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Variability in Nitrate Uptake Kinetics of Phytoplankton Communities in a Mediterranean Coastal Lagoon

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Nitrate uptake kinetics of different phytoplankton communities exhibit great variability over a 1-year period. Classical saturation kinetics accompanied by an induction phase are shown by blooms of *Chaetoceros* sp. in low nitrate waters. Biphasic kinetics, with transition points between 10 and 50 µM, are shown by blooms of *Skeletonema costatum* and flagellates, while unsaturated kinetics (up to 100 µM) are shown by *Thalassiosira* blooms and flagellate blooms. The latter also exhibit surge uptake of nitrate in situations of negative growth rates and rather high ammonium levels. The uptake patterns of the three diatom genera are similar to those observed in cultures or consistent with known internal nitrate accumulation characteristics. Based on the kinetic patterns presented, it is apparent that, for nitrate concentrations near 50 µM such as in upwelling areas, nitrate uptake will be underestimated in present models of nitrate assimilation by a factor of almost 2 for *S. costatum* and a factor of about 3 for *Thalassiosira* sp.

Keywords: nitrate uptake; variable kinetics; *Chaetoceros*; *Thalassiosira*; variable *Ks*

Introduction

Recent studies of nitrate uptake in laboratory cultures of unicellular algae (Ower & Cresswell, 1986; Collos *et al.*, 1992; Watt *et al.*, 1992) have revealed biphasic kinetics, with a first transition near 60 µM for *Skeletonema costatum* and *Thalassiosira weissflogii*, 150 µM for *Ankistrodesmus falcatus*, and 1100 µM in *Chlamydomonas reinhardtii*. These kinetics are similar to findings in higher plants (Pace & McClure, 1986; Siddiqi *et al.*, 1990), but differ from previous work on unicellular algae. This paper reports on the nitrate uptake kinetics by natural communities of phytoplankton in a coastal lagoon where nitrate concentrations are generally low, but can reach 70 µM seasonally (Picot *et al.*, 1990), and occasional blooms of *S. costatum* take place (Tournier & Pichot, 1987). Current models of new production (Dugdale & MacIsaac, 1971; Kiefer & Kremer, 1981; Coste *et al.*, 1982; Fasham *et al.*, 1990) consider that nitrate uptake is saturated near 10 µM. The present work studies the kinetics of nitrate uptake in a much wider range of nitrate concentrations in order to see if previous findings on laboratory cultures can be extended to natural populations of marine phytoplankton.

Materials and methods

Samples were taken from Thau Lagoon, a large coastal lagoon located in southern France. Surface water was sampled between 0800 and 0900 h local time by immersing 20 l polycarbonate bottles below the surface. These samples were taken to a shore-based field laboratory immediately. Within 1 h of sampling, the water was dispensed in six 2·5 l polycarbonate bottles and enriched with ^{15}N-labelled sodium nitrate at the following final concentrations: 1, 5, 10; 20, 50 and 100 µM. They were incubated at the surface of the lagoon for 4 h (generally from 1000 to 1400 h). An additional 8 l bottle was used at 100-µM enrichment and aliquots were taken every hour over 4 h in order to test the constancy of uptake with time (Harrison *et al.*, 1989). Samples were filtered using GF/F membranes, dried for 24 h at 60 °C and stored desiccated. Particulate nitrogen and isotopic analyses were carried out according to Owens and Rees (1989) with Roboprep/Tracermass instrumentation. Uptake rate calculations used Equation 4 in Collos (1987) which yields uptake rates unbiased by other nitrogen sources. Values for half-saturation constants for nitrate uptake (*Ks*)

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TABLE 1. Initial nutrients, microalgal biomass levels and growth rates

Date	Nitrate (μM)	Nitrite (μM)	Ammonium (μM)	Silicate (μM)	Chl *a* (μg l^{-1})	PN (μM)	μ (h^{-1})
23 March 1993	0·59	0·17	0·33	2·9	1·1	2·1	0·116
20 April 1993	1·29	0·10	0·85	4·3	0·4	2·0	0·018
18 May 1993	0·45	0·17	0·96	4·7	1·2	2·3	0·038
15 June 1993	0·21	0·08	0·14	5·9	0·7	1·9	0·022
12 August 1993	U	U	0·46	10·1	2·1	2·6	0·236
7 September 1993	0·41	0·10	2·20	12·6	1·0	1·6	−0·004
5 October 1993	1·49	0·43	1·65	20·6	1·1	1·1	0·078
2 November 1993	3·37	0·32	2·98	15·6	0·4	1·1	−0·042
30 November 1993	4·00	0·17	0·39	14·5	11·4	5·5	0·034
1 February 1994	0·55	0·09	0·97	11·4	0·6	1·5	−0·024

U, undetectable; Chl *a*, chlorophyll *a*; PN, particulate nitrogen; μ, particulate nitrogen-based growth rate.

were calculated by a non-linear regression based on Marquardt (1963).

Samples for nutrients were frozen after collection, except for ammonium which was fixed by the addition of reagents in the field according to Aminot (1983) and for silicate which was stored in polyethylene bottles at 4 °C until analysis within a few weeks. Nitrate, nitrite, soluble reactive phosphorus (SRP) and reactive silicate were measured by segmented flow analysis according to the procedures of Tréguer and Le Corre (1975). Detection limits were 0·05, 0·005, 0·03 and 0 1 μM for nitrate, nitrite, SRP and silicate, respectively.

For chlorophyll *a* analyses, 20 ml were filtered onto GF/F membranes with a differential vacuum of less than one-third atmosphere. The samples were kept at −20 °C until analysis. Samples were extracted by grinding in 90% acetone. The extracts were left for 24 h in the dark at 4 °C and analysed by fluorometry (Holm-Hansen *et al.*, 1965).

Growth rates were based on changes in particulate nitrogen during the incubation period, which took into account all nitrogen sources. They were computed as in Guillard (1973):

$$Ke = (\ln (PN_1/PN_0))/(t_1 - t_o) \text{ in units of time}^{-1} \quad (1)$$

Results

Initial conditions and phytoplankton growth rates are shown in Table 1. Water temperature ranged from 7·1 °C in February to 23·5 °C in August. Salinity ranged from 29·3 in late November to 38·5 in September. Nitrate was generally low, except in late November following heavy rains. Ammonium was variable, with high concentrations (1–3 μM) corresponding to negative growth rates of phytoplankton. The highest chlorophyll *a* (Chl *a*) and particulate nitrogen (PN) levels were also observed in late November following the input of nitrate from the watershed. Chl *a* and PN were significantly correlated ($r^2 = 0\ 885$). Most of the phytoplankton (83–96% in cell numbers) consisted of *Ostreococcus tauri* (Courties *et al.*, 1994), but, according to fractionation measurements, this group represented only 21–44% of the total biomass in terms of Chl *a*. Other phytoplankton groups or genera of diatoms are presented in Table 2.

Figure 1 shows representative trends in cumulative nitrate uptake as a function of time, and illustrates the three known patterns of uptake for such nutrients (Collos, 1983; Harrison *et al.*, 1989): induced (May and August), constant (November) and surge (February, June and September) uptake [the latter following the terminology of Conway *et al.* (1976)]. This classification has been used because it has implications on the interpretation of the shape of the uptake *vs.* concentration curves. While constant uptake was encountered most frequently (linear regressions between cumulative nitrate uptake and time yielded coefficients of determination ranging from 0·861 to 0·998), there were two instances of induced uptake (May and August) where uptake increased with duration of incubation (Figure 1). In one of those instances (August), initial nitrate was undetectable (Table 1). In both cases, *Chaetoceros* was dominant in cell numbers other than *O. tauri*. Surge uptake, characterized by an elevated uptake rate at the beginning of incubation, followed by a reduction in rate with time, occurred in four cases [February, June, September (Figure 1) and early November (data now shown)]. This uptake pattern corresponded in most cases to a dominance in phytoflagellates (Table 2), negative growth rates in terms of PN (Table 1), and rather high ammonium levels (1–3 μM).

TABLE 2. Relative abundances (in numbers) of phytoplankton groups other than *Ostreococcus tauri*

Date	Chaet. sp.	Skel. cost.	Thal.	Other diatoms	Eugl.	Crypt.	Perid.	Flag.
23 March 1993	p					+		+ +
20 April 1993	p					r	r	+ +
18 May 1993	+ +					+		r
15 June 1993	+	+ +		+		r		r
12 August 1993	+ +	+		r	r	r	r	+
7 September 1993	+			r	r	r	r	+ +
5 October 1993	r			r	+	+	+	+ +
2 November 1993	p			p	p	p	r	+ +
30 November 1993	r		+ +			r	r	+
1 February 1994		p				r		+ +

Chaet. sp., *Chaetoceros* sp.; Skel. cost., *Skeletonema costatum*; Thal., *Thalassiosira* sp.; Eugl., Euglenophyceae (mostly *Eutrepsiella* sp.); Crypt., Cryptophyceae; Perid., Peridinians; Flag. phytoflagellates <5 µm and other individual cells; + +, dominant; +, common; r, rare; p, present.

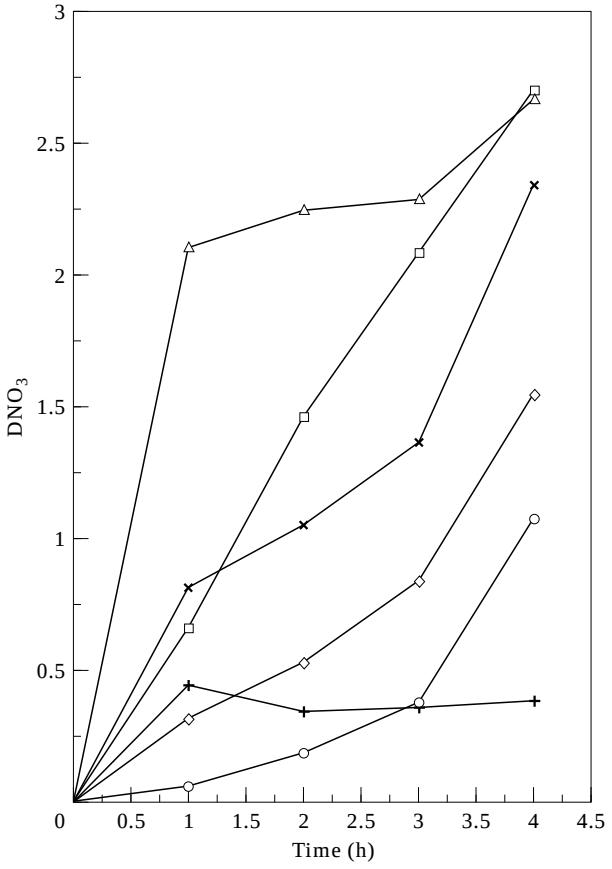

FIGURE 1. Cumulative nitrate uptake (in µM) as a function of time. ○, August; □, November; △, February (× 100); ◇, May (× 10); +, September (× 10); ×, June (× 40).

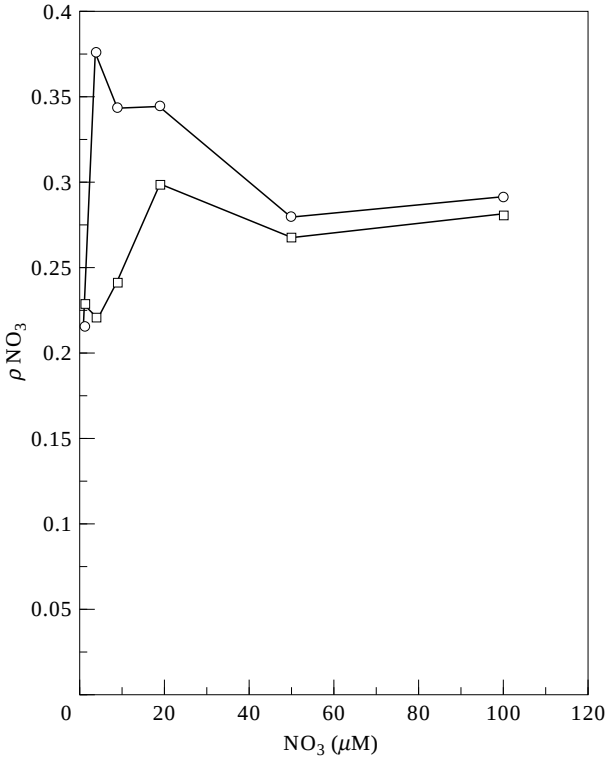

FIGURE 2. Nitrate uptake (in µM h^{-1}) as a function of nitrate concentration. Classical kinetics. Dominant genus: *Chaetoceros*. ○, August; □, May (× 10).

Nitrate uptake as a function of nitrate concentration could be classified in three categories: classical (Michaelis-Menten), biphasic and unsaturated uptake. The classical pattern (Figure 2) exhibited saturation of uptake near 5–20 µM (May and August), and possibly a decrease at the highest concentrations. This corresponded each time with a bloom of *Chaetoceros* sp. which were numerically dominant (Table 2). These two cases also corresponded to the induced kinetics shown in Figure 1. The biphasic type (Figure 3) showed a first plateau between 10 and

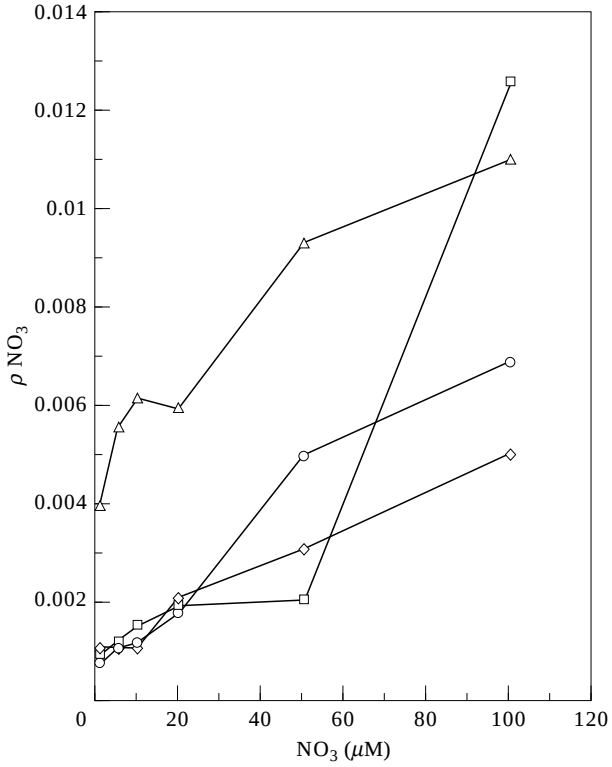

FIGURE 3. Nitrate uptake (in μM h^{-1}) as a function of nitrate concentration. Biphasic kinetics. Dominant species: *Skeletonema costatum* in June, flagellates on other dates. ○, February; □, April; △, June; ◇, October.

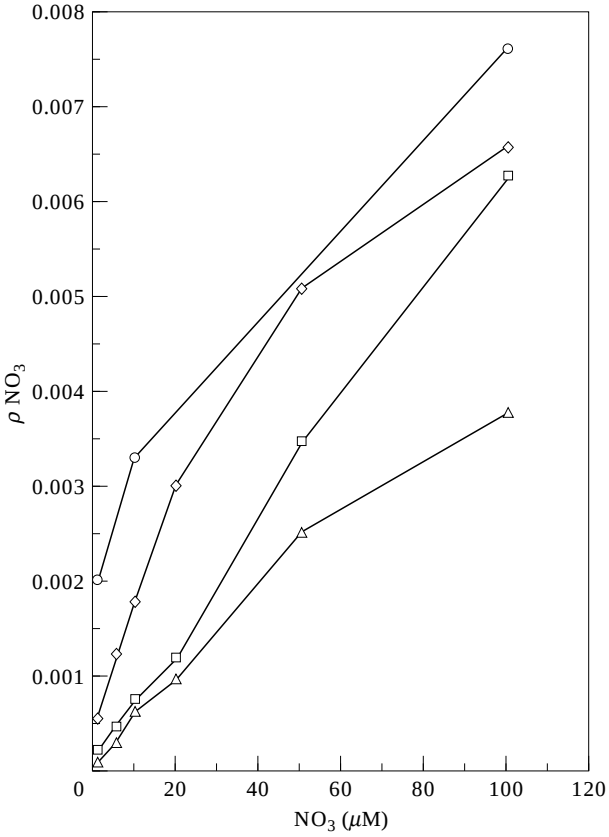

FIGURE 4. Nitrate uptake (in μM h^{-1}) as a function of nitrate concentration. Unsaturated kinetics. November values have been divided by 100. Dominant genus: *Thalassiosira* on 30 November, flagellates on other dates. ○, March; □, September; △, 2 November; ◇, 30 November.

50 μM, then uptake increased again, without reaching an apparent plateau (February, April, June and October communities). This corresponded with either a *S. costatum* bloom (June) or flagellates blooms (February, April and October). Finally, the unsaturated uptake type (Figure 4) was found in March, September and November, with a practically linear relationship between nitrate uptake and concentration in the range 1–100 μM, or with slight curvature, but without obvious saturation. These were due to blooms of *Thalassiosira* (30 November) or flagellates (March, September and 2 November).

Discussion

In considering uptake processes, it is the available cell surface which is important as a controlling variable. No direct estimate of cell surface is available here, but Chl *a* can be used as an indirect estimate, as in Chan (1980): ‘ since the chloroplasts of most diatoms and many dinoflagellates are located towards the periphery of the cell . . ., it is likely that the amount of chloroplast material would be proportional to the cell surface area ’. As *O. tauri* always represent less than 44% of the phytoplankton biomass in terms of Chl *a*, uptake trends will be discussed in terms of the other phytoplankton groups because of their larger size and biomass. This is consistent with a recent study by Li (1995) who found that eukaryotic algae (large cells) represented only 10% of the total by numbers but 68% on the basis of productivity.

Distortions in uptake curves result when the uptake rate is not constant with time during the incubation period (Conway *et al.*, 1976; Collos, 1983; Harrison *et al.*, 1989). This was the case in several instances in the present study, when cumulative nitrate uptake was not related to time in a linear manner (Figure 1). In the case of surge uptake, such a phenomenon will lead to an underestimation of uptake at high substrate levels, so that some of the uptake curves shown here in either the biphasic (February) or the unsaturated (September and early November) mode may have presented even higher values at the highest substrate level, if instantaneous uptake rates could have been measured. Inversely, for samples in which uptake

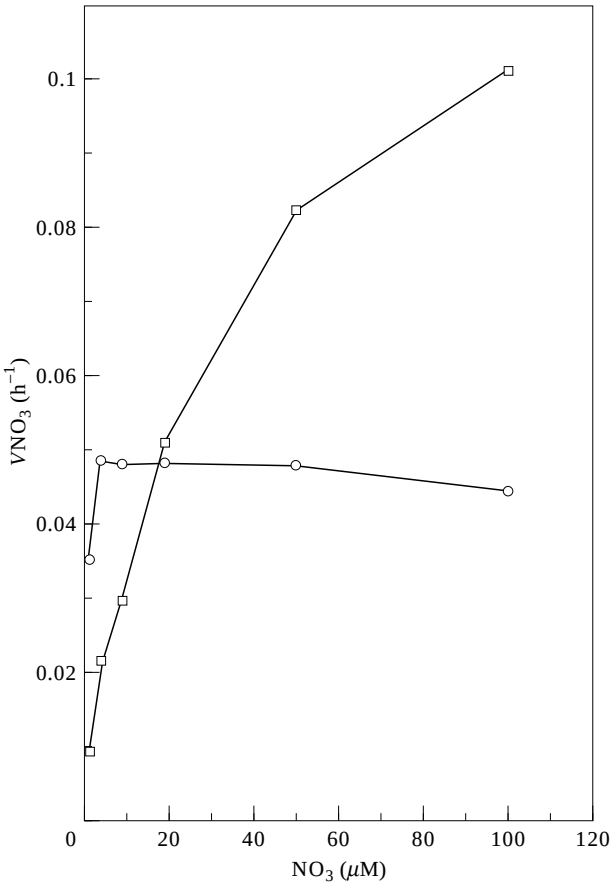

FIGURE 5. Specific nitrate uptake (h^{-1}) as a function of nitrate concentration for two different communities dominated by *Chaetoceros* (August, ○) and *Thalassiosira* (November, □).

increased with time (May and August, Figure 1), the plateau is higher than it should have been for instantaneous estimates of uptake. But overall, the trends are not greatly modified by those biases, i.e. the underestimate of *Vmax* in the first case is only reinforcing the trend of unsaturated uptake in Figures 3 and 4, and the overestimate of *Vmax* in the second case does not suppress the plateau in Figure 2. For the other data, however, there is no reason to believe that the trends are due to methodological artefacts.

The results obtained illustrate a great variety of uptake patterns over the study period. These appear to be related to taxonomic groups in some instances. For example, the correspondence between *Chaetoceros* blooms in May and August and classical saturated uptake (Figure 2) is most striking. This can be related to the observations that *Chaetoceros* does not exhibit rapid nutrient uptake in culture (Conway & Harrison, 1977), and does not accumulate large pools of internal nitrate (Collos, 1982; Dortch, 1982). In the same way, the biphasic uptake patterns exhibited in culture by *S. costatum* (Collos *et al.*, 1992) also seem to occur in nature (Figure 3, June sample). Concerning *Thalassiosira*, the practically linear relationship between uptake and nitrate concentration (Figure 4, 30 November sample) is very similar to that shown by the same genus in culture (Collos *et al.*, 1992) at a similar growth rate (0·89 day^{-1} *vs.* 0·82 day^{-1} in the present case). The lack of saturation at high nitrate levels for these two diatoms can also be related to their ability to accumulate large internal nitrate pools, in contrast to *Chaetoceros* (Dortch *et al.*, 1984). Diffusion-controlled kinetics are unlikely here as internal nitrate concentrations are generally in the millimolar range in phytoplankton (Dortch *et al.*, 1984; Marsot *et al.*, 1992).

The only environmental variable related to the PN-based growth rate was water temperature. Otherwise, the only data which could be discussed in terms of seasonal succession of different groups of phytoplankton are presented in Figure 5. Two contrasting situations and corresponding responses of the nitrate uptake system are shown. One is the late spring situation with very low to undetectable nitrate levels. Values of *Ks* for *Chaetoceros*-dominated communities were 0·33 µM in August and 0·36 µM in May (not shown in Figure 5), and were similar to values found by Eppley *et al.* (1969) for *Chaetoceros gracilis* in laboratory cultures. In contrast, *Thalassiosira*-dominated communities occurred in late November, following a period of heavy rains and nitrate input through the watershed. The *Ks* value was 53 µM, which is much higher than values reported for the same genus (Eppley *et al.*, 1969; Carpenter & Guillard, 1971), but similar to the value of 64 µM calculated from data on *Thalassiosira weissflogii* growing at 0·40 day^{-1} (Collos *et al.*, 1992) using a larger range of substrate concentrations.

The two contrasting patterns shown in Figure 5 are consistent with current concepts of interspecific competition for nutrients (Dugdale, 1967; Eppley *et al.*, 1969; Doyle, 1975). Note, however, that for a substrate value of 4 µM (observed on 30 November) for the *Thalassiosira* bloom, Figure 5 predicts that *Chaetoceros* should outcompete *Thalassiosira*. This apparent paradox can be explained by the fact that the curve obtained for this genus probably reflects its previous nutrient history characterized by higher nutrient levels (due to heavy rainfall in our case). *Ks* is known to change on time scale of days upon nitrogen depletion (Eppley *et al.*, 1969; Collos, 1980).

The simultaneous occurrence of negative growth rates, based on PN changes, and uptake of nitrate in surface waters during the day have been observed previously (Collos *et al.*, 1989), along with increased

ammonium concentrations. Degradation of PN could also explain the high ammonium levels encountered. The results of this study, obtained with incubation periods centred about noon, where negative growth rates corresponded to ' dominance ' of flagellates only are supported by the findings of Nelson and Brand (1979). In laboratory cultures, they found negative growth rates in the middle of the light period for flagellates, but not for diatoms.

Concerning the consequences of the present results on estimates of nitrate uptake in the oceans, some models of new production (Dugdale & MacIsaac, 1971; Kiefer & Kremer, 1981; Coste *et al.*, 1982; Fasham *et al.*, 1990) consider that nitrate uptake (or growth rate in cases where nitrate is the only nitrogen source) is saturated near 10 µM. While this may be a correct assumption in some cases, such as in blooms of *Chaetoceros* sp. (Figure 2), such models may require substantial modifications for other situations. For example, in upwelling systems, nitrate levels up to 50 µM have been observed (Kokkinakis & Wheeler, 1987). For those nitrate concentrations, nitrate uptake will be underestimated in such models by a factor of almost 2 for *S. costatum* and a factor of about 3 for *Thalassiosira* sp.

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