Vol. 225: 169–176, 2002 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published January 11

Ecological advantages of toxin production by the dinoflagellate *Alexandrium minutum* under phosphorus limitation

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ABSTRACT: Unialgal and mixed cultures of the toxic dinoflagellate *Alexandrium minutum* and the non-toxic dinoflagellate *Prorocentrum micans* were cultured under phosphate (P) limitation (<1 µM), in the presence or absence of the copepod *Acartia clausi*. The aim was to determine the possible effects of interspecific competition, predation and nutrient limitation on the production of Paralytic Shellfish Poisoning (PSP) toxin by *A. minutum*. The growth rate of *A. minutum* was higher in the cultures of *A. minutum* and copepods than in the mixed cultures of *A. minutum* and *P. micans*, which indicates that *A. minutum* was more negatively affected by interspecific competition than by predation. Toxin content per cell in *A. minutum* increased in all cultures, but toxin production rate was higher in cultures with *P. micans* and/or *A. clausi*. Toxin concentration in *A. minutum* increased as PO_4^{3-} concentration diminished. In the mixed cultures with copepods, analyses of toxin content in the copepods showed that at cell toxin contents lower than approximately 20 fmol cell^{-1}, mainly cells of *A. minutum* were ingested by copepods, whereas at higher toxins content per cell, copepods fed mainly on *P. micans*. We conclude that one of the possible advantages of toxin production by *A. minutum* under P limitation is to enhance interspecific competition, by redirecting grazing pressure to non-toxic phytoplankton species. It would allow a possibly low competitive ability of *A. minutum* to be offset under low nutrient concentrations.

KEY WORDS: Dinoflagellates · Phosphorus · Nutrients · Toxins · Copepods · Predation · Interspecific competition

INTRODUCTION

It is not yet clear why some marine dinoflagellates produce toxins. Although toxins are supposed to act as feeding deterrents, many studies have shown that toxic algae are ingested by grazers (Huntley et al. 1986, Ives 1987, Carlsson et al. 1995, Turriff et al. 1995, Teegarden & Cembella 1996, Dutz 1998, Maneiro et al. 2000, Frangópulos et al. 2000). Other explanations for toxicity other than grazing deterrence include precursors for subcellular organelles, cell-wall degradation products, nucleic acid synthesis, nitrogen storage, and inhibition of competing co-occurring phytoplankton species (see Turner & Tester 1997). One of the most recent explanations is that some toxins may serve as pheromones (Wyatt & Jenkinson 1997).

Toxin synthesis in marine phytoplankton species is not a constitutive component of algal metabolism, but both toxin content and toxin composition of algae are influenced by environmental growth conditions (Plumley 1997). Cell toxin content of dinoflagellates has been shown to be associated with changes in temperature (Ogata et al. 1987, Anderson et al. 1990), salinity (Parkhill & Cembella 1999), light (Ogata et al. 1987, Parkhill & Cembella 1999) and nitrogen (N) and phosphorous (P) availability (Boyer et al. 1987, Anderson et

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al. 1990). Although all these studies have shown that cellular toxicity of dinoflagellates can be enhanced under a wide range of different environmental conditions, as toxin production is closely coupled to growth rate and there is also a linkage between growth rate and the abiotic factors, the direct link between these abiotic factors and toxin production can not be clearly separated from the effect of growth on toxin production (Anderson et al. 1990).

The exception to this linkage between toxin production and cell division is the enhanced toxin production observed in some toxic dinoflagellates under nutrient limitation (Anderson et al. 1990). A connection between nutrient limitation and enhanced cell toxicity has been observed in dinoflagellates that produce Paralytic Shellfish Poisoning (PSP) toxins (Boyer et al. 1987, Anderson et al. 1990, Béchemin et al. 1999, John & Flynn 2000), Diarrhetic Shellfish Poisoning (DSP) toxins (Johansson et al. 1996), and in other toxic marine phytoplankton species such as haptophytes (Johansson & Granéli 1999a,b).

As dinoflagellates seem to produce more toxins under nutrient limitation, toxin production could be an adaptation evolved to offset the negative effects of interspecific competition. Therefore, in addition to abiotic factors, cellular toxicity could also be affected by biotic factors. However, the combined effect of nutrient limitation, interspecific competition and predation on dinoflagellate toxin production has not been reported.

The toxic dinoflagellate *Alexandrium minutum*, the non-toxic dinoflagellate *Prorocentrum micans* and the copepod *Acartia clausi* often co-occur in Ría de Vigo, Spain. To test the possible effects of interspecific competition, predation and nutrient limitation on toxin production of *A. minutum* several experiments were carried out with unialgal and mixed cultures of the toxic and non-toxic dinoflagellates cultured under nutrient limiting conditions, in the presence or absence of the copepod *A. clausi*.

MATERIALS AND METHODS

Zooplankton collection. Zooplankton were collected by vertically integrated tows from a depth of 20 m to the surface, at a field station 39 m deep located in Ría de Vigo, Spain (42° 13.3′ N, 8° 47.7′ W). Samples were transported within 2 h of collection to the laboratory, and adults females of *Acartia clausi* were sorted out for the experiments.

Experimental design. The non-axenic strain of *Alexandrium minutum* (AL 1V) and the strain of *Prorocentrum micans* used in this study were isolated from the Galician rias and came from long-established populations cultured in the Instituto Español de Oceanografía, Vigo, Spain. This toxic strain of *A. minutum* only contains gonyautoxins 1, 2, 3 and 4 (GTX1-4) (Franco et al. 1994). Filtering efficiency is a function of the diameter of the algal species; for *Acartia clausi* feeding on *A. minutum* and *P. micans* it is 100% (Donaghay & Small 1979).

Cultures of 75 ml of diluted K medium in 100 ml beakers with *Alexandrium minutum* (ALEX, 12 replicates), *A. minutum* and 1 copepod (ALEX-1C, 20 replicates), *A. minutum* and *Prorocentrum micans* (ALEX-PRO, 12 replicates), *A. minutum*, *P. micans* and 1 copepod (ALEX-PRO-1C, 100 replicates), and *A. minutum*, *P. micans* and 3 copepods (ALEX-PRO-3C, 20 replicates) were grown at 15°C on a 12:12 h light:dark cycle and with an illumination of 50 µmol photon m^{-2} s^{-1} provided by 4 cool-white fluorescent tubes. Replicates (n = 80) of the ALEX-PRO-1C experimental culture were used to for the estimation of toxin content in the copepods. The culture medium was prepared with aged natural sea water (salinity of 33.1‰) filtered through GF/F Whatman filters and autoclaved. As PSP toxins are nitrogenous compounds (approx. 33% of PSP toxin weight is NH_4^+) and, hence, N is necessary for toxin biosynthesis (Flynn et al. 1994), the initial concentrations of NH_4^+ and NO_3^- were high enough to avoid N limitation during the experiment. Mean ± SE of the initial nutrient concentrations (in µM) were 1.19 ± 0.02 for PO_4^{3-}, 198.71 ± 0.15 for NO_3^- and 5.53 ± 0.11 for NH_4^+.

Every 3 d cell abundance, toxins content per cell and the concentration of NH_4^+, NO_3^- and PO_4^{3-} were analyzed for each replicate. Copepod toxin content was also analyzed every 3 d but the first value was analyzed on the sixth day of the experiment to allow copepods to acclimate. Samples of 5 and 2 ml of each experimental replicate were removed for toxin analyses and cell abundance estimation, respectively. Cell densities were determined by counting cell number present in 1 ml in a Sedgewick-Rafter chamber. Algal cells removed from 3 replicates of each experimental culture were pooled and filtered on pre-combusted 13 mm GF/C Whatman filters for toxin analyses. Therefore, the volume filtered on each filter for toxin analyses was 15 ml. The filtered water was used to estimate nutrient concentrations. The concentrations of NH_4^+, NO_3^- and PO_4^{3-} in the media were analyzed with a Technicon AAII auto-analyzer. The filters with the material for toxin analyses were stored at –80°C in ultracentrifuge plastic tubes and lyophilized.

Toxin analyses. To estimate cell toxin content of *Alexandrium minutum*, 400 µl of 0.05 M acetic acid was added to the lyophilized material and the sample was homogenized using a pipette tip adapted to fit the shape of the vial. The sample was shaken followed by freezing twice. Finally, the extract was centrifuged twice at 400 × *g* for 10 min, after which 200 µl of the supernatant was carefully collected with a Hamilton syringe, and stored at –20°C.

From the replicates of the ALEX-PRO-1C experimental culture, between 15 and 17 copepods were transferred to distilled water and without delay, all the copepods were collected with a known volume of distilled water (no higher than 40 µl). Copepods were stored at −80°C in ultracentrifuge plastic tubes and lyophilized. Acetic acid (125 µl, 0.05 M) was added to the lyophilized material followed by the same steps described above.

Analysis of the gonyautoxins by high-performance liquid chromatography with fluorescence detection (HPLC-FD) was performed following a modification of the method of Oshima et al. (1989) described by Franco & Fernández (1993). Chromatographic profiles of *Alexandrium minutum* cells were determined by duplicate injections of 90 µl of extract (diluted with 0.05 M acetic acid, as necessary). Chromatographic profiles of copepods were determined by a single injection of 90 µl of the extracts. Toxins from the Certified Reference Material Program of the National Research Council of Canada (Halifax) were used as standards.

Toxicity of *Alexandrium minutum*, in saxitoxin equivalents (STXeq), was calculated from the HPLC chromatograms. The toxin concentrations were multiplied by a toxin-specific conversion factor to yield toxicity. The specific toxicity conversion factors of the individual toxins were adopted from Oshima (1995) based upon empirical mouse bioassay data determined using purified standards, and assuming the conversion factor of 1 mouse unit (MU) = 0.23 µg STXeq for the ddy mouse strain: 567.6 (GTX1), 205.2 (GTX2), 364.3 (GTX3) and 414.7 (GTX4).

Calculation of specific growth rate and net toxin production rate. A specific growth rate μ (d^{-1}) between successive sampling days for each kind of experimental culture was calculated using the equation:

$$\mu = \frac{\ln(N_1 / N_0)}{t_1 - t_0}$$

where N_1 and N_0 are cell concentrations at time t_1 and $t_{0,}$ respectively. In those experimental concentrations with copepods no correction has been made of losses of cells due to predation. The difference between the specific growth rate in ALEX-PRO and ALEX-PRO-1C for both *Alexandrium minutum* and *Prorocentrum micans* were used an indicator of grazing pressure on both algal species.

Toxin content (fmol $cell^{-1}$) was multiplied by N_t to yield T_t, the total toxin concentration (fmol toxin ml^{-1} culture) at time t. Net toxin production rate R_{tox} (fmol toxin $cell^{-1}$ d^{-1}) was calculated as mentioned by Anderson et al. (1990) using the following equation:

$$R_{tox} = \mu \frac{(T_1 - T_0)}{(N_1 - N_0)}$$

RESULTS

Table 1 shows final concentrations of NH_4^+, NO_3^- and PO_4^{3-} in each kind of experimental culture. The cultures were not N limited during the experiment, whereas P limitation occured. To compare these nutrient concentrations with those usually found in the field in this region, data from weekly samples collected from Ría de Vigo in 1997 at station 42° 14.6′ N, 8° 48.5′ W, using a hose divided from 0 to 5 m, 5 to 10 m and 10 to 15 m depth, are shown (Centro de Control do Medio Mariño, Xunta de Galicia). The annual mean ± SD and range of the nutrient concentrations (µM) were 0.91 ± 0.59 from 0.01 to 2.99 (n = 156) for NH_4^+, 4.01 ± 4.19 from 0.01 to 14.1 (n = 159) for NO_3^-, and 0.37 ± 0.19 from 0.04 to 0.79 (n = 157) for PO_4^{3-}.

Fig. 1 shows cell density of *Alexandrium minutum* and *Prorocentrum micans* in the experimental cultures. Daily copepod mortality was approximately 3%. Each replicate was not taken into account if any of the copepods died, and for this reason on Day 12 of the experiment there were no replicates left from culture ALEX-PRO-3C. The highest cell abundance of *A. minutum* was obtained in culture ALEX, whereas the low-

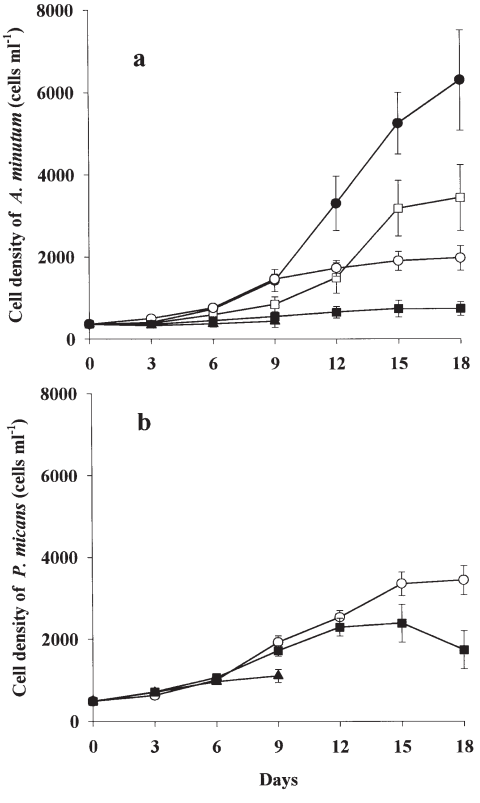

Fig. 1. Cell density (mean ± SE, cells ml^{-1}) of (a) *Alexandrium minutum* and (b) *Prorocentrum micans* in the experimental cultures. (●) ALEX, (□) ALEX-1C, (○) ALEX-PRO, (■) ALEX-PRO-1C and (▲) ALEX-PRO-3C

Table 1. Inorganic nutrient concentrations (µM, mean ± SE) at the end of the experiment (Day 18) for each kind of experimental culture

	ALEX	ALEX-1C	ALEX-PRO	ALEX-PRO-1C
NH_4^+	2.97 ± 0.06	2.88 ± 0.18	2.83 ± 0.10	2.77 ± 0.11
NO_3^-	88.05 ± 3.45	112.86 ± 10.81	83.03 ± 5.40	103.60 ± 1.00
PO_4^{3-}	0.19 ± 0.02	0.15 ± 0.02	0.13 ± 0.01	0.10 ± 0.01

est values were observed in culture ALEX-PRO-1C. This suggests that *A. minutum* was negatively affected by both interspecific competition and predation. In the mixed culture ALEX-PRO the growth of *P. micans* was higher than *A. minutum*. The growth of *A. minutum* was higher in ALEX-1C than in ALEX-PRO, which indicates that *A. minutum* was more negatively affected by interspecific competition than by predation. Finally, the abundances of *P. micans* in ALEX-PRO and in ALEX-PRO-1C were similar until Day 12 of the experiment, when the abundance of *P. micans* was lower in culture ALEX-PRO-1C. Therefore, at the beginning of the experiment copepods fed mainly on *A. minutum*, whereas at the end of the experiment cells of *P. micans* were also ingested by copepods.

Fig. 2 shows toxic concentration per cell of *Alexandrium minutum* in the experimental cultures. An analysis of covariance (ANCOVA) taking time into account as covariable, showed that toxic content per cell was lower in ALEX than in ALEX-PRO ($F_{1,52}$ = 6.8, p = 0.012). This suggests that toxin production in *A. minutum* was affected by interspecific competition. Fig. 3 shows PO_4^{3-} decrease in all experimental cultures. Although there were no significant differences between ALEX and ALEX-PRO ($F_{1,36}$ = 41.1, p = 0.16), PO_4^{3-} decreased earlier in ALEX-PRO. Moreover, PO_4^{3-} decrease in ALEX-PRO-1C was significantly higher than in ALEX-1C ($F_{1,38}$ = 41.1, p < 0.001). Therefore, it seems that in those mixed cultures with both dinoflagellate species, *A. minutum* became P-stressed earlier than *Prorocentrum micans*.

Toxin production was also affected by predation because toxic content per cell was significantly higher in those experimental cultures with copepods than without copepods: ALEX and ALEX-1C ($F_{1,52}$ = 6.0, p = 0.018), ALEX-PRO and ALEX-PRO-1C ($F_{1,52}$ = 7.5, p = 0.008). There were no significance differences in the concentration of NH_4^+, time taken as the covariable, between ALEX and ALEX-1C (ANCOVA, $F_{1,24}$ = 0.07,

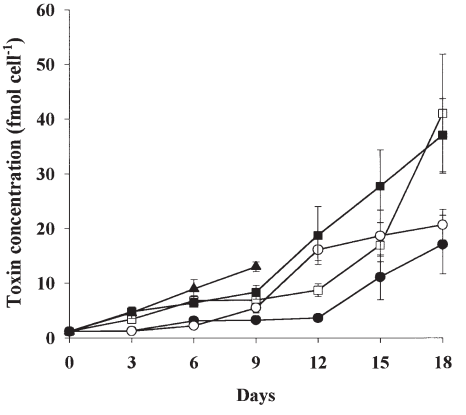

Fig. 2. *Alexandrium minutum.* Toxin concentration per cell (mean ± SE, fmol cell^{-1}) in the experimental cultures. Symbols as in Fig. 1

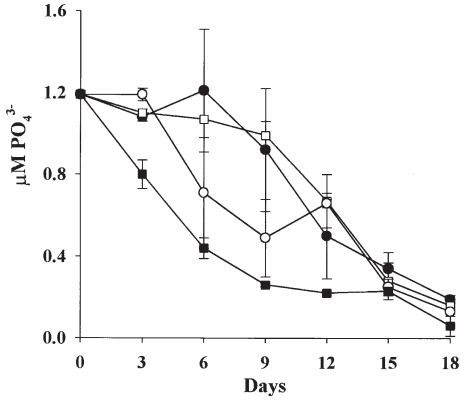

Fig. 3. Phosphate concentration (mean ± SE, µM) in the experimental cultures. Symbols as in Fig. 1

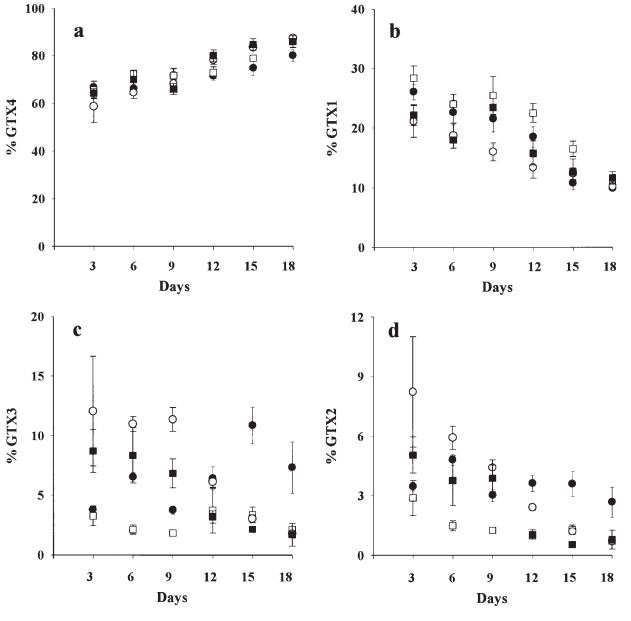

Fig. 4. *Alexandrium minutum.* Specific toxic composition as percentage of gonyautoxins (GTX1-4) in the experimental cultures. Symbols as in Fig. 1

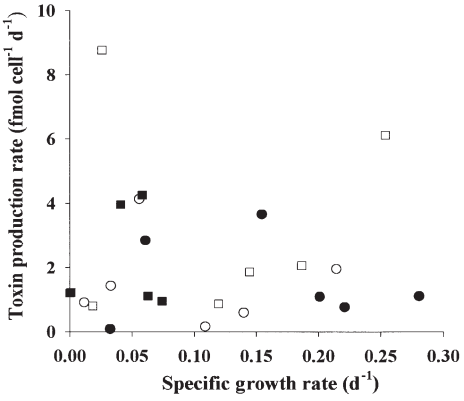

Fig. 5. *Alexandrium minutum.* Relationship between toxin production rate (fmol cell^{-1} d^{-1}) and specific growth rate (d^{-1}) in the experimental cultures. All SEs were <0.45 for toxin production rate and <0.3 for specific growth rate. Symbols as in Fig. 1

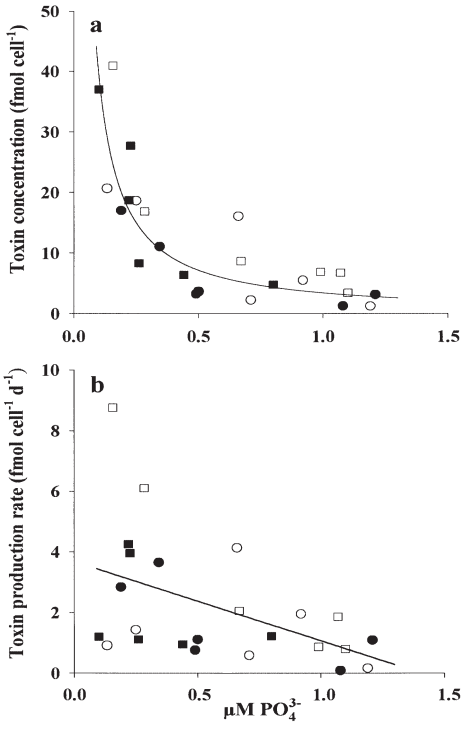

Fig. 6. *Alexandrium minutum.* Relationships between (a) mean toxin content per cell (fmol cell^{-1}) and (b) toxin production rate (fmol cell^{-1} d^{-1}) with mean PO_4^{3-} concentration (µM). All SEs for toxin content per cell were <10.9 and <0.08 for PO_4^{3-} concentration. Symbols as in Fig. 1

p = 0.789) and between ALEX-PRO and ALEX-PRO-1C ($F_{1,24}$ = 0.06, p = 0.81). Therefore, the increase of toxin content per cell in those experimental cultures with copepods was not due to the ammonia excreted by the copepods.

Fig. 4 shows that specific toxic composition of *Alexandrium minutum* varied over the type of experiment but the change was similar in all experimental cultures. The percentage of GTX4 increased whereas the percentages of GTX1, GTX2 and GTX3 diminished. However, the combined contribution of the isomers GTX1-GTX4 (~97%) and GTX2-GTX3 (~3%) remains relatively constant over time in all the experimental concentrations.

Fig. 5 shows that the enhanced toxin production observed in all cultures was not associated with growth of *Alexandrium minutum* (slope different from zero, $F_{1,22}$ = 0.005, p = 0.945). Toxin concentration in *A. minutum* was significantly related to PO_4^{3-} concentration. There were significant relationships between PO_4^{3-} concentration (P) and both toxin content per cell (T) (lnT = 1.232 – 1.062 lnP, r^2 = 0.68, $F_{1,22}$ = 48.2, p < 0.001, Fig. 6a) and R_{tox} (R_{tox} = 3.689–2.625 P, r^2 = 0.23, $F_{1,22}$ = 6.5, p = 0.018, Fig. 6b).

Copepod toxic content shown in Table 2 confirms that copepods fed on *Alexandrium minutum.* However, ingestion of *A. minutum* cells decreased as toxin content per cell increased. According to cell toxin content of *A. minutum* and toxins found in copepods, cells of *A. minutum* ingested by copepods diminished after Day 9, coinciding with a significant increase in toxin content per cell in *A. minutum* (Fig. 2). Fig. 7 shows the difference between the specific growth rate in ALEX-PRO and ALEX-PRO-1C for both *A. minutum* and *Pro-*

Table 2. Specific toxin composition for gonyautoxins (GTX1-4), total toxin per copepod (as the combined GTX1, GTX2, GTX3 and GTX4) and total toxicity potency of *Acartia clausi* on different experiment days. The equivalent of *Alexandrium minutum* cells according to cell toxic content at that time and total toxin per copepod is also shown. On Day 18 of the experiment there were not enough copepods for toxin analysis

Day	GTX4	GTX1	GTX3	GTX2	Total toxin per copepod	Toxicity per copepod	Equivalent in cells
		(pmol copepod^{-1})			(pmol copepod^{-1})	(pg STXeq copepod^{-1})	of *A. minutum*
6	1003.9	585.1	23.1	28.1	1640.2	0.76	257
9	2264.1	1080.8	42.4	75.7	3463.1	1.58	416
12	665.2	314.7	12.2	12.8	1005.0	0.46	54
15	1105.9	529.5	18.8	30.9	1685.2	0.77	61

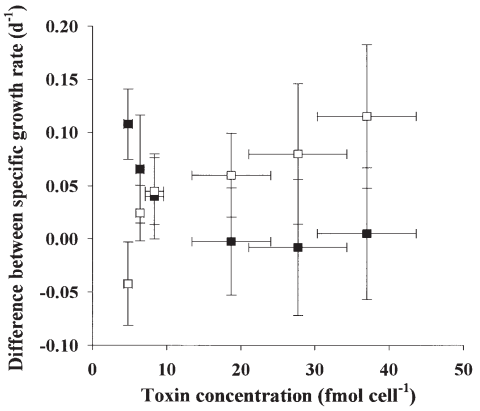

Fig. 7. Relationships between the difference of the specific growth rates of (■) *Alexandrium minutum* and (□) *Prorocentrum micans* in ALEX-PRO and ALEX-PRO-1C (d^{-1}, mean ± SE) and toxin concentration of *Alexandrium minutum* (fmol $cell^{-1}$, mean ± SE)

rocentrum micans. The specific growth rate in ALEX-PRO is higher than in ALEX-PRO-1C for each dinoflagellate species if ingested by the copepods. Therefore, grazing pressure of *Acartia clausi* on *P. micans* increased ($F_{5,54}$ = 4.3, p = 0.002) whereas on *A. minutum* de-creased ($F_{5,64}$ = 5.02, p < 0.001) as cellular toxicity of *A. minutum* increased.

DISCUSSION

The results of this study suggest that one of the possible ecological advantages of *Alexandrium minutum* is producing toxins to increase grazing pressure on alternative non-toxic competitors under phosphorus limitation. Under N or P limitation, some toxic dinoflagellates are probably outcompeted. To increase toxin production would provide the dinoflagellates with an opportunity to compete with otherwise superior algal species under the prevailing nutrient limiting conditions. This possible strategy of producing toxins as a mechanism to enhance interspecific competition under nutrient limitation implies two premises: (1) grazers must be capable of discriminating against toxic cells; (2) toxic dinoflagellates may be poor competitors in low nutrient concentrations, which would justify the necessity to have an alternative adaptation to offset the ecological disadvantages of low competitive ability in low nutrient concentrations.

The success of the toxin production strategy of dinoflagellates under nutrient limiting conditions should depend on the presence of grazers and on the capacity of grazers to recognize and to reject toxic cells. It has been shown that copepods can discriminate against toxic dinoflagellates, either by trial and error consumption of harmful phytoplankton, allowing copepods to learn which species should be avoided (Uye & Takamatsu 1990), or by behavioral rejection prior to ingestion (Teegarden 1999). Shaw et al. (1997) showed that PSP toxins and okadaic acid acted as feeding deterrents for the copepod *Tigriopus californicus*. Our results support the hypothesis that dinoflagellate toxins can act as feeding deterrents, because as cellular toxicity of *Alexandrium minutum* increased, *Acartia clausi* ingested a lower amount of cells of *A. minutum*, but increased grazing pressure on *Prorocentrum micans*. The differences in cell toxin content between cultures with and without copepods, could simply be due to copepods feeding selectively on cells of *A. minutum* with a lower toxicity. However, it is necessary to point out that many studies have shown that grazers ingest toxic algae (see Turner et al. 1998). It could be due to low cellular toxicity and/or the incapacity of grazers to detect toxins. As suggested by Teegarden (1999), the presence of toxins could confer the definite advantage of reducing that dinoflagellate's palatability or desirability compared to a non-toxic phytoplankton species, but this does not cause them to avoid or to be selected against by all grazer species. In cases where toxic dinoflagellates are ingested by grazers, this negative effect can be compensated for by reduced reproductive success of grazers due to ingested toxins (Frangópulos et al. 2000).

As mentioned, the second premise is that dinoflagellates should be poor competitors under nutrient limitation. Nutrient uptake affinity (K_s) is considered an index of species competitive ability at low nutrient concentrations. Smayda (1997) showed that K_s values for NH_4^+, NO_3^- and PO_4^{3-} uptake by dinoflagellates were substantially higher than diatom coefficients. As species with low K_s constants are supposed to be favored under low nutrient conditions, diatoms should be expected to outcompete dinoflagellates under low nutrient supply rates. Yamamoto & Tarutani (1996) carried out a study with 2 potential competitors that often co-occur in Mikawa Bay (Japan), the toxic dinoflagellate *Alexandrium tamarense* and the non-toxic diatom *Skeletonema costatum*. They showed that the specific phosphate uptake rate (uptake rate divided by cellular phosphorous content) was much lower in *A. tamarense* than in *S. costatum* and furthermore, specific growth rate of *S. costatum* was higher than of *A. tamarense* over a range of PO_4^{3-} between 1 and 12 µM. It indicated that *A. tamarense* was clearly in a more disadvantaged position to proliferate than *S. costatum* in an environment of any phosphate concentration. Other studies have also shown that toxic dinoflagellates are poor competitors compared to Prymnesiophyceae (Riegman et al. 1996) and even compared to non-toxic dinoflagellates (Cannon 1996). As in mixed cultures without

copepods where the growth rate of *Prorocentrum micans* was higher than *Alexandrium minutum*, it might be interpreted as a lower nutrient uptake affinity of toxic dinoflagellates than non-toxic dinoflagellates. However, to prove this hypothesis it would be necessary to generate normalised growth rate curves versus nutrient concentrations for both algal species.

All the studies mentioned above clearly showed that dinoflagellates are poor competitors under nutrient limitation. However, blooms of toxic dinoflagellate species are frequent in nutrient-depleted waters. Smayda (1997) suggested that toxin production could be an adaptation evolved to offset the ecological disadvantages of dinoflagellates with low nutrient affinity. The enhanced toxin production observed in *Alexandrium minutum*, and in other toxic marine phytoplankton species as nutrient concentration diminished, seems to corroborate his hypothesis. Since PSP toxins are nitrogenous compounds, in PSP producing dinoflagellates, a reduced amount of toxin per cell is observed under N limitation (Boyer et al. 1987, Anderson et al. 1990), but toxin production is higher under P limitation (Boyer et al. 1987, Anderson et al. 1990, also this study). Moreover, it is important to point out that, in agreement with our results, Anderson et al. (1990) showed that this enhanced toxin production in *Alexandrium* spp. under P limitation was not an indirect consequence of nutrient influence on growth rate. This lack of relationship between specific growth rate and toxin production rate could be due to toxins being synthesized from low molecular weight metabolites, while cell growth is a much more complex process. Our experimental design has the problem that algae were grown at high NO_3^- concentrations (Table 1) as compared to the field nutrient concentrations. John & Flynn (2000) mentioned that in high-N systems factors causing a cell-density-dependent cessation of growth may adversely affect cell physiology and hence, they suggested that experiments using more than 100 µM of N may give rise to unrepresentative physiological responses. However, Anderson et al. (1990) observed in batch-culture experiments similar toxin content per cell in *Alexandrium* spp. cultured with NO_3^- initial concentrations of 880 and 20 µM. As the lowest NO_3^- concentration used by Anderson et al. (1990) is closer to field concentrations, high toxin production in PSP producing dinoflagellates could also be observed with the nutrient concentrations found in the field. In those marine toxic phytoplankton species which produce toxins with a low elemental N and P composition, cellular toxicity increases both under N and P limitation (Johansson et al. 1996, Granéli et al. 1998, Johansson & Granéli 1999a,b). This link between toxin production and nutrient availability could explain why cultured *Alexandrium* cells are typically less toxic than those collected from natural populations in the same region (Cembella 1998). When the strain of *Alexandrium minutum* used in this study was isolated from the field, toxin content per cell was ~18 fmol (Franco et al. 1994). However, as the algae have been cultured for a long time under optimal conditions, toxin content per cell of this long-established population is now only about 1.1 fmol.

If the ecological significance of toxin production was only to deter grazers, toxin content per cell would be high under any nutrient concentration. The linkage between toxin production and nutrient concentration indicates that toxin production is also related to interspecific competition. Although some toxin-producing algal species excrete their toxins into the surrounding water (see Johansson & Granéli 1999a), gonyautoxins are intracellular compounds (Anderson & Cheng 1988, Doucette & Anderson 1993) and hence, toxins produced by *Alexandrium minutum* could not inhibit the growth of potential competitors by the excretion of toxins into the water. Therefore, we concluded that in addition to feeding avoidance, another ecological advantage of toxin production by *Alexandrium minutum* under P limitation is to offset interspecific competition by redirecting grazing pressure onto non-toxic phytoplankton species that are potential competitors.

Acknowledgements. We thank Andrew G. Bauder and José Franco for useful comments on the manuscript. This research was supported by Project MAR96-1822, a grant from the Chilean Government to M.F., an FPU grant to I.M., a grant from FUNDACIÓN PROVIGO to I.R. and a grant from AECI to A.R.V. We thank Charo Carballo for useful help with nutrient analyses. Nutrient concentrations were kindly provided by Centro de Control do Medio Mariño (Xunta de Galicia).

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Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: November 6, 2000; Accepted: April 24, 2001
Proofs received from author(s): December 13, 2001