominated by diatoms. This is consistent with the trend observed by Crespo et al. (2006) in a 1-year time-series of phytoplankton community structure in the Ría de Vigo. However, Crespo et al. (2006) reported a much larger dinoflagellate bloom than in this study. The association of diatoms with upwelling is regularly observed in the Iberian (Figueiras & Rios, 1993), NW African (Estrada & Blasco, 1985), Benguela (Fawcett et al., 2007) and California currents (Lassiter et al., 2006). In the Iberian system, this association has been described by a linear correlation between diatom biovolume (or biomass) and the upwelling index (Figueiras & Rios, 1993).

Since HAB species were generally a small component of the phytoplankton community, it was difficult to determine whether they displayed particular nitrogen uptake strategies or not. However, the occurrence of *Dinophysis acuta*, *D. caudata* and *Gymnodinium catenatum* exclusively during the downwelling season, concurrently with high NH_4^+ concentrations and regeneration rates and very low f-ratios, suggests that their growth was supported mainly by regenerated NH_4^+. Their abundance in terms of biomass may have also been higher than that suggested by their numerical abundance, since these species have large cell sizes. This study showed that urea was also a significant source of nitrogen supporting the growth of these dinoflagellate communities.

The ‘typical’ nitrogen uptake scenario expected for upwelling systems is the dominance of new production (f-ratio >0.5) during upwelling events and a switch to regenerated production (f-ratio <0.5) during downwelling (or upwelling relaxation) events (Dugdale et al., 1990). This relationship between upwelling strength and f-ratio has been reported for the Benguela (Seeyave et al., 2009) and in the Iberian (Álvarez-Salgado et al., 2002) upwelling systems. However, the results from the present study in the Ría de Vigo have shown that hourly-scaled f-ratios were generally <0.5 during both the upwelling and downwelling periods and that NH_4^+ was the principal source of available nitrogen. However, an ƒ-ratio >0.5 was measured on one occasion, at 12 m depth, when a pulse of upwelling occurred and the NO_3^- concentration increased to 6.8 µmol N L^{-1} at the thermocline, stimulating $\rho(NO_3^-)$, which increased to >0.2 µmol N L^{-1} h^{-1}. Unfortunately, no subsequent measurements were performed, therefore the timing and spatial extent of the sampling may have missed some high $\rho(NO_3^-)$ episodes that would have been more typical of a moderate to strong upwelling scenario.

Estimates of new production for the shelf region of the Iberian upwelling system produced an upwelling season-averaged ƒ-ratio of 0.20 over the shelf and 0.33 within the rías (Arístegui et al., 2009), both indicating a high proportion of regenerated production (Álvarez-Salgado et al., 2002). Although these results are not directly comparable with those obtained in the present study due to differences in methods and in the spatial and temporal scales on which the estimates are based, all results suggest that phytoplankton growth during the upwelling season is not supported exclusively by NO_3^-. Similarly low ƒ-ratios (0.03 - 0.38) were measured in the nutrient-impoverished surface layer in the Portuguese upwelling area off Cape Sines, whereas higher ratios (0.52 - 0.82) were measured below the nutricline (Slawyk et al., 1997), although this study did not measure urea uptake, which would probably have lowered the ratios. Another study on the north-west Iberian shelf measured ƒ-ratios between 0.5 and 0.7 in an upwelling region, and around 0.4 (without urea) and <0.1 (with urea) in an oligotrophic offshore filament (Joint et al., 2001). Closer to this study region, Bode et al. (2004a) measured ƒ-ratios of 0.6 and 0.7 (averaged for low- and high-production periods, respectively) in 80m-deep water off the coast of A Coruña.

A very limited number of N uptake measurements have been conducted actually within the Galician Rias. ƒ-ratios reported by Bode et al. (2005) for the Ria de Ferrol were higher than in the present study during both upwelling and downwelling seasons, between 0.6 and 0.9 at the surface in both July and September, although water-column integrated values were lower in September (0.3-0.5), due to both increasing $\rho(NH_4^+)$ and decreasing $\rho(NO_3^-)$ with depth (A. Bode pers. comm., after revision of data from Bode et al., 2005). If ρ(urea) had been included in their ƒ-ratio calculations, these could have been significantly lower, particulary since they measured higher dissolved organic nitrogen

(DON) than dissolved inorganic nitrogen (DIN) concentrations during the summer months, underlining the potential importance of DON as a source of nitrogen to phytoplankton. Urea can be an important source of nitrogen for phytoplankton growth, as shown in the present study where ρ(urea) was on average 27 ± 16 % of total nitrogen uptake, and as shown by the difference in the *ƒ*-ratios published by Joint et al. (2001) with and without including urea uptake in the calculation. Bode et al. (2004a) measured urea uptake on 3 occasions during their study off A Coruña, and although the *ƒ*-ratio did decrease on one of these occasions to ~0.5, the difference was less pronounced than for the current study or for Joint et al. (2001). The relative importance of urea as a source of N for phytoplankton growth in the Iberian upwelling system therefore appears highly variable, and difficult to resolve due to the paucity of measurements. The *ƒ*-ratios in the present study were low compared to the California upwelling system (Dugdale et al., 2006), and towards the lower end of the range published for the Benguela (Probyn, 1992). They were however comparable to values measured during upwelling relaxation in the Benguela (Seeyave et al., 2009). This could be due to the topographic difference between the ria and open shelf waters where measurements were made in the Benguela and California systems. This has implications for the hydrography and nutrient environments of the different systems, whereby the rías can remain stratified during weak to moderate upwelling, particularly in its outer reaches, leading to surface nutrient depletion, whereas upwelling on the open shelf tends to cause stronger mixing and higher surface nutrient concentrations (as shown by the higher *ƒ*-ratios reported by Joint et al. (2001) and Bode et al. (2004a) during upwelling).

PN-specific rates were particularly high in June (0.026 ± 0.004 h^{-1}) relative to September (0.005 ± 0.001 h^{-1}) and relative to values obtained in the Benguela (0.006 ± 0.0004 $^{-1}$) (unpublished data). However, due to the relatively low biomass, this did not lead to higher ρ(N), which was of the same order of magnitude as in the Benguela. According to Dugdale et al. (1990), specific nitrate uptake [$V(NO_3^-)$] is a function of ambient NO_3^- and if biomass accumulation occurs as a result of the “shift-up” in $V(NO_3^-)$, then $\rho(NO_3^-)$ will increase non-linearly with $V(NO_3^-)$. Here, $V(NO_3^-)$ and $\rho(NO_3^-)$ were linearly correlated (data not shown), indicating that no biomass accumulation had occurred. This low realisation of potential new production was also observed at Point

Conception in the California current and attributed to strong advection and turbulence (Dugdale et al., 2006). In this study, although the water column was stratified, the positive estuarine circulation that prevails during upwelling causes organic matter export out of the ría (Estrada, 1984; Figueiras et al., 1994), which could explain the low biomass accumulation. Grazing, which is particularly high in the rías due to mussel cultivation (Fernández-Reiriz et al., 2007) and the presence of microheterotrophs during summer (Figueiras & Pazos, 1991b), will also strongly control phytoplankton biomass (Teixeira et al., 2011; Bode et al., 2004b).

Nitrate uptake rates in June were similar to those reported by Bode et al. (2004a), but one order of magnitude higher than those measured in the Ría de Ferrol in both June and September [A. Bode, pers. comm., revision of data originally published in Bode et al., 2005]. But it must be noted that in the Ría de Ferrol, measurements were based on 24 h incubations, which therefore included dark uptake, unlike in this study. This could contribute significantly to the difference in uptake rates. In contrast, nitrate uptake was several-fold lower than in the California (Dugdale et al., 2006), Benguela (Probyn, 1992) and the Cap Blanc upwelling region (Dugdale et al., 1990). This could be an effect of the normalisation of nitrogen uptake rates to particulate nitrogen and possible abundance of detrital particulate nitrogen in the Ría. Normalisation to chl-a would no doubt reduce these differences since chl-a concentrations in the Ría were lower than in the other upwelling systems.

Ammonium uptake rates were within the range of those measured by Bode et al. (2004a) and several-fold higher than those measured by Bode et al. (2004b). During the downwelling event, $r(NH_4^+)$ was generally higher than $\rho(NH_4^+)$ whereas during the upwelling event it was generally lower. The higher regeneration rates during downwelling are consistent with previous studies (e.g. Varela et al., 2003) and with the higher ambient NH_4^+ concentrations measured during this period. However, Varela et a. (2003) reported that uptake and regeneration were coupled and thus NH_4^+ did not accumulate in the coastal area off the Ria de Vigo. Their results, however, were depth-averaged and included a station outside the mouth of the ria, which could explain this difference. Urea uptake rates have been measured in very few other studies. They were up to two orders of magnitude higher than those reported by Bode et al. (2004a), who despite these low rates found that ρ(urea) exceeded ρ(NH_4^+) (but not ρ(NO_3^-)) on the occasions when both were measured. Generally, N uptake rates were at least one order of magnitude higher than those reported for experiments conducted further offshore on the continental shelf (Slawyk et al., 1997). Bode et al. (2005) measured higher ρ(NO_3^-) relative to ρ(NH_4^+) in July, when NO_3^- concentrations were higher than NH_4^+ (although still <1 μmol N L^{-1}), but the opposite in September, when NH_4^+ concentrations were higher, suggesting that the source of nitrogen used was determined by the relative concentration of each nitrogen source, rather than by preference. In the present study, NH_4^+ appeared to be taken up preferentially to NO_3^- in both seasons, and irrespective of ambient concentrations of each N source. Nitrate uptake was particularly low at high NH_4^+ concentrations (>0.5 μmol N L^{-1}), suggesting that ρ(NO_3^-) was inhibited by NH_4^+. Both preferential uptake of NH_4^+ relative to NO_3^- and inhibition of NO_3^- uptake by NH_4^+ have been widely reported (see review by Dortch (1990)). These phenomena are linked to the lower energetic cost of NH_4^+ assimilation relative to NO_3^-, which must first be reduced intracellularly to NO_2^- then to NH_4^+ before the latter can be synthesised into amino acids and proteins. Nitrogen uptake kinetics parameters can indicate preference, whereby a higher V_m for NH_4^+ than for NO_3^- would suggest preference for NH_4^+ over NO_3^-. The presence of NH_4^+ in NO_3^- kinetics experiments, however, can potentially cause inhibition of NO_3^- uptake and bias the results (Dortch, 1990; Collos et al., 2004).

To address this, a nitrogen uptake kinetics experiment was carried out on a mixed diatom assemblage in June, with an ambient NH_4^+ concentration of 0.33 μmol N L^{-1}. This was below the range of concentrations generally thought to inhibit NO_3^- uptake (Dortch, 1990), therefore the obtained ratio V_m(NH_4^+): V_m(NO_3^-) of 12.8 should indicate a genuine preference for NH_4^+, rather than inhibition. The ratio of α(NH_4^+): α(NO_3^-) showed that NH_4^+ was also preferred at limiting concentrations, although the preference was more strongly expressed at saturating concentrations. Urea was also preferred over NO_3^- at saturating concentrations, confirming the potential importance of regenerated nitrogen for phytoplankton growth in this system.

The V_m(NH_4^+): V_m(NO_3^-) ratio was several-fold higher than in other upwelling systems (Table 2), due to the particularly high V_m(NH_4^+) measured in the present study. This value was more than one order of magnitude higher than any V_m reported in Table 2, although V_m values of a similar order of magnitude have been measured in cultures (Cochlan et al., 2008; Yamamoto et al. 2004). There do not appear to be any methodological reasons that could have been responsible for these very high uptake rates, and the uptake rates did follow Michaelis-Menten kinetics, ruling out the possibility that the samples could have been contaminated. Furthermore, the incubation length was sufficiently long to avoid the bias introduced by “surge uptake” on the calculated uptake rates (Collos et al., 1997). Therefore, it seems that the phytoplankton population present was genuinely capable of very high nitrogen (and particularly NH_4^+) uptake, if the substrate was present in sufficiently high concentrations. Furthermore, the $\rho(NH_4^+)$ values for this experiment were 0.10-0.99 µmol N L^{-1} h^{-1}, which was similar to the range of $\rho(NH_4^+)$ values reported by Bode et al. (2004b) at ambient concentrations between 0.1 and 1.0 µmol N L^{-1}, indicating that these rates were not unrealistic.

5. Conclusions

The two surveys carried out in the Ría de Vigo showed contrasting situations in terms of hydrography, nutrient concentrations, community structure and nitrogen uptake. Toxic dinoflagellates were present during the period of downwelling-favourable winds, when phytoplankton growth was supported primarily by ammonium. This was observed particularly towards the head of the ría, where NH_4^+ concentrations were highest. Urea was also an important source of nitrogen. This reliance on regenerated N is consistent with the trend identified for HABs in upwelling systems by Kudela et al. (2010). Phytoplankton showed a preference for NH_4^+ over NO_3^- or possibly inhibition of $\rho(NO_3^-)$ by NH_4^+. During the period of upwelling-favourable winds, the water column was stratified and nutrients were depleted above the thermocline, because upwelling was not strong enough to mix the water column. The phytoplankton community was fairly typical of summer upwelling, largely dominated by diatoms. Because of the low ambient NO_3^- concentrations, phytoplankton growth was still supported primarily by recycled nitrogen, although to a lesser extent than during downwelling. An upwelling pulse at the end of the survey led to NO_3^--dominated nitrogen uptake at the thermocline, thus indicating the potential for new production under stronger upwelling conditions. Maximum potential new production was not realised due to organic matter export out of the ría, possibly combined with grazing control. Nitrogen uptake kinetics showed that during this period NH_4^+ was preferred over NO_3^- and the phytoplankton community was able to exploit rapid increases in NH_4^+ concentration. Potentially toxic *Pseudo-nitzschia* species were present, as well as *Dinophysis acuminata*, showing that the upwelling season can potentially be conducive to HABs as well as the downwelling season.

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Table 1. Ambient concentrations and uptake rates of NO_3^-, NH_4^+ and urea, particulate nitrogen concentrations, *f*-ratios and NH_4^+ regeneration rates at various stations along the ría in (a) September 2006 and (b) June 2007.

Table 2. Comparison of nitrogen uptake kinetics parameters from this and other studies in upwelling systems. Measurements from this study are in bold. a: Kudela et al. (2008a); b: Seeyave et al. (2009); c: Kudela et al. (2008b); d: Kudela & Cochlan (2000); e: Dortch & Postel (1989); f: Seeyave (2009).

Figure 1. Map of NW Spain showing the four Rías Baixas (left-hand panel) and detailed map of the Ría de Vigo (right-hand panel) showing the CTD stations B0-B5 (closed circles) and the meteorological stations (open circles).

Figure 2. Southerly wind components averaged 6-hourly in (a) September 2006 and (b) June 2007. Horizontal bars indicate the sampling periods.

Figure 3. Temperature (b,e,h,k) and salinity (c,f,i,l) contour plots obtained from MiniBAT deployments along longitudinal transects (a,d,g,j) of the ria on 26 and 30 September 2006 and 25 and 28 June 2007.

Figure 4. Vertical profiles of NO_3^- and NH_4^+ concentrations in September 2006 at stations B5, B3 and B2.

Figure 5. Vertical profiles of NO_3^- and NH_4^+ concentrations in June 2007 at stations B5, B3 and B2.

Figure 6. Chl-a profiles obtained from CTD fluorescence measurements in September 2006 (a,b,c) and June 2007 (d,e,f) at stations B5, B3 and B2. Note the difference in scale between 2006 and 2007.

Figure 7. Concentrations of diatoms, dinoflagellates and flagellates (mean of concentrations at 3 and 10 m, with error bars representing standard errors) at stations B5, B3 and B2 on (a) 26 September 2006, (b) 30 September 2006, (c) 25 June 2007 and (d) 28 June 2007.

Figure 8. Nitrogen uptake kinetics measured on a water sample collected from 2 m depth at station B3 on 28 June 2007. Note the different scale in (a).

Table 1. Ambient concentrations and uptake rates of NO_3^-, NH_4^+ and urea, *f*-ratios and NH_4^+ regeneration rates at various stations along the ría in (a) September 2006 and (b) June 2007.

Date	**Station**	**Depth**	**Ambient conc. (µmol N l^{-1})**			**Uptake (µmol N l^{-1} h^{-1})**				**f-ratio**	**r(NH_4)**
			NH_4	NO_3	urea	ρ(NH_4)	ρ(NO_3)	ρ(urea)	Total ρ(N)		µmol N l^{-1} h^{-1}
a.											
26-Sep	B5	0	0.62	1.58	0.68	0.037	0.012	0.023	0.071	0.16	0.091
26-Sep	B3	0	0.95	1.84	0.28	0.046	0.006	0.012	0.064	0.10	0.034
26-Sep	B2	0	3.17	1.53	1.30	0.038	0.005	0.016	0.058	0.08	n.d.
27-Sep	B5	0	0.72	1.01	0.74	0.035	0.006	0.016	0.057	0.10	0.274
28-Sep	B3(1)	0	1.68	2.36	0.37	0.039	0.006	0.008	0.053	0.11	0.058
28-Sep	B3(2)	0	1.71	2.62	0.80	0.038	0.013	0.015	0.066	0.19	0.048
29-Sep	B2(1)	0	4.61	2.39	2.29	0.059	0.005	0.024	0.088	0.06	0.451
29-Sep	B2(2)	0	3.85	2.03	1.30	0.063	0.005	0.028	0.096	0.05	0.168
b.											
25-Jun	B2	0	0.44	0.30	0.41	0.092	0.071	0.115	0.278	0.26	n.d.
25-Jun	B2	10	2.23	1.12	0.60	0.188	0.019	0.033	0.240	0.08	0.092
25-Jun	B3	0	0.26	0.08	0.15	0.126	0.030	0.042	0.198	0.15	0.102
26-Jun	B0	0	0.26	0.04	0.10	0.139	0.033	0.033	0.206	0.16	0.145
26-Jun	B5	0	0.33	0.40	0.21	0.127	0.034	0.024	0.185	0.18	0.235
27-Jun	B3	0	0.23	0.08	0.13	0.148	0.114	0.072	0.334	0.34	0.194
28-Jun	B2	12	2.47	6.83	0.35	0.104	0.229	0.039	0.371	0.62	0.014
28-Jun	B3	0	0.33	0.52	0.08	0.104	0.043	0.181	0.328	0.13	0.002

Table 2. Comparison of nitrogen uptake kinetics parameters from this and other studies. Measurements from this study are in bold, those from other upwelling systems are highlighted in grey. a: Kudela et al. (2008a); b: Seeyave et al. (2009); c: Kudela et al. (2008b); d: Kudela & Cochlan (2000); e: Fan et al. (2003); f: Sahlsten (1987); g: Dortch & Postel (1989); h: Chang et al. (1995); i: Seeyave (2009).

Species	Location	V_{max} ($x10^{-3}$ h^{-1})			K_s (µmol l^{-1})			α = V_{max}/K_s			$\frac{V_{max}(NH_4^+)}{V_{max}(NO_3^-)}$	$\frac{α(NH_4^+)}{α(NO_3^-)}$	$\frac{V_{max}(urea)}{V_{max}(NO_3^-)}$	α(
		NO_3^-	NH_4^+	Urea	NO_3^-	NH_4^+	Urea	NO_3^-	NH_4^+	Urea				
MONOSPECIFIC BLOOMS														
Dinoflagellates														
Akashiwo sanguinea	California	5.2	15.1	7.2	1.00	2.37	0.43	5.2	6.4	16.7	2.9	1.2	1.4	
Alexandrium catenella	Benguela	>17.5	14.9	3.5	nd	2.52	0.65	nd	5.9	5.4	<0.9	nd	<0.2	
Cochlodinium spp.	California	0.9	>4.0	2.1*	1.00	nd	4.06*	0.9	0.3	0.8*	4.4	nd	2.3	
Dinophysis acuminata	Benguela	3.5	13.9	6.2	0.79	0.67	0.53	4.4	20.7	11.7	4.0	4.7	1.8	
Lingulodinium polyedrum	California	3.9	8.1	10.6	0.47	0.59	0.99	8.2	13.7	10.7	2.1	1.7	2.8	
Prorocentrum minimum	Choptank Estuary (Chesapeake Bay)	53.8	868.6	492.6	7.12	5.09	16.84	7.6	170.6	29.3	16.2	22.6	9.2	
Diatoms														
Pseudo-nitzschia	Benguela	15.0	18.0	4.9	1.21	1.34	nd	12.4	13.4	nd	1.2	1.1	0.3	
MIXED ASSEMBLAGES														
	Central North Pacific gyre	3.0	16.0	16.0	0.03	0.03	0.02	100.0	533.3	800.0	5.3	5.3	5.3	
	Washington coast upwelling	5.8	6.8	4.6	0.05	0.71	0.78	116.0	9.6	5.9	1.2	0.1	0.8	
Mixed diatoms	**Ría de Vigo**	**26.2**	**335.9**	**67.7**	**0.37**	**3.36**	**0.95**	**70.8**	**100.0**	**71.3**	**12.8**	**1.4**	**2.6**	
Mixed dinoflagellates	Western New Zealand	13.8	20.7	12	1.1	0.5	0.5	12.5	41.4	24.0	1.5	3.3	0.9	
	Neuse Estuary (N. Carolina)	4.0	52.9	5.77	0.54	2.38	0.37	0.6	10.4	0.3	13.3	18.6	1.4	
Diatoms + dinoflagellates	Benguela	3.5	14.6	4.4	0.82	0.62	nd	4.3	23.5	nd	4.2	5.5	1.3	
	Benguela	24.0	6.2	3.2	8.24	0.53	0.21	2.9	11.7	15.2	0.3	4.0	0.1	
	Fal Estuary	7.0	15.5	nd	3.00	1.55	nd	2.3	10.0	nd	2.2	4.3	nd	

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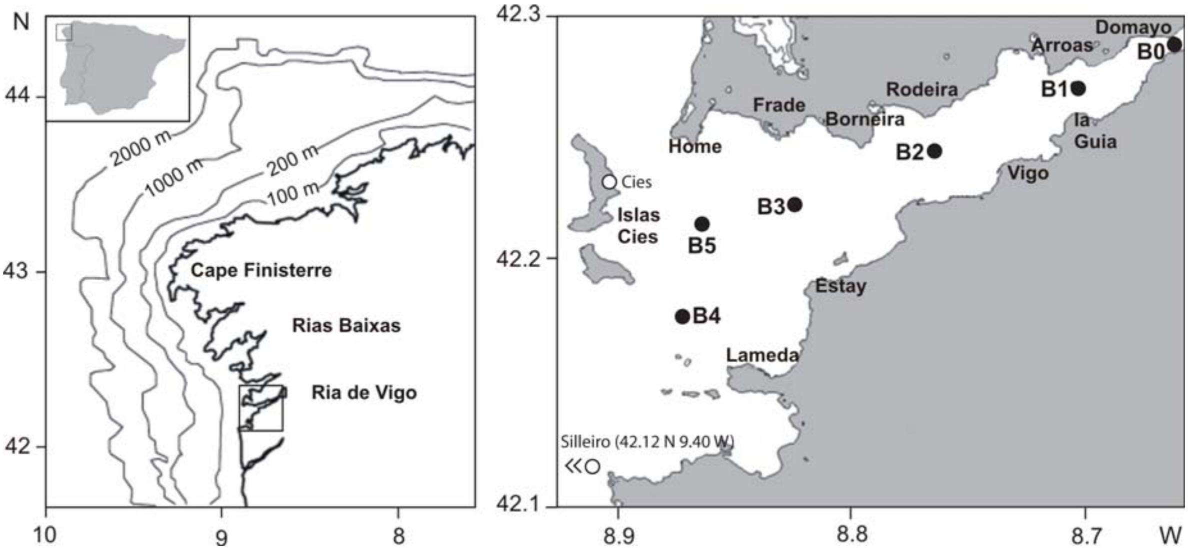

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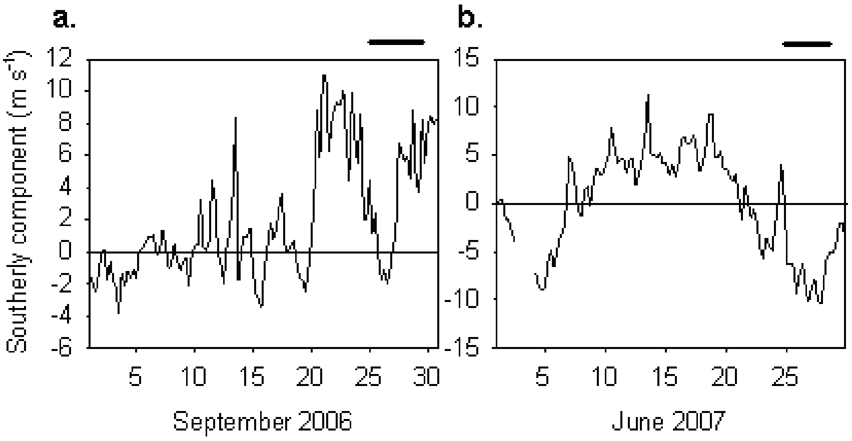

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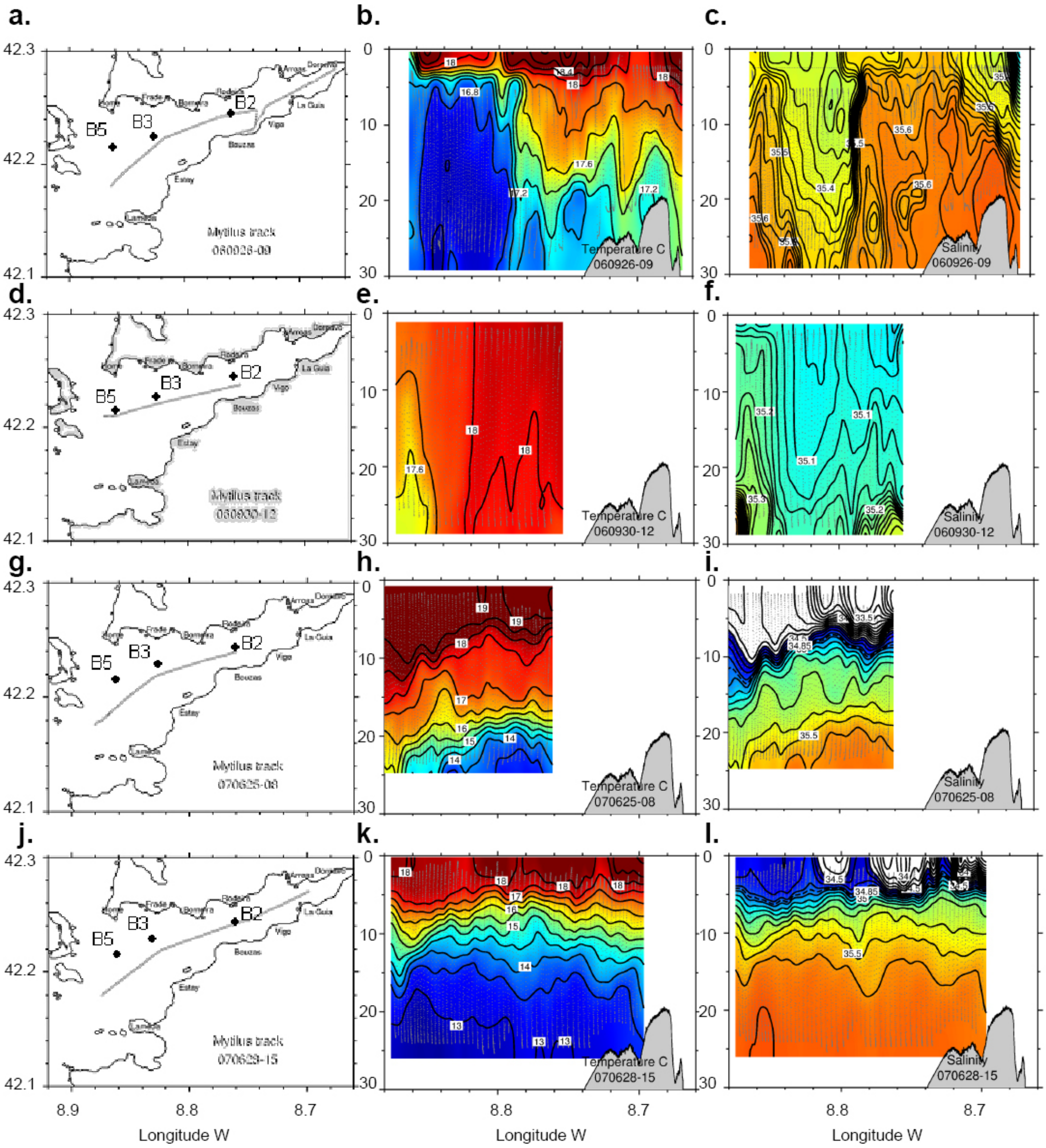


Figure 4. Vertical profiles of NO_3^- and NH_4^+ concentrations in September 2006 at stations B5, B3 and B2.

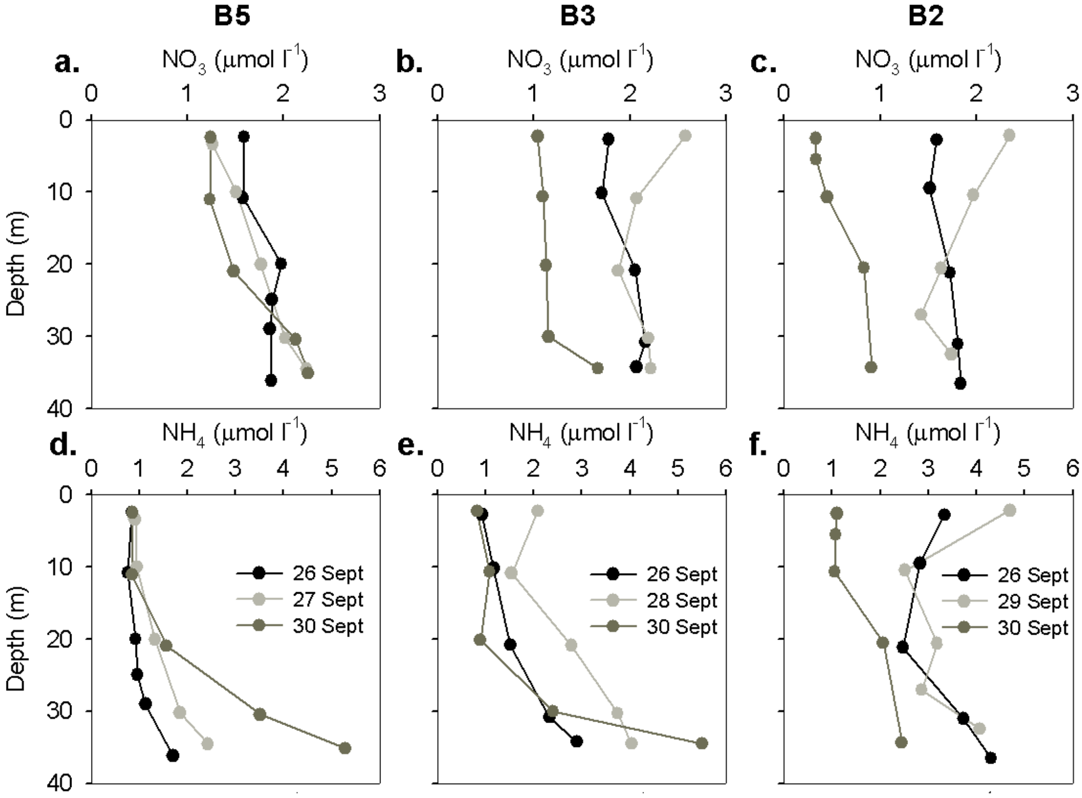
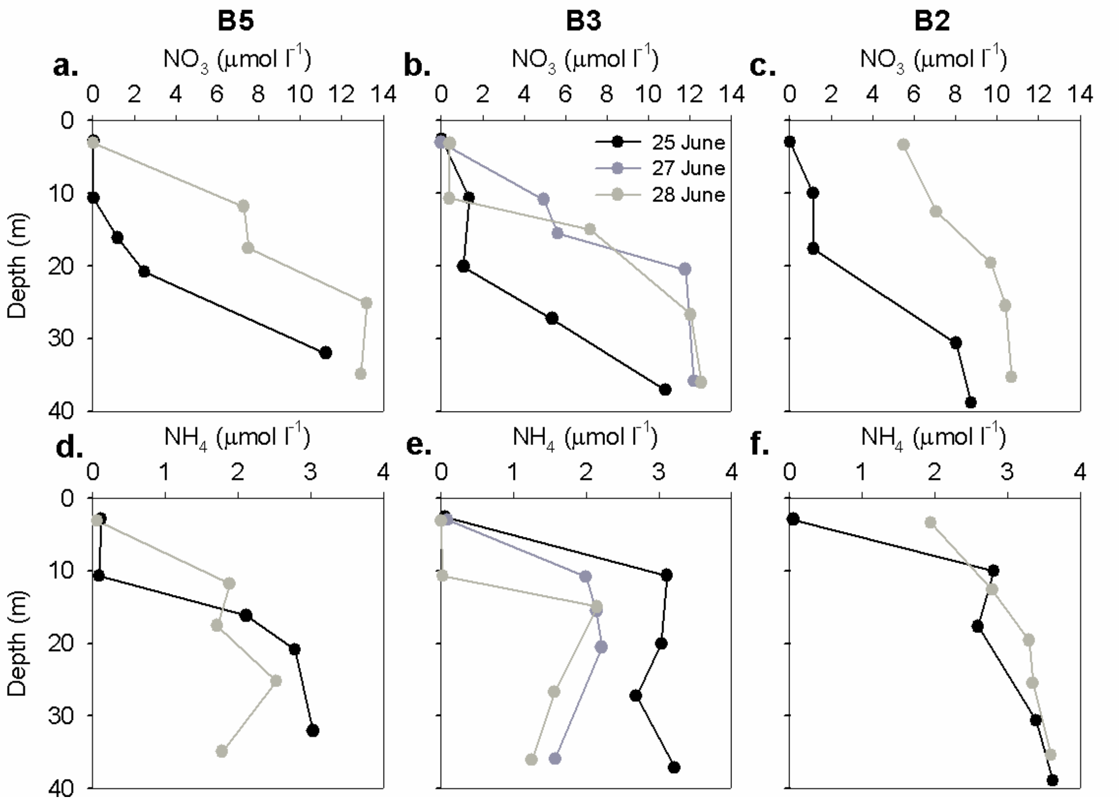


Figure 5. Vertical profiles of NO_3^- and NH_4^+ concentrations in June 2007 at stations B5, B3 and B2.



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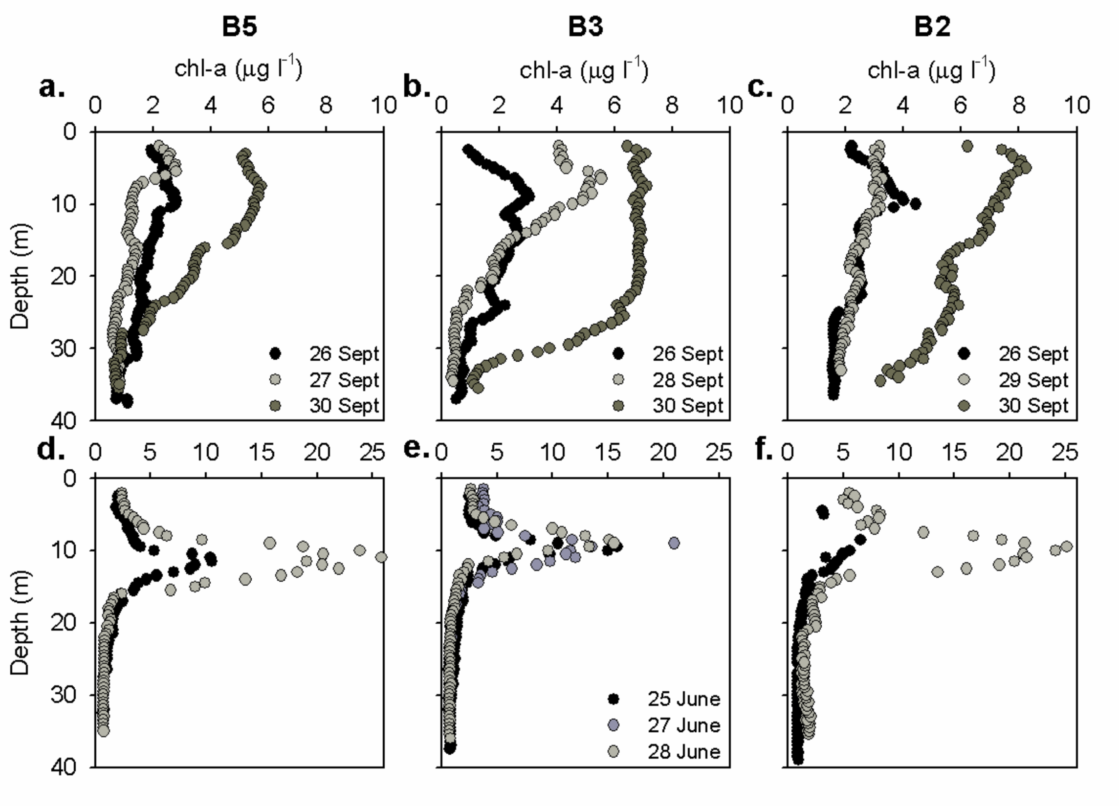

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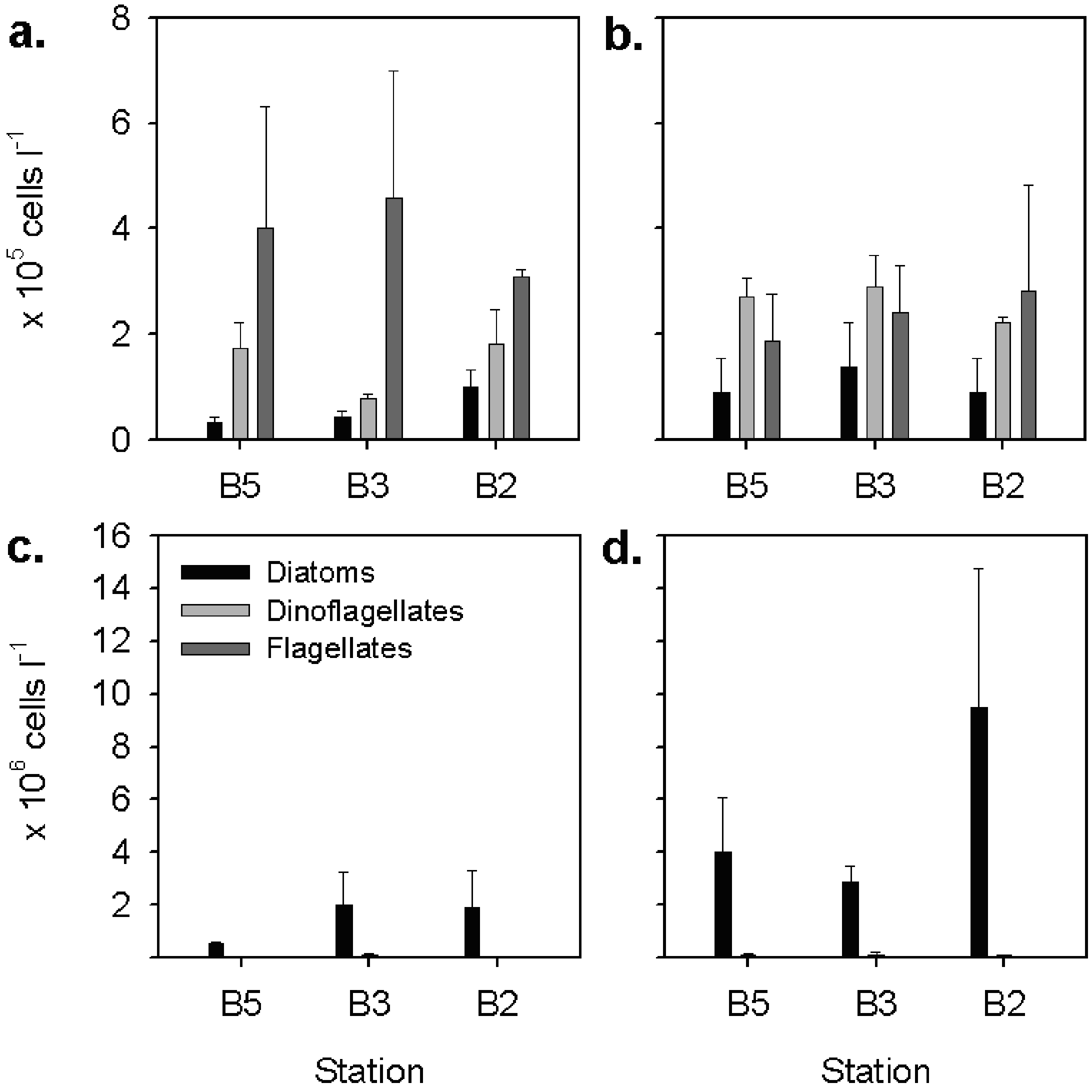

Figure 8. Nitrogen uptake versus ambient concentration fitted to the Michaelis-Menten equation for uptake kinetics using SigmaPlot (Jandel Scientific). Note the different scale in (a).