

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

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23

1 **Abstract**

2

3 The Galician Rías, situated in the Iberian upwelling system, are regularly affected by
4 blooms of toxic dinoflagellates, which pose serious threats to the local mussel farming
5 industry. These tend to occur towards the end of summer, during the transition from
6 upwelling to downwelling favourable seasons, when cold bottom shelf waters in the rías
7 are replaced by warm surface shelf waters. Nitrate, ammonium and urea uptake rates
8 were measured in the Ría de Vigo during a downwelling event in September 2006 and
9 during an upwelling event in June 2007. In September the ría was well mixed, with a
10 downwelling front observed towards the middle of the ría and relatively high nutrient
11 concentrations (1.0-2.6 $\mu\text{mol L}^{-1}$ nitrate; 1.0-5.6 $\mu\text{mol L}^{-1}$ ammonium; 0.1-0.8 $\mu\text{mol L}^{-1}$
12 phosphate; 2.0-9.0 $\mu\text{mol L}^{-1}$ silicic acid) were present throughout the water column.
13 Ammonium represented more than 80 % of the nitrogenous nutrients, and the
14 phytoplankton assemblage was dominated by dinoflagellates and small flagellates. In
15 June the water column was stratified, with nutrient-rich, upwelled water below the
16 thermocline and warm, nutrient-depleted water in the surface. At this time, nitrate
17 represented more than 80 % of the nitrogenous nutrients, and a mixed diatom assemblage
18 was present. Primary phytoplankton production during both events was mainly sustained
19 by regenerated nitrogen, with ammonium uptake rates of 0.035-0.063 $\mu\text{mol N L}^{-1} \text{ h}^{-1}$ in
20 September and 0.078-0.188 $\mu\text{mol N L}^{-1} \text{ h}^{-1}$ in June. Although *f*-ratios were generally low
21 (<0.2) in both June and September, a maximum of 0.61 was reached in June due to higher
22 nitrate uptake (0.225 $\mu\text{mol N L}^{-1} \text{ h}^{-1}$). Total nitrogen uptake was also higher during the
23 upwelling event (0.153-0.366 in June and 0.053-0.096 $\mu\text{mol N L}^{-1} \text{ h}^{-1}$ in September).
24 Nitrogen uptake kinetics demonstrated a strong preference for ammonium and urea over
25 nitrate in June. This study underlined the importance of regenerated production
26 (including organic nitrogen) in the Ría de Vigo in supporting both harmful algal bloom
27 communities during the downwelling season, but also (to a lesser extent) diatom
28 communities during stratified periods of weak to moderate upwelling.

29

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

1 **1. Introduction**

2
3 Nitrogen is generally recognised as being the nutrient limiting primary production in
4 coastal marine ecosystems (Dugdale, 1967; Ryther & Dunstan, 1971; Howarth & Marino,
5 2006). Furthermore, nitrogen inputs to coastal waters are increasingly thought to be
6 implicated in the reported global increase in Harmful Algal Blooms (HABs) (Anderson et
7 al., 2002), in particular due to the increase in dissolved organic nitrogen (Glibert et al.,
8 2006). Nitrogen uptake measurements provide valuable information on the relative
9 contributions of new and regenerated forms of nitrogen to primary production. Such
10 measurements are particularly important for understanding the ecology of HABs,
11 especially in upwelling systems, which are characterised by large fluctuations in nitrate
12 (NO_3^-) concentrations. A number of nitrogen uptake measurements have been made in
13 the California and Benguela upwelling systems (Dugdale et al., 1990; Probyn, 1992;
14 Dugdale et al., 2006; Seeyave et al., 2009). In the Iberian upwelling system some
15 previous measurements of nitrogen uptake and regeneration have been reported in shelf
16 waters (Slawyk et al., 1997, Joint et al., 2001; Bode et al., 2004a,b; Bode et al., 2005) and
17 a few reported in the rias (Bode et al., 2005; Varela et al., 2003). Published f -ratios have
18 been calculated either from direct measurements using ^{15}N (but most of these have not
19 included urea), or estimated from the NO_3^- flux into the euphotic zone caused by
20 upwelling (Alvarez-Salgado et al., 2002), or based on satellite-derived primary
21 production estimates (Aristegui et al., 2009). The relatively low seasonally-averaged
22 ratios (0.20-0.33) derived from the latter study were attributed to low continental nutrient
23 inputs, low nutrient concentrations in the source water, low average coastal winds and the
24 importance of heterotrophy and therefore nutrient regeneration (Aristegui et al., 2006).

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

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

15 The abundance of diatoms is positively correlated to upwelling (Figueiras & Rios,
16 1993), and HABs tend to occur during downwelling events in late summer-early autumn
17 (Fraga et al., 1988; Figueiras et al., 1994). The horizontal distribution of diatoms and
18 dinoflagellates also reflects the intensity of upwelling or stratification along the rías, with
19 diatoms dominating towards the interior, where upwelling is strongest, whereas
20 dinoflagellates tend to occur in the outer, more stratified parts of the rías (Tilstone et al.,
21 1994). The apparent increase in blooms of certain HAB species in the last 4 decades has
22 been attributed to enhanced eutrophication of the rías as a result of increased sewage
23 discharges, expansion of the mussel farms and increases in forest fires (Wyatt & Reguera,
24 1989), as well as a decrease in the duration and average intensity of the upwelling season
25 (Alvarez-Salgado et al., 2008).

26 No consensus has yet been reached regarding the mechanisms leading to HAB
27 development in the rías (Pitcher et al., 2010). Some studies have supported the hypothesis
28 of advection of offshore populations into the rías (Fraga et al., 1993; Sordo et al., 2000),
29 whereas others have suggested *in situ* HAB development (Fraga et al., 1990; Figueiras &
30 Pazos, 1991a; Pazos et al., 1995; Figueiras et al., 1998). In any case, downwelling is
31 thought to favour motile species such as *Gymnodinium catenatum*, which can maintain

1 themselves in the surface layer (Fraga et al., 1988; Figueiras et al., 1994; Fermin et al.,
2 1996). HABs can also develop during weak to moderate upwelling, which raises the
3 nutricline without being sufficiently intense to mix the entire water column (Figueiras &
4 Rios, 1993). In this situation, dinoflagellates can undertake diel vertical migrations that
5 allow them to exploit the high nutrient concentrations at the nutricline during the night
6 and photosynthesise during the day in the surface layer (Figueiras & Fraga, 1990; Fraga
7 et al., 1992; Fraga et al., 1999). Using a box model, Rios et al. (1995) suggested that
8 diatom growth was sustained by nitrate during the upwelling season, whereas autumn
9 dinoflagellate populations relied on ammonium as their main source of nitrogen.

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

16 **2. Materials and methods**

18 *2.1. Sampling*

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

28 Continuous measurements of temperature, salinity and chl-a fluorescence were also
29 made on surface water collected underway (2.5 m depth). Continuous vertical profiles of
30 these parameters were carried out at various stations along the ría (Figure 1) using a
31 Seabird Electronics 911+ CTD system coupled with a Seatech fluorometer mounted on a

1 sampling rosette fitted with 12-L Niskin bottles. Seawater samples were collected from 3-
2 6 depths in acid-washed and Milli-Q rinsed 5- or 10-L carboys for routine chl-a and
3 nutrient analyses among other parameters. These were stored in the dark until transported
4 ashore (within <5 hours). At some stations water was only collected from the underway
5 supply (2.5 m). Water for ^{15}N incubations and associated nitrate, ammonium and urea
6 analyses was collected from ~3 m in both sampling periods and occasionally from the
7 chl-a maximum (10-12 m) in 2007.

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

13
14 Figure 1

15 16 2.2. *Nutrients and phytoplankton*

17
18 Nutrient samples (nitrate NO_3^- , nitrite NO_2^- , ammonium NH_4^+ , phosphate HPO_4^{2-} and
19 silicic acid $\text{Si}(\text{OH})_4$) were analysed within ~6 h of being collected in both years using an
20 Alpkem autoanalyser following the method of Hansen & Grasshoff (1983) as modified
21 by Mouriño & Fraga (1985) and Álvarez-Salgado et al. (1992). Ammonium was also
22 measured using the fluorometric (o-Phthaldialdehyde, OPA) method of Holmes et al.
23 (1999) for the samples that were incubated for NH_4^+ uptake determinations. After reagent
24 addition, samples were incubated overnight in the dark and fluorescence was determined
25 on a Turner Designs TD700 fluorometer. Urea was determined manually on fresh
26 samples following the diacetylmonoxime thiosemicarbazide method of Mulvenna &
27 Savidge (1992) adapted to room temperature using reaction times of 72-96 h (Goeyens et
28 al., 1998) in 2006, but following the method of Grasshoff et al. (1999) in 2007. Precisions
29 were $<0.05 \mu\text{mol N L}^{-1}$ for all nutrients.

30 Chl-a concentrations were determined by fluorometry after filtering 100 mL of
31 seawater through 25 mm GF/F filters (Welschmeyer, 1994). Samples for phytoplankton

1 preserved in Lugol's iodine were settled overnight and counted under an inverted
2 microscope and identified to species level, when possible, as previously described in
3 Crespo et al. (2006).

4 5 2.3. Nitrogen uptake

6
7 For each incubation, water was decanted into two 0.5-L and one 1-L Nalgene
8 polycarbonate bottles. The 0.5-L samples were inoculated with stock solutions of $K^{15}NO_3$
9 and urea [$CO(^{15}NH_2)_2$] and the 1-L sample with $^{15}NH_4Cl$. All stock solutions had a
10 concentration of $1\ \mu mol\ N\ mL^{-1}$ and ^{15}N purities were 99.6, 99.1 and 99.7 % for $K^{15}NO_3$,
11 $CO(^{15}NH_2)_2$ and $^{15}NH_4Cl$, respectively. The volume of ^{15}N spike in each case aimed to
12 achieve a final concentration of approximately 10 % of the ambient nutrient
13 concentration. However, at very low NO_3^- concentrations ($<0.05\ \mu mol\ L^{-1}$), the aqueous
14 enrichments were sometimes as high as 93 %. Therefore the correction for high spike
15 addition of Eppley et al. (1977) was applied to some of the uptake rates (see below).
16 Immediately after spiking the NH_4^+ sample, exactly 0.5 L was transferred to a separate
17 0.5-L polycarbonate bottle for incubation, while the remaining 0.5L was filtered through
18 a 47-mm Whatman precombusted GF/F filter to measure time zero aqueous ^{15}N
19 enrichment (R_0) in the filtrate. Subsamples were also taken from the filtrate for later
20 analyses of ambient NO_3^- , NH_4^+ and urea.

21 Samples were incubated in a grey plastic box placed on-deck, maintained at *in situ*
22 temperature by a flow of surface water. For subsurface samples, 50 % shading was
23 provided by a nylon mesh. Incubations lasted for between 1h30 and 2h in 2006 and 2h30-
24 3h in 2007 and took place between 10:00 and 14:00. Incubations were terminated by
25 filtration onto pre-combusted GF/F filters, which were then rinsed with filtered seawater
26 and dried at 60 °C overnight. Filtration of $^{15}NO_3^-$ and ^{15}N -urea spiked samples was onto
27 25-mm Whatman GF/F filters, whereas the $^{15}NH_4^+$ spiked samples were filtered onto 47-
28 mm Whatman GF/F filters using a different system that allowed clean collection of the
29 filtrate for later isotopic dilution analyses. Aqueous enrichment at the start and end of the
30 incubations was measured on filtrates from the start and end of each incubation. These
31 were frozen for later recovery of aqueous NH_4 by diffusion onto ashed halved 25-mm

1 GF/F filters (Probyn 1987). Filters were processed and analysed in the same way as the
2 ^{15}N uptake samples to determine the parameters R_0 and R_t in Equation 3 of Glibert et al.
3 (1982).

4 Uptake rates were calculated from equations 1-3 of Dugdale & Wilkerson (1986)
5 corrected for isotopic dilution of $^{15}\text{NH}_4^+$ by regenerated $^{14}\text{NH}_4$ according to Glibert et al.
6 (1982) in September 2006, and for the NH_4^+ samples in June 2007. The equation of
7 Eppley et al. (1977) was applied to all NO_3^- and urea measurements in June 2007 for
8 consistency. Ammonium recycling was calculated from the Blackburn-Caperon model
9 (Blackburn, 1979; Caperon et al., 1979) since the NH_4^+ concentration always changed
10 during the incubation.

11 A nitrogen uptake kinetics experiment was carried out on 28 June 2007, on water
12 collected from 2 m depth at station B3. Water collected from the CTD was decanted into
13 eighteen 75-mL Sterilin Iwaki culture flasks. Six 75-mL samples were spiked with
14 different volumes of 10 % enriched $1 \text{ mmol N L}^{-1} \text{ NO}_3^-$ solution, another 6 with 10 %
15 enriched $1 \text{ mmol N L}^{-1} \text{ NH}_4^+$ solution and the remaining 6 with 10 % enriched 2 mmol N
16 L^{-1} urea solution to obtain final concentrations between 0.6 and $30 \text{ } \mu\text{mol N L}^{-1}$ for NO_3^- ,
17 between 0.3 and $30 \text{ } \mu\text{mol N L}^{-1}$ for NH_4^+ and between 0.2 and $60 \text{ } \mu\text{mol N L}^{-1}$ for urea.
18 The experiment was carried out in the same incubator as the standard uptake incubations
19 and the incubation lasted 2h30. Incubations were terminated by filtration onto 25-mm
20 precombusted GF/F filters and the filters were processed in the same way as for the
21 standard uptake experiments. The PN-specific uptake rates were plotted against
22 concentration of each nitrogen species and fitted to the Michaelis-Menten equation for
23 uptake kinetics using SigmaPlot (Jandel Scientific) to derive the parameters K_m (half-
24 saturation constant) and V_{max} (maximum uptake rate).

1 **3. Results**

2 3 *3.1. Hydrographic setting*

4
5 Figures 2 & 3

6
7 In September, southerly winds were predominant (Figure 2a) and the water column
8 was relatively well mixed, as a consequence of downwelling (Figure 3b,c). The
9 downwelling front, indicated by vertical temperature and salinity isolines, was observed
10 in the vicinity of station B2 (Figure 3b,c, see also Romera-Castillo et al., 2011). By the
11 end of the survey, the water column had warmed and salinity had dropped, and both
12 horizontal and vertical gradients were weak (Figure 3e,f). In June, no water was upwelled
13 during the 2 weeks prior to the survey, due to predominantly southerly winds. Winds
14 switched to upwelling-favourable northerly flow during the 3 days preceding the survey,
15 although with relatively weak components ($< 4 \text{ m s}^{-1}$). Thus upwelling was not strong
16 enough to mix the entire water column, and consequently the surface layer remained
17 stratified. A thermocline was observed between 10 and 20 m (Figure 3h,i), showing
18 positive estuarine circulation, with a warm, less saline surface layer flowing out of the ría
19 ($T = 18\text{-}20 \text{ }^{\circ}\text{C}$, $S = 33.1\text{-}34.9$) and colder, more saline water ($T = 13\text{-}15 \text{ }^{\circ}\text{C}$, $S = 35.4\text{-}$
20 35.9) flowing into the ría at depth. By 28 June the thermocline was uplifted to $\sim 5\text{-}10 \text{ m}$
21 following a pulse of upwelling, with surface temperatures of $\sim 18 \text{ }^{\circ}\text{C}$ and salinities of
22 $33.0\text{-}34.7$ (Figure 3k,l).

23 24 *3.2. Nutrients*

25
26 Figure 4

27
28 In September, NO_3^- concentrations were relatively homogeneous throughout the
29 water column, displaying an increase with depth of $< 1 \text{ } \mu\text{mol L}^{-1}$ (Figure 4). Little
30 horizontal variation was observed at the start of the survey, however at the end
31 concentrations were up to $1.3 \text{ } \mu\text{mol L}^{-1}$ higher at B5 relative to B2. Ammonium

1 concentrations were highest at B2, with concentrations ranging from 1.1 to 4.7 $\mu\text{mol L}^{-1}$
2 at the surface, whereas at B3 they ranged from 0.8 to 2.1 $\mu\text{mol L}^{-1}$ and at B5 they
3 remained $\sim 0.9 \mu\text{mol L}^{-1}$. Concentrations increased with depth, to maxima of 5.3, 5.5 and
4 4.3 $\mu\text{mol L}^{-1}$ at B5, B3 and B2, respectively. Phosphate and Si(OH)_4 profiles were very
5 similar to NH_4^+ profiles, displaying the same spatial and temporal variations (data not
6 shown).

7

8 Figure 5

9

10 In June, all nutrient concentrations were very low at the surface at the start of the
11 survey ($< 0.05 \mu\text{mol L}^{-1} \text{NO}_3^-$, $\leq 0.1 \mu\text{mol L}^{-1} \text{NH}_4^+$, Figure 5). Concentrations increased
12 with depth to maximum values of 11.2 $\mu\text{mol L}^{-1} \text{NO}_3^-$ and 3.6 $\mu\text{mol L}^{-1} \text{NH}_4^+$. By the end
13 of the survey, concentrations had increased, consistent with a rising pycnocline caused by
14 upwelling. Maximum surface concentrations increased to 5.5 $\mu\text{mol L}^{-1} \text{NO}_3^-$ and 1.9 μmol
15 $\text{L}^{-1} \text{NH}_4^+$.

16

17 *3.3. Chl-a and phytoplankton community structure*

18

19 Figure 6

20

21 Chl-a concentrations were relatively low in September 2006 (Figure 6a,b,c),
22 particularly at the start of the survey ($< 5 \mu\text{g L}^{-1}$). At this time there was little horizontal
23 variation between stations B5 and B2. By 30 September chl-a had increased and showed
24 a horizontal gradient, with maximum concentrations of 5.8, 7.1 and 8.1 $\mu\text{g L}^{-1}$ at B5, B3
25 and B2, respectively (Figure 6). At the start of the survey, chl-a was relatively
26 homogeneous throughout the water column, whereas on 30 Sept, chl-a concentrations had
27 increased above initial values in the upper 15-30 m. In June 2007, a pronounced
28 maximum developed with upwelling at ~ 10 m depth (Figure 6d,e,f). Maximum
29 concentrations were 10.5, 15.8 and 6.5 $\mu\text{g L}^{-1}$ at B5, B3 and B2, respectively on 25 June.
30 On 28 June they had increased at B5 and B2, to 25.8 and 25.1 $\mu\text{g L}^{-1}$, respectively,
31 whereas at B3 concentrations remained largely unchanged. Surface concentrations were

1 not significantly different from those measured during the September survey, although
2 concentrations in the sub-surface maximum at 10 m were significantly higher (Mann-
3 Whitney U-test, $p < 0.05$).

4 5 Figure 7

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

18 In June, the phytoplankton community in the top 10 m at stations B5, B3 and B2 was
19 dominated by diatoms, which represented 95 to 99 % of total phytoplankton cells, with
20 concentrations as high as 9.5×10^6 cells L^{-1} (Figure 7c,d). Dinoflagellate concentrations
21 only reached a maximum of 0.12×10^6 cells L^{-1} , representing up to 5 % of total
22 phytoplankton cells, whereas small flagellates reached 0.04×10^6 cells L^{-1} (2 % of total
23 cell concentration). The main diatom species were *Chaetoceros* spp., *Leptocylindrus* spp.,
24 *Nitzschia* cf. *americana* and *Skeletonema costatum*. *Pseudo-nitzschia* cf. *delicatissima*
25 and *P.* cf. *seriata*, two groups potentially including toxic species, and the toxic
26 dinoflagellate *Dinophysis acuminata* were also present. *P.* cf. *seriata* was most abundant
27 on 25 June (maximum 237.6×10^3 cells L^{-1} at B3), whereas *P.* cf. *delicatissima* was most
28 abundant on 28 June (maximum 72.8×10^3 cells L^{-1} at B5). *Dinophysis acuminata* was
29 most abundant at B3, where it formed a sub-surface maximum at 10 m (15×10^3 cells L^{-1})
30 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 $\rho(\text{N})$], followed by urea (15 to 32 %), whereas $\rho(\text{NO}_3^-)$ contributed <20 % (Table 1). Total $\rho(\text{N})$ 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 $\mu\text{mol N L}^{-1} \text{h}^{-1}$, $\rho(\text{urea})$ from 0.008 to 0.028 $\mu\text{mol N L}^{-1} \text{h}^{-1}$ and $\rho(\text{NO}_3^-)$ from 0.005 to 0.013 $\mu\text{mol N L}^{-1} \text{h}^{-1}$. Hourly-scaled f -ratios were very low, ranging from 0.05 to 0.19. f -ratios were lowest at the inner station B2 (<0.1), due to the very high contribution of $\rho(\text{NH}_4^+)$, 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 $\rho(\text{N})$, with uptake rates ranging from 0.078 to 0.188 $\mu\text{mol N L}^{-1} \text{h}^{-1}$. During stratified periods when surface NO_3^- was depleted, $\rho(\text{NO}_3^-)$ was lower than $\rho(\text{NH}_4^+)$ and often lower than $\rho(\text{urea})$. Surface NO_3^- uptake rates ranged from 0.001 to 0.043 $\mu\text{mol N L}^{-1} \text{h}^{-1}$, representing 8-26 % total $\rho(\text{N})$. 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 $\rho(\text{N})$. However, $\rho(\text{NO}_3^-)$ was measured at 12 m at B2, following the upwelling pulse, and in this case it was 2-fold higher than $\rho(\text{NH}_4^+)$, reaching 0.225 $\mu\text{mol N L}^{-1} \text{h}^{-1}$. Urea was also an important source of nitrogen, particularly at the surface at B3, with uptake rates reaching 0.161 $\mu\text{mol N L}^{-1} \text{h}^{-1}$. Highest total $\rho(\text{N})$ was measured at the central stations B3 and B2 (0.153-0.366 $\mu\text{mol N L}^{-1} \text{h}^{-1}$), whereas it was 0.153 and 0.158 $\mu\text{mol N L}^{-1} \text{h}^{-1}$ at the outer and inner stations, respectively. Total $\rho(\text{N})$ 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 $\rho(\text{N})$ was significant for all 3 nitrogen sources (Mann-Whitney U-test, $p < 0.05$); it was 3-fold for $\rho(\text{NH}_4^+)$, 7-fold for $\rho(\text{NO}_3^-)$ and 6-fold for $\rho(\text{urea})$. f -ratios were generally lower than expected for the upwelling season, as they were always <0.2 in the surface. The f -ratio reached 0.61 at 12 m following the upwelling pulse on 28 June.

1

2 Figure 8

3

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

12 Ammonium regeneration rates [$r(\text{NH}_4^+)$] were highly variable, ranging from 0.034 to
13 $0.451 \mu\text{mol N L}^{-1} \text{h}^{-1}$ in September and from 0.002 to $0.235 \mu\text{mol N L}^{-1} \text{h}^{-1}$ in June (Table
14 1). Although $r(\text{NH}_4^+)$ was on average higher in September ($0.161 \pm 0.060 \mu\text{mol N L}^{-1} \text{h}^{-1}$)
15 relative to June ($0.112 \pm 0.032 \mu\text{mol N L}^{-1} \text{h}^{-1}$), this difference was not statistically
16 significant (Student's t-test, $p > 0.05$). There was no significant correlation between NH_4^+
17 uptake and regeneration. Regeneration rates were generally higher than uptake rates in
18 the September survey, but mostly lower than or similar to uptake rates during the June
19 survey.

20

21 Table 2

22

1 4. Discussion

2
3 The hydrographic conditions that prevailed in the Ría de Vigo in September 2006 and
4 June 2007 were typical of the downwelling and upwelling seasons, respectively. The
5 phytoplankton communities present during the two surveys were also fairly typical of the
6 downwelling and upwelling seasons, whereby the downwelling community was
7 dominated by a mixture of dinoflagellates and flagellates, and the upwelling community
8 was dominated by diatoms. This is consistent with the trend observed by Crespo et al.
9 (2006) in a 1-year time-series of phytoplankton community structure in the Ría de Vigo.
10 However, Crespo et al. (2006) reported a much larger dinoflagellate bloom than in this
11 study. The association of diatoms with upwelling is regularly observed in the Iberian
12 (Figueiras & Rios, 1993), NW African (Estrada & Blasco, 1985), Benguela (Fawcett et
13 al., 2007) and California currents (Lassiter et al., 2006). In the Iberian system, this
14 association has been described by a linear correlation between diatom biovolume (or
15 biomass) and the upwelling index (Figueiras & Rios, 1993).

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

25 The 'typical' nitrogen uptake scenario expected for upwelling systems is the
26 dominance of new production (f -ratio >0.5) during upwelling events and a switch to
27 regenerated production (f -ratio <0.5) during downwelling (or upwelling relaxation)
28 events (Dugdale et al., 1990). This relationship between upwelling strength and f -ratio
29 has been reported for the Benguela (Seeyave et al., 2009) and in the Iberian (Álvarez-
30 Salgado et al., 2002) upwelling systems. However, the results from the present study in
31 the Ría de Vigo have shown that hourly-scaled f -ratios were generally <0.5 during both

1 the upwelling and downwelling periods and that NH_4^+ was the principal source of
2 available nitrogen. However, an f -ratio >0.5 was measured on one occasion, at 12 m
3 depth, when a pulse of upwelling occurred and the NO_3^- concentration increased to 6.8
4 $\mu\text{mol N L}^{-1}$ at the thermocline, stimulating $\rho(\text{NO}_3^-)$, which increased to $>0.2 \mu\text{mol N L}^{-1}$
5 h^{-1} . Unfortunately, no subsequent measurements were performed, therefore the timing
6 and spatial extent of the sampling may have missed some high $\rho(\text{NO}_3^-)$ episodes that
7 would have been more typical of a moderate to strong upwelling scenario.

8 Estimates of new production for the shelf region of the Iberian upwelling system
9 produced an upwelling season-averaged f -ratio of 0.20 over the shelf and 0.33 within the
10 rías (Aristegui et al., 2009), both indicating a high proportion of regenerated production
11 (Álvarez-Salgado et al., 2002). Although these results are not directly comparable with
12 those obtained in the present study due to differences in methods and in the spatial and
13 temporal scales on which the estimates are based, all results suggest that phytoplankton
14 growth during the upwelling season is not supported exclusively by NO_3^- . Similarly low
15 f -ratios (0.03 - 0.38) were measured in the nutrient-impoverished surface layer in the
16 Portuguese upwelling area off Cape Sines, whereas higher ratios (0.52 - 0.82) were
17 measured below the nutricline (Slawyk et al., 1997), although this study did not measure
18 urea uptake, which would probably have lowered the ratios. Another study on the north-
19 west Iberian shelf measured f -ratios between 0.5 and 0.7 in an upwelling region, and
20 around 0.4 (without urea) and <0.1 (with urea) in an oligotrophic offshore filament (Joint
21 et al., 2001). Closer to this study region, Bode et al. (2004a) measured f -ratios of 0.6 and
22 0.7 (averaged for low- and high-production periods, respectively) in 80m-deep water off
23 the coast of A Coruña.

24 A very limited number of N uptake measurements have been conducted actually
25 within the Galician Rías. f -ratios reported by Bode et al. (2005) for the Ria de Ferrol
26 were higher than in the present study during both upwelling and downwelling seasons,
27 between 0.6 and 0.9 at the surface in both July and September, although water-column
28 integrated values were lower in September (0.3-0.5), due to both increasing $\rho(\text{NH}_4^+)$ and
29 decreasing $\rho(\text{NO}_3^-)$ with depth (A. Bode pers. comm., after revision of data from Bode et
30 al., 2005). If $\rho(\text{urea})$ had been included in their f -ratio calculations, these could have been
31 significantly lower, particularly since they measured higher dissolved organic nitrogen

1 (DON) than dissolved inorganic nitrogen (DIN) concentrations during the summer
2 months, underlining the potential importance of DON as a source of nitrogen to
3 phytoplankton. Urea can be an important source of nitrogen for phytoplankton growth, as
4 shown in the present study where $\rho(\text{urea})$ was on average 27 ± 16 % of total nitrogen
5 uptake, and as shown by the difference in the f -ratios published by Joint et al. (2001)
6 with and without including urea uptake in the calculation. Bode et al. (2004a) measured
7 urea uptake on 3 occasions during their study off A Coruña, and although the f -ratio did
8 decrease on one of these occasions to ~ 0.5 , the difference was less pronounced than for
9 the current study or for Joint et al. (2001). The relative importance of urea as a source of
10 N for phytoplankton growth in the Iberian upwelling system therefore appears highly
11 variable, and difficult to resolve due to the paucity of measurements. The f -ratios in the
12 present study were low compared to the California upwelling system (Dugdale et al.,
13 2006), and towards the lower end of the range published for the Benguela (Probyn,
14 1992). They were however comparable to values measured during upwelling relaxation in
15 the Benguela (Seeyave et al., 2009). This could be due to the topographic difference
16 between the ria and open shelf waters where measurements were made in the Benguela
17 and California systems. This has implications for the hydrography and nutrient
18 environments of the different systems, whereby the rías can remain stratified during weak
19 to moderate upwelling, particularly in its outer reaches, leading to surface nutrient
20 depletion, whereas upwelling on the open shelf tends to cause stronger mixing and higher
21 surface nutrient concentrations (as shown by the higher f -ratios reported by Joint et al.
22 (2001) and Bode et al. (2004a) during upwelling).

23 PN-specific rates were particularly high in June ($0.026 \pm 0.004 \text{ h}^{-1}$) relative to
24 September ($0.005 \pm 0.001 \text{ h}^{-1}$) and relative to values obtained in the Benguela ($0.006 \pm$
25 0.0004 h^{-1}) (unpublished data). However, due to the relatively low biomass, this did not
26 lead to higher $\rho(\text{N})$, which was of the same order of magnitude as in the Benguela.
27 According to Dugdale et al. (1990), specific nitrate uptake [$V(\text{NO}_3^-)$] is a function of
28 ambient NO_3^- and if biomass accumulation occurs as a result of the “shift-up” in $V(\text{NO}_3^-)$
29), then $\rho(\text{NO}_3^-)$ will increase non-linearly with $V(\text{NO}_3^-)$. Here, $V(\text{NO}_3^-)$ and $\rho(\text{NO}_3^-)$ were
30 linearly correlated (data not shown), indicating that no biomass accumulation had
31 occurred. This low realisation of potential new production was also observed at Point

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

9 Nitrate uptake rates in June were similar to those reported by Bode et al. (2004a), but
10 one order of magnitude higher than those measured in the Ría de Ferrol in both June and
11 September [A. Bode, pers. comm., revision of data originally published in Bode et al.,
12 2005]. But it must be noted that in the Ría de Ferrol, measurements were based on 24 h
13 incubations, which therefore included dark uptake, unlike in this study. This could
14 contribute significantly to the difference in uptake rates. In contrast, nitrate uptake was
15 several-fold lower than in the California (Dugdale et al., 2006), Benguela (Probyn, 1992)
16 and the Cap Blanc upwelling region (Dugdale et al., 1990). This could be an effect of the
17 normalisation of nitrogen uptake rates to particulate nitrogen and possible abundance of
18 detrital particulate nitrogen in the Ría. Normalisation to chl-a would no doubt reduce
19 these differences since chl-a concentrations in the Ría were lower than in the other
20 upwelling systems.

21 Ammonium uptake rates were within the range of those measured by Bode et al.
22 (2004a) and several-fold higher than those measured by Bode et al. (2004b). During the
23 downwelling event, $r(\text{NH}_4^+)$ was generally higher than $\rho(\text{NH}_4^+)$ whereas during the
24 upwelling event it was generally lower. The higher regeneration rates during
25 downwelling are consistent with previous studies (e.g. Varela et al., 2003) and with the
26 higher ambient NH_4^+ concentrations measured during this period. However, Varela et al.
27 (2003) reported that uptake and regeneration were coupled and thus NH_4^+ did not
28 accumulate in the coastal area off the Ria de Vigo. Their results, however, were depth-
29 averaged and included a station outside the mouth of the ria, which could explain this
30 difference. Urea uptake rates have been measured in very few other studies. They were
31 up to two orders of magnitude higher than those reported by Bode et al. (2004a), who

1 despite these low rates found that $\rho(\text{urea})$ exceeded $\rho(\text{NH}_4^+)$ (but not $\rho(\text{NO}_3^-)$) on the
2 occasions when both were measured. Generally, N uptake rates were at least one order of
3 magnitude higher than those reported for experiments conducted further offshore on the
4 continental shelf (Slawyk et al., 1997). Bode et al. (2005) measured higher $\rho(\text{NO}_3^-)$
5 relative to $\rho(\text{NH}_4^+)$ in July, when NO_3^- concentrations were higher than NH_4^+ (although
6 still $<1 \mu\text{mol N L}^{-1}$), but the opposite in September, when NH_4^+ concentrations were
7 higher, suggesting that the source of nitrogen used was determined by the relative
8 concentration of each nitrogen source, rather than by preference. In the present study,
9 NH_4^+ appeared to be taken up preferentially to NO_3^- in both seasons, and irrespective of
10 ambient concentrations of each N source. Nitrate uptake was particularly low at high
11 NH_4^+ concentrations ($>0.5 \mu\text{mol N L}^{-1}$), suggesting that $\rho(\text{NO}_3^-)$ was inhibited by NH_4^+ .
12 Both preferential uptake of NH_4^+ relative to NO_3^- and inhibition of NO_3^- uptake by NH_4^+
13 have been widely reported (see review by Dortch (1990)). These phenomena are linked to
14 the lower energetic cost of NH_4^+ assimilation relative to NO_3^- , which must first be
15 reduced intracellularly to NO_2^- then to NH_4^+ before the latter can be synthesised into
16 amino acids and proteins. Nitrogen uptake kinetics parameters can indicate preference,
17 whereby a higher V_m for NH_4^+ than for NO_3^- would suggest preference for NH_4^+ over
18 NO_3^- . The presence of NH_4^+ in NO_3^- kinetics experiments, however, can potentially cause
19 inhibition of NO_3^- uptake and bias the results (Dortch, 1990; Collos et al., 2004).

20 To address this, a nitrogen uptake kinetics experiment was carried out on a mixed
21 diatom assemblage in June, with an ambient NH_4^+ concentration of $0.33 \mu\text{mol N L}^{-1}$. This
22 was below the range of concentrations generally thought to inhibit NO_3^- uptake (Dortch,
23 1990), therefore the obtained ratio $V_m(\text{NH}_4^+): V_m(\text{NO}_3^-)$ of 12.8 should indicate a genuine
24 preference for NH_4^+ , rather than inhibition. The ratio of $\alpha(\text{NH}_4^+): \alpha(\text{NO}_3^-)$ showed that
25 NH_4^+ was also preferred at limiting concentrations, although the preference was more
26 strongly expressed at saturating concentrations. Urea was also preferred over NO_3^- at
27 saturating concentrations, confirming the potential importance of regenerated nitrogen for
28 phytoplankton growth in this system.

29 The $V_m(\text{NH}_4^+): V_m(\text{NO}_3^-)$ ratio was several-fold higher than in other upwelling
30 systems (Table 2), due to the particularly high $V_m(\text{NH}_4^+)$ measured in the present study.
31 This value was more than one order of magnitude higher than any V_m reported in Table 2,

1 although V_m values of a similar order of magnitude have been measured in cultures
2 (Cochlan et al., 2008; Yamamoto et al. 2004). There do not appear to be any
3 methodological reasons that could have been responsible for these very high uptake rates,
4 and the uptake rates did follow Michaelis-Menten kinetics, ruling out the possibility that
5 the samples could have been contaminated. Furthermore, the incubation length was
6 sufficiently long to avoid the bias introduced by “surge uptake” on the calculated uptake
7 rates (Collos et al., 1997). Therefore, it seems that the phytoplankton population present
8 was genuinely capable of very high nitrogen (and particularly NH_4^+) uptake, if the
9 substrate was present in sufficiently high concentrations. Furthermore, the $\rho(\text{NH}_4^+)$
10 values for this experiment were 0.10-0.99 $\mu\text{mol N L}^{-1} \text{ h}^{-1}$, which was similar to the range
11 of $\rho(\text{NH}_4^+)$ values reported by Bode et al. (2004b) at ambient concentrations between 0.1
12 and 1.0 $\mu\text{mol N L}^{-1}$, indicating that these rates were not unrealistic.
13

1 **5. Conclusions**

2

3 The two surveys carried out in the Ría de Vigo showed contrasting situations in terms of
4 hydrography, nutrient concentrations, community structure and nitrogen uptake. Toxic
5 dinoflagellates were present during the period of downwelling-favourable winds, when
6 phytoplankton growth was supported primarily by ammonium. This was observed
7 particularly towards the head of the ría, where NH_4^+ concentrations were highest. Urea
8 was also an important source of nitrogen. This reliance on regenerated N is consistent
9 with the trend identified for HABs in upwelling systems by Kudela et al. (2010).

10 Phytoplankton showed a preference for NH_4^+ over NO_3^- or possibly inhibition of $\rho(\text{NO}_3^-)$
11 by NH_4^+ . During the period of upwelling-favourable winds, the water column was
12 stratified and nutrients were depleted above the thermocline, because upwelling was not
13 strong enough to mix the water column. The phytoplankton community was fairly typical
14 of summer upwelling, largely dominated by diatoms. Because of the low ambient NO_3^-
15 concentrations, phytoplankton growth was still supported primarily by recycled nitrogen,
16 although to a lesser extent than during downwelling. An upwelling pulse at the end of the
17 survey led to NO_3^- -dominated nitrogen uptake at the thermocline, thus indicating the
18 potential for new production under stronger upwelling conditions. Maximum potential
19 new production was not realised due to organic matter export out of the ría, possibly
20 combined with grazing control. Nitrogen uptake kinetics showed that during this period
21 NH_4^+ was preferred over NO_3^- and the phytoplankton community was able to exploit
22 rapid increases in NH_4^+ concentration. Potentially toxic *Pseudo-nitzschia* species were
23 present, as well as *Dinophysis acuminata*, showing that the upwelling season can
24 potentially be conducive to HABs as well as the downwelling season.

25

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27

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

4
5 Table 2. Comparison of nitrogen uptake kinetics parameters from this and other studies in
6 upwelling systems. Measurements from this study are in bold. a: Kudela et al. (2008a); b:
7 Seeyave et al. (2009); c: Kudela et al. (2008b); d: Kudela & Cochlan (2000); e: Dortch &
8 Postel (1989); f: Seeyave (2009).

9
10 Figure 1. Map of NW Spain showing the four Rías Baixas (left-hand panel) and detailed
11 map of the Ría de Vigo (right-hand panel) showing the CTD stations B0-B5 (closed
12 circles) and the meteorological stations (open circles).

13
14 Figure 2. Southerly wind components averaged 6-hourly in (a) September 2006 and (b)
15 June 2007. Horizontal bars indicate the sampling periods.

16
17 Figure 3. Temperature (b,e,h,k) and salinity (c,f,i,l) contour plots obtained from
18 MiniBAT deployments along longitudinal transects (a,d,g,j) of the ría on 26 and 30
19 September 2006 and 25 and 28 June 2007.

20
21 Figure 4. Vertical profiles of NO_3^- and NH_4^+ concentrations in September 2006 at stations
22 B5, B3 and B2.

23
24 Figure 5. Vertical profiles of NO_3^- and NH_4^+ concentrations in June 2007 at stations B5,
25 B3 and B2.

26
27 Figure 6. Chl-a profiles obtained from CTD fluorescence measurements in September
28 2006 (a,b,c) and June 2007 (d,e,f) at stations B5, B3 and B2. Note the difference in scale
29 between 2006 and 2007.

30
31 Figure 7. Concentrations of diatoms, dinoflagellates and flagellates (mean of
32 concentrations at 3 and 10 m, with error bars representing standard errors) at stations B5,
33 B3 and B2 on (a) 26 September 2006, (b) 30 September 2006, (c) 25 June 2007 and (d)
34 28 June 2007.

35
36 Figure 8. Nitrogen uptake kinetics measured on a water sample collected from 2 m depth
37 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. ($\mu\text{mol N l}^{-1}$)			Uptake ($\mu\text{mol N l}^{-1} \text{h}^{-1}$)				f-ratio	r(NH_4) $\mu\text{mol N l}^{-1} \text{h}^{-1}$
			NH_4	NO_3	urea	$\rho(\text{NH}_4)$	$\rho(\text{NO}_3)$	$\rho(\text{urea})$	Total $\rho(\text{N})$		
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	$V_{\max} (\times 10^{-3} \text{ h}^{-1})$			$K_s (\mu\text{mol l}^{-1})$			$\alpha = V_{\max}/K_s$			$V_{\max}(\text{NH}_4^+)$	$\alpha(\text{NH}_4^+)$	$V_{\max}(\text{urea})$	α
		NO_3^-	NH_4^+	Urea	NO_3^-	NH_4^+	Urea	NO_3^-	NH_4^+	Urea	$V_{\max}(\text{NO}_3^-)$	$\alpha(\text{NO}_3^-)$	$V_{\max}(\text{NO}_3^-)$	α
MONOSPECIFIC BLOOMS														
<u>Dinoflagellates</u>														
<i>Akashiwo sanguinea</i>	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	
<i>Alexandrium catenella</i>	Benguela	>17.5	14.9	3.5	nd	2.52	0.65	nd	5.9	5.4	<0.9	nd	<0.2	
<i>Cochlodinium spp.</i>	California	0.9	>4.0	2.1*	1.00	nd	4.06*	0.9	0.3	0.8*	4.4	nd	2.3	
<i>Dinophysis acuminata</i>	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	
<i>Lingulodinium polyedrum</i>	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	
<i>Prorocentrum minimum</i>	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	
<u>Diatoms</u>														
<i>Pseudo-nitzschia</i>	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	Ria 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	
	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	
Mixed dinoflagellates	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	
	Benguela	3.5	14.6	4.4	0.82	0.62	nd	4.3	23.5	nd	4.2	5.5	1.3	
Diatoms + dinoflagellates	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	

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

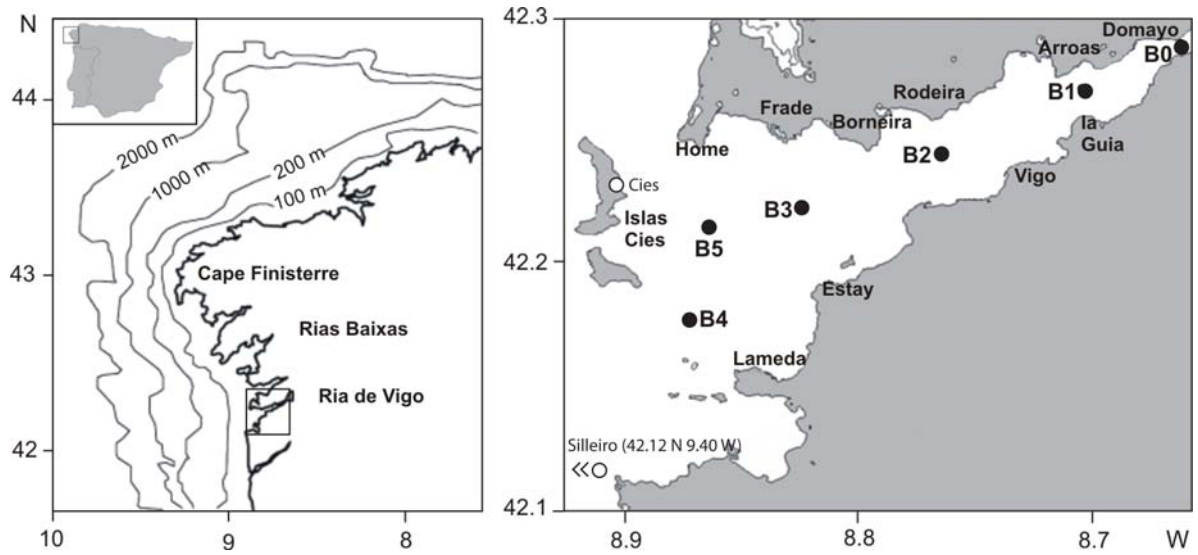


Figure 2. Southerly wind components averaged 6-hourly in (a) September 2006 and (b) June 2007. Horizontal bars indicate the sampling periods.

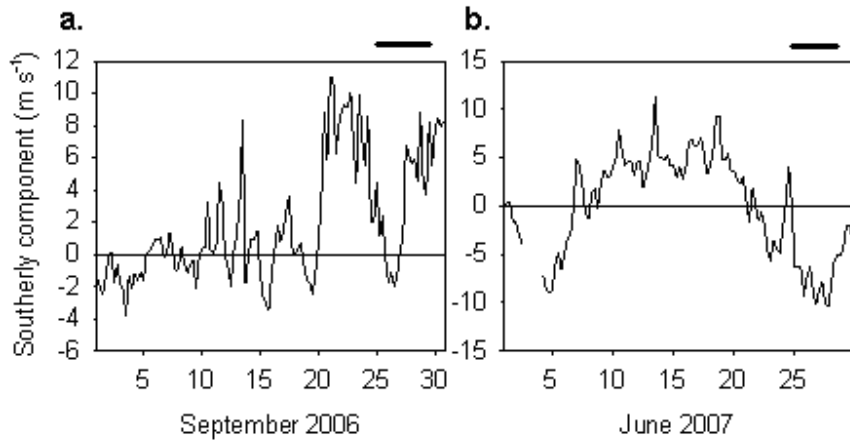


Figure 3. Temperature (b,e,h,k) and salinity (c,f,i,l) contour plots obtained from MiniBAT deployments along longitudinal transects (a,d,g,j) of the ria on 26 and 30 September 2006 and 25 and 28 June 2007.

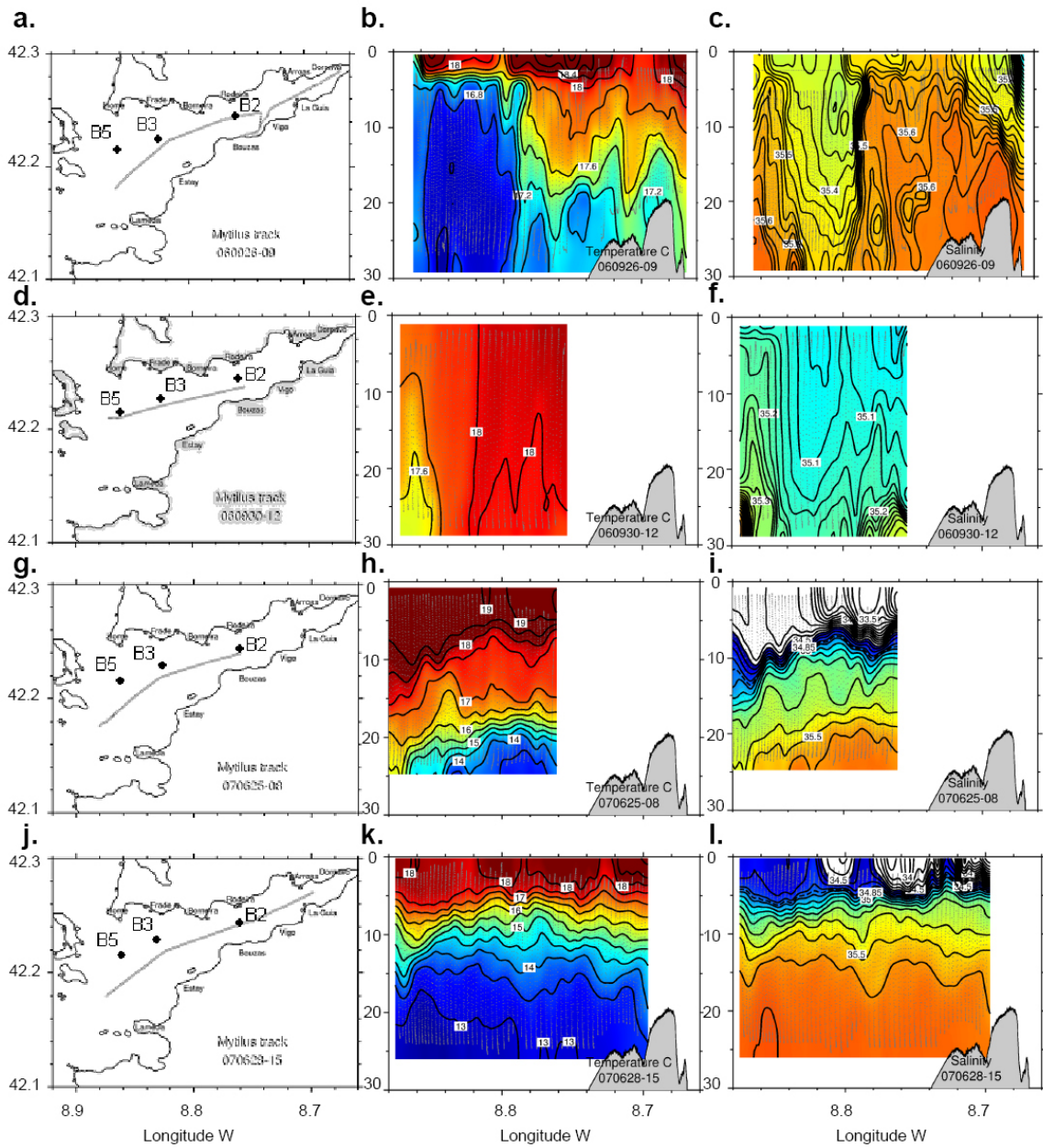


Figure 4. Vertical profiles of NO_3^- and NH_4^+ concentrations in September 2006 at stations B5, B3 and B2.

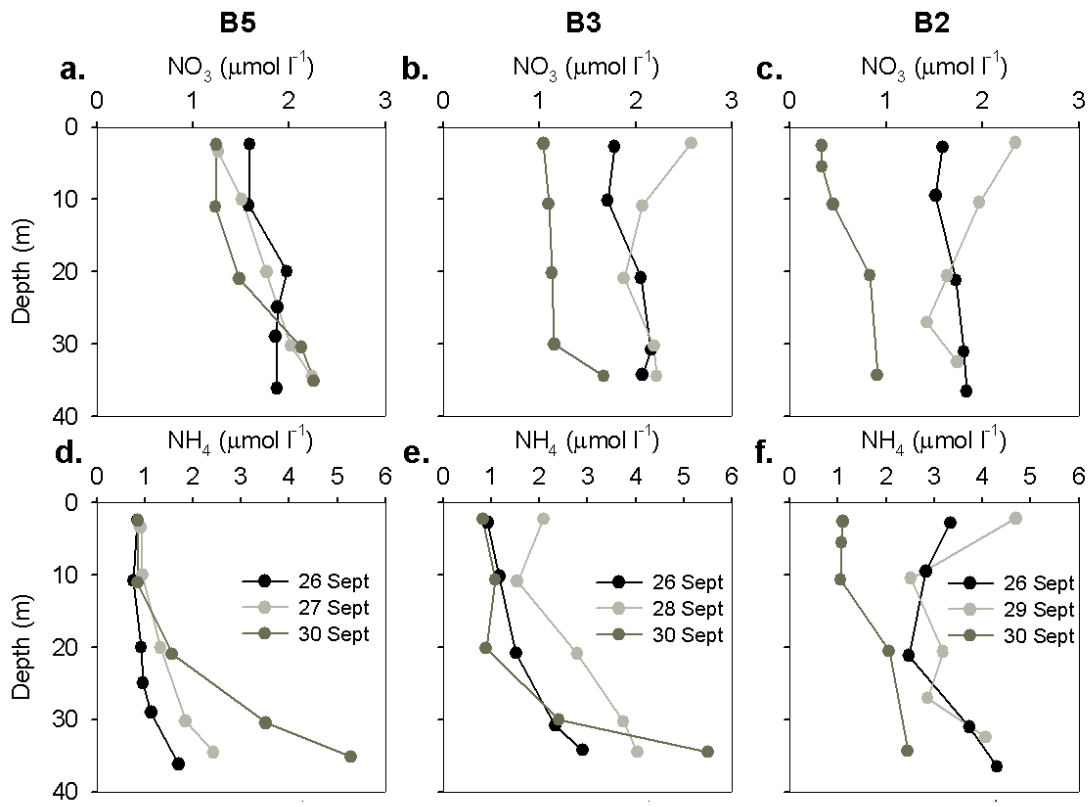


Figure 5. Vertical profiles of NO_3^- and NH_4^+ concentrations in June 2007 at stations B5, B3 and B2.

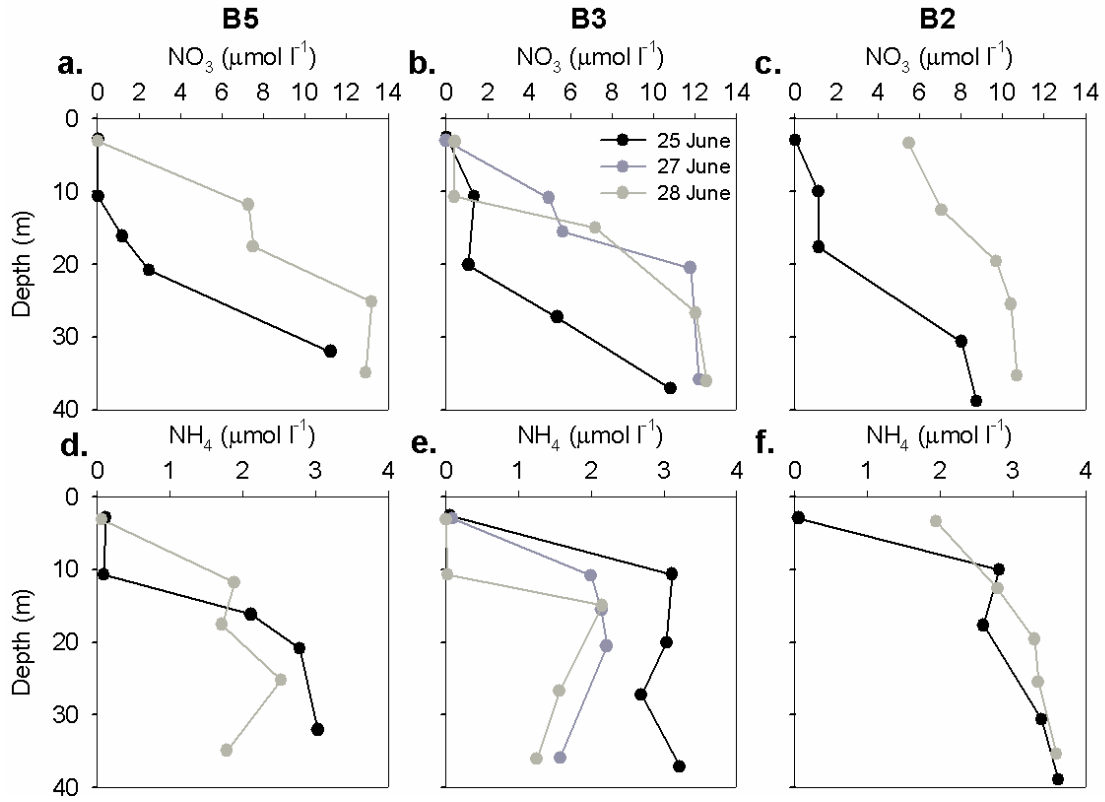


Figure 6. Chl-a profiles obtained from CTD fluorescence measurements in September 2006 and June 2007 at stations B5, B3 and B2. Note the difference in scale between 2006 and 2007.

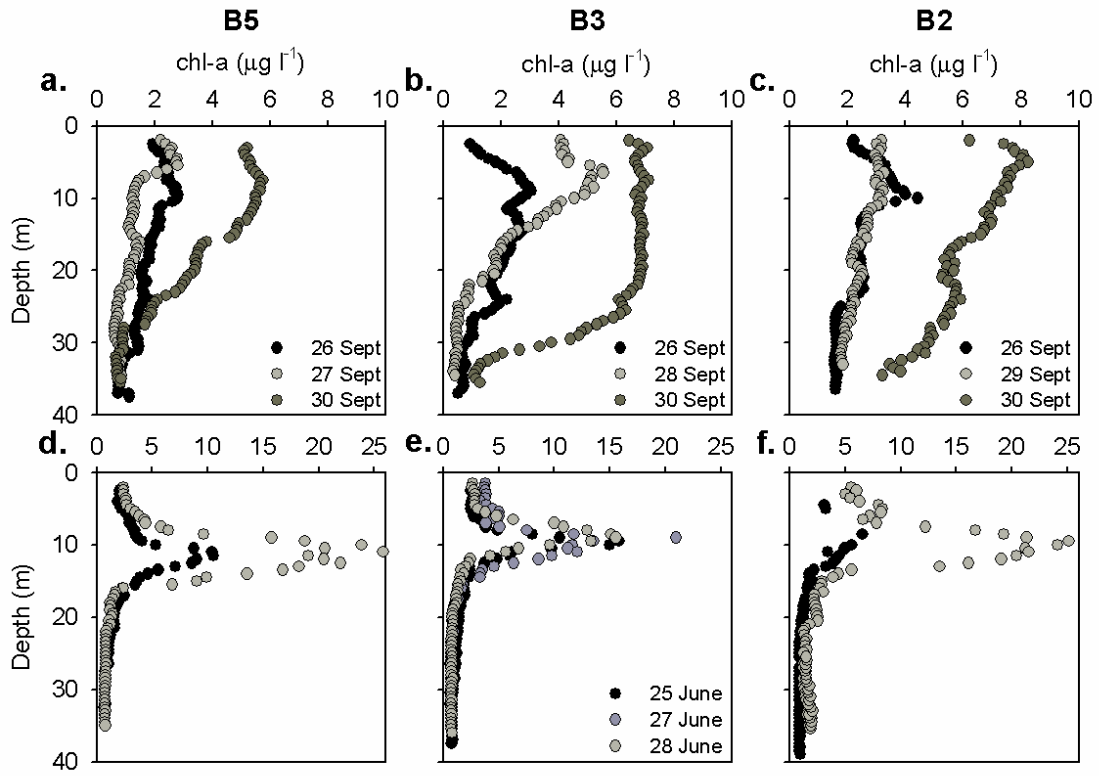


Figure 7. Concentrations of diatoms, dinoflagellates and flagellates (mean of concentrations at 3 and 10 m, with error bars representing standard errors) at stations B5, B3 and B2 on (a) 26 September 2006, (b) 30 September 2006, (c) 25 June 2007 and (d) 28 June 2007.

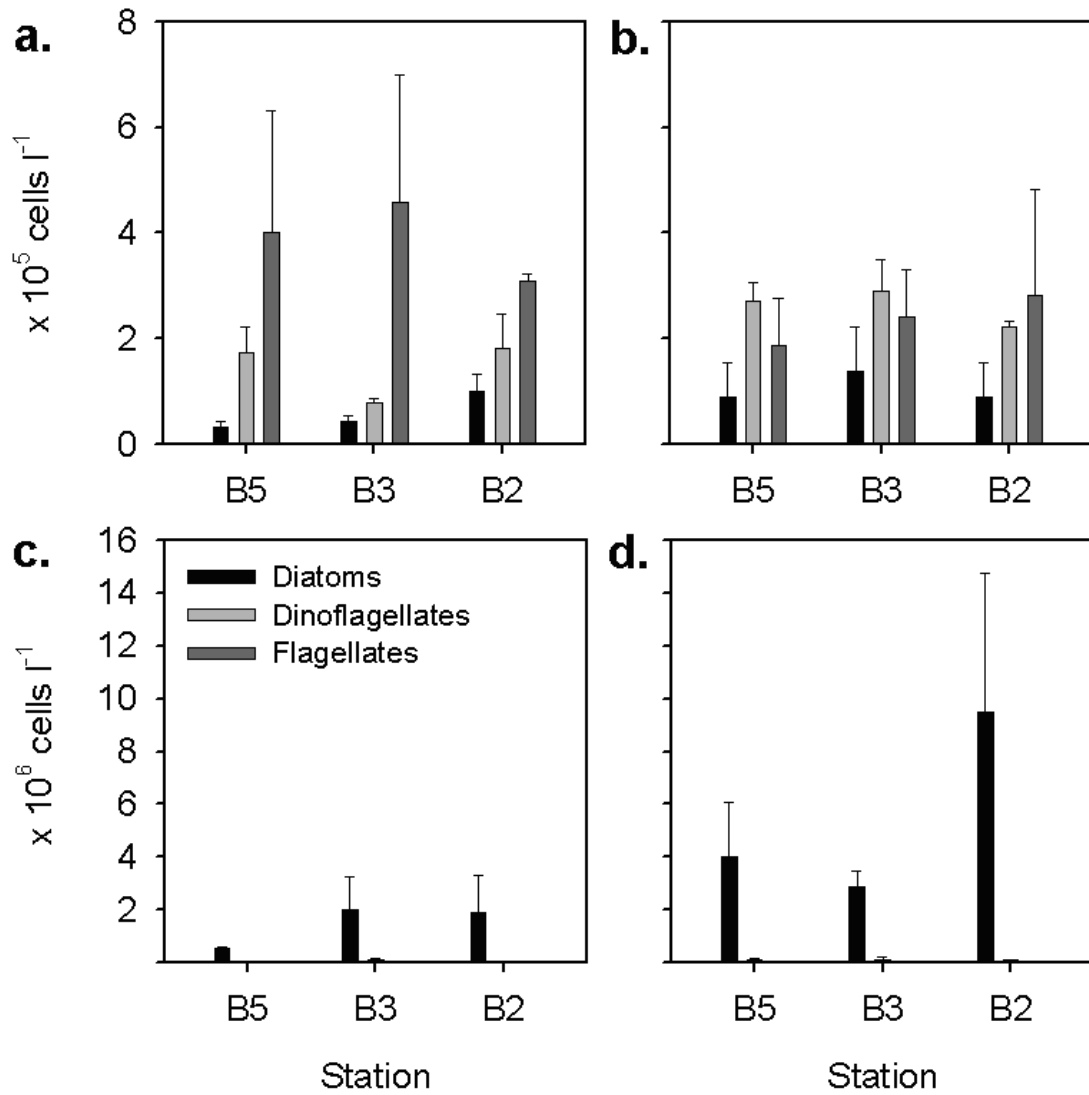


Figure 8. Nitrogen uptake versus ambient concentration fitted to the Michaelis-Menten equation for uptake kinetics using SigmaPlot (Jandel Scientific). Note the different scale in (a).

