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# Nitrogen nutrition in assemblages dominated by *Pseudo-nitzschia* spp., *Alexandrium catenella* and *Dinophysis acuminata* off the west coast of South Africa

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ABSTRACT: A study was carried out in the southern Benguela upwelling system in 2006 and 2007 to establish how the nutrient regime determines community succession and the selection of harmful algal bloom species. In March 2006, *Pseudo-nitzschia* spp. reached concentrations of $13 \times 10^6$ cells $l^{-1}$, representing 80% of the total estimated phytoplankton biomass, while chlorophyll *a* (chl *a*) reached 57 µg $l^{-1}$. High rates of $NO_3^-$ uptake ($\rho_{NO_3^-}$, maximum 0.56 µmol N $l^{-1}$ $h^{-1}$) led to $NO_3^-$ depletion (<0.1 µM). However, cell numbers remained high for several days, sustained by regenerated nitrogen, with *f*-ratios dropping from 0.79 to 0.12. In March 2007, a bloom of *Alexandrium catenella* ($4.5 \times 10^5$ cells $l^{-1}$, 77% of the phytoplankton biomass) occurred, with surface chl *a* concentrations of 26 µg $l^{-1}$. $NO_3^-$ concentrations were high (10 to 17 µM), sustaining high $\rho_{NO_3^-}$ (maximum 0.61 µmol N $l^{-1}$ $h^{-1}$) and *f*-ratios up to 0.87. In April, *Dinophysis acuminata* reached $3.1 \times 10^4$ cells $l^{-1}$ (91% phytoplankton biomass), with $NO_3^-$ concentrations <0.5 µM and *f*-ratios <0.1, indicative of regenerated production. Nutrient uptake kinetics showed that *Pseudo-nitzschia* spp. displayed the highest maximum specific uptake rates ($15.0 \times 10^{-3}$ and $18.0 \times 10^{-3}$ $h^{-1}$ for $NO_3^-$ and $NH_4^+$, respectively). *D. acuminata* displayed the highest affinity for $NH_4^+$, as shown by its higher α (slope of the nutrient uptake vs. concentration curve) of $20.7 \times 10^{-3}$, compared to $13.4 \times 10^{-3}$ and $5.9 \times 10^{-3}$ $h^{-1}$ (µmol N $l^{-1}$)$^{-1}$ for *Pseudo-nitzschia* spp. and *A. catenella*, respectively. *D. acuminata* was an affinity strategist, successful in nitrogen-depleted waters, whereas both *Pseudo-nitzschia* spp. and *A. catenella* were velocity strategists, better adapted to utilising high $NO_3^-$ concentrations during upwelling pulses.

KEY WORDS: Benguela upwelling system · Harmful algal blooms · Nutrient uptake · Nutrient kinetics · Dinoflagellates · Diatoms



## INTRODUCTION

Harmful algal blooms (HABs) are a regular occurrence on the west coast of South Africa, within the southern Benguela upwelling system, in late austral summer (February to April). HAB species in the Benguela include both high biomass producers, whose blooms can cause oxygen depletion and hydrogen sulfide production, and toxin producers, which are potential vectors of paralytic, diarrhetic and amnesic shellfish poisoning (PSP, DSP, ASP) (Pitcher & Calder 2000). PSP is typically attributed to *Alexandrium catenella* (Pitcher & Calder 2000), although a bloom of *A. minutum* in Cape Town harbour has recently been reported (Pitcher et al. 2007), indicating the potential threat posed by this species. In South Africa, DSP was first reported in 1991 on the west coast and attributed to *Dinophysis acuminata* (Pitcher et al. 1993b). *Dinophysis* spp. cell concentrations up to $4 \times 10^4$ cells $l^{-1}$ were recorded in March/ April 2005 off Lambert's Bay in association with okadaic acid (OA) concentrations of up to 0.4 µg $l^{-1}$ (Fawcett et al. 2007). *Pseudo-nitzschia* spp. (hereafter *Pseudo-nitzschia*) blooms also occur in the southern Benguela and were linked with high concentrations of domoic acid (up to

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3 µg $l^{-1}$) for the first time in March 2006 off Lambert's Bay (Fawcett et al. 2007), although no cases of ASP have yet been reported.

The HAB taxa that occur in the southern Benguela also form blooms in the other eastern boundary upwelling systems. For example, *Dinophysis acuminata* and *D. acuta* blooms occur in the Iberian upwelling system (Reguera et al. 1993), blooms of *Pseudo-nitzschia pseudodelicatissima*, *P. australis* and *P. multi-series* occur in the California upwelling system (Buck et al. 1992, Adams et al. 2000, Trainer et al. 2001) and *Alexandrium catenella* blooms occur in the Humboldt (Avaria 1979) and California systems (Nishitani & Chew 1988). HABs around the South African coast are closely associated with the upwelling system, as they are generally restricted to the west of Cape Agulhas (Fig. 1) (Pitcher & Calder 2000). They are particularly frequent north of Cape Columbine, where the broad southern Namaqua shelf favours stratification (Fig. 1). Blooms tend to occur towards the end of the upwelling season (March to April), when reduced wind stress and increased thermal stratification favour dinoflagellates

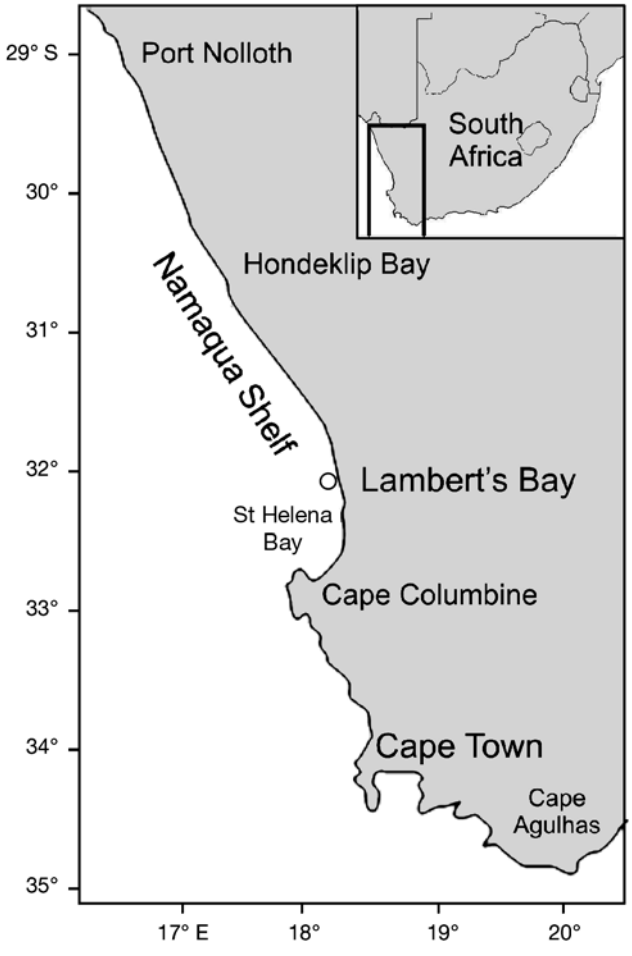


Fig. 1. West Coast of South Africa, showing the sampling station 3.5 km off Lambert's Bay (O)

(Pitcher et al. 1993a). Within these periods, community succession is dictated by local wind patterns, whereby upwelling-favourable winds and a well-mixed water column tend to favour diatoms, whereas upwelling relaxation is conducive to dinoflagellate blooms (Fawcett et al. 2007) and can lead to their onshore advection (Pitcher & Boyd 1996, Pitcher et al. 1998). The persistence of stratified conditions, however, leads to a decline in biomass and a shift in community composition towards small flagellates, in particular coccolithophorids (Pitcher & Boyd 1996, Pitcher et al. 1998, Fawcett et al. 2007). The link between HABs, upwelling relaxation and onshore advection has also been reported for the Iberian (Tilstone et al. 1994) and Californian (Trainer et al. 2002) upwelling systems.

According to Margalef's Mandala (Margalef 1978), the seasonal shift from diatoms in spring to dinoflagellates in summer occurs along a gradient of decreasing turbulence and nutrient availability. However, the 'red tide sequence' occurs under the anomalous combination of low turbulence/high nutrient conditions (Margalef et al. 1979), which fits with the apparent low affinity of dinoflagellates for nitrogen uptake relative to diatoms (Smayda 1997, Collos et al. 2005). However, HABs in upwelling systems tend to occur at the end of the upwelling season under stratified and nutrient-depleted conditions (Tilstone et al. 1994, Pitcher & Boyd 1996); therefore, they must have evolved adaptive strategies allowing survival under such conditions. In addition to the 4 adaptations suggested by Smayda (1997) (nutrient retrieval migrations, mixotrophy, secretion of allelochemicals and allelopathy), utilisation of recycled nitrogen sources may be significant in situations where 'new' nitrogen is depleted.

It has been hypothesised that diatoms may express a preference for nitrate ($NO_3^-$) over ammonium ($NH_4^+$) (Malone 1980), and several studies have shown that flagellates (Glibert et al. 1982a, Probyn 1985) and dinoflagellates can be sustained by regenerated nitrogen, e.g. *Alexandrium catenella* in the Thau Lagoon, France (Collos et al. 2007) and HAB assemblages including *Dinophysis acuta* and *Gymnodinium catenatum* in the Iberian upwelling system (Rios et al. 1995). However, laboratory studies have shown preferences for either $NO_3^-$ or $NH_4^+$ (as shown by parameters of nutrient uptake kinetics for $NO_3^-$ and $NH_4^+$) in both diatom and dinoflagellate species (Dortch 1990). Furthermore, the nitrogen status of the cell can influence its preference for either form of nitrogen (Collos & Slawyk 1979) or its ability to compete for a specific nutrient (Riegman et al. 1996). *Pseudo-nitzschia* blooms on the United States West coast have been attributed to either elevated $NO_3^-$ (Horner et al. 2000, Marchetti et al. 2004) or $NH_4^+$ concentrations (Trainer et al. 2007). Field populations of *P. australis* from Cali-

fornia were found to grow equally well on $NO_3^-$, $NH_4^+$, or urea (Howard et al. 2007); however, *P. australis* cultures exhibited lower growth rates supported by urea relative to $NO_3^-$ and $NH_4^+$ and a preference for $NO_3^-$ (higher maximum uptake rates $v_{max}$ and lower half-saturation constants $K_s$) relative to recycled nitrogen forms (Cochlan et al. 2008).

We hypothesise that the success of HAB species in the southern Benguela at the end of the upwelling season could be attributable to their ability to utilise alternative nitrogen sources or to their high affinity for $NO_3^-$ under low $NO_3^-$ conditions.

To test this hypothesis, regular measurements of nitrogen uptake rates ($NO_3^-$, $NH_4^+$ and urea) were performed in conjunction with hydrographic and other biological measurements during ~3 wk sampling periods in 2006 and 2007 off Lambert's Bay on the West coast of South Africa. In addition, experiments in nutrient uptake kinetics were performed during blooms of *Pseudo-nitzschia*, *Alexandrium catenella* and *Dinophysis acuminata*.

## MATERIALS AND METHODS

**Sampling.** Sampling was carried out between 7 and 23 March 2006 and between 20 March and 11 April 2007 at the same station, 3.5 km off Lambert's Bay (32° 05.020' S, 18° 16.010' E; Fig. 1) in ~50 m water.

Vertical profiles of temperature and fluorescence were established using a Seabird Electronics Seacat CTD coupled with a Wetstar fluorometer. Discrete water samples were taken daily from 5 depths (0, 5, 10, 15, 20 m) for chlorophyll *a* (chl *a*) analyses. Samples for $^{15}N$ incubations were taken at 2 depths: at the surface (0 m) and a subsurface depth that was 5 m on all dates in 2007 and on most dates in 2006, execept for 10 and 11 March 2006 where it was 10 m. Samples for phosphate ($PO_4^{3-}$), silicic acid [$Si(OH)_4$, hereafter Si], nitrite ($NO_2^-$), $NO_3^-$, $NH_4^+$ and urea concentrations were only taken at 2 depths in 2006, but at all 5 depths in 2007. Phytoplankton counts were performed on surface samples only. All water was collected using 5 l Niskin bottles and transported ashore in dark containers within 1 h of collection. Incubations and nutrient and chl *a* analyses were performed ashore in Lambert's Bay, whereas the more time-consuming analyses (e.g. $NH_4^+$ recovery and mass spectrometry) were carried out at a later date either in Cape Town or Southampton (UK). Wind data were obtained from a weather station exposed to maritime wind conditions, situated at 97 m altitude and 8.5 km from the sampling station (South African Weather Service).

**Statistical data analysis.** Where comparisons were made between groups of data (e.g. 2006 vs. 2007), Student's *t*-tests were applied, provided that variances within each group were not significantly different (as determined by a 2-tailed *F*-test). Paired *t*-tests were used for comparing simultaneous surface and subsurface measurements or measurements of different parameters made on the same water sample. The non-parametric Mann-Whitney *U*-test was employed when variances were significantly different. The level of confidence used to determine significance was 95% ($p < 0.05$); therefore, the p-value is only shown when higher levels of confidence were achieved.

**Nutrients.** All nutrients were determined manually within 2 h of collection after filtration through 25 mm Whatman GF/F filters. $NO_3^-$ concentrations were determined colourimetrically after reduction to $NO_2^-$ on a cadmium column and corrected for ambient $NO_2^-$ (Nydahl 1976). Concentrations of Si, $PO_4^{3-}$, $NH_4^+$, $NO_2^-$ and urea were measured following Grasshoff et al. (1999) and scaled to 5 ml sample volumes.

**Chl *a* and phytoplankton concentrations.** Water samples (100 ml) collected for the determination of chl *a* concentrations were filtered onto 25 mm Whatman GF/F filters, ground and extracted in 9 ml of 90% acetone for at least 24 h at –20°C in the dark, then centrifuged for 10 min. Duplicate measurements were carried out in association with nutrient uptake incubations. Fluorescence was determined using a Turner Designs 10-AU fluorometer calibrated with commercial chl *a* (Sigma). Fluorescence readings were taken before and after acidification with 2 drops 10% HCl to correct for phaeopigments (Parsons et al. 1984).

Phytoplankton samples (100 ml) were preserved in 2.5% buffered formalin and counted using inverted microscopy (Utermöhl 1958). Identification was to species level where possible. Three species of *Pseudo-nitzschia* were enumerated, including *P. australis* and 2 unidentified species. The most abundant of these 2 ('Species 1') had an apical axis of 38 µm (±6 µm, n = 20) and a transapical axis of 3.4 µm (±0.6 µm, n = 20). 'Species 2' only represented 0 to 16% of the total *Pseudo-nitzschia* concentrations and was a small species with a transapical axis of <3 µm. For the experiments in nutrient uptake kinetics, biomass estimates were made to determine the dominance of *Pseudo-nitzschia*, *Alexandrium catenella* and *Dinophysis acuminata* in the 3 experiments, respectively. Cell volumes were calculated from cell measurements and stereometric shapes (Hillebrand et al. 1999). Biovolume-to-carbon conversions were then applied following the equations of Menden-Deuer & Lessard (2000):

$$\text{Dinoflagellates: } C_{cell} = 0.760 \times V^{0.819} \quad (1a)$$

$$\text{Diatoms: } C_{cell} = 0.288 \times V^{0.811} \quad (1b)$$

where $C_{cell}$ is cellular carbon content (pg C cell$^{-1}$) and $V$ is cell volume (µm$^3$).

Due to the low number of species in these experiments and their consistent cell sizes, these estimates were considered to be robust.

Multivariate analyses were performed on the count data using PRIMER (V. 5). Between-station similarities were calculated using the Bray–Curtis similarity index based on standardised cell concentrations after square root transformation. The similarity matrices obtained were then used to perform cluster analyses and multidimensional scaling (MDS). The last station in 2007 was distinct from all others at the 60% similarity level in the cluster analysis. Since the objective was to identify similarities within clusters of stations, it was excluded from the MDS analysis. Similarity percentage (SIMPER) analysis was performed on standardised count data after square root transformation, to determine species' contribution to similarity within the clusters identified at the 60% similarity level. The Shannon diversity index (Shannon 1948) was calculated (using PRIMER) from:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i \quad (2)$$

where $S$ is the number of species and $p_i$ is the proportion of each species $i$ relative to total phytoplankton abundance.

**Nutrient uptake.** ***Standard nutrient uptake incubations:*** Two 1 l and one 2 l water samples taken from the surface and subsurface were dispensed into Nalgene polycarbonate bottles. The 1 l samples were inoculated with stock solutions of 1 mmol $^{15}N\ l^{-1}$ (Prochem, BOC), as either $K^{15}NO_3$ (99.6 at.%) or $CO(^{15}NH_2)_2$ (99.1 at.%), whereas the 2 l sample was inoculated with $^{15}NH_4Cl$ (99.7 at.%). Aqueous enrichments after spike addition were, on average, 23.1% (±19.7%) in 2006 and 21.4% (±16.3%) in 2007. Samples were incubated in Perspex incubation tubes for 3 h (generally starting between 11:15 and 12:00 h) at simulated *in situ* temperatures (11 to 17°C) and irradiances (50% shading was achieved with neutral density filters [Lee] for the subsurface samples). Incubations were terminated by filtration onto 47 mm precombusted GF/F filters (Whatman), which were then rinsed with artificial seawater and dried (75°C, overnight). Between 5 and 10 small (5 mm diameter) discs were punched out of each filter, depending on the amount of material present, and pelletted into tin capsules (Pellican Scientific 5 × 9 mm). Isotopic composition and particulate nitrogen (PN) concentrations were determined on a GV Instruments IsoPrime stable isotope mass spectrometer interfaced with a EuroVector Euro EA elemental analyser.

Uptake rates were calculated from Eqs. (1) to (3) of Dugdale & Wilkerson (1986), corrected for isotopic dilution of $^{15}NH_4^+$ by regenerated $^{14}NH_4^+$ according to Glibert et al. (1982b).

Filtrates from the start and end of each incubation were returned frozen to the Marine & Coastal Management laboratory in Cape Town, where aqueous $NH_4^+$ was recovered by diffusion onto ashed and halved 25 mm GF/F filters (Probyn 1987) with $KHSO_4$ as the trapping agent. Filters were processed and analysed in the same way as the $^{15}N$ uptake samples to determine aqueous enrichment at the start ($R_0$) and end ($R_t$) of the incubations.

***Nutrient uptake kinetics:*** On 16 March 2006, an experiment was undertaken on a sample from 5 m water depth dominated by *Pseudo-nitzschia* (80% total estimated phytoplankton carbon biomass, ~57% of

Table 1. Phytoplankton species' contribution to total between-station similarity derived from SIMPER analysis performed on clusters defined at the 60% similarity level and mean (±SD) Shannon diversity indices ($H'$), surface temperatures, $NO_3^-$ concentrations and $f$-ratios for each cluster (assemblage type)

| Assemblage type | Dates | Species | Percent contribution to total similarity | $H'$ | Temp. (°C) | $NO_3^-$ (µM) | $f$-ratio |
|---|---|---|---|---|---|---|---|
| I | 20, 21, 28 Mar 2007 | *Skeletonema costatum* | 31.6 | 1.5 ± 0.4 | 11.5 ±0.3 | 16.5 ± 6.3 | 0.56 ± 0.46 |
| | | *Alexandrium catenella* | 21.3 | | | | |
| | | *Thalassiosira* spp. | 16.2 | | | | |
| | | *Scrippsiella trochoidea* | 11.5 | | | | |
| II | 4, 5, 8–10 Apr 2007 | *Gymnodinium* spp. | 93.1 | 0.3 ± 0.3 | 16.3 ± 0.4 | 0.2 ± 0.2 | 0.07 ± 0.02 |
| | | *Dinophysis acuminata* | 6.2 | | | | |
| III | 22–27, 29–31 Mar, 2–3 Apr 2007 | *S. costatum* | 29.2 | 1.1 ± 0.3 | 13.6 ± 1.4 | 4.9 ± 8.4 | 0.25 ± 0.16 |
| | | *Chaetoceros* spp. | 28.6 | | | | |
| | | *Pseudo-nitzschia* spp. | 18.5 | | | | |
| | | *Bacteriastrum* spp. | 11.0 | | | | |
| IV | 1, 6, 7 Apr 2007 | *Chaetoceros* spp. | 72.0 | 0.4 ± 0.1 | 16.2 ± 0.9 | 0.3 ± 0.4 | 0.15 ± 0.12 |
| | | *Pseudo-nitzschia* spp. | 11.3 | | | | |
| V | 7–23 Mar 2006 | *Pseudo-nitzschia* spp. | 61.5 | 0.7 ± 0.2 | 12.7 ± 1.3 | 9.8 ± 9.6 | 0.53 ± 0.26 |
| | | *Chaetoceros* spp. | 34.8 | | | | |

which was *P. australis*). In 2007, experiments were undertaken on surface samples dominated by *Alexandrium catenella* (77%) on 21 March and by *Dinophysis acuminata* (91%) on 8 April.

Subsamples were dispensed from a 5 l sample into eighteen 250 ml bottles (rinsed with sample water) and spiked with different volumes of 10% enriched $KNO_3$, $NH_4Cl$ and urea solutions (1 mmol N $l^{-1}$). Ambient concentrations are shown in Table 2 and nutrient additions were as follows (mean ± SE, n = 3): 0.19 (0.11), 0.48 (0.27), 1.99 (1.55), 4.85 (2.66), 8.73 (3.25) and 20.16 (3.65) µmol N $l^{-1}$ for $NO_3^-$; 0.13 (0.04), 0.28 (0.06), 0.63 (0.20), 2.13 (0.04), 5.32 (0.11) and 19.58 (2.14) µmol N $l^{-1}$ for $NH_4^+$; and 0.13 (0.04), 0.28 (0.06), 0.63 (0.19), 2.15 (0.06), 5.39 (0.15) and 16.90 (2.68) µmol N $l^{-1}$ for urea. The aqueous enrichment was <10% at the lowest concentrations, which was sometimes problematic when the ambient nutrient concentration was high (see 'Results'). Replication and a greater number of treatments (particularly in the range of subsaturating concentrations) would have been preferable; however, this was not possible due to logistical constraints. Notwithstanding these shortcomings, the $r^2$ values obtained for the regressions (0.88 to 1.00) and the p-values for the parameters (Table 3) provide confidence in these estimates.

Samples were incubated for 3 h under surface temperature and light conditions, then filtered onto 25 mm precombusted GF/F filters. The proportion of added $^{15}N$ incorporated into the particulate phase during the incubations ranged from 18 to 52% (Expt 1), 37 to 55% (Expt 2) and 6 to 23% (Expt 3) at the lowest nitrogen addition; therefore, substrate depletion was not an issue. Samples were analysed in the same way as the standard uptake samples, except that no correction was made for isotope dilution in the $NH_4^+$ experiments. According to Dugdale & Wilkerson (1986), this should only be problematic (i.e. lead to an overestimation of $K_s$) for incubation lengths >6 h.

Plots of nutrient uptake versus concentration were fitted to the Michaelis–Menten equation (Dugdale 1967) by iterative, non-linear least-squares regression (SigmaPlot, Jandel Scientific) such that:

$$\nu = \nu_{max} S/(K_s + S) \quad (3)$$

where ν is the particulate nitrogen (PN)-specific uptake rate ($h^{-1}$), $\nu_{max}$ is the maximum PN-specific uptake rate ($h^{-1}$), $S$ is the substrate concentration (µmol N $l^{-1}$) and $K_s$ is the half-saturation constant (µmol N $l^{-1}$). The initial slope of the curve α, used as an indicator of nutrient affinity at concentrations $<K_s$, was calculated from $\alpha = \nu_{max}/K_s$ (Healey 1980).

Table 2. Cell concentrations of the dominant phytoplanktion species and initial particulate organic nitrogen (PON) and ambient $NO_3^-$, $NH_4^+$ and urea concentrations in the 3 nutrient uptake kinetics experiments

| Expt | Dominant species | Cell conc. ($\times 10^3$ cells $l^{-1}$) | PON (µmol N $l^{-1}$) | Ambient conc. (µmol N $l^{-1}$) | | |
|---|---|---|---|---|---|---|
| | | | | $NH_4^+$ | $NO_3^-$ | Urea |
| 1 | *Pseudo-nitzschia* | 7980 | 13.7 | 0.18 | 0.09 | 1.68[a] |
| 2 | *Alexandrium catenella* | 304 | 34.8 | 0.16 | 16.89[a] | 0.27 |
| 3 | *Dinophysis acuminata* | 31 | 7.2 | 0.42 | 0.47 | 0.23 |

[a]Ambient concentration hindered estimation of the kinetics parameters

Table 3. Parameters of nutrient uptake kinetics $\nu_{max}$ and $K_s$, means (SE), derived from Fig. 8. The initial slope of the curve, α, is the ratio $\nu_{max}/K_s$. Maximum uptake and α are particulate nitrogen (PN)-specific or cell-specific. *$p < 0.05$; n.d.: not determined

**PN specific**

| Expt | Dominant species | $\nu_{max}$ ($\times 10^{-3}$ $h^{-1}$) | | | $K_s$ (µmol N $l^{-1}$) | | | α ($\times 10^{-3}$ $h^{-1}$ [µmol N $l^{-1}$]$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $NO_3^-$ | $NH_4^+$ | Urea | $NO_3^-$ | $NH_4^+$ | Urea | $NO_3^-$ | $NH_4^+$ | Urea |
| 1 | *Pseudo-nitzschia* | 15.0 (0.4)* | 18.0 (0.3)* | 4.9 (0.3)[a] | 1.21 (0.15)* | 1.34 (0.07)* | n.d. | 12.4 | 13.4 | n.d. |
| 2 | *Alexandrium catenella* | n.d. | 14.9 (0.8)* | 3.5 (0.2)* | n.d. | 2.52 (0.36)* | 0.65 (0.12)* | n.d. | 5.9 | 5.4 |
| 3 | *Dinophysis acuminata* | 3.5 (0.2)* | 13.9 (0.2)* | 6.2 (0.6)* | 0.79 (0.26) | 0.67 (0.06)* | 0.53 (0.22) | 4.4 | 20.7 | 11.7 |

**Cell specific**

| Expt | Dominant species | $\nu_{max}$ (pmol N $cell^{-1}$ $h^{-1}$) | | | $K_s$ (µmol N $l^{-1}$) | | | α (pmol N $cell^{-1}$ $h^{-1}$ [µmol N $l^{-1}$]$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $NO_3^-$ | $NH_4^+$ | Urea | $NO_3^-$ | $NH_4^+$ | Urea | $NO_3^-$ | $NH_4^+$ | Urea |
| 1 | *Pseudo-nitzschia* | 0.02 | 0.03 | 0.01 | 1.21 | 1.34 | n.d. | 0.02 | 0.02 | n.d. |
| 2 | *A. catenella* | n.d. | 1.30 | 0.31 | n.d. | 2.52 | 0.65 | n.d. | 0.52 | 0.48 |
| 3 | *D. acuminata* | 0.74 | 2.90 | 1.30 | 0.79 | 0.67 | 0.53 | 0.94 | 4.33 | 2.45 |

[a]Estimated from the mean of $\nu_{urea}$ at 4 saturating concentrations

In Expt 1, calculations of $v_{urea}$ at the 2 lower urea concentrations were not reliable due to a high ambient concentration (1.7 µmol N $l^{-1}$), which resulted in very low enrichments (1 to 2%) in these treatments; therefore, it was not possible to estimate $K_s$ in this case. However, since no increase in v was observed at 4 concentrations between 2.7 and 17.0 µmol N $l^{-1}$, $v_{max}$ of urea could be estimated from the mean of these 4 measurements. The ambient $NO_3^-$ concentration in Expt 2 was very high (16.9 µM); therefore, no estimate could be made for either $K_s$ or $v_{max}$ of $NO_3^-$.

Cell-specific maximum uptake rates (µmol N $cell^{-1}$ $h^{-1}$) were estimated from:

$$v_{max(cell)} = v_{max} \times PN/n$$

where PN is the estimated nitrogen biomass (µmol N $l^{-1}$) of the dominant species (= total PN × % dominance) and $n$ is the cell concentration of the dominant species (cells $l^{-1}$). The dominance of the species of interest was never 100%; therefore, these conversions to cellular $v_{max}$ are only estimates. Given the high proportion of the dominant species, we consider our calculations to be reasonable estimates that will allow us to make some inferences on the effect of cell size.

## RESULTS

### Hydrographic conditions

Temperatures in both years fluctuated (~11 to 17°C) as cold (11 to 12°C) upwelling pulses alternated with warmer (14 to 17°C) relaxation/downwelling cycles.

In 2006, the sampling period was characterised on 3 occasions by surface warming (to 16°C on the first occasion, then 14°C), with a thermocline apparent at 5 to 10 m depth (Fig. 2a). In between these periods, upwelling pulses introduced colder (11 to 12°C) water to the surface and the water column was well mixed. Strong southerly winds corresponded to upwelling events and northerly or weaker southerly winds were concurrent with downwelling and stratification (Fig. 2c). Most of the sampling period was characterised by southerly winds, with the exception of 18 to 19 March. Southerly winds were strongest immediately after this period, when water temperatures were lowest (<11°C) and the water column was very well mixed.

In 2007, recently upwelled water (~11°C) was present at the surface at the start of the survey. Northerly winds prevailed until 23 March, allowing surface tem-

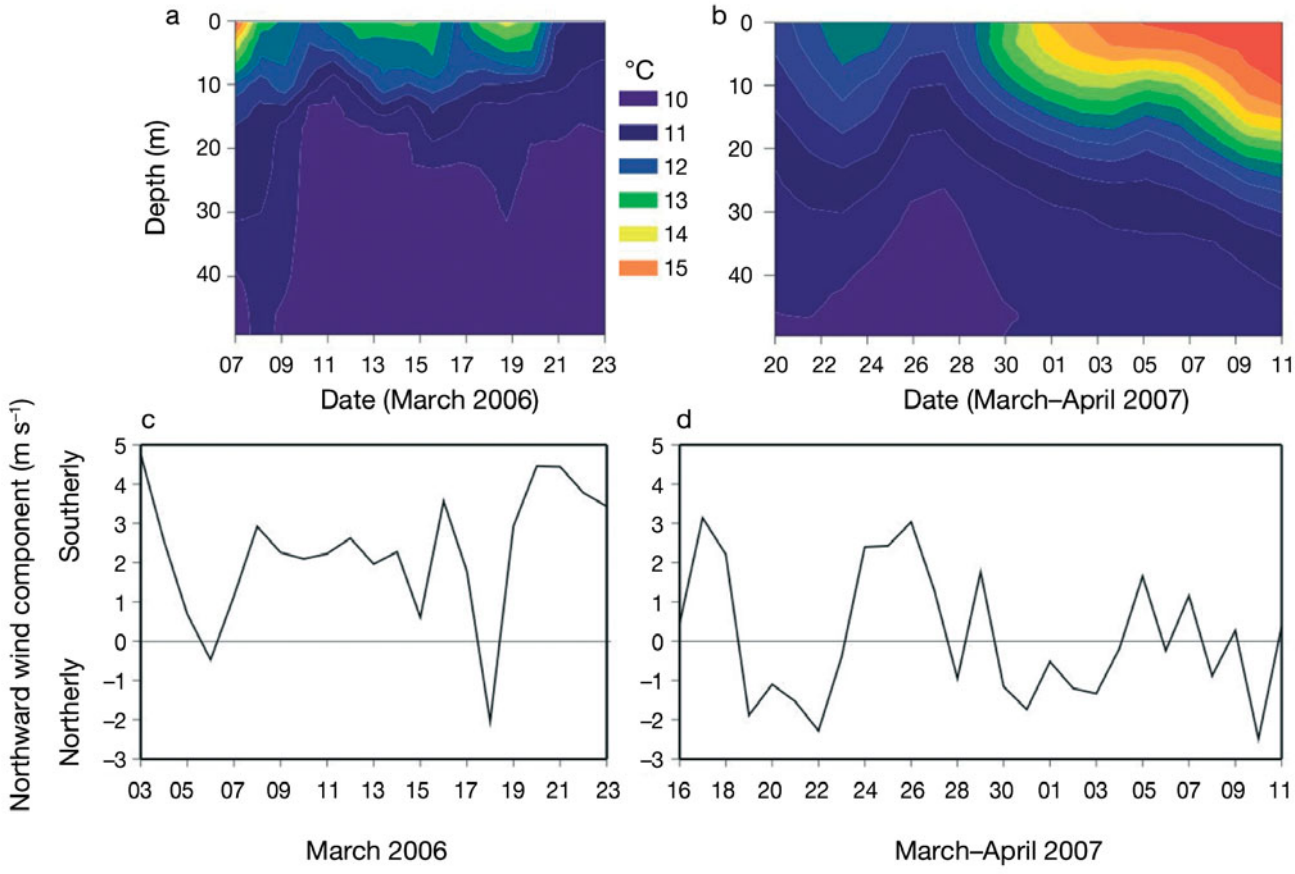


Fig. 2. Temperature contour plots obtained from daily CTD casts in (a) 2006 and (b) 2007, and daily averaged northward wind components in (c) 2006 and (d) 2007

peratures to warm to 14°C before wind reversal caused another pulse of upwelling to decrease surface temperatures to 11°C (Fig. 2b). Northerly winds then prevailed for most of the remaining period (Fig. 2d), and the water column became strongly stratified with surface temperatures increasing from 12 to 17°C over ~8 d and remaining between 16 and 17°C thereafter.

### Nutrients

Similar patterns were observed in both years, with surface nutrients fluctuating between high concentrations (17 to 25 µM $NO_3^-$, 17 to 49 µM Si, 2 to 3 µM $PO_4^{3-}$) during the upwelling periods and very low concentrations (<0.8 µM $NO_3^-$, <4.0 µM Si, <1.2 µM $PO_4^{3-}$) during the stratified periods (Fig. 3). Overall, $NO_3^-$, $PO_4^{3-}$ and Si concentrations were significantly higher (119, 75 and 45%, respectively) in 2006 than in 2007 (Student's *t*-test for $NO_3^-$ and $PO_4^{3-}$, Mann-Whitney *U*-test for Si, $p < 0.01$). On the other hand, $NH_4^+$ concentrations were, on average, 74% higher in 2007 than in 2006, although this difference was due to the presence of a few very high concentrations in 2007 and was not statistically significant. On average, urea concentrations were similar in both years.

$NH_4^+$ concentrations ranged from 0.1 to 1.5 µM at the surface and from 0.3 to 1.7 µM at the subsurface in 2006 (Fig. 3e,g), and from 0.1 to 4.2 µM at 0 m and from 0.05 to 4.4 µM at 5 m in 2007 (Fig. 3f,h). Concentrations were significantly higher (52%) at the subsurface than at the surface in 2006 (paired *t*-test); however, the difference (15%) was not significant in 2007.

Urea concentrations ranged from 0.2 to 1.0 µmol N $l^{-1}$ at the surface and from 0.5 to 3.1 µmol N $l^{-1}$ in the subsurface in 2006 (Fig. 3e,g) and from 0.2 to 1.3 µmol N $l^{-1}$ at the surface and from 0.4 to 3.7 µmol N $l^{-1}$ in the subsurface in 2007 (Fig. 3f,h). Concentrations in both years were significantly higher (250 and 254%, respectively) at the subsurface than at the surface (paired *t*-test, $p < 0.01$).

### Chl *a*

In both years, chl *a* usually displayed a maximum at 5 to 10 m depth, although the highest concentration of 57 µg $l^{-1}$ in 2006 was measured at the surface (Fig. 4a,b). Maximum concentrations at 5 m were 44 µg $l^{-1}$ in 2006 and 64 µg $l^{-1}$ in 2007.

Generally, chl *a* concentrations increased during periods of stratification following wind reversal (Figs. 2 & 4a,b). However, in April 2007, the persistence of warm, stratified conditions led to a decline in chl *a*, which remained at <5 µg $l^{-1}$ for the last 10 d of the survey (Fig. 4b). In 2006, stratification did not persist for longer than 4 d and chl *a* concentrations were always >8 µg $l^{-1}$, whereas in 2007, concentrations dropped occasionally below 1 µg $l^{-1}$. Overall, chl *a* concentrations were significantly higher (98%) in 2006 than in 2007 (Student's *t*-test, $p < 0.01$).

### Phytoplankton community structure

In 2006, diatoms were always dominant, representing 96 to 100% of the total phytoplankton numerical abundance (Fig. 4c), whereas in 2007, dinoflagellate dominance (65% on 21 March, 100% on 4 to 5 and on 8 to 10 April), alternated with diatom dominance (71 to 100% on 20 and 22 to 25 March, on 29 March to 3 April and 6 to 7 April) (Fig. 4d). Cluster analysis performed on both years' data combined revealed that, at the 60% similarity level, all 2006 stations clustered together, whereas the 2007 stations were divided into 4 clusters or assemblage types (Fig. 5).

In 2006, the dominant diatom species were *Pseudo-nitzschia* (up to 85%) and *Chaetoceros* spp. (up to 41%) (Fig. 4e), and these 2 taxa together were responsible for 96% of similarity between the 2006 stations (assemblage Type V; Table 1). *Pseudo-nitzschia* 'Species 1' was the most abundant, representing 45 to 93% of the total *Pseudo-nitzschia* concentrations, followed by *P. australis* with 0 to 50%. The contribution of dinoflagellates was low (maximum 4% of the total cell concentration on 20 March), and these were composed mainly of *Prorocentrum micans*, *P. triestinum* and *Scrippsiella trochoidea* (Fig. 4g).

In 2007, the dominant diatom species were *Skeletonema costatum* (28 to 85%), *Chaetoceros* spp. (8 to 93%) and *Pseudo-nitzschia* (2 to 30%) (Fig. 4f). These 3 taxa were collectively responsible for 76% similarity within assemblage Type III, whereas *Pseudo-nitzschia* spp. and *Chaetoceros* spp. alone were responsible for 83% similarity within Type IV (Table 1). The dominant dinoflagellate species was *Alexandrium catenella* at the start of the survey (48% cell concentration, 77% biomass on 21 March); however, the cluster that was characterised by *A. catenella* (Type I) was also defined by the presence of *S. costatum* (Table 1). *Gymnodinium* spp. were dominant on 4 to 5 and on 9 to 10 April (92 to 100% total cell numbers), and these stations formed assemblage Type II together with 8 April, which was numerically co-dominated by *Dinophysis acuminata* (44%) and *Gymnodinium* sp. (54%) (Fig. 4h), although in terms of biomass it was dominated by *D. acuminata* (91%).

The Shannon diversity index (*H'*) was broadly related to the upwelling/relaxation cycles, with the highest values occurring in nutrient-rich, newly upwelled

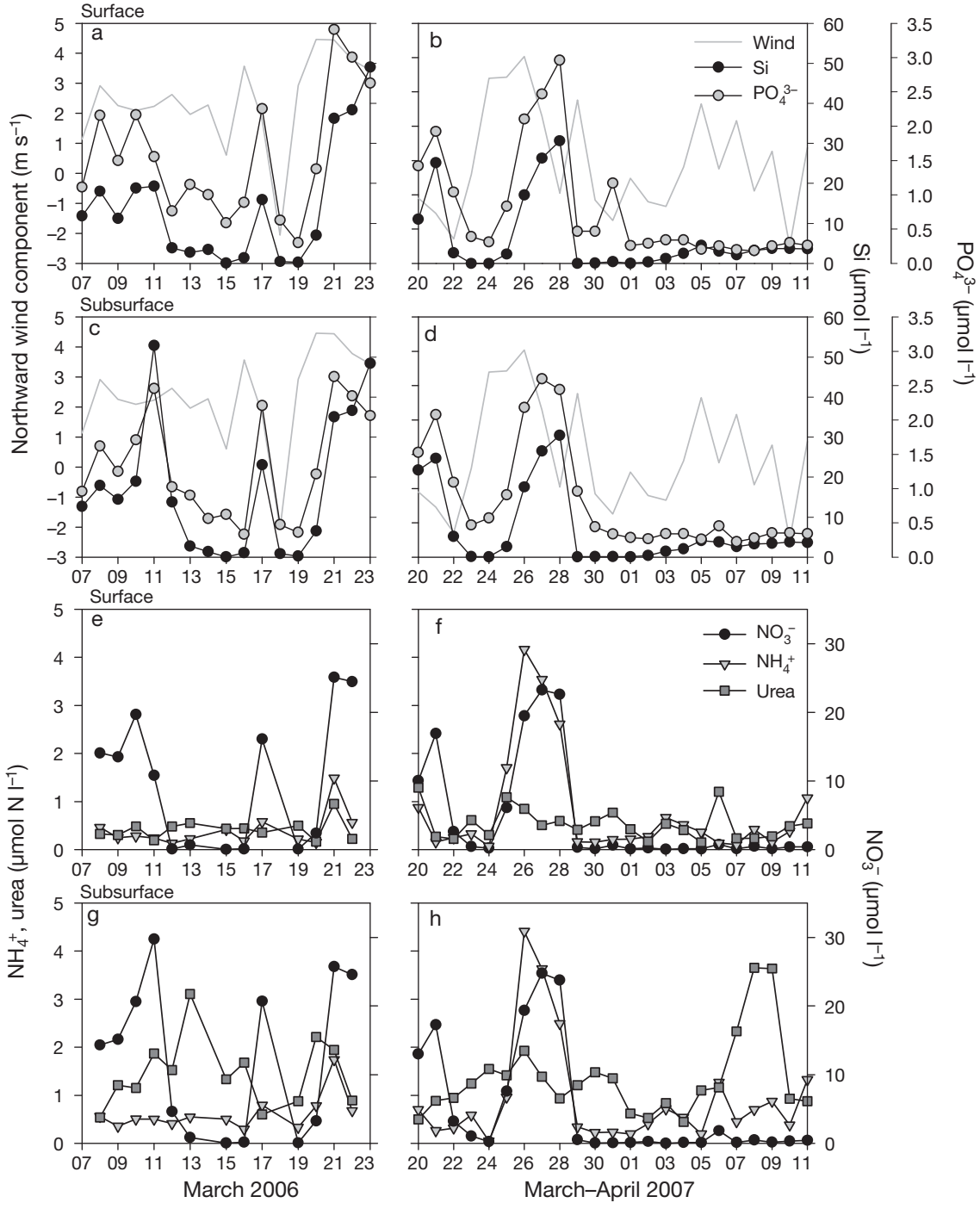


Fig. 3. Northward wind components and concentrations of Si and $PO_4^{3-}$ at the surface in (a) 2006 and (b) 2007 and in the subsurface in (c) 2006 and (d) 2007; concentrations of $NO_3^-$, $NH_4^+$ and urea at the surface in (e) 2006 and (f) 2007 and in the subsurface in (g) 2006 and (h) 2007

waters (assemblage Types I, III & V) and the lowest occurring in nutrient-poor, stratified waters (Table 1).

Type I assemblages were associated with the lowest average temperatures, which were 9 to 29% lower than for the other types (these differences were significant in all cases except with Type V, as shown by either Student's *t*-test or Mann-Whitney's *U*-test), and with average $NO_3^-$ concentrations up to 2 orders of magnitude higher than those of the other types (Table 1). Type II was associated with the warmest temperatures and the lowest $NO_3^-$ concentrations (significantly different from all other types except IV). Types III and V occurred at intermediate temperatures and $NO_3^-$ concentrations (Table 1).

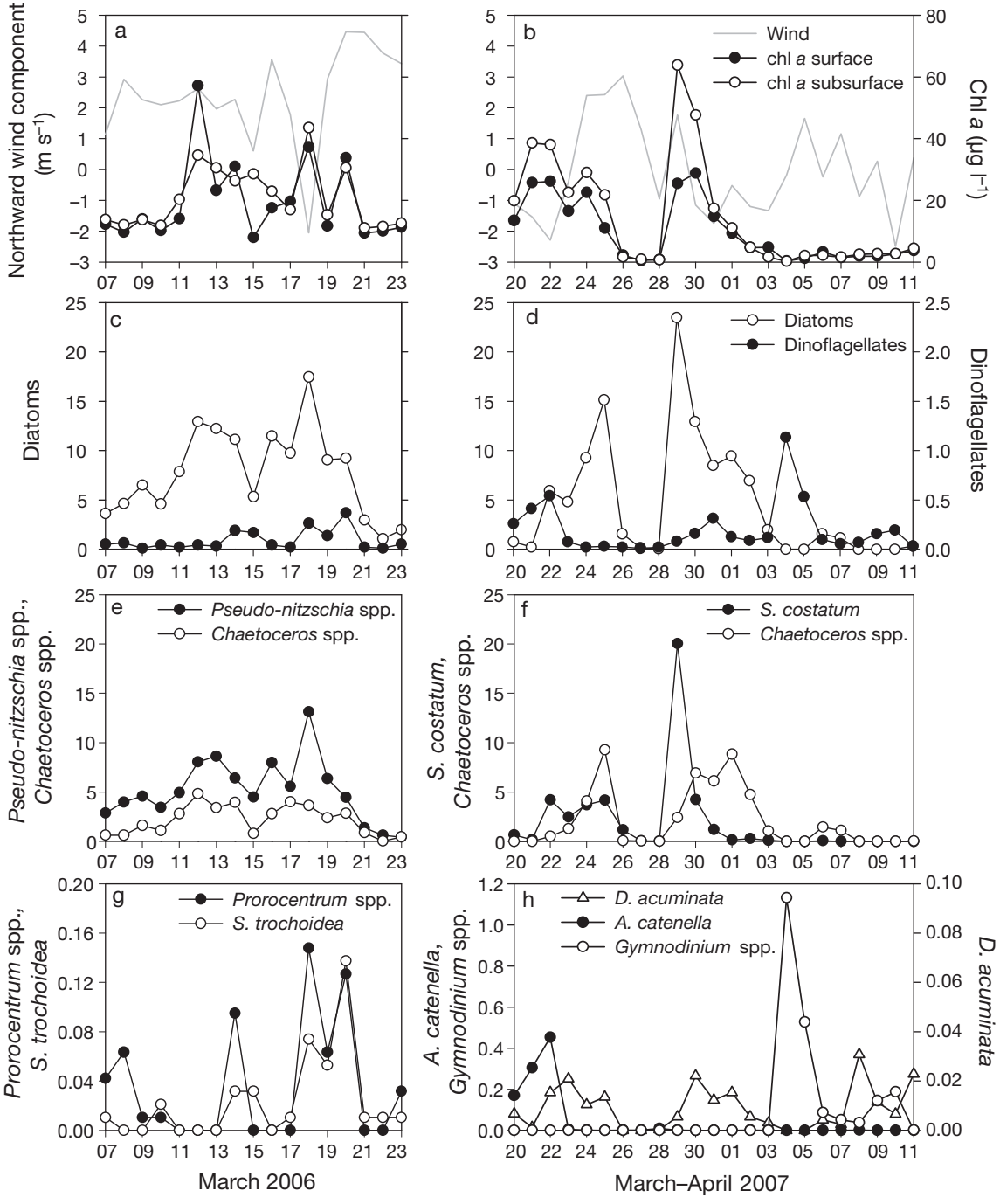


Fig. 4. Chlorophyll *a* (chl *a*) concentrations and northward wind components in (a) 2006 and (b) 2007; total diatom and dinoflagellate concentrations (×10$^6$ cells l$^{-1}$) in (c) 2006 and (d) 2007; concentrations (×10$^6$ cells l$^{-1}$) of the main diatom species in (e) 2006 and (f) 2007 and of the main dinoflagellate species in (g) 2006 and (h) 2007. Full taxonomic names: see Table 1

### Nutrient uptake

Rates of nutrient uptake broadly followed the upwelling/relaxation cycles, where absolute nitrogen uptake ($\rho_N$) was dominated by $\rho_{NO_3^-}$ during upwelling and by $\rho_{NH_4^+}$ and $\rho_{urea}$ during stratified, $NO_3^-$-depleted periods. On average, $\rho_{NO_3^-}$ was significantly higher (142%) in 2006 than in 2007, whereas $\rho_{NH_4^+}$ was significantly lower (17%, $p < 0.01$).

In 2006, $\rho_{NO_3^-}$ was 0.02 to 0.40 µmol N l$^{-1}$ h$^{-1}$ at the surface and 0.01 to 0.55 µmol N l$^{-1}$ h$^{-1}$ at the subsurface, whereas $\rho_{NH_4^+}$ was 0.04 to 0.15 at 0 m and 0.03 to

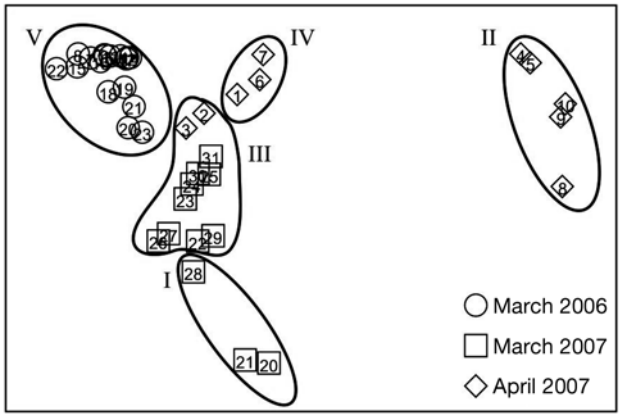


Fig. 5. MDS plot for months in 2006 and 2007 combined. Clusters identified at the 60% similarity level are circled and labelled (I to V) as in Table 1. Symbol shape represents month and year and labels represent day of the month

0.21 µmol N $l^{-1}$ $h^{-1}$ at the subsurface and $\rho_{urea}$ was 0.01 to 0.06 at the surface and 0.01 to 0.11 µmol N $l^{-1}$ $h^{-1}$ at the subsurface (Fig. 6a,c). Overall, $\rho_{NO_3^-}$ was significantly higher than $\rho_{NH_4^+}$ (112%) and $\rho_{urea}$ (278%) at both depths, and $\rho_{NH_4^+}$ was significantly (79%) higher than $\rho_{urea}$ (paired *t*-test, $p < 0.01$).

In 2007, $\rho_{NO_3^-}$ was particularly high for the *Alexandrium catenella*-dominated assemblage on 21 March (0.61 µmol N $l^{-1}$ $h^{-1}$ at the surface, 0.53 µmol N $l^{-1}$ $h^{-1}$ at the subsurface), but was generally low for the rest of the survey (<0.25 µmol N $l^{-1}$ $h^{-1}$ at the surface, <0.29 µmol N $l^{-1}$ $h^{-1}$ at the subsurface) (Fig. 6b,d). $NH_4^+$ uptake was <0.23 µmol N $l^{-1}$ $h^{-1}$ at the surface and <0.24 µmol N $l^{-1}$ $h^{-1}$ at the subsurface, and $\rho_{urea}$ was <0.08 µmol N $l^{-1}$ $h^{-1}$ at the surface and <0.18 µmol N $l^{-1}$ $h^{-1}$ at the subsurface (Fig. 6b,d). Overall, $\rho_{NH_4^+}$ was significantly higher (137%) than $\rho_{urea}$ at both depths (paired *t*-test, $p < 0.001$).

The alternating dominance of new ($NO_3^-$) and regenerated ($NH_4^+$ and urea) nitrogen uptake during the upwelling/downwelling cycles was reflected in the variable *f*-ratios [$= \rho_{NO_3^-} / (\rho_{NO_3^-} + \rho_{NH_4^+} + \rho_{urea})$ (Eppley & Peterson 1979)] of 0.11 to 0.84 at the surface and 0.10 to 0.80 at the subsurface in 2006 (Fig. 6e) and 0.06 to 0.87 at the surface and 0.03 to 0.79 at the subsurface in 2007 (Fig. 6f). Overall, *f*-ratios were significantly higher in 2006 than in 2007 (Student's *t*-test, $p < 0.01$). There was a significant linear correlation with wind (previous day northward component) at both depths in 2006 ($n = 26$, $p < 0.01$) and in 2007, after removal of 21 to 22 March, which represented the unusual combination of high *f*-ratio and northerly wind ($n = 28$) (Fig. 7).

Some significant differences in $\rho_N$ (as shown by Student's *t*-test or Mann-Whitney's *U*-test as appropriate) were observed between the different assemblage types. Type I had the lowest average $\rho_{NH_4^+}$ (2- to 3- fold lower than other types) and the highest $\rho_{NO_3^-}$ (2- to 30-fold higher), although with only 2 measurements in that group it was not possible to prove the significance of these differences. Type II assemblages displayed on average 2.5-fold higher $\rho_{NH_4^+}$ than Type I and significantly lower $\rho_{NO_3^-}$ than Types III, IV and V (3- to 18-fold). Consequently, Type II assemblages displayed significantly (3- to 7-fold) lower *f*-ratios than Assemblage Types III and V. Type III assemblages displayed 50% lower *f*-ratios than did Type V.

### Nutrient uptake kinetics

Maximum specific uptake rates ($v_{max}$) for both *Pseudo-nitzschia*- and *Dinophysis acuminata*-dominated assemblages were higher for $NH_4^+$ than for $NO_3^-$ (21% and 4-fold, respectively) (Fig. 8, Table 3). For *Pseudo-nitzschia*, the estimate of $v_{max}$ for urea derived from averaging urea at the 4 highest (saturating) concentrations was ~3-fold lower than $v_{max}$ for $NH_4^+$ and $NO_3^-$. *D. acuminata* also displayed lower (2-fold) $v_{max}$ for urea than for $NH_4^+$, but 2-fold higher than for $NO_3^-$. All 3 species displayed similar $v_{max}$ for $NH_4^+$ ($13.9 \times 10^{-3}$ to $18.0 \times 10^{-3}$ $h^{-1}$), and *Pseudo-nitzschia* displayed a 5-fold higher $v_{max}$ for $NO_3^-$ than *D. acuminata*. On the other hand, *D. acuminata* displayed the highest $v_{max}$ for urea, 27 and 77% higher than for *Pseudo-nitzschia* and *A. catenella*, respectively.

On a per cell basis, $v_{max}$ increased with size, from *Alexandrium catenella* to *Dinophysis acuminata* for $NH_4^+$ and urea, and was higher in *D. acuminata* than in *Pseudo-nitzschia* for $NO_3^-$ (Table 3).

Half-saturation constants ($K_s$) were lowest for *Dinophysis acuminata* for all 3 nutrients and for urea in *Alexandrium catenella* (<1 µmol N $l^{-1}$), whereas they were >1 µmol N $l^{-1}$ for *Pseudo-nitzschia* and $NH_4^+$ in *A. catenella* (Table 3). Furthermore, $K_s$ was higher for $NH_4^+$ than for $NO_3^-$ in *Pseudo-nitzschia*, whereas the opposite was observed for *D. acuminata*; $K_s$ was lower for urea than for $NH_4^+$ in *A. catenella*, whereas the opposite was observed in *D. acuminata*. These values are given for comparison with earlier studies that did not report α (the initial slope of the curve); however, the present study focussed on α-values as a measure of affinity.

*Pseudo-nitzschia* and *Alexandrium catenella* displayed little variability in α between N sources. On a nitrogen basis, α for $NH_4^+$ was ~2-fold higher for *Pseudo-nitzschia* than for *A. catenella*, but on a per cell basis, α was 1 order of magnitude higher for *A. catenella* (Table 3). The highest α-value was measured for $NH_4^+$ in *Dinophysis acuminata* and, on a per cell basis, the highest α-values were those measured for all nitrogen sources in *D. acuminata*.

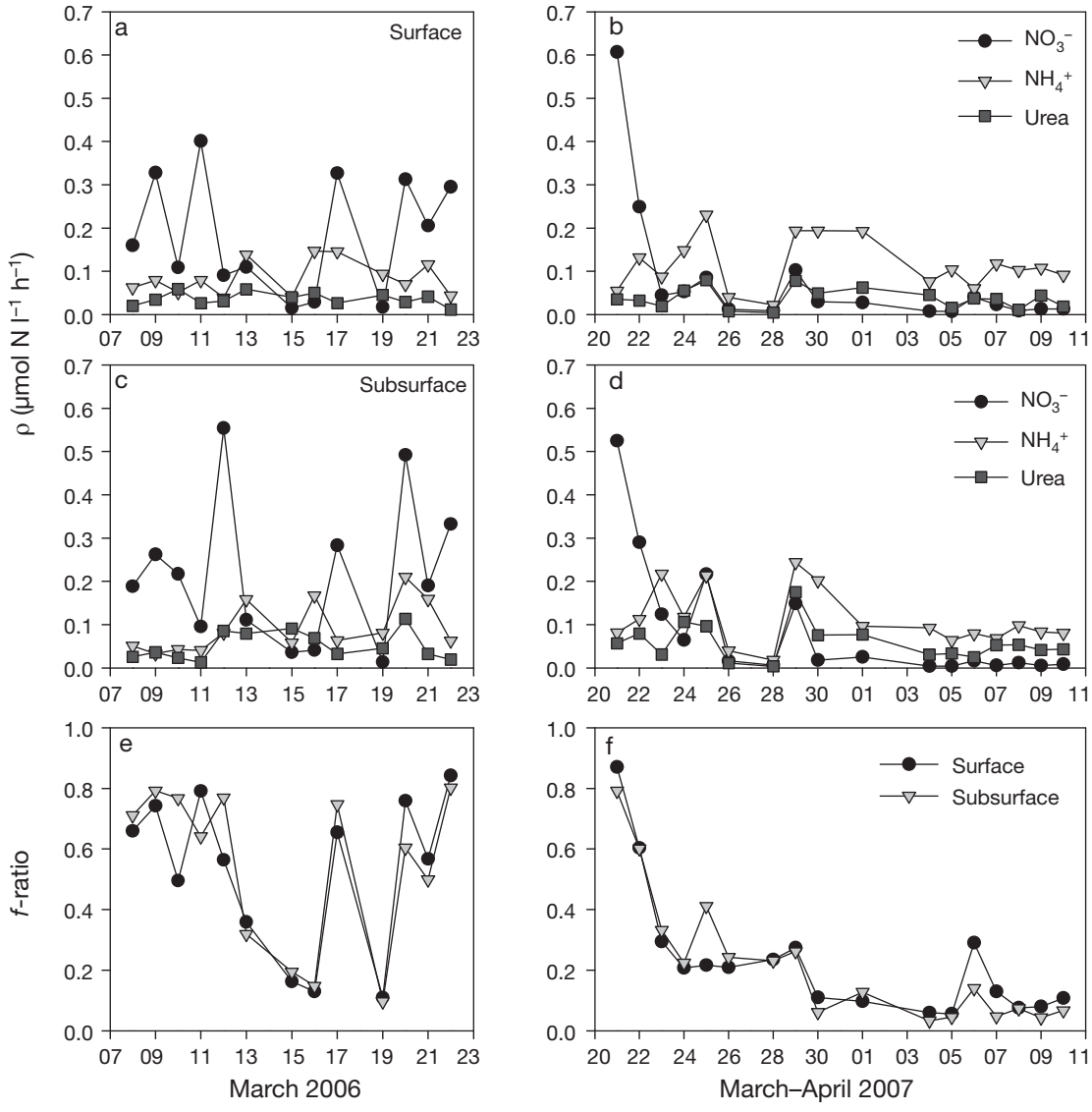


Fig. 6. Surface uptake rates of $NH_4^+$, $NO_3^-$ and urea in (a) 2006 and (b) 2007; subsurface uptake rates in (c) 2006 and (d) 2007; and *f*-ratios at both depths in (e) 2006 and (f) 2007

## DISCUSSION

Although the occurrence of HABs along the west coast of South Africa is clearly linked to wind forcing and water column stability (Pitcher et al. 1998, Probyn et al. 2000, Pitcher & Nelson 2006, Fawcett et al. 2007), little is known about the nitrogen nutrition of HAB species during upwelling/quiescent cycles.

The different assemblage types identified by cluster analysis could, to a certain extent, be linked with nutrient regime. For example, Type I (*Alexandrium catenella*/*Skeletonema costatum*) was associated with recently upwelled water, i.e. lower temperatures and generally higher $NO_3^-$ concentrations. Although Type V (*Pseudo-nitzschia*) assemblages appeared to be favoured by high $NO_3^-$ concentrations, they were still able to sustain their growth by utilising recycled nitrogen when $NO_3^-$ became limiting and occurred at temperatures ranging from 11 to 14°C. Types II (*Gymnodinium* spp./*Dinophysis acuminata*) and IV (*Chaetoceros* spp.), on the other hand, were associated with warm, stratified conditions, low $NO_3^-$ concentrations and low *f*-ratios. Type III (mixed diatoms) was present under intermediate temperature and $NO_3^-$ conditions.

### *Alexandrium catenella*

The *Alexandrium catenella* bloom was present before the start of our survey in 2007; therefore, we do not

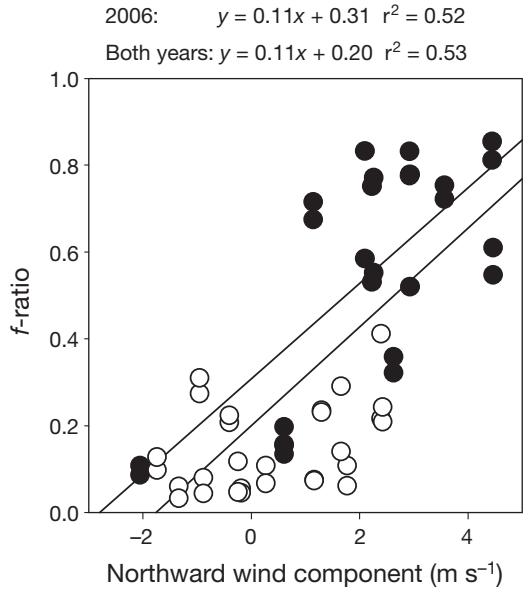


Fig. 7. *f*-ratio versus northward wind component in 2006 (●) and 2007 (○). Data are from both incubation depths. Linear correlations were significant in 2006 and for both years combined (but not in 2007 alone)

know under which conditions it was initiated. However, it displayed a very high $\rho_{NO_3^-}$ (0.61 µmol N $l^{-1}$ $h^{-1}$) and *f*-ratio (0.87), and the concentration of *A. catenella* cells dropped rapidly when $NO_3^-$ became depleted, indicating that high $NO_3^-$ concentrations would have been required to sustain this bloom concentration. Also, the second pulse of upwelling (25 March) reintroduced *A. catenella* cells, confirming the link between upwelling/high $NO_3^-$ and the presence of *A. catenella*. Although it became more abundant over the next 3 d, it then disappeared from the community, possibly because of competitive exclusion by diatoms but most likely due to alongshore (and to a lesser extent onshore) advection and decreasing $NO_3^-$ concentrations. *A. catenella* is known to form cysts in the Lambert's Bay area (Joyce & Pitcher 2004), and encystment can occur in response to nutrient starvation (Anderson et al. 1984), so its disappearance could be attributable to encystment and sinking in response to nutrient depletion. Nitrate uptake by the *A. catenella* bloom (measured on 21 March 2007) was the highest measured in both years, higher than the maximum measured rate in the Benguela for the period from 1983 to 1991 (Probyn 1992) and within the range (0.6 to 1.1 µmol N $l^{-1}$ $h^{-1}$) of theoretical maximum uptake rates calculated by Probyn (1992). Nitrate uptake was also higher than maximum values measured in the Californian, Peruvian and northwestern African upwelling systems (Dugdale et al. 1990). However, our maximum $\rho_{NO_3^-}$ was only ~17 % of that measured in the Ría de Ferrol within the Iberian upwelling system (Bode et al. 2005).

Studies of *Alexandrium catenella* blooms and cultures have yielded contradictory results with respect to their nitrogen requirements. For example, very high $NO_3^-$ requirements (>880 µM) (Siu et al. 1997) and high $K_s$ for growth (3.3 to 7.7 µM) (Matsuda et al. 1999) have been measured in cultures of *A. catenella*. In the Mediterranean, blooms were associated with high $NO_3^-$ and $NH_4^+$ concentrations on the northeast Spanish coast (Bravo et al. 2008), whereas $NH_4^+$ and urea were the main nitrogen sources fuelling a bloom in the Thau Lagoon on the French coast (Collos et al. 2007). A similar dichotomy exists for *A. minutum*: a bloom in Cape Town harbour displayed extremely high $\rho_{NH_4^+}$ (up to 1.1 µmol N $l^{-1}$ $h^{-1}$) but very low $\rho_{NO_3^-}$ (<0.05 µmol N $l^{-1}$ $h^{-1}$) (Pitcher et al. 2007), whereas the $NO_3^-$ requirement of an *A. minutum* bloom in the Penze Estuary (northwest France) was calculated at 184 µM, with an $NH_4^+$ requirement of only 25 µM (Maguer et al. 2004).

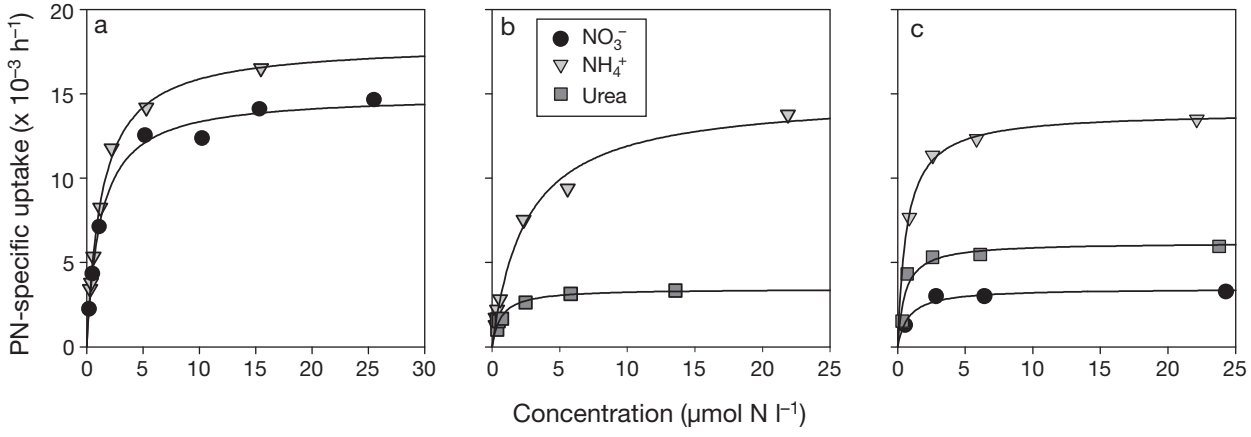


Fig. 8. Nutrient uptake versus concentration data fitted to the Michaelis-Menten equation using iterative, non-linear regression. Experiments were performed on (a) 16 March 2006, (b) 21 March 2007 and (c) 8 April 2007 on assemblages dominated by (a) *Pseudo-nitzschia*, (b) *Alexandrium catenella* and (c) *Dinophysis acuminata*. PN: particulate nitrogen

Measurements of $\rho_N$ in the field provide limited information on nutritional preference because in upwelling systems, $\rho_{NO_3^-}$ increases as a 'shift-up' response to increased $NO_3^-$ concentrations supplied by upwelling (Dugdale et al. 1990, 2006). This is demonstrated in our study by the significant correlation between *f*-ratios and wind direction, used as an indicator of upwelling, hence $NO_3^-$ concentration (Fig. 7). Furthermore, the relative preference index (RPI, McCarthy et al. 1977) is also biased by ambient concentrations, particularly in upwelling systems where $NO_3^-$ can be much more abundant than $NH_4^+$ (Stolte & Riegman 1996). The parameters of uptake kinetics can provide valuable information on the nutritional preferences of a given species (Dugdale 1967, Dortch 1990) and the potential outcome of interspecific competition for nutrients (Eppley et al. 1969), although they too can vary in response to nitrogen starvation (MacIsaac & Dugdale 1969, Collos 1980) and to elevated ambient nitrogen concentrations (Caperon & Meyer 1972, Collos et al. 2005).

In our study, $\nu_{max}$ was at least 17% higher for $NO_3^-$ than for $NH_4^+$ (based on $\nu_{NO_3^-}$ measured at ambient concentration) and 5-fold higher than for urea, whereas $\alpha$ for $NH_4^+$ and urea were low, indicating that *Alexandrium catenella* was a poor competitor for those substrates at low concentrations ($<K_s$) measured during the bloom. In the Thau Lagoon, on the other hand, *A. catenella* displayed a preference for $NH_4^+$, as shown by higher $\nu_{max}$ and $\alpha$ for $NH_4^+$ than for $NO_3^-$ (Collos et al. 2004). Although $\nu_{max}$ in our study was 4- and 17-fold lower than that in the Thau for $NH_4^+$ and urea, respectively, $K_s$ was also lower and as a result $\alpha$ was similar for $NH_4^+$ and 4-fold higher for urea in our study, indicating that *A. catenella* was a relatively poor competitor for low ambient nitrogen in both regions (including $NO_3^-$ in the Thau). The lower $\nu_{max}$ and $K_s$ for $NH_4^+$ and urea in the Benguela could be a result of natural selection for low-nutrient-adapted cell lines (Doyle 1975) in an environment where $NH_4^+$ and urea concentrations are often <1 µmol N $l^{-1}$, whereas they can be as high as 8 and 4 µmol N $l^{-1}$ in the Thau (Collos et al. 2007).

Of the 5 studies carried out on HAB dinoflagellates in the upwelling systems presented in Table 4 (Kudela & Cochlan 2000, Kudela et al. 2008a,b), *Alexandrium catenella* in the present study was the only one that expressed a preference for $NO_3^-$ over $NH_4^+$, and its $\nu_{max}$ for $NO_3^-$ was higher than those of the other species. The $\nu_{max}$ of *A. catenella* for $NH_4^+$ was similar to that of *Akashiwo sanguinea*, which was the highest out of the 6 studies, although its $\alpha$ value for $NH_4^+$ was at the low end of the spectrum, indicating that it was a better competitor for $NH_4^+$ at high concentrations. On the other hand, *A. catenella* displayed low values for both $\nu_{max}$ and $\alpha$ for urea, indicating that it was a poor

Table 4. Comparison of the parameters of nutrient uptake kinetics determined in the present study with parameters from the literature. To enable comparison, studies were selected only if they included both $\nu_{max}$ and $K_s$ (in the same units as in our study) and targeted either the same species as in our study or an upwelling system. n.d. = not determined

| | Cell size (µm³) | Expt type | $\nu_{max}$ | | | $K_s$ | | | α | | | Temp. (°C) | Source |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $NO_3^-$ | $NH_4^+$ | Urea | $NO_3^-$ | $NH_4^+$ | Urea | $NO_3^-$ | $NH_4^+$ | Urea | | |
| **Dinoflagellates** | | | | | | | | | | | | | |
| *Akashiwo sanguinea* | 20 000–100 000 | Field | 5.2 | 15.1 | 7.2 | 1.00 | 2.37 | 0.43 | 5.2 | 6.4 | 16.7 | 15.1 | Kudela et al. (2008a) |
| *Alexandrium catenella* | 14 000 | Field | >17 | 14.9 | 3.5 | n.d. | 2.52 | 0.65 | n.d. | 5.9 | 5.4 | 11.2 | Present study |
| *A. catenella* | 14 000 | Field | 24.0 | 64.0 | 61.0 | 4.60 | 8.40 | 43.90 | 5.2 | 7.6 | 1.4 | 20.0 | Collos et al. (2004) |
| *Cochlodinium* spp. | 10 000–50 000 | Field | 0.9 | >4.0 | 2.1[a] | 1.00 | n.d. | 4.06[a] | 0.9 | 0.3 | 0.8* | 15.0 | Kudela et al. (2008b) |
| *Dinophysis acuminata* | 13 800 | Field | 3.5 | 13.9 | 6.2 | 0.79 | 0.67 | 0.53 | 4.4 | 20.7 | 11.7 | 15.8 | Present study |
| *Lingulodinium polyedrum* | 16 800–63 000 | Field | 3.9 | 8.1 | 10.6 | 0.47 | 0.59 | 0.99 | 8.2 | 13.7 | 10.7 | 15.0 | Kudela & Cochlan (2000) |
| **Diatoms** | | | | | | | | | | | | | |
| *Pseudo-nitzschia* | 450 | Field | 15.0 | 18.0 | 4.9 | 1.21 | 1.34 | n.d. | 12.4 | 13.4 | n.d. | 12.6 | Present study |
| *P. australis* | 1400–4600 | Culture | 105.3 | 80.0 | n.d. | 2.82 | 5.37 | n.d. | 37.3 | 14.9 | n.d. | 15.0 | Cochlan et al. (2008) |
| **Flagellates** | | | | | | | | | | | | | |
| *Heterosigma akashiwo* | 300–900 | Culture | 18.0 | 28.0 | 2.9 | 1.47 | 1.44 | 0.42 | 12.2 | 19.4 | 6.9 | 20.0 | Herndon & Cochlan (2007) |

[a]Mean of 2 measurements carried out on different dates

competitor for urea at both high and low concentrations. Higher $\nu_{max}$ for $NH_4^+$ and similar (if not lower) $\nu_{max}$ for $NO_3^-$ and urea were measured in cultures of the raphidophyte *Heterosigma akashiwo* isolated from the California upwelling system (Herndon & Cochlan 2007) (Table 4). The higher α measured for *H. akashiwo* indicated that it was also a better competitor for $NH_4^+$ at limiting concentrations, which is consistent with the hypothesis that small flagellates express a preference for $NH_4^+$ (Glibert et al. 1982a, Probyn 1985). Overall, *A. catenella* in the present study displayed characteristics typically attributed to diatoms.

Nitrate concentrations in the other studies were low; therefore, the higher $\nu_{max}$ for $NO_3^-$ in the Benguela could be explained by acclimation to a higher ambient concentration, which is mediated by an increase in the number of uptake sites on a cell's surface (Caperon & Meyer 1972).

Temperature can also influence variability in the uptake kinetics of $NH_4^+$ and $NO_3^-$, whereby $\nu_{max}$ and α for $NH_4^+$ are positively correlated with temperature and α for $NO_3^-$ is negatively correlated with temperature (Lomas et al. 1996, Fan et al. 2003). The lower temperature in the Benguela compared to that in the other studies (Table 4) could contribute to lowering $\nu_{max}$ and α for $NH_4^+$; however, the large differences in α for urea were due to other factors, since urea uptake is thought to be independent of temperature (Fan et al. 2003). In the latter case, interspecific differences were probably the most significant.

### *Pseudo-nitzschia*

*Pseudo-nitzschia* Species 1 was dominant throughout the 2006 survey and present in very high concentrations ($>10^6$ cells $l^{-1}$ except on the last 3 d), whereas *P. australis* was less abundant, although reaching concentrations $>10^6$ cells $l^{-1}$ on several occasions and representing up to 50% of the total *Pseudo-nitzschia* cell numbers when total concentrations were lowest. Upwelling-induced high $NO_3^-$ concentrations (>10 µM) supported high uptake rates, up to 0.40 µmol N $l^{-1}$ $h^{-1}$ at the surface, with maximum uptake ~30% higher than that measured in *Pseudo-nitzschia* populations in the Juan de Fuca eddy (Washington coast, USA) (Marchetti et al. 2004). Highest cell concentrations were reached during periods of wind relaxation/reversal, during which $NO_3^-$ became depleted in the surface layer. *Pseudo-nitzschia* was able to maintain its population size ($5 \times 10^6$ to $9 \times 10^6$ cells $l^{-1}$) and to remain dominant despite the depletion of $NO_3^-$ and Si and the increase in dinoflagellate abundance. This could be due to the lower cellular nitrogen requirements owing to its smaller cell size relative to that of dinoflagellates, as predicted by the positive correlation between the minimum cell-specific nitrogen quota and cell volume (Aksnes & Egge 1991, Litchman et al. 2007). In addition, the ability to take up $NH_4^+$ (up to 0.15 µmol N $l^{-1}$ $h^{-1}$) would have increased its competitive advantage when $NO_3^-$ became limiting.

PN-specific $\nu_{max}$ was higher for $NH_4^+$ relative to both dinoflagellate species and for $NO_3^-$ relative to *Dinophysis acuminata*, although, on a per-cell basis, $\nu_{max}$ was lower relative to the dinoflagellate species due to its smaller cell size (Table 3), consistent with the $V_{cell}$–$\nu_{max}$ relationship derived by Litchman et al. (2007). This trend of lower cell-specific but higher biomass-specific $\nu_{max}$ seems to hold true for diatoms as a whole relative to dinoflagellates (Litchman et al. 2007). The higher biomass-specific $\nu_{max}$ may be due to an increased number of smaller uptake sites per unit cell surface area (Caperon & Meyer 1972, Aksnes & Egge 1991). Thus, when cell size effects are eliminated, *Pseudo-nitzschia* appears to be a 'velocity' strategist (Sommer 1984), as is generally the case for small, r-selected diatoms (Litchman et al. 2007).

*Pseudo-nitzschia* had a high $\nu_{max}$ and high α for both $NO_3^-$ and $NH_4^+$ relative to the dinoflagellate species in Table 4, indicating that *Pseudo-nitzschia* was a good competitor at both high and low concentrations, and this was reflected in its ability to thrive during both upwelling and relaxation.

However, $\nu_{max}$ and α for $NO_3^-$ were lower in our study than in a *Pseudo-nitzschia australis* culture isolated from the Californian upwelling system (Cochlan et al. 2008) (Table 4). This could be explained by the nutrient history of the culture (Fan et al. 2003), which was grown on 70 µM $NO_3^-$ as the sole nitrogen source. Although the medium was $NO_3^-$-depleted prior to starting the experiments, the cells were not nitrogen-starved; therefore, regulation of $\nu_{max}$ and α in response to nitrogen depletion had most likely not yet taken place. The same order of preference was observed in the present study and in culture experiments using *P. multiseries* (Radan 2008) and *P. cuspidata* (Auro 2007), showing a general trend in preference for $NH_4^+$, as is often observed in phytoplankton due to the lower energetic cost of $NH_4^+$ assimilation relative to $NO_3^-$ (Dortch 1990).

The Californian *Pseudo-nitzschia australis* also had a higher $\nu_{max}$ for $NH_4^+$ than that in our study, even though it was not preconditioned with $NH_4^+$. If *Pseudo-nitzschia* in the California system responds as well to high $NH_4^+$ concentrations as it does in culture, this would support the hypothesis that high anthropogenic $NH_4^+$ concentrations (>12 µM) are responsible for blooms of *P. pseudodelicatissima* on the Washington coast (Trainer et al. 2007). The *P. australis* culture had

a similar α to that in our study, indicating that the Californian strain was more competitive at high $NH_4^+$ but not at limiting $NH_4^+$ concentrations.

### *Dinophysis acuminata*

Although *Gymnodinium* spp. were responsible for 93% similarity within Type II, *Dinophysis acuminata* co-occurred with it at 3 of the 5 stations; hence, we assume that the 2 taxa were adapted to similar environmental conditions. Type II communities occurred under highly stratified, $NO_3^-$-depleted (0.1 to 0.5 µM) conditions and were even more dependent on recycled nitrogen than Types III and IV (mixed diatoms), with *f*-ratios of ~0.1. These species conform with the traditional concept of dinoflagellates being adapted to low nutrient concentrations and turbulence (Margalef 1978). *D. acuta* and *G. catenatum*–dominated communities present during the downwelling season in the Iberian upwelling system also rely on $NH_4^+$ (Rios et al. 1995).

*Dinophysis acuminata* displayed a higher $v_{max}$ and α for $NH_4^+$ and urea than for $NO_3^-$, as did most dinoflagellate species in Table 4. These species expressed a preference for recycled nitrogen and were better competitors for it at both saturating and limiting concentrations.

*Dinophysis acuminata* displayed lower $v_{max}$ for $NO_3^-$ than all other species in Table 4 except *Cochlodinium* spp. (Kudela et al. 2008b), indicating that *D. acuminata* was not able to compete for high concentrations of $NO_3^-$, hence its appearance in the phytoplankton community after a long period of $NO_3^-$ depletion. Maximum uptake of $NH_4^+$ and urea was towards the middle of the range for dinoflagellates, whereas α was higher for $NH_4^+$ than in all other species including most diatoms. This suggests that *D. acuminata* is an 'affinity strategist' (Sommer 1984), well adapted to growing at very low $NH_4^+$ and urea concentrations, although it was less competitive at low $NO_3^-$.

The difference in α for $NH_4^+$ is also consistent with the higher temperature observed during the *Dinophysis acuminata* bloom, which would favour a higher α (Fan et al. 2003), although it is difficult to disentangle temperature effects from species-specific differences.

## CONCLUSIONS

The southern Benguela is a highly dynamic region influenced by local wind patterns that drive upwelling/relaxation cycles on timescales of days. This, in turn, influences community structure and the occurrence of HABs (Pitcher et al. 1998). The present study revealed extremely high variability in phytoplankton community structure in response to changes in the upwelling regime. We have sought to elucidate the nutritional characteristics of different HAB species and their link to variable nutrient regimes.

The toxic diatom *Pseudo-nitzschia* was favoured by upwelling and was able to rapidly utilise the high $NO_3^-$ concentrations supplied by upwelling. Biomass accumulation occurred during wind relaxation, and *Pseudo-nitzschia* switched to $NH_4^+$ as its main source of nitrogen as $NO_3^-$ became depleted. Due to its small size, PN-specific $v_{max}$ was high, as was α; therefore, it was competitive at both saturating and limiting $NO_3^-$ and $NH_4^+$ concentrations.

*Alexandrium catenella* bloomed at high $NO_3^-$ concentrations, displayed high *f*-ratios and appeared to have a high requirement for $NO_3^-$ since it disappeared when $NO_3^-$ became depleted. The higher $v_{max}$ for $NO_3^-$ indicated a preference for $NO_3^-$ over recycled nitrogen, while the opposite has been observed in other dinoflagellate species in the California upwelling system, as well as for *A. catenella* blooms in the Thau Lagoon. We hypothesise that these differences were most likely due to differences in nutrient history and/or interspecific differences, although temperature differences were also considered.

*Dinophysis acuminata* bloomed under low $NO_3^-$ concentrations and had low *f*-ratios and a higher $v_{max}$ for $NH_4^+$ and urea than for $NO_3^-$, indicative of a preference for recycled nitrogen. Its high α values indicated a high affinity for recycled nitrogen sources, particularly $NH_4^+$, but a low affinity for $NO_3^-$, when compared with a range of HAB species. This high affinity for $NH_4^+$ would have given it a competitive advantage under stratified conditions, despite its low affinity for $NO_3^-$.

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