

Nitrogen and phosphorus requirements of an *Alexandrium minutum* bloom in the Penzé Estuary, France

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Abstract

During an intense (up to 33×10^6 cells L^{-1}) *Alexandrium minutum* bloom in the Penzé estuary (France), total NO_3 , NH_4 , and PO_4 requirements of the bloom were, respectively, 184, 25, and 20 $\mu\text{mol } L^{-1}$, with peak uptake rates of 43, 6, and 4.8 $\mu\text{mol } L^{-1} d^{-1}$. The measured ambient concentrations of NH_4 and PO_4 were far short of this peak demand, whereas those of NO_3 were far in excess, indicating that NO_3 supply is important for sustaining the bloom. Comparison of the measured NO_3 uptake rates with advective fluxes indicates that a reduction of NO_3 concentrations in river waters to $<200 \mu\text{mol } L^{-1}$ would be necessary to contain the bloom in the Penzé estuary. The role of NO_3 was restricted to sustenance of the bloom, whereas warm conditions resulting in a water column stability seem to have triggered the bloom, and a self-shading, probably coupled with a phosphorus limitation, caused its decline.

Because of their impact on the economy of culture fisheries and human health at local and regional scales, incidences of blooms of toxic dinoflagellate *Alexandrium* spp. and the conditions leading to their recurrence have drawn a wide scientific interest in the last two decades. While a stability of the water column induced by thermal (Giaccobe et al. 1996) or haline (Cannon 1990) stratification or a mechanical accumulation, as at the front (Townsend et al. 2001), provides a physical setting for a rapid increase in cell numbers, several environmental factors could trigger and sustain the blooms. Successful germination of cysts (Garcès et al. 1999), seasonal temperature maximum (Erard-Le Denn 1997), reduction of salinity (Anderson et al. 1983), abundant light (Rasmussen and Richardson 1989), a large supply of inorganic nutrients from allochthonous sources (Anderson et al. 2002), and high dissolved organic nitrogen and phosphorus loads (Glibert et al. 2001) are some such factors. Incidences of toxic blooms near sources of river waters (Hallegraeff et al. 1988) and, in recent years, near sites of agricultural (Desalos 1999) and urban wastewater discharge

(Cannon 1990), high concentrations of ambient nutrients and high NO_3/PO_4 ratios (Hessen et al. 1997) have led to the conclusion that a large initial stock of dissolved inorganic nitrogen (N), especially nitrate, would be needed to sustain the bloom through its life cycle. Whether the high ambient nutrient concentrations are in fact adequate to support massive *Alexandrium* blooms, however, still remains unanswered, mainly because the absolute N and phosphorus (P) requirements of a bloom are not known.

Alexandrium minutum appeared for the first time in the Brittany estuaries in the late 1980s (Erard-Le Denn 1997). The Morlaix Bay ($48^{\circ}5'–45'N$; $3^{\circ}49'–59'W$), and in particular the Penzé estuary draining into it, is the most affected, with the bloom recurring almost every year around June (Desalos 1999). This predictability gave us an opportunity to measure N- and P-uptake rates of the bloom during the course of its full cycle, along with other physical, chemical, and biological parameters. With these data, we intend to answer three questions: What are the total N and P requirements of a bloom?; can the allochthonous supply (in the present case, the Penzé river waters) satisfy these requirements?; and, to what extent should this supply be reduced if the bloom were to be prevented from fully developing?

Material and methods

Our previous observations in 1997 and 1998 (Morin et al. 2000) showed that the *A. minutum* bloom in the Penzé es-

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Table 1. Environmental parameters at sites of highest cell densities of *A. minutum* in the Penzé estuary.

Year	Cell density ($\times 10^6 \text{ L}^{-1}$)	Temp ($^{\circ}\text{C}$)	Salinity	NO_3 ($\mu\text{mol L}^{-1}$)	NH_4 ($\mu\text{mol L}^{-1}$)	PO_4 ($\mu\text{mol L}^{-1}$)
1997	18	17.3	27.7	187	3.6	2.9
1998	0.6	16.3	28	526	11.5	2.5
1999	33	18.9	26.1	274	0.4	1.5

tuary usually occurs in June. Hence, nine samplings, with a frequency of 2 d when the bloom was in progress, were made between 25 May and 5 July 1999. During each sampling, samples were obtained from a 1-m depth in waters of ~ 26 salinity (located by measurements with a portable Seabird CTD), in which location the development of the bloom was at its maximum (Erard-Le Denn 1997; Morin et al. 2000). On each sample, the following measurements were made: cell counts, concentrations of chlorophyll *a* (Chl *a*), particulate organic nitrogen (PON), nitrate, ammonium and phosphate, uptake of nitrate, ammonium, and phosphate, and regeneration of ammonium.

Nitrate and PO_4 were measured in a Technicon autoanalyzer (Tréguer and Le Corre 1975) and NH_4 by the indophenol blue method (Koroleff 1970), with analytical precisions of $\pm 0.1 \mu\text{mol L}^{-1}$ for NO_3 and $\pm 0.02 \mu\text{mol L}^{-1}$ for the other two nutrients in the range of concentrations measured. Samples meant for measurements of PON, Chl *a*, and N uptake were prefiltered through 200- μm mesh. Particulate matter for PON was recovered on preignited Whatman GF/F filter pads, and the PON content was measured in a Perkin-Elmer model 240 elemental analyzer (coefficient of variation 5%). Chl *a* was measured fluorometrically in a Turner Designs fluorometer, with a precision of $\pm 0.05 \mu\text{g L}^{-1}$. Phytoplankton cell counts of Lugol-preserved samples were made with an inverted microscope. Along with CTD measurements of temperature and salinity, the photosynthetically active radiation (PAR) was measured with a quantum sensor (Biospherical Instruments) mounted on the CTD.

Nitrate and NH_4 uptake rates were measured with ^{15}N -labeled substrates and NH_4 regeneration, using the isotope dilution method. Tracers with 99% ^{15}N enrichment were added at $\sim 10\%$ of ambient concentrations, but not at concentrations lower than an absolute concentration of $0.02 \mu\text{mol L}^{-1}$. The amount of tracer added was between 10.7% and 13.6% of the total dissolved NH_4 pool and between 9.4% and 11.9% of the total dissolved NO_3 pool. Samples dispensed in polycarbonate bottles were incubated under simulated in situ conditions for 2–3 h around local noon. At the end of the incubation, a fraction of the sample was filtered through 30- and 15- μm filters, and the remaining sample was placed directly onto preignited GF/F filter pads. Fractional filtration was done in order to obtain a realistic estimate of N uptake by *A. minutum*, which is normally distributed in the size range of 16–27 μm (Erard-Le Denn 1997).

NH_4 from the filtrate was recovered by diffusion in basic pH (8.5). Ratios of $^{15}\text{N} : ^{14}\text{N}$ were measured in an emission spectrometer (Sopra Instruments, model GS1), and the N-uptake rates were calculated using the equation of Dugdale and Wilkerson (1986). Uptake rates of NH_4 were corrected for isotopic dilution (Glibert et al. 1982), with the dilution

factor ranging from 1 to 1.4 (average 1.1). NH_4 regeneration rates were calculated either with the equation of Glibert et al. (1982) or that of Laws (1984), depending upon whether the NH_4 concentrations of the incubation medium remained unchanged or not.

Uptake of P was measured with ^{33}P by inoculating the samples with $\text{H}_2^{33}\text{PO}_4$ ($1 \mu\text{Ci L}^{-1}$). Unlike the N uptake, only two fractions (total and $< 10 \mu\text{m}$) were considered. The experimental design was also modified so that each time, P uptake was measured in samples with natural ambient concentrations and those enriched with NaH_2PO_4 at $32 \mu\text{mol P L}^{-1}$. The ratio $V_{\text{max}} : V_{\text{trace}}$ indicates the degree of P limitation, if any. All P-uptake measurements were done in triplicate. As the half-life of ^{33}P was short, the initial radioactivity was measured from aliquots withdrawn immediately after the addition of labeled P. After an incubation period of 3–4 h, the samples were filtered successively onto 10- and 0.6- μm Nucleopore filters. The radioactivity retained on the filters was measured in a Packard Tricarb 1600 TR scintillation counter.

Results

A. minutum in the Penzé Estuary showed a preference to salinities greater than 20, with the greatest densities measuring between 26 and 28 (Table 1). From a few thousand cells per liter on 9 June, the bloom attained the highest concentration of 33×10^6 cells L^{-1} on 22 June, and by the end of June, this value had declined to less than 50×10^3 cells L^{-1} (Fig. 1). *A. minutum* was contained mostly in the 15–30- μm fraction and at the peak of the bloom constituted $\sim 90\%$ of the cell counts in this fraction. At its peak, *A. minutum* dominated more than 60% of the total cell counts, with the remaining cell counts composed of *Nitzschia longissima* (12%) and *Chaetoceros* sp. (23%). The decline of the *A. minutum* bloom was accompanied by a nanoplankton bloom (148×10^6 cells L^{-1} on 5 July).

Changes of the concentrations of Chl *a*, PON, NO_3 , PO_4 , and NH_4 (Figs. 2, 3) occurred, as expected, when a massive phytoplankton bloom developed. Chl *a* and PON increased, respectively, from $10 \mu\text{g L}^{-1}$ and $30 \mu\text{mol L}^{-1}$ before the bloom to $80 \mu\text{g L}^{-1}$ and $210 \mu\text{mol L}^{-1}$ on 22 June. This increase (62–64%) was observed mainly in the 15–30- μm fraction. Nitrate concentrations decreased through $45 \mu\text{mol L}^{-1}$, but those of PO_4 decreased only marginally, by $0.2 \mu\text{mol L}^{-1}$. Even at the peak of the bloom, residual concentrations of NO_3 were in excess of $200 \mu\text{mol L}^{-1}$, leading to $\text{NO}_3 : \text{PO}_4$ ratios of > 100 . Such high residual concentrations, especially of nitrate, are unusual and different from those noted in other areas in which the water column becomes stripped of its nutrients at the peak of the bloom. Concen-

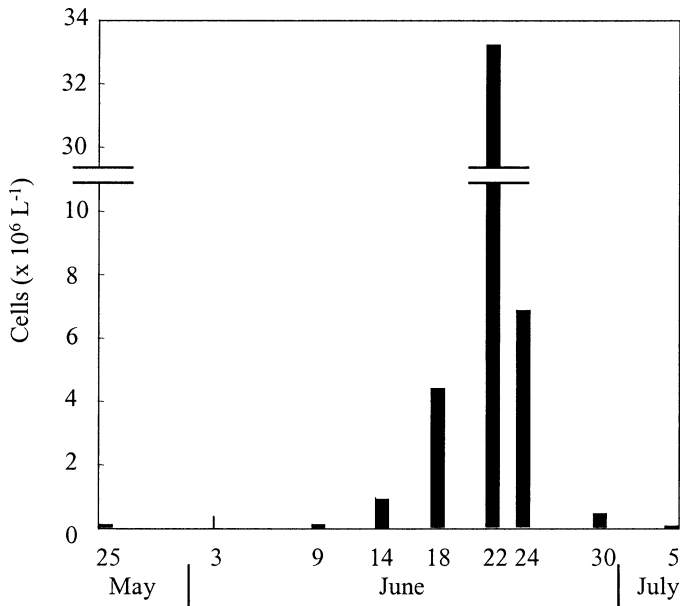


Fig. 1. Changes in cell densities of *A. minutum* during the bloom in 1999.

trations of NH₄, however, decreased more than an order of magnitude, from about 8.8 to 0.35 μmol L⁻¹.

The bloom development was accompanied by a sharp increase in N uptake, from 600 nmol L⁻¹ h⁻¹ on 14 June to 3,700 nmol L⁻¹ h⁻¹ on 22 June (Fig. 4a,b). Ammonium uptake constituted 30–70% of total N uptake until *A. minutum*

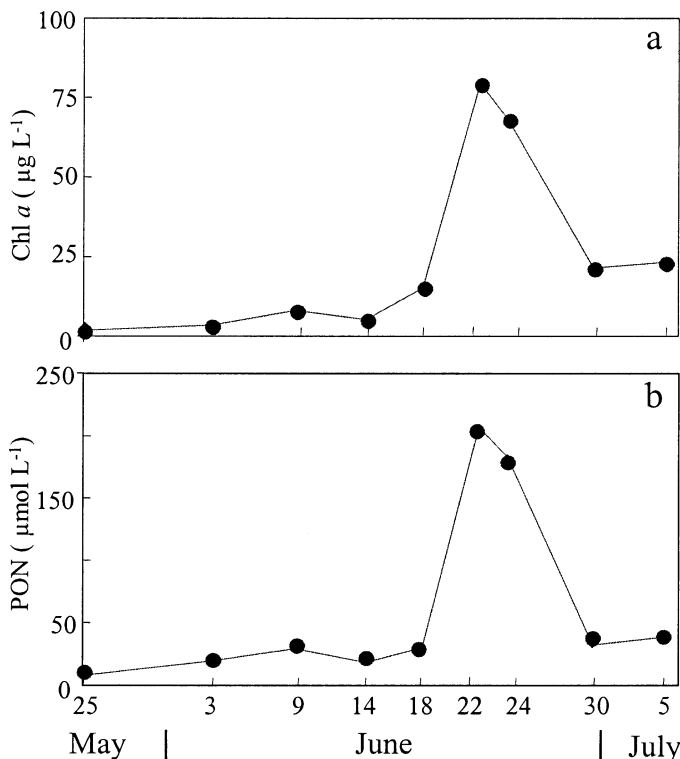


Fig. 2. Changes in concentrations of a) chlorophyll *a* and b) PON during the *A. minutum* bloom.

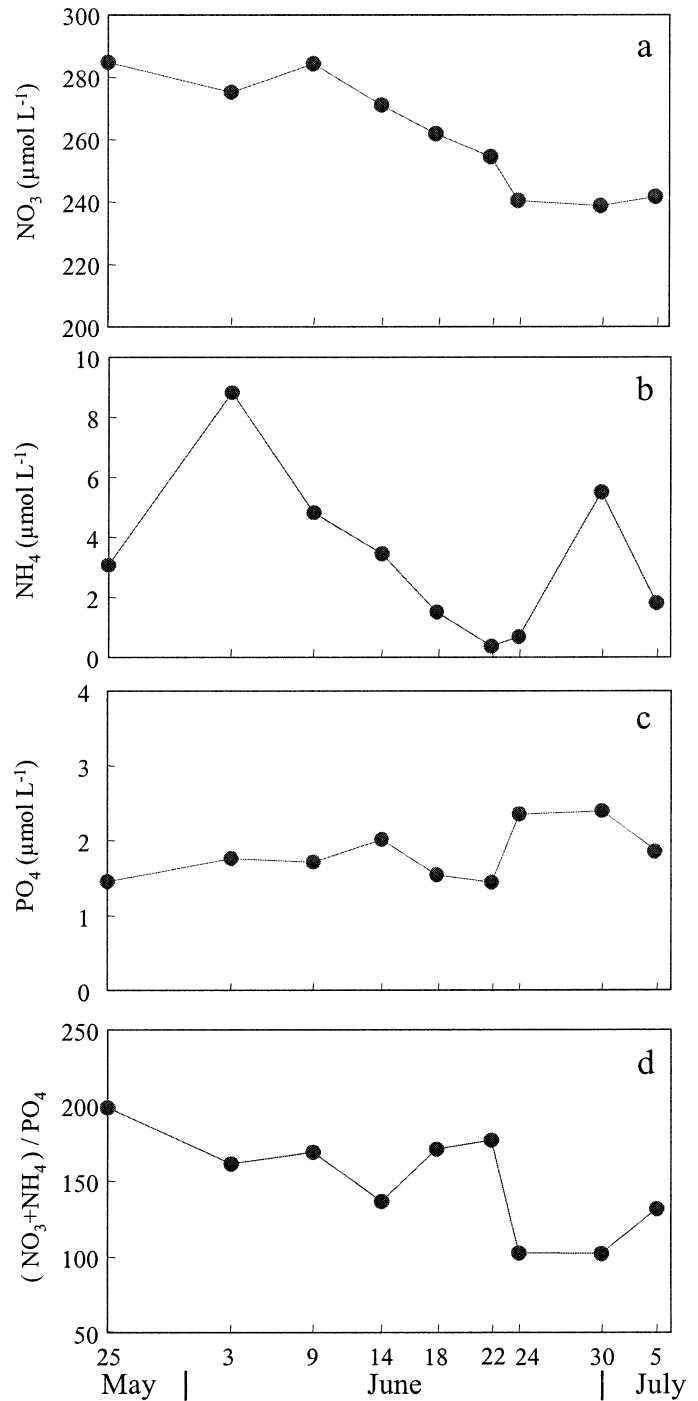


Fig. 3. Changes in ambient concentrations of a) nitrate, b) ammonium, c) phosphate, and d) DIN :DIP ratios during the *A. minutum* bloom.

cell concentrations reached 1 × 10⁶ L⁻¹. As the intensity of the bloom began to increase, NH₄ was rapidly replaced as an N source by NO₃ (85–91% of total N uptake), and only when the bloom began to decline did the proportion of NH₄ uptake increase again. Nitrate, therefore, was essential to sustain the bloom. Ammonium regeneration rates (100–125 nmol L⁻¹ h⁻¹) during the bloom (Fig. 4d) were not substan-

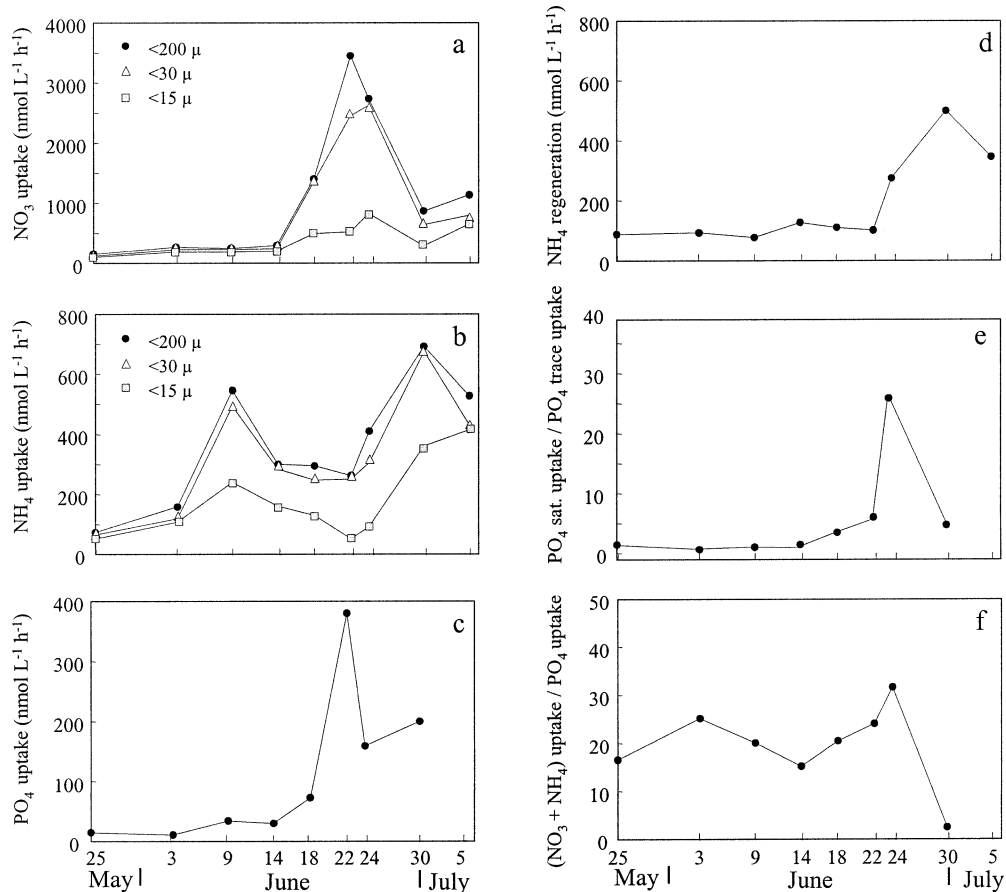


Fig. 4. Changes in a) nitrate-uptake rate, b) ammonium-uptake rate, c) phosphate-uptake rate, d) ammonium regeneration rate, e) ratio of uptake of saturation to trace uptake of phosphate, and f) N:P uptake ratios.

tially higher than those noted before the bloom (77–93 nmol L⁻¹ h⁻¹). Even the addition of NH₄ regenerated to the ambient concentrations of NH₄ during the bloom; the latter could not have supported more than a few hours of uptake.

Phosphorus uptake also increased sharply with the bloom development, from 15 to 380 nmol L⁻¹ h⁻¹ (Fig. 4c). The $V_{\max} : V_{\text{trace}}$ ratios remained generally close to 1 until a few days before the peak of the bloom, when the ratio increased to above 30 (Fig. 4e). Nitrogen (NO₃ plus NH₄):P ratios of uptake rates normalized to Chl *a* were consistently above 15 during the entire course of the bloom (Fig. 4f).

Discussion

Blooms of *A. minutum* have been known to occur often in nutrient-rich coastal waters such as ports, estuaries, and bays (Delgado et al. 1990; Townsend et al. 2001), indicating a relationship between sudden and excessive N (and P) loading and initiation of the bloom. In the Morlaix Bay, however, nutrient loading is not a critical parameter for initiation of the bloom. Brittany estuaries are a classical example of heavy application of N fertilizers in the hinterland and of their runoff into the lower rivers, estuaries, and coastal waters (Desalos 1999), with NO₃ content of river waters in

excess of 500–600 μmol L⁻¹ and N:P ratios ranging from 65 in summer to >300 in winter, as far back as the 1980s (Wafar 1981). Nitrate content of the river waters is generally greater than 400 μmol L⁻¹ even in summer (Wafar 1981), and in the salinity range within the estuary where *A. minutum* blooms, it was higher than 150 μmol L⁻¹ in June. Almost all *A. minutum* blooms in the Brittany coastal area have been known to occur toward the end of spring, suggesting an important role for temperature (Desalos 1999). Our measurements (Morin et al. 2000) showed that peak cell densities occurred at water temperatures of >16°C (Table 1) with a proportionality worth mentioning. The data for 1999 also show clearly that the bloom rapidly develops when the tide tends toward neap and the stability index ($\Delta\sigma/\Delta z$) of the water column increases sharply and remains at this level for several days (Fig. 5b). Warm conditions coupled with high water column stability, and not nutrients, would therefore favor initiation of *A. minutum* blooms in the Brittany estuaries. As vegetative cells were rarely seen in other months, the seed population for the bloom could only have come from the resting cysts (>100 (g dry sediment)⁻¹) (Erard-Le Denn et al. 1997), and the cue for their germination could have been the warm temperature, comparable with the 16°C threshold observed by Cannon (1993), along with a

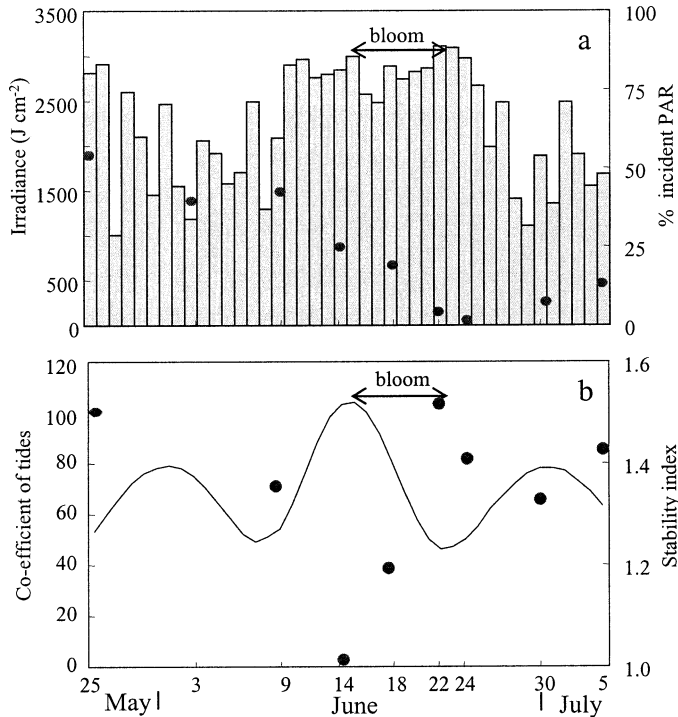


Fig. 5. Changes in a) surface incident radiation (vertical bars) and % incident PAR (closed circles) at 1-m depth during the *A. minutum* bloom and b) tidal heights (line) and the stability index (circles).

stimulation by light (Anderson et al. 1987) that downwells to the sediment level in the Penzé estuary at this time of the year. In fact, surface-incident radiation levels were the highest ($>2,500 \text{ J cm}^{-2}$) for several days before the bloom got underway (Fig. 5a).

Nutrients, on the other hand, would be essential for maintenance of the bloom. The high ambient concentrations and uptake rates of NO_3^- relative to those of NH_4^+ and the high $V_{\text{max}}:V_{\text{trace}}$ ratios of P uptake during the peak of the bloom indicates that among the three nutrients, it is the supply of NO_3^- that maintains the bloom. With our data on N uptake, it is possible to calculate the amount of NO_3^- that would be required to support the bloom, provided it could be demonstrated that the bloom develops in situ and is not a product of mechanical accumulation, as has been suggested at times (Garcès et al. 1999). This was done by calculating, with the doubling rate of 0.45 d^{-1} obtained for a strain of *A. minutum* from these waters (Erard-Le Denn 1997), the increase in cell numbers from an initial density of $0.13 \times 10^6 \text{ cells L}^{-1}$ on 9 June and comparing them with the actual counts. The enumerated densities were lower than the predicted ones (Fig. 6), indicating that in situ growth to concentrations in excess of $30 \times 10^6 \text{ cells L}^{-1}$ is possible, provided there is no limitation from external factors. The doubling rate calculated with our data was 0.43 d^{-1} . This is comparable with the 0.45 d^{-1} (Erard-Le Denn 1997) and 0.47 d^{-1} (Piumsomboon et al. 2001) obtained from exponentially growing cultures of *A. minutum*.

The total N and P required to support a bloom of this size can be calculated with our data on uptake rates. At the peak

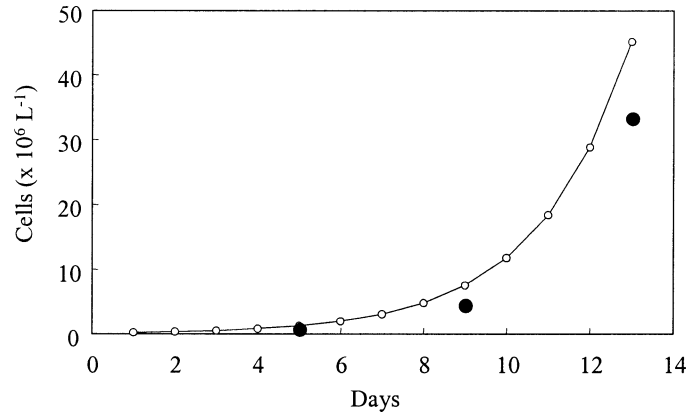


Fig. 6. Predicted increase in cell density during the *A. minutum* bloom (open circles and line) and the observed densities (filled circles).

of the bloom, the 15–30- μm fraction was almost entirely ($\sim 90\%$) composed of *A. minutum*. NO_3^- and NH_4^+ uptake rates measured in this fraction were $1,926$ and $200 \text{ nmol L}^{-1} \text{ h}^{-1}$, respectively. With *A. minutum* density of $18.59 \times 10^6 \text{ cells L}^{-1}$ in this fraction, this would give NO_3^- and NH_4^+ uptake rates of 103.6×10^{-6} and $10.7 \times 10^{-6} \text{ nmol cell}^{-1} \text{ h}^{-1}$. As the samples for P-uptake measurements were not fractionated in a similar way, the total P uptake was scaled down to the proportion of the N uptake in the 15–30- μm fraction and then divided by the density of *A. minutum* cells in this fraction. This gave a P-uptake rate of $11.4 \times 10^{-6} \text{ nmol cell}^{-1} \text{ h}^{-1}$.

Nitrate, NH_4^+ , and PO_4 required by the bloom was calculated as

$$\text{DIN(P)} = \sum_{t=0}^{t=n} C_t \times v$$

where n is the duration of the bloom (from $0.13 \times 10^6 \text{ cells L}^{-1}$ on 9 June to $0.5 \times 10^6 \text{ cells L}^{-1}$ on 30 June), C_t is the concentration of cells on a given day, and v is the uptake rate d^{-1} . C_t was calculated with a doubling rate of 0.43 d^{-1} until the bloom was at its highest density and with a decay constant of -0.52 ($r^2 = 0.97$; $n = 4$) when the bloom was on the decline. Conversion of the hourly rates of uptake to daily rates was done with a factor of 12 for NO_3^- and PO_4 and a factor of 16 for NH_4^+ , the latter giving allowance for the reduced dark uptake.

Total NO_3^- and NH_4^+ requirements for the bloom, respectively, were in the order of 184 and $25 \mu\text{mol L}^{-1}$, with peak values of 43 and $6 \mu\text{mol L}^{-1} \text{ d}^{-1}$ (Fig. 7). In the case of P, it was $20 \mu\text{mol L}^{-1}$, with peak uptake rate of $4.8 \mu\text{mol L}^{-1} \text{ d}^{-1}$. Although the measured ambient NH_4^+ and PO_4 concentrations fall far short of this peak demand, ambient NO_3^- concentrations were greatly in excess at any time (Fig. 3).

The collapse of the bloom could not have been caused by a lack of N. The N:P concentration ratios were, even at the peak of the bloom, around 100, and the biomass-normalized N:P uptake ratios were, except for a single instance after the bloom, up to two times greater than the Redfield ratio, indicating a large sufficiency of N. Nor could a lack of stability of the water column have been the cause, since the

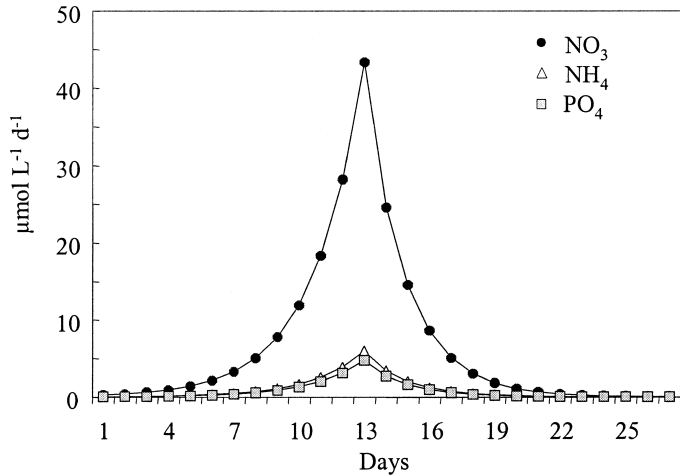


Fig. 7. Predicted quantities of nitrate, ammonium, and phosphate required for the *A. minutum* bloom.

stratification was still marked beyond peak bloom growth. The PAR measured at 1 m below the surface, however, decreased dramatically, from about 50% at the beginning of the bloom to about 1–2% two days after the peak cell density (Fig. 5a). This was much less than a PAR of 10% of incident radiation needed for dinoflagellate growth (MacIsaac and Dugdale 1972) and implies that self-shading could have been the cause of the decline. Phosphorus could also have played a role, since the $V_{\max} : V_{\text{trace}}$ ratio of P uptake increased to 25 at the peak of the bloom from near-unity in the early stages of the bloom. Besides, even though the changes in the concentrations of PO_4 during the bloom were not substantial, the PO_4 that was available ($1.44 \mu\text{mol L}^{-1}$) at the peak of the bloom could not have been adequate to satisfy the demand ($4.8 \mu\text{mol L}^{-1} \text{d}^{-1}$).

Instances in which an increase in dissolved organic nitrogen (DON) component may have favored development of some blooms have been reported (Anderson et al. 2002). We have not measured DON concentrations in Penzé river waters during the bloom but consider, based on two observations, that it is unlikely that DON or any of its assimilable forms could have been important in sustaining the bloom in the present case. In the first instance, the increase of PON ($\sim 180 \mu\text{mol L}^{-1}$) during the bloom was, giving allowances for loss by sedimentation, on the same order as consumption of inorganic N ($210 \mu\text{mol L}^{-1}$). In the second instance, initial availability of DON for uptake is not as important as that of NO_3 —DON concentrations in the freshwater segment of the adjacent Morlaix River waters draining the same agriculture basin in previous years (Wafar 1981), or in the salinity range of 25–35 within the Morlaix estuary during the study period (Colobert-Le Floch 2001), were at no time higher than 10–20% of those of NO_3 . Apart from the fact that the DON concentrations were too low to support the N requirements of the bloom, it is also unlikely that the bloom could have used all the DON fractions, since many of the DON compounds are highly refractory and are not readily used by phytoplankton (Anderson et al. 2002). A continuous replenishment of inorganic N, especially NO_3 , would therefore be required to reconcile the difference between the calculated

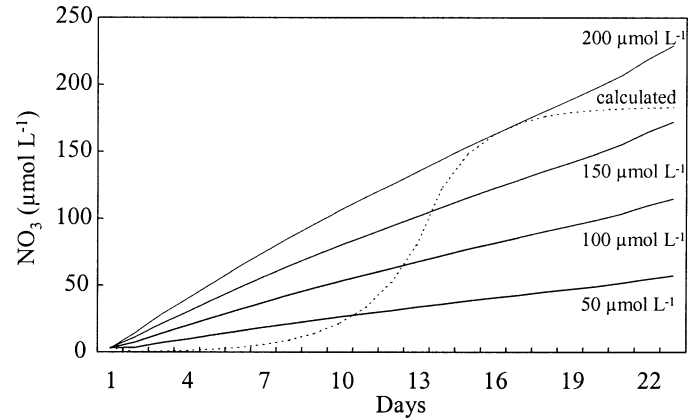


Fig. 8. Comparison of nitrate required by the *A. minutum* bloom and the quantities of nitrate supplied to the bloom at varying initial ($50 \mu\text{mol L}^{-1}$ to $200 \mu\text{mol L}^{-1}$) concentrations in Penzé river waters.

quantity of NO_3 required for the bloom ($184 \mu\text{mol L}^{-1}$) and the apparent decrease in the ambient concentrations of NO_3 ($45 \mu\text{mol L}^{-1}$) from the beginning to the end of the bloom.

The increase in the concentrations of NO_3 within a unit volume of the estuarine water on mixing with river water during the period of the bloom can be calculated as

$$\sum_{t=0}^{t=n} [(RFt \times Mt) + (V \times Ct)/(RF + V)] - Ct$$

where RF is the river discharge rate, V is the basin volume, and M and C are the concentrations of NO_3 , respectively, in river and seawater.

V at a tidal height of 3 m was computed as 1.8×10^9 liters and C at $t = 0$ was taken as $3 \mu\text{mol L}^{-1}$, a concentration typical of the coastal waters of Morlaix Bay in late spring (Wafar 1981). Using the river discharge rates ($1\text{--}2 \text{ m}^3 \text{ s}^{-1}$) measured during the bloom period and varying the concentrations of NO_3 in river waters from 50 to $200 \mu\text{mol L}^{-1}$, quantities of net accumulation of NO_3 within a liter of estuarine water can be generated (Fig. 8). Comparison of these with total NO_3 utilization calculated for the bloom shows that a substantial reduction in the concentration of NO_3 in the river waters to below $200 \mu\text{mol L}^{-1}$ would be necessary if the bloom were to be contained. An assumption that is implicit in these calculations is that the flushing of the estuary is unimportant during the course of the bloom. This is a reasonably tenable assumption, since the freshwater discharge by Brittany rivers, especially in summer, is much lower than the tidal prism volume, with flow ratios (of freshwater discharge in a tidal cycle to tidal prism volume), for example, of the Morlaix River being on the order of 0.001 to 0.004 (Wafar 1981). With an average flow of $1 \text{ m}^3 \text{ s}^{-1}$ from the Penzé river, about 21 d will be required for a complete flushing, which is greater than the length of time needed for the exponential growth phase of the bloom.

Fertilizer application on land is a major non-point source of N pollution to lower rivers, estuaries, and coastal waters (Anderson et al. 2002). The Brittany estuaries are a pioneer example of heavy input of N nutrients from fertilizer appli-

cation in the hinterland) and their runoff into the coastal waters, with NO_3 content of river waters in excess of 500–600 $\mu\text{mol L}^{-1}$ and N:P ratios generally >100 . Containment, if not total eradication, of *A. minutum* blooms would be possible if a three- to fourfold reduction in NO_3 transported by the river waters could be envisaged. Elsewhere, the scales may not be the same as in Brittany rivers, but among the factors that are known to initiate and sustain a toxic bloom, control of the input of NO_3 is certainly within reach of current technologies and management tools.

References

- ANDERSON, D. M., S. W. CHISHOLM, AND C. J. WATRAS. 1983. Importance of life cycle events in the population dynamics of *Gonyaulax tamarensis*. *Mar. Biol.* **76**: 79–189.
- , P. M. GLIBERT, AND J. M. BURKHOLDER. 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition and consequences. *Estuaries* **25**: 704–726.
- , C. D. TAYLOR, AND E. V. ARMBRUST. 1987. The effect of darkness and anaerobiosis on dinoflagellate cyst germination. *Limnol. Oceanogr.* **32**: 340–351.
- CANNON, J. A. 1990. Development and dispersal of red tide in the Port River, South Australia, p. 110–115. In E. Graneli, B. Sundstrom, L. Edler, and D. M. Anderson [eds.], *Toxic marine phytoplankton*. Elsevier.
- . 1993. Growth in culture of the toxic dinoflagellate *Alexandrium minutum* from the Port River, South Australia, p. 741–746. In T. J. Smayda and Y. Shimizu [eds.], *Toxic phytoplankton blooms in the sea*. Elsevier.
- COLOBERT-LE FLOCH, I. 2001. Absorption et régénération de l'azote dans les systèmes côtiers: Réponse à des apports massifs de nitrate. Thèse de doctorat, Univ. de Bretagne occidentale, France.
- DELGADO, M., M. ESTRADA, J. CAMP, J. V. FERNANDEZ, M. SANTMARTI, AND C. LLETI. 1990. Development of a toxic *Alexandrium minutum* Halim (Dinophyceae) bloom in the harbour of Sant Carles de la Rapita (Ebro Delta, northwestern Mediterranean). *Sci. Mar.* **54**: 1–7.
- DESALOS, D. 1999. *Alexandrium minutum* dans les estuaires nord-finistériens: Eaux colorées et toxicité des coquillages en relation avec quelques facteurs naturels. Mémoire pour l'obtention du Diplôme d'Agronomie, Ifremer, France.
- DUGDALE, R. C., AND F. P. WILKERSON. 1986. The use of ^{15}N to measure nitrogen uptake in eutrophic oceans; Experimental considerations. *Limnol. Oceanogr.* **31**: 673–689.
- ERARD-LE DENN, E. 1997. *Alexandrium minutum*. Efflorescences toxiques dans les eaux côtières françaises, p. 53–65. In B. Berland and P. Lassus [eds.], *Repère Océan*, V. 13.
- , M. L. COCHARD, AND J. LE GRAND. 1997. Etude sur l'utilisation des kystes d'*Alexandrium minutum* à la prévision des efflorescences. Rapport PNEAT, Ifremer, France convention No. 97.5.440912.
- GARCÉS, E., M. MASO, AND J. CAMP. 1999. A recurrent and localized dinoflagellate bloom in a Mediterranean beach. *J. Plankton Res.* **21**: 2373–2391.
- GIACOBBE, M. G., F. D. OLIVA, AND G. MAIMONE. 1996. Environmental factors and seasonal occurrence of the dinoflagellate *Alexandrium minutum*, a PSP potential producer, in a Mediterranean lagoon. *Estuarine Coastal Shelf Sci.* **42**: 539–549.
- GLIBERT, P. M., F. LIPSCHULTZ, J. J. MCCARTHY, AND M. A. ALTABET. 1982. Isotope dilution models of uptake and ammonium by marine plankton. *Limnol. Oceanogr.* **27**: 639–650.
- , R. MAGNIEN, M. W. LOMAS, J. ALEXANDER, C. FAN, E. HARAMOTO, M. TRICE, AND T. M. KANA. 2001. Harmful algal blooms in the Chesapeake and coastal bays of Maryland, USA: Comparison of 1997, 1998, and 1999 events. *Estuaries* **24**: 875–883.
- HALLEGRAEFF, G. M., D. A. STEFFENSEN, AND R. WETHERBEE. 1988. Three estuarine Australian dinoflagellates that can produce paralytic shellfish toxins. *J. Plankton Res.* **10**: 533–541.
- HESSEN, D. O., A. HINDAR, AND G. HOLTAN. 1997. The significance of nitrogen runoff for eutrophication of freshwater and marine recipient. *Oceanology* **26**: 312–320.
- KOROLEFF, F. 1970. Direct determination of ammonia in natural waters as indophenol blue. *Int. Council Exploration Sea* **9**: 19–22.
- LAWS, E. A. 1984. Isotope dilution models and the mystery of vanishing ^{15}N . *Limnol. Oceanogr.* **29**: 379–386.
- MACISAAC, J. J., AND R. C. DUGDALE. 1972. Interactions of light and nitrogen in controlling nitrogen uptake in the sea. *Deep-Sea Res.* **19**: 209–232.
- MORIN, P., E. ERARD-LE DENN, J. F. MAGUER, C. MADEC, C. VIDEAU, J. LE GRAND, AND E. MACÉ. 2000. Etudes des causes de prolifération de microalgues toxiques en mer. Cas d'*Alexandrium*. Rapport scientifique à l'agence de l'eau Loire Bretagne, Convention no. 7.98.9476, 135 p.
- PIUMSOMBOON, A., C. SONGROOP, A. KUNGSUWAN, AND P. POLPUNTHIN. 2001. Species of the dinoflagellate genus *Alexandrium* (Gonyaulacales) in the Gulf of Thailand, p. 12–15. In G. M. Hallegraeff, S. I. Blackburn, C. J. Bolch, and R. J. Lewis [eds.], *Harmful algal blooms 2000*. IOC, Unesco.
- RASMUSSEN, J., AND K. RICHARDSON. 1989. Response of *Gonyaulax tamarensis* to the presence of pycnocline in an artificial water column. *J. Plankton Res.* **11**: 747–762.
- TOWNSEND, D. W., N. R. PETTIGREW, AND A. C. THOMAS. 2001. Offshore blooms of the red tide dinoflagellate *Alexandrium* sp. in the Gulf of Maine. *Cont. Shelf Res.* **21**: 347–369.
- TRÉGUER, P., AND P. LE CORRE. 1975. Manuel d'analyse des sels nutritifs dans l'eau de mer (Utilisation de l'Autoanalyseur II Technicon), 2nd ed. Univ. de Bretagne Occidentale, France.
- WAFAR, M. V. M. 1981. Nutrients, primary production and dissolved and particulate organic matter in well-mixed temperate coastal waters (Bay of Morlaix—western English Channel). Doctoral Thesis, Univ. of Paris.

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