

SHORT COMMUNICATION

Nitrogenous nutrition of the potentially toxic diatom *Pseudonitzschia pungens* f. *multiseries* Hasle

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Abstract. The potentially toxic diatom *Pseudonitzschia pungens* f. *multiseries* was grown on different sources of nitrogen in batch cultures. Ammonium did not support growth at concentrations >200 μM , and even lowered the growth rate, when it was supplied in addition to growth-saturating nitrate concentrations. This seemed to be a combined effect of inhibition of nitrate uptake and direct ammonia toxicity. Urea, glutamine and nitrite were used readily by *P.pungens*.

Harmful phytoplankton blooms have become a serious problem to aquaculture, showing signs of a global epidemic in the last few years (Smayda, 1990; Hallegraeff, 1993). In the autumn of 1987, more than 100 people in Canada had to be treated clinically because of poisoning symptoms. In this incident, the toxic amino acid domoic acid was produced by *Pseudonitzschia pungens* f. *multiseries* Hasle (Bates *et al.*, 1989). Meanwhile, other species of this genus have been shown to be potentially toxic: *Pseudonitzschia pseudodelicatissima* Hasle (Martin *et al.*, 1990), *P.australis* Frenguelli (Fritz *et al.*, 1992), *P.delicatissima* Heiden (Worms *et al.*, 1991) and *P.serriata* (Cleve) Peragallo (Lundholm *et al.*, 1994).

Nitrogen seems to play an important role in the development of blooms of *P.pungens* (Smith *et al.*, 1990) and nitrogen limitation inhibits toxin production (Bates *et al.*, 1991). Nitrogen occurs in several available forms in the marine environment: as inorganic ammonium, nitrite or nitrate, or as organic urea, amino acids, pyrimidines and purines. The interactions of NH_4^+ and NO_3^- have been of particular interest during the last 30 years (see the review by Dortch, 1990). Growth-inhibiting effects of ammonium have been reported by various authors (e.g. Natarajan, 1970; Thomas *et al.*, 1980); indeed, Bates *et al.* (1993) have reported lowered growth rates of *P.pungens* f. *multiseries* at concentration levels >220 μM NH_4^+ .

Here, we discuss experiments that were designed to obtain information about the nitrogenous nutrition of *P.pungens* f. *multiseries*.

Clone KP 84 of *P.pungens* was used in all experiments (courtesy of R.Pocklington, Canada). The medium was based on f/2 (Guillard and Ryther, 1962) in artificial seawater (according to Lyman and Fleming, 1940), with the main nutrients N, P and Si being altered for the particular needs of every experiment. The concentration of Si was elevated to 150 μM , P was 35 μM , thus indicating P limitation only when the total N concentration exceeded 525 μM , regarding a stoichiometric ratio of N:P of ~15:1 (Table I). The components of the artificial seawater and the

Table I. Design of the experiments. PFD is photon flux density

No.	Volume (ml)	Equipment	PFD ($\mu\text{E m}^{-2} \text{s}^{-1}$)	Nitrogen
I	2000	batch culture in glass bottles, continually stirred	230	400 $\mu\text{M NH}_4^+$ or 800 $\mu\text{M NO}_3^-$
II	2000	as above	230	400 $\mu\text{M NO}_3^-$ and 7–150 $\mu\text{M NH}_4^+$
III	150	batch culture in Erlenmeyer flasks, shaken daily	25/230	300 $\mu\text{M NH}_4^+$ or 200 $\mu\text{M NO}_3^-$
IV	100	as above	25	200 μM nitrite or 100 μM urea or 100 μM glutamine
V	150	as above	25	40 $\mu\text{M NH}_4^+$ and 5–600 $\mu\text{M NO}_3^-$

medium were autoclaved separately (126°C, 40 min). Urea, glutamine, ammonium and silica solutions were sterilized by filtration (0.2 μm filter), and then added to the autoclaved medium. Table I also lists the equipment used and the chosen photon flux densities (delivered by Osram Biolux and Fluora lamps in a 14 h:10 h bright–dark cycle). Although the cultures were non-axenic, sterile techniques were applied during inoculation and sampling.

The batch cultures were sampled three times a week, and cell and nutrient concentrations were analysed. For counting, the samples were fixed with Lugol's iodine, filled into sedimentation chambers and counted to 400 cells under an inverted microscope, which is reported to give a 95% confidence interval of $\pm 10\%$ (Utermöhl, 1958). A non-linear regression (Statgraphics 6.1) of the counting results with the integrated form of the logistic growth curve
$$N_t = \frac{K}{1 + ([K - N_0]N_0)^{-r}}$$
 gave estimates for the carrying capacity (K) (cells ml^{-1}) and the initial growth rate (r) (day^{-1}).

The nutrients were determined using methods described in Grasshoff *et al.* (1983), except for silicate which was analysed according to Strickland and Parsons (1972). The photon flux densities (PFD) were measured with a LiCor 186 photometer with a spherical receptor.

With 400 μM ammonium as the sole N source, the growth rate (r) and the carrying capacity (K) of *P. pungens* were much lower than with 800 μM nitrate (Figure 1). Additions of ammonium to media already containing limiting concentrations of nitrate resulted in lowered growth curves. Both r and K were declining functions of the ammonium concentration (Figure 2). A moderate growth-inhibiting effect of ammonium started at 20 $\mu\text{M NH}_4^+$ and was more pronounced at higher concentrations. (It has to be taken into account that the algae may have switched to P limitation at the highest N levels.)

On the other hand, addition of nitrate to flasks with 40 $\mu\text{M NH}_4^+$ led to an increase in the growth rate with increasing nitrate concentrations (Figure 3). At lower PFDs the growth with nitrate did not change significantly ($r = 0.49$ at 230 $\mu\text{E m}^{-2} \text{s}^{-1}$, $r = 0.54$ at 25 $\mu\text{E m}^{-2} \text{s}^{-1}$) (Table II). In cultures with ammonium, there was no measurable growth or an irregular growth pattern, as also described by Subba Rao and Wohlgeschaffen (1990). In ammonium-containing media, the cells developed a lobed shape and stopped forming chains, but close stacks of up to 10 cells

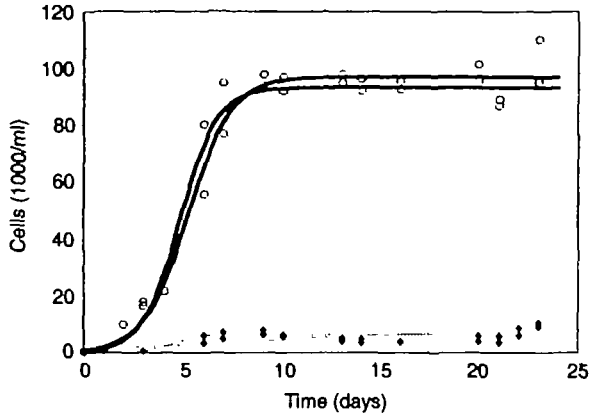


Fig. 1. Logistic growth curve of *P.pungens* f. *multiseries* with ammonium (dotted line) or nitrate (solid line) as sole N source.

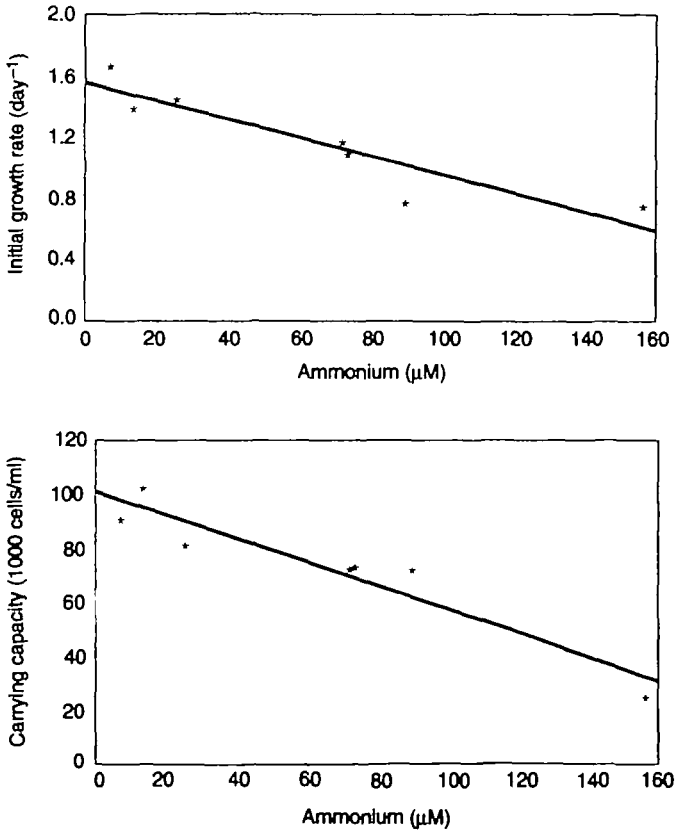


Fig. 2. Initial growth rate (r) and carrying capacity (K) of *P.pungens* f. *multiseries* growing with 400 μM nitrate and added ammonium concentrations.

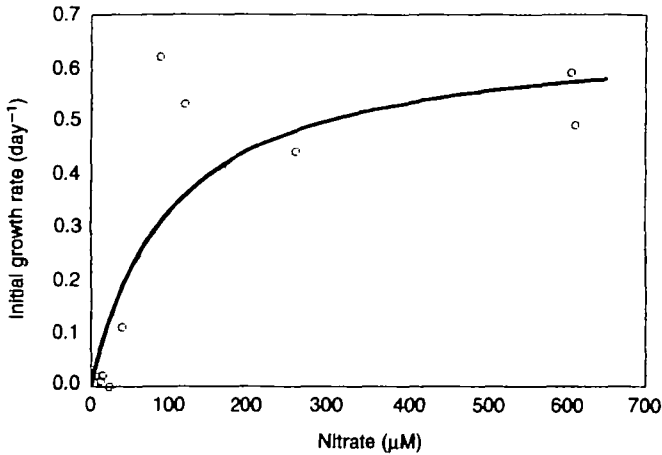


Fig. 3. Growth rate (r) of *P.pungens* f. *multiseries* growing with 40 μM ammonium and added nitrate concentrations.

were observed. The remaining chains formed circles instead of stiff needles.

Growth rates with nitrite, glutamine and urea were quite similar to those with nitrate. The growth rates were not correlated with the amount of electrons needed for the assimilation of the particular N source (Table II).

Among all examined N sources, only ammonium seemed to have negative effects on the growth of *P.pungens*, depending on the NH_4^+ concentration and the $\text{NH}_4^+:\text{NO}_3^-$ ratio in the culture media (Figures 2 and 3). Dortch (1990) pointed out that the inhibition of nitrate uptake by ammonium is not as complete as claimed by some authors and is often confounded by a preference for ammonium uptake. Without discussing the physiological mechanisms in detail, the results presented here can be explained as follows. Ammonium inhibited or at least slowed down the nitrate uptake at high $\text{NH}_4^+:\text{NO}_3^-$ ratios and thus led to lower growth rates. At the given pH (8.4 ± 0.2), $\sim 10\%$ of the ammonium existed in non-dissociated form (ammonia, NH_3 ; see Warren, 1962). Previous studies have shown toxic physiological effects of NH_3 (Azov and Goldman, 1982). Therefore, *P.pungens* was barely able to grow with ammonium as the sole N source in high concentrations. With low and non-toxic NH_4^+ concentrations, growth took place and the growth rate increased with lower $\text{NH}_4^+:\text{NO}_3^-$ ratios (Figure 3) until the maximum growth rate was reached. At very low $\text{NH}_4^+:\text{NO}_3^-$ ratios, the inhibition of nitrate uptake may have been overcome by the high nitrate concentrations, as already suggested by Carpenter and Dunham (1985) and Pennock (1987). In the case of growth-saturating nitrate concentrations, NH_4^+ only decreased the growth rate.

Compared to other diatoms, the deleterious effect of ammonium on *P.pungens* start at lower concentrations than observed so far: *Nitzschia closterium* (now called *Cylindrotheca closterium*) is inhibited at 4 mM (ZoBell, 1935) and the neritic diatoms *Asterionella japonica* (now called *Asterionella* (or *Asterionellopsis*) *glacialis*), *Skeletonema costatum* and *Chaetoceros affinis* are not affected by 200 μM NH_4^+ (Thomas *et al.*, 1980). Benthic diatoms of the Ems Estuary were unaffected at up to 500 μM ammonium (Admiraal, 1977). Only Natarajan (1970) reported slight

Table II. Initial growth rates (with SE) of *P. pungens* f. *multiseriis* with different N sources and at different photon flux densities (PFD)

N source	$\mu\text{M N}$	PFD ($\mu\text{E m}^{-2} \text{s}^{-1}$)	Specific growth rate (day^{-1})	SE
Ammonium	300	230	— ^a	—
Ammonium	300	25	0.36 ^b	0.11
			0.33 ^b	0.11
Nitrate	200	230	0.42	0.10
			0.55	0.23
			0.54	0.16
			0.46	0.15
Nitrate	200	25	0.66	0.08
			0.42	0.05
Glutamine	200	25	0.83	0.19
			0.65	0.14
Urea	200	25	0.52	0.08
			0.60	0.10
			0.38	0.10
Nitrite	200	25	0.42	0.06
			0.42	0.07
			0.38	0.07

^a No growth observable.

^b Growth in ribbon-shaped colonies, with deformed cell walls, see the text.

effects on the photosynthetic rate above 65 μM , but a 90% reduction was not measured until >3 mM. Other clones of *P. pungens* have been tested by Bates *et al.* (1993), indicating a growth-impairing effect starting at 220 μM . Thus, clone KP 84 seems to be rather sensitive to ammonium. This is another hint at the clonal differences within the genus *Pseudonitzschia* (cf. Lundholm *et al.*, 1994).

These results also show that the toxic effects of ammonium on *P. pungens* do not depend on irradiance and, moreover, that the growth rate is not affected by the significant light reduction. This is another point favouring the hypothesis that *P. pungens* might be adapted to low PFD. This is also documented by Bates and Léger (1992) and Sommer (1994), who reported the competitive displacement of *P. pungens* to low-light conditions.

The concentrations of ammonium offered in the batch cultures were much higher than they are in the ocean, but in coastal and estuarine waters concentrations up to 150 μM are sometimes reached (Admiraal, 1977; Thomas *et al.*, 1980). Disregarding clonal variability, the observed growth inhibition of *P. pungens* may affect the distribution of this species in ammonium-rich waters. Blooms of *P. pungens* f. *multiseriis* have until now occurred in winter, i.e. under unfavourable growth conditions. The extraordinary intolerance to high ammonium concentrations and the demand of high nitrate concentrations may play their role together with the competitive displacement to low-light seasons (Sommer, 1994). Ammonium concentrations are usually lower in winter, simultaneously agricultural drainage leads to an increased nitrate concentration (cf. Smith *et al.*, 1990), while irradiance is low. To prove this scenario, the nutrients during bloom conditions have to be measured and the until now non-toxic nominate form of *P. pungens* has to be subjected to the same experiments, because f. *pungens* often precedes f. *multiseriis* in the seasonal succession of species (e.g. Lange *et al.*, 1992; Taylor *et al.*, 1994).

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