Harmful Marine Algal Blooms - P. Lassus, G. Arzul, E. Erard, P. Gentien, C. Marcaillou


# Nitrogen And Phosphorus Limitation In Cultured *Alexandrium minutum* Halim Does Not Promote Toxin Production

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## Abstract

Studies of a high- and low-toxin strains of *Alexandrium minutum* isolated from Ría de Vigo fail to indicate profound C-N physiology differences in amino acid synthesis, biomass, growth rates or pigmentation, which might explain the 10 to 20 fold differences in PSP toxin content. Maximum toxin synthesis occurs during N-refeeding of N-deprived cells and thereafter toxin content covaries with free intracellular arginine. For both strains, toxin synthesis and toxin content on a cell or biomass basis decline during N or P deprivation. Implications for changes in the toxin content of field populations are discussed.

## Introduction

*A. minutum* is widely distributed in the Mediterranean and NE Atlantic (Erard-Le Denn, 1991). It forms red tides and PSP outbreaks in the Rías Altas (Blanco et al., 1985), and is associated with PSP near to quarantine levels in the Rías Bajas. Two *A. minutum* strains isolated from Ría de Vigo, AL1V (highly toxic) and AL2V (weakly toxic) were employed. The physiology of recovery from N-deprivation, with a period of exponential growth, and subsequent deprivation of N or P were studied.

## Materials & Methods

*A. minutum* strains AL1V and AL2V (IEO, Vigo) were grown in modified K-media (Keller and Guillard,1985) with no Tris, glycerophosphate, or ammonium. Experimental flasks contained different nutrient regimes: low N:P medium (100 $\mu$M nitrate, 20 $\mu$M phosphate) and high N:P medium (300 $\mu$M nitrate, 5 $\mu$M phosphate). Cultures were started with N-deprived inocula (elevated C:N ratios) and grown at 15°C in a 12h/12h light/dark cycle of 200 $\mu mol.m^{-2}.s^{-1}$. Samples were taken for 15 days for estimates of cell number (microscopy), C-N biomass (elemental analysis), pigments (HPLC, Zapata et al., 1987), intracellular amino acids (HPLC, Flynn and Flynn, 1992), and toxins (HPLC, Franco and Fernández-Vila, 1993) calibrated against standards quantified by A. Cembella. The experiment was performed twice with similar results.

## Results

In comparison with the more toxic AL1V, AL2V cells had up to twice the biomass and a higher C:N ratio during exponential growth (7 vs 5), but grew at

a similar maximum rate on a C basis (0.25 $d^{-1}$). Despite of differences between strains with respect to pigmentation (AL2V had smaller ratios of chlorophyll c2 and peridinin to chlorophyll a) these are of insufficient magnitude to enable their reliable use in field situations. Pigment quantity and quality were not reliable as indicators of biomass or nutrient status. Amino acid profiles were similar for both strains: arginine was the most important free amine in terms of N. Concentrations of amino acids, initially higher for AL1V, declined in response to both N- and P-deprivation. The amounts of the nonprotein amine-X (Flynn et al., 1993), were insignificant throughout the batch culture cycle. Concentrations of glutamine were always high, even after N-deprivation. These factors suggest that *A. minutum*, and probably *Alexandrium* spp. in general, do not have a typical dinoflagellate amino acid metabolism. In both strains, toxin composition varied only slightly during N- or P-deprivation, with the composition altering from 94% to 97% GTX4 (the remainder being GTX1 > GTX3 > GTX2).

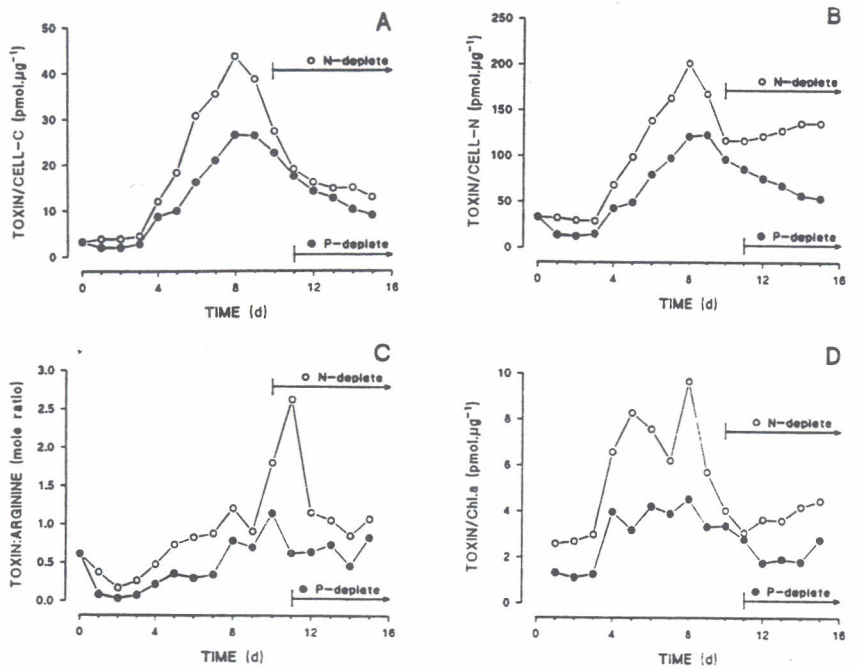


Figure 1. Changes in toxin content for cells of *Alexandrium minutum* strain AL1V expressed on (A) cell-C, (B) cell-N, (C) free intracellular arginine content, and (D) chlorophyll a bases. Cells in the low-nitrate culture (open symbols) became progressively more N-deprived from day 10, and the low-phosphate cells (closed symbols) more P-deprived from day 11.

Figure 1 shows changes in toxin content for AL1V on different bases; these cultures exhausted nitrate on day 10 or phosphate on day 11. Comparative data for the low toxin strain, AL2V, are given in Figure 2.

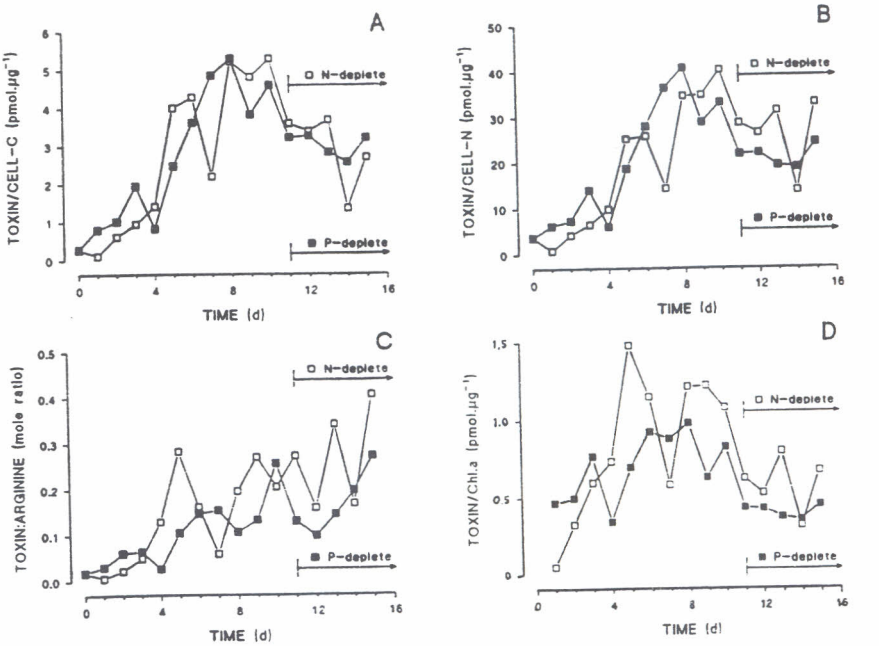


Figure 2. As Figure 1 but for *Alexandrium minutum* strain Al2V (low toxin strain). Cells became increasingly N or P-deprived (low-nitrate or low-phosphate cultures respectively) from day 11.

Toxin/cell-C for AL1V (Fig. 1A) peaked value around day 8 whereas peak toxin/cell was attained by day 6; cells of this strain became smaller during late exponential growth. AL2V (Fig. 2A) showed a similar pattern, but there was no significant difference between the toxicity of the two cultures. Toxin/cell-N for the low-nitrate cells of AL1V (Fig. 1B) remained constant once nitrate was exhausted, while it declined in the phosphate deprived cells which continued to assimilate nitrate-N. In AL2V (Fig. 2B), toxin/cell-N became constant for both N and P-deprived cells. These events suggest a rôle for P as well as for N toxin synthesis. The mole ratio of toxin: arginine in AL1V (Fig. 1C) declined during N-refeeding at the start of the experimental period; this reflects an increase in arginine content during that period. For the N-deprived cells of AL1V at day 10, toxin:arginine rose as arginine declined on exhaustion of the N-sources, before recovering, presumably due to amino acid cycling. For the P-deprived cells of AL1V the ratio remained constant (Fig. 1C) because both arginine and toxin cell contents declined together. The ratio of toxin:arginine in AL2V (Fig. 2C) was around 25% of that for AL1V. This reflects a difference in toxin content of an order of magnitude but also a smaller arginine content. Fig. 1D shows changes in toxin/Chl*a* for AL1V; the values varied by a factor of up to 5 for AL1V.

When the variation due to AL2V is included (Fig. 2D), toxin/Chla for these isolates of *Alexandrium minutum* varied by a factor around 25.

## Discussion

Differences in growth rates, CN composition, pigmentation and free amino acid content appear inadequate to explain the 10-20 fold difference in toxin content between strains. Unless there are significant differences in the compartmentalization of metabolites such as arginine, then the different ability to synthesize toxins would appear to be profound and not linked simply to the availability of those metabolites. Pigment analyses by HPLC, while adequate to detect marker pigments for dinoflagellates, are inadequate to differentiate between highly toxic and weakly toxic strains of *A. minutum*. Neither may they be used to determine biomass, or to give indications of nutritional status or toxin content.

The responses of *A. minutum* to changes in nutrient status suggest that toxin synthesis is associated with intracellular free arginine concentrations. Factors which promote elevated arginine may lead to enhanced toxin synthesis and vice versa (Fig. 3). Both N-deprivation and P-deprivation have detrimental effects on amino acid synthesis and on the size and composition of the intracellular free amino acid pool leading to a decline in arginine levels and toxin synthesis (Fig. 3B). Although the detrimental effect of N-deprivation on toxin synthesis has been noted before, the adverse effect of P-deprivation on *A. minutum* contradicts the enhancement of toxicity during P-deprivation reported by others (Boyer et al., 1987; Anderson et al., 1990) for other *Alexandrium* spp. This may indicate species-specific variation associated with differences in toxin composition, or some factor associated with nutrient history or experiment design.

The enhancement of toxin content by the relief of N-deprivation, and the detrimental effects of N- and P-deprivation have implications for the toxin content of field populations. Natural *A.minutum* populations may encounter conditions which enhance their N-status, such as high ammonium levels. Ammonium uptake is very rapid compared with nitrate and may be cause a more rapid response in toxin synthesis. Growth of *A. minutum* is associated with run-off and the formation of haloclines in the Galician Rías (Blanco et al., 1985) and in the Mediterranean (Delgado et al., 1990). Concentrations of ammonium may exceed 10 $\mu$M under such conditions (especially near mussel rafts). Such conditions may be expected to be favourable for toxin synthesis (Fig. 3A). Nutrient enrichment prior to stratification seems a common factor in the development of dinoflagellate blooms. At the beginning of such growths, nutrient levels are presumably relatively high and toxin synthesis may be high also. But, large accumulations of algae may remove nutrients rapidly and, depending on the N:P ratio, lead to varying degrees of N and/or P-stress and an associated decrease in toxin synthesis (Fig. 3B). Subsequent improvements of nutrient status may be expected to affect amino acid synthesis and hence toxin synthesis (Fig. 3A). Of unknown importance for toxin synthesis is the change in C-N status associated with vertical migration. Preliminary studies on other dinoflagellates

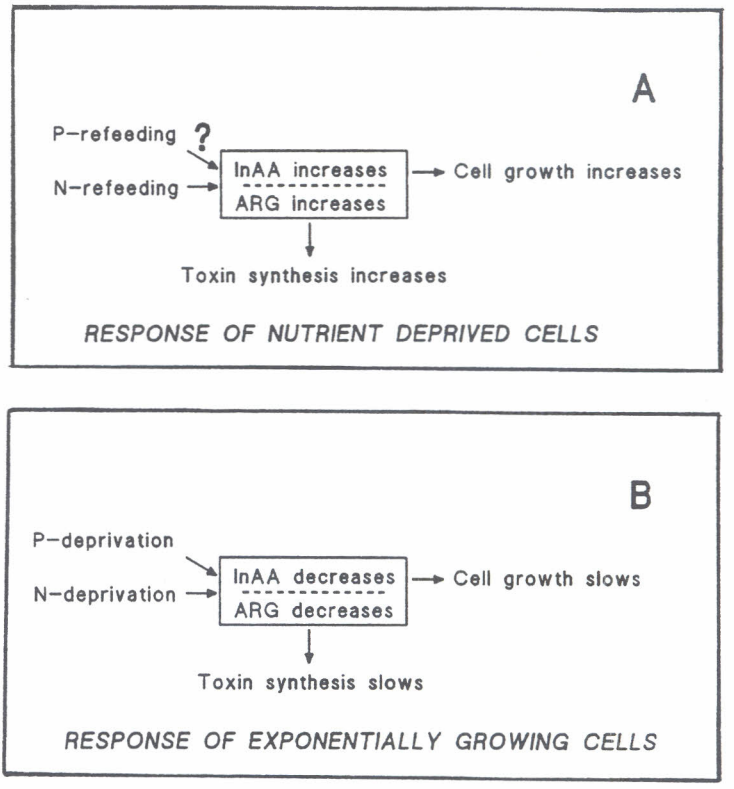


Figure 3. Suggested interactions between nutrient status, the content of intracellular free amino acids (InAA), arginine (ARG), and toxin during, (A) the encounter by nutrient-deprived cells of nutrients, and (B) during nutrient-deprivation of exponentially growing cells.

(Flynn et al.,1993) suggest significant shifts in the composition of intracellular amino acids nitrogen assimilation in the dark phase of growth which would stimulate uptake at a nutricline (Paasche et al., 1984). This, in part, probably reflects the need for concurrent photosynthesis if N is to be completely assimilated. If this is so for toxic species, then one may then expect associated changes in rates of toxin synthesis. Any or all of these events may be expected to have profound effects on the level of toxicity in individual cells by affecting the amino acid pool. The events leading to the growth of toxic *Alexandrium*, together with any subsequent encounter with elevated nutrients, may therefore be expected to play important rôles in determining the toxicity of individual cells and of the whole population.

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