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Effects of the toxic dinoflagellate *Karlodinium* sp. (cultured at different N/P ratios) on micro and mesozooplankton*

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SUMMARY: An experimental study was carried out to investigate whether two potential predators such as *Oxyrrhis marina* (phagotrophic dinoflagellate) and *Acartia margalefi* (copepoda: calanoida) had different responses when feeding on toxic (*Karlodinium* sp.-strain CSIC1-) or non-toxic (*Gymnodinium* sp1) dinoflagellates with a similar shape and size. Both prey were cultured at different N/P ratios (balanced N/P = 15, and P-limited N/P > 15) to test whether P-limitation conditions could lead to depressed grazing rates or have other effects on the predators. Both predators ingested the non-toxic *Gymnodinium* sp1, and low or non-ingestion rates were observed when incubated with *Karlodinium* sp. The dinoflagellate *O. marina* did not graze at all on *Karlodinium* sp. at N/P > 15 and very little at NP = 15, as its net growth rates were always negative when feeding on *Karlodinium* sp. cultured under P-limitation conditions. *A. margalefi* had lower ingestion rates when feeding on *Karlodinium* sp. grown at N/P = 15 than when feeding *on Gymnodinium* sp1, and did not graze on P-limited *Karlodinium* sp. Nevertheless, feeding on *Karlodinium* sp. grown under N/P =15 or N/P > 15 did not have any paralyzing or lethal effect on *A. margalefi* after 24 h. Finally, a direct effect on the viability of *A. margalefi* eggs was detected when healthy eggs were incubated for 5 days in the presence of *Karlodinium* sp. grown under N/P =15 or N/P > 15, producing a decrease in viability of 20% and 60% respectively.

Keywords: micro- and mesozooplankton (*Oxyrrhis marina* and *Acartia margalefi*), toxic and non-toxic dinoflagellate (*Karlodinium* sp., *Gymnodinium* sp1) grazing rates, P-limitation, predator mortality, *Karlodinium* sp., egg viability.

RESUMEN: EFECTOS DE UN DINOFLAGELADO TÓXICO SOBRE SUS DEPREDADORES. – Se estudió experimentalmente la respuesta de dos depredadores tales como *Oxyrrhis marina* (dinoflagelado fagotrófico) y *Acartia margalefi* (copepoda: calanoida) sobre dos dinoflagelados de tamaño y morfología similares, uno tóxico (*Karlodinium* sp. -cepa CSIC1-) y otro no tóxico (*Gymnodinium* sp1). Ambas presas fueron cultivadas a diferentes cocientes N/P para comprobar si la limitación por fósforo potenciaba el carácter disuasorio de *Karlodinium* sp., lo que induciría a menores tasas de ingestión y a la mortalidad de los depredadores. Ambos ingirieron el dinoflagelado no tóxico *Gymnodinium* sp1 y la depredación era baja o nula cuando fueron incubados con *Karlodinium* sp. *O. marina* no ingirió *Karlodinium* sp. en condiciones de limitación de P (N/P > 15) y las tasas de ingestión fueron muy bajas o nulas en condiciones de N/P =15. Las tasas de crecimiento siempre fueron negativas cuando *Karlodinium* sp. fue cultivado a N/P > 15. *A. margalefi* presentó tasas de ingestión mas bajas sobre *Karlodinium* sp. que sobre *Gymnodinium* sp1 y la depredación sobre *Karlodinium* sp. cultivado en condiciones de N/P > 15 fue despreciable. La ingestión sobre *Karlodinium* sp. cultivado a cocientes N/P = 15 ó N/P > 15 no tuvo ningún efecto paralizante o letal sobre *A. Margalefi* después de 24 h de incubación. Sin embargo, huevos de *A. margalefi* incubados con *Karlodinium* sp. a cocientes N/P = 15 ó N/P > 15 sufrieron una disminución del 20% o del 60%, respectivamente, de su viabilidad.

Palabras clave: micro y mesozooplancton (*Oxyrrhis marina* y *Acartia margalefi*), dinoflagelados tóxicos y no tóxicos (*Karlodinium* sp., y *Gymnodinium* sp1), tasas de depredación, limitación por fósforo, mortalidad del depredador, viabilidad de huevos.

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INTRODUCTION

The importance of micro- and mesozooplankton in controlling and terminating harmful algal blooms (HABs, i.e. formed by toxic species, mostly dinoflagellates containing PSP, DSP or fish-killing toxins) is highly site and species specific (see review by Turner and Tester 1997). For mesozooplankton (200 μm to 20 mm, mostly copepods) with relatively slow response times (weeks), the impact on HABs seems to be mostly constrained to the initial phases of the blooms, when the population of dinoflagellates is still developing (Watras *et al.*, 1985; Uye 1986; Calbet *et al.,* 2003). At this state, any toxic (i.e. deterrence of predator activity) or allelopathic effect on other algae (Fistarol *et al.*, 2004; Suikkanen *et al.*, 2004), could be crucial in deciding the fate of the emerging bloom. However, microzooplankton, with response times in the order of days, can react faster than mesozooplankton and impact the populations of HABs not only at the beginning of the bloom, but also when the bloom is fully developed (Turner and Anderson, 1983, Calbet *et al.*, 2003). Both types of predators, however, can be affected (damaged, killed) by toxic or otherwise undesirable cells reacting against them by selecting non-toxic species and hence favouring the proliferation of HABs even more (Teegarden, 1999, Vaqué *et al.*, 2003). Furthermore, deleterious effects on predators seem to be enhanced when toxic dinoflagellates are growing in nutrient-limited conditions that increase dinoflagellate toxin content (Boyer *et al.,* 1987, Guisande *et al.*, 2002).

In the NW Mediterranean the population dynamics of HAB species since 1994 has revealed the recurrence of *Karlodinium* sp. (CSIC1) blooms (>10^4 cells ml^{-1} (Delgado and Alcaraz, 1999), formerly identified as *Gyrodinium corsicum*) over 7 years, coinciding with periods of water stability during winter (Garcés *et al.,* 1999). These blooms have been associated with large fish and zooplankton kills in the area (Delgado *et al.*, 1995; Garcés *et al.*, 1999; Vila *et al.,* 2001, Vila and Masó 2005). Furthermore, Delgado and Alcaraz (1999) showed that *Acartia grani* incubated with a high concentration (>3000 cells ml^{-1}) of the wild toxic dinoflagellate were killed in a few hours. In addition, since the Mediterranean waters are often P-limited, the toxicity of *Karlodinium* sp. (CSIC1) is probably enhanced by its potential predators.

Within the multidisciplinary BIOHAB project (Biological control of Harmful algal Blooms), we tested the potential impact of predators on the cultured *Karlodinium* sp. (CSIC1) at low cell concentration, growing at balanced (Redfield) nutrient and under P-limited conditions. We studied whether two selected potential predators are capable of ingesting *Karlodinium* sp., which would contribute to maintaining the toxic community at a very low or null concentration. For these purposes, we carried out an experimental study to test whether: (i) predators like *Oxyrrhis marina* (phagotrophic dinoflagellate) and *Acartia margalefi* (copepod), common in the study area, showed different responses when feeding on the toxic dinoflagellate *Karlodinium* sp. (CSIC1), or on the non-toxic dinoflagellate *Gymnodinium* sp1 of a similar size, and (ii) any negative effects on predators were enhanced when the dinoflagellate prey was cultured under P-limitation conditions., We estimated the ingestion rates for both grazers, and also determined the growth rates for *Oxhyrris marina*. Finally, for *A. margalefi* we tested egg viability by incubating healthy eggs with *Gymnodinium* sp1 or *Karlodinium* sp cultured at N/P =15 or N/P > 15.

Due to the scarcity of studies dealing with the biological control of the fish killer *Karlodinium* sp. growing under P-limitation conditions, the present study will contribute to clarifying the role and response of its potential grazers

MATERIAL AND METHODS

Experimental organisms

The copepod *Acartia margalefi* (550 μm) and the phagotrophic dinoflagellate *Oxyrrhis marina* (25 μm) were isolated from Barcelona harbour in 2003 and 1995 respectively. Strains of *Gymnodinium* sp1 and *Karlodinium* sp. (CSIC1), hereafter *Karlodinium* sp., were isolated from Alfacs Bay (Ebro Delta River, Mediterranean Sea) in 1995. All these organisms (copepods, and dinoflagellates) were maintained in the laboratory of the Institut de Ciències del Mar. Based on Scanning Electronic Microscopy observations, *Karlodinium* sp. was initially identified as *Gyrodinium corsicum* by Delgado *et al.* (1995). However, recent work using molecular techniques indicates that this organism is co-specific with *Karlodinium* sp. (E. Garcés, pers. com). *Karlodinium* sp. and *Gymnodinium* sp1 (both with a similar size ~14 μm) were cultured in F/2 media under a balanced (Redfield) N/P ratio (N/P = 15), and under P-limitation (N/P = 136), in controlled

TABLE 1. – Initial concentration (mean ± SD, n=3) of the two dinoflagellates at different NP ratios.

Dinoflagellates	Treatment	Initial concentrations (cells ml^{-1})
Karlodinium sp.	N/P =15	2517 ± 204 1027 ± 43 322 ± 59 130 ± 33
Gymnodinium sp1	N/P =15	2164 ± 415 1390 ± 226 450 ± 74 140 ± 7
Karlodinium sp.	N/P >15	2374 ± 227 1173 ± 107 328 ± 13 95 ± 7
Gymnodinium sp1	N/P >15	2290 ± 128 1314 ± 65 410 ± 94 104 ± 12

light and temperature conditions (14:10 h light/dark, and 18ºC) over 21 days. Hereafter, balanced N/P ratio will be referred to as N/P = 15, and P-limitation as N/P > 15.

Experimental design

Exponential phase cultures of *Karlodinium* sp. or *Gymnodinium* sp1 were diluted conveniently with F/2 media in four 1 litre batches for each dinoflagellate and condition (different N/P ratios) achieving nominal concentrations of 100, 300, 1200, 2500 cel ml^{-1}. Triplicate 75 ml tissue bottles were filled up with the different concentrations of dinoflagellate cultures (*Karlodinium* sp. or *Gymnodinium* sp1) and fixed immediately with acid lugol (2% final concentration) in order to make the initial concentration (Table 1). *O. marina* was cultured with *Rhodomonas salina* and the culture was diluted with filtered sea water until a concentration of 7500 cells ml^{-1}. Then, triplicate 75 ml tissue bottles were filled up with the four different concentrations of each dinoflagellate grown in the two nutrient conditions and were used as controls without predators. The same experimental set up was repeated twice, in one set we added 1 ml containing 7500 cells of *O. marina* (100 cells ml^{-1}, final concentration) and immediately after, we fixed 10 ml with acid lugol (2% final concentration) to measure the initial concentration of *O. marina*. In the other set, three *A. margalefi*, cultured with a mixture of *R. salina* and *O. marina*, were added to each bottle. Each species of prey with and without predators were incubated over 24 hours in a controlled light and temperature chamber (14:10 light/dark cycle and 18ºC). The incubation bottles were maintained in a slowly rotating plankton wheel (0.2 rpm). After 24 h all triplicates were also preserved with acid lugol (2% final concentration) and settled down for 24 hours in 50-ml sedimentation chambers. Dinoflagellates were counted in an inverted microscope (400×, Zeiss). At t_0 and t_{24} we enumerated the abundance *of Karlodinium* sp., *Gymnodinium* sp1 and the phagotrophic dinoflagellate *O. marina*. At the end of the experiment, the *A. margalefi* were checked for activity and monitored for 24h using a stereomicroscope. In order to estimate the possible direct effects of *Karlodinium* sp. on the viability of copepod eggs, about 100 females of *A. margalefi* from the laboratory culture were selected and fed "ad libitum" with a culture of *O. marina*. The eggs laid by the females in 24 h (~2000 eggs) were distributed equally into 4 Petri dishes (~500 eggs per plate). In each Petri dish we added a solution containing 2000 cell ml^{-1} of *Karlodinium* sp. or *Gymnodinium* sp1, each of which was previously cultured either at P-limited or in a nutrient-balanced medium. The Petri dishes were incubated at 18ºC and a 14:10 h light/dark period in a temperature and light controlled room during 5 days. The hatched eggs in each of the four conditions were counted daily.

Data analysis

- Specific growth rates of *Karlodinium* sp. and *Gymnodinium* sp1 were calculated as follows:

$$\mu_{np} = 1/t * \text{Ln} (\text{Ab dinof}_t / \text{Ab dinof}_0)$$
(without predators)
$$\mu_p = 1/t * \text{Ln} (\text{Ab dinof}_t / \text{Ab dinof}_0)$$
(with predators),

where μ_{np} is the specific gross growth rate (without predators) and μ_p is the specific net growth rate of dinoflagellates (with predators), t is the incubation time in days, Ab dinof$_t$ and Ab dinof$_0$ are the abundance of dinoflagellates at the end and at the beginning of the experiments respectively.

- The number of dinoflagellate cells produced can be calculated as follows:

Cells produced ml^{-1} d$^{-1}_{(np)}$ =
= Ab dinof$_0$ * ($e^{\mu np * t}$) - Ab dinof$_0$ (without predators)
Cells produced ml^{-1} d$^{-1}_{(p)}$ =
= Ab dinof$_0$ * ($e^{\mu p * t}$) - Ab dinof$_0$ (with predators)

- Specific growth rates (μ_{om}) of *O. marina* when incubated with *Karlodinium* sp. or *Gymnodini*um sp 1 were calculated as:

$$\mu_{om} = 1/t * \text{Ln (Ab } O.\ marina_{\ t} / \text{ Ab. } O.\ marina_{\ 0})$$

t is the incubation time in days, Ab *O. marina*$_t$ and Ab *O. marina*$_0$ are the abundance of *O. marina* at the end and at the beginning of the experiments respectively.

- Grazing rates on *Karlodinium* sp. and *Gymnodinium* sp1

Grazing rates were calculated as the difference between the specific gross growth rate and specific net growth rate (μ_{np} - μ_p).

- The ingestion rate was calculated as follows:

Dinof. grazer^{-1} t^{-1} =
Cells prod. ml^{-1} t$^{-1}_{(np)}$ - Cells prod. ml^{-1} t$^{-1}_{(p)}$/ Ab grazer

Where Dinof. grazer^{-1} t^{-1} are the number of *Karlodinium* sp. or *Gymnodinium* sp1 ingested per *O. marina* or *A. margalefi* per time unit; Cells prod. ml^{-1} t$^{-1}_{(np)}$, is the number of dinoflagellate cells produced without predators and Cells prod. ml^{-1} t$^{-1}_{(p)}$ is the number of dinoflagellate cells produced with predators. Ab grazer, is the initial abundance of the corresponding grazer (*O. marina* or *A. margalefi*).

RESULTS

The specific growth rate of *O. marina* was maximal when it was incubated with the highest concentration of *Gymnodinium* sp1 cultured at N/P = 15 (Fig. 1A). *O. marina* always showed null or negative growth rates when it was incubated with *Karlodinium* sp grown at N/P =15 or under P-limitation at any concentration (Fig. 1). Significantly lower growth rates of *O. marina* were observed when it was incubated with the highest concentration of *Karlodinium* sp. compared to the maximal density of *Gymnodinium* sp1, either at NP >15 (ANOVA, n = 6, P= 0.02) or NP =15 (ANOVA, n = 6, P = 0.02). Ingestion rates of *O. marina* were higher (4.5 ± 1.75 cells *O. marina* d^{-1}) when feeding on the greatest *Gymnodinium* sp1 concentration under a

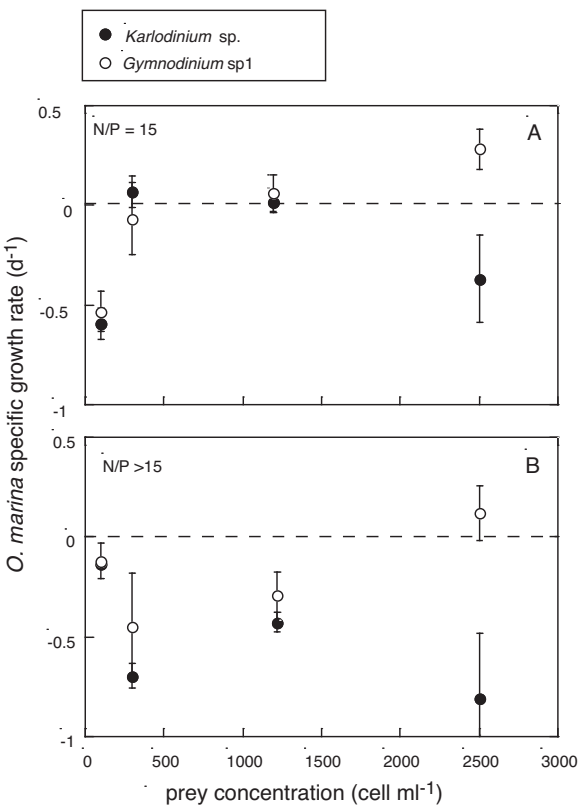

FIG. 1. – Specific growth rate *Oxyrrhis marina* incubated at different dinoflagellate concentrations (*Karlodinium* sp. (CSIC1) and *Gymnodinium* sp1). (A) N/P balanced and (B) P-limitation conditions. (n=3, errors bars: SD).

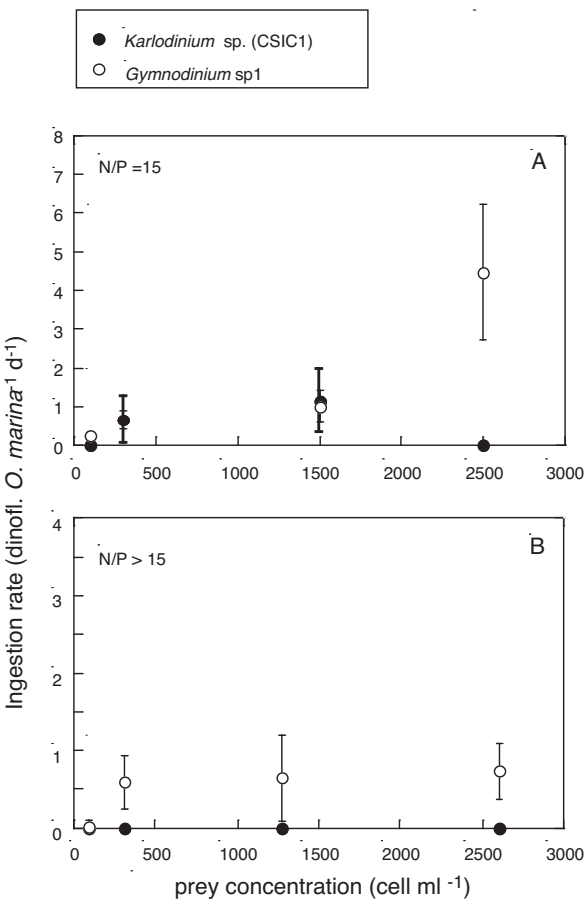

FIG. 2. – Ingestion rates of *Oxyrrhis marina* feeding at different dinoflagellate concentrations (*Karlodinium* sp. (CSIC1) and *Gymnodinium* sp1). (A) N/P balanced and (B) P-limitation conditions. (n=3, errors bars: SD).

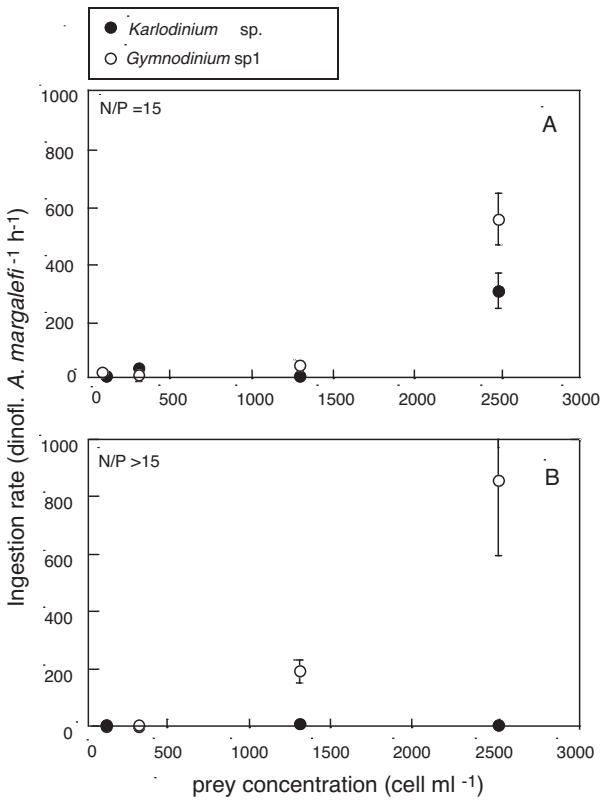

FIG. 3. – Ingestion rate of *Acartia margalefi* feeding at different dinoflagellate concentrations (*Karlodinium* sp. (CSIC1) and *Gymnodinium* sp1). (A) N/P balanced and (B) P-limitation conditions. (n=3, errors bars: SD).

balanced N/P ratio (Fig. 2A) than under P-deficient conditions (Fig. 2B). Ingestion rates were negligible or null feeding on *Karlodinium* sp. grown under both N/P conditions (Fig. 2). *A. margalefi* showed similar ingestion rates for *Gymnodinium* sp1 at both N/P ratios and lower ingestion rates for P-deficient *Karlodinium* sp. than for *Karlodinium* sp. grown at N/P = 15 (Fig. 3). In summary, both potential predators, when incubated with the highest concentration of each prey (~2500 cel ml^{-1}), presented null ingestion rates for *Karlodinium* sp. and maximum rates for *Gymnodinium* sp1 (Figs. 2, 3).

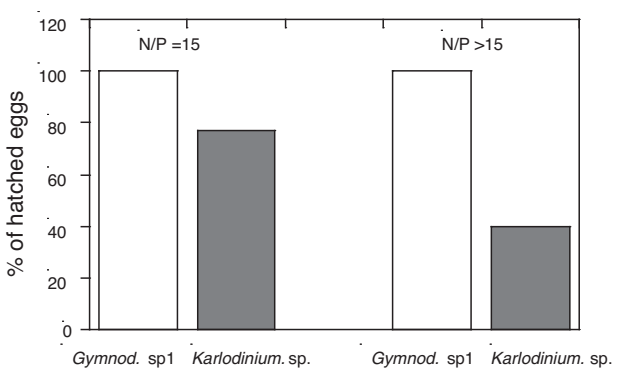

FIG. 4. – Percentage of hatched eggs of *Acartia margalefi* incubated with 2000 cells ml^{-1} of *Karlodinium* sp. (CSIC1) and *Gymnodinium* sp1 grown under N/P balanced (NP = 15) and P-limitation conditions (N/P > 15).

The toxic dinoflagellate *Karlodinium* sp. either cultured at N/P =15 or N/P > 15 had a direct effect on the egg viability of *A. margalefi* after 5 days exposure (Fig. 4). Thus, 100% of eggs were hatched when incubated with *Gymnodini*um sp1. This percentage decreased dramatically after eggs were exposed to *Karlodinium* sp. The number of non-hatched eggs was maximal (60%) when incubated with *Karlodinium sp*. cultured at N/P > 15 (Fig. 4).

DISCUSSION

The toxic dinoflagellate *Karlodinium* sp., common in the north-western Mediterranean, has similar characteristics to the widely distributed species *Karlodinium micrum* on the US Atlantic coast (Deeds *et al.,* 2003; Jonhson *et al.,* 2003). Both dinoflagellates have adverse consequences for different organisms such as fish, dinoflagellates, fungi, mesozooplankton, etc. (Delgado and Alcaraz, 1999; Deeds *et al.,* 2003). In a previous study, the toxic non-cultured *Karlodinium* sp. (formerly *Gyrodinium corsicum*, see Fernández-Tejedor *et al.,* 2003) had paralyzing and lethal effects on the copepod *Acartia grani* at ~3000 cells ml^{-1} after only a few hours of exposure (Delgado and Alcaraz 1999). However, in the present study at a slightly lower *Karlodinium* sp. concentration, we did not observe any direct lethal effects on another copepod of the same genus, *A. margalefi*. These contrasting results may be due to differences in the toxin content of the cultured and wild strain of *Karlodinium* sp. For instance, intracellular levels of toxins can vary within a single algal clone, depending on culture age and conditions (e.g. Turner and Tester, 1997) and it is not uncommon for toxicity to decrease over time in algal cultures (Cembella and Therriault, 1998; and Burkholder *et al.,* 2001). We could not determine the toxic substances produced by *Karlodinium* sp. but the results of the experiments indicate some deleterious effects on predators: On one hand, null or negative growth rates of *O. marina* when incubated with any concentration of *Karlodinium* sp., and on the other hand, the reduction in the viability of *A. margalefi* eggs (when healthy eggs were incubated with *Karlodinium* sp. Deeds *et al.* (2002) have recently isolated polar lipid-like compounds with hemolytic, cytotoxic, and ichthyotoxic properties from several *Karlodinium micrum* strains. Likewise, Fernandez –Tejedor *et al.* (2003) have found similar toxic prop-

erties in the same species that we used for our experiment. Those compounds could be related to the adverse effects caused by high concentrations of this organism. However, the proper characterization of the toxins of *Karlodinium* sp. and the mechanisms involved in zooplankton mortality reported in the experiments conducted in Alfacs Bay (Delgado and Alcaraz, 1999) are still open questions.

The experiments presented here, as well as the previous study carried out by Vaqué *et al*. (2003), indicate that micro and mesozooplankton, when incubated with the highest dinoflagellate concentration, show lower ingestion rates for *Karlodinium* sp. than for a similar but non-toxic dinoflagellate, when prey were cultured under balanced N/P ratios (Figs. 2-3). The low ingestion rate of *Karlodinium* sp. when the concentration did not exceed 1500 cells ml^{-1} (0.02 -1.2 cells *O. marina*$^{-1}$ d^{-1}, Fig. 2) was similar to those obtained in Chesapeake Bay by Johnson *et al*. (2003) who suggested that *O. marina* could be a potential grazer of *Karlodinium micrum*. In any case, we think that the role of grazers, such as *O. marina*, concerning such prey could not prevent its proliferation. In addition, when *Karlodinium* sp. was cultured under P-limitation conditions, we detected even higher negative effects on the predators (Figs. 1-3). Moreover, the non-toxic *Gymnodinium* sp1 was consumed less by *O. marina* than when it was grown at an N/P balanced ratio, and consequently a lower *O. marina* growth rate was observed. *A. margalefi* showed similar feeding rates for *Gymnodinium* sp1 with and without P-limitation conditions and null ingestion for *Karlodinium* sp. under P-limitation at any concentration. Cells growing under P-limitation conditions would probably have some structural deficiencies, or palatability effects that could be detected by the predators. However the higher ingestion rates of both predators for *Gymnodinium* sp1 than for *Karlodinium* sp. (when concentration was ~2500 cel ml^{-1}) at P-limitation conditions suggest that *Karlodinium* sp. releases deleterious compounds. In addition, viability of *A. margalefi* eggs decreased dramatically when healthy eggs were incubated with this toxic dinoflagellate at N/P >15 (Fig. 4). Otherwise, over 24 h incubation *A. margalefi* feeding on *Karlodinium* sp. under P-deficient conditions did not show any apparent paralyzing effects or mortality of adults (Delgado and Alcaraz, 1999). There are very few studies dealing with the effect of *Karlodinium* sp. "type" living under P-limitation conditions. For instance, *Gyrodinium* cf. *aureolum*, also associated with fish and shellfish kills, showed a higher toxic effect on *Mytilus* sp. embryos under P-limitation (Gentien *et al*., 1991). We do not know which mechanisms (under phosphorous limitation) enhance these negative effects. There could be enhanced toxin production or an accumulation of cell toxin content when division rates are more depressed by P-limitation than toxin production. However, Anderson *et al*. (1990) observed that in *Alexandrium* spp grown under P-limitation conditions, toxin production was enhanced independently from the dinoflagellate growth rate.

Field observations show that blooms of toxic dinoflagellate species are frequent in nutrient depleted waters (Guisande *et al*., 2002), and toxin production could be an adaptation evolved to offset the ecological disadvantages of dinoflagellates with low nutrient affinity (Smayda, 1997). The enhancement of adverse effects on its predators due to P-limitation may be relevant in Mediterranean waters such as those of the Alfacs Bay, in which *Karlodinium* sp. has been repeatedly detected. According to the time series of simultaneous data of nutrient and dinoflagellate concentration in the same area, Olivos (2000) and Camp *et al*. (2003) observed a tendency towards a significant average phosphorus limitation (N/P=35).

The recurrent incidence of *Karlodinium* sp. blooms in the NW Mediterranean Sea (Delgado *et al*., 1995; Garcés *et al*., 1999; Fernandez-Tejedor *et al*., 2003), requires further research that combines both field and experimental studies in order to determine the appropriate approach for controlling their growth and unwanted toxic effects.

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REFERENCES

Anderson, DM., P.T.O Cheng, J.J. Sullivan, S. Hall and C. Lee. – 1990. Dynamics and physiology of saxitoxin production by the dinoflagellate *Alexandrium minutum* spp. *Mar. Biol*., 104: 511-524.

Boyer, G.L., J.J. Sullivan, R.J. Anderson, P.J. Harrison and

F.J.R.Taylor. – 1987. Effects of nutrient limitation on toxic production and composition in the marine dinoflagellate *Protogonyaulax tamarensis*. *Mar. Biol*., 96: 123-128.
Burkholder, J.M., H.B. Glasgow and N. Deamer-Melia, – 2001. Overview and present status of the toxic *Pfiesteria complex* (Dinophyceae). *Phycologia,* 40: 186-214.
Calbet, A., D. Vaqué, J. Felipe, M. Vila, M.M. Sala, M. Alcaraz and M. Estrada. – 2003. Relative grazing impact of microzooplankton and mesozooplankton on a bloom of the toxic dinoflagellate Alexandrium minutum. *Mar. Ecol. Prog. Ser*., 259: 303-309.
Camp, J., E. Flo and M. Masó. – 2003. Monitoring program of quality control of water littoral waters. Final report. Compilation of results 1994-2001. Catalan water Society and Institut of Marine Sciences (CMIMA-CSIC). 127 pp.
Cembella, A.D. and J.C. Therriault. – 1998. Comparative toxicity of cultured isolates and natural populations of *Protogonyaulax tamarensis* (Lebour) Taylor from the St. Lawrence. *J. Shellfish Res*., 7: 152.
Deeds, J.R., D.E. Terlizzi, J.E. Adolf, D.K Stoecker and A.R. Place. – 2002. Toxic activity from cultures of *Karlodinium micrum* (=*Gyrodinium galatheanum*) (Dynophyceae)-a dinoflagellate associated with fish mortalities in an estuarine aquaculture facility. *Harmful Algae*., 21: 169-189.
Deeds, J.R., J.P.Y. Kao, R.E. Hoesch and A.R. Place. – 2003. Toxic mode of action of KmxT2, a new fish-killing toxin from *Karlodinium micrum* (Dinophyceae). *Second Symposium on Harmful Marine Algae*. Woods Hole Oceanographic Institution (USA). December 9-13. Abstracts book: 6p.
Delgado, M., J.V. Fernandez, E. Garcés, E. Matamoros and J. Camp. – 1995. Blooms of a dinoflagellate belong to the genus *Gyrodinium* in Alfacs Bay (Ebro Delta), associated to fish mortalities. *Proccedings of the Fifth National Congress of Aquaculture*. Eds: Castelló, F. and A. Calderer. San Carles de la Ràpita. 10-13 May 1995.
Delgado, M. and M. Alcaraz. – 1999. Interactions between red tide microalgae and herbivorous zooplankton: the noxious effects of *Gyrodinium corsicum* (Dinophyceae) on *Acartia grani* (Copepoda: Calanoida). *J. Plankton Res*., 21: 2361-2371.
Fernandez-Tejedor, M., E. Garcés, J. Camp, A. Penna and M. Zapata. – 2003. *Karlodinium micrum* (=*Gyrodinium galatheanum*) an ichthyotoxic dinoflagellate in Alfacs bay. *Plankton Symposium*. Vigo (Spain). October 9-13.
Fernandez-Tejedor, M., M.A. Soubrier-Pedreño, P. Riobó, and E. Cacho. – 2003. Toxicidad "*in vivo*" e "*in vitro*" de los cultivos de *Gyrodinium corsicum*. VIII Reunión Iberica sobre Fitoplancton tóxico y biotoxinas. Universidad de La Laguna (2003).
Fistarol, G.O., C. Legrand, E. Selander, C. Hummert, W. Soilte and E. Graneli. – 2004. Allelopathy in *Alexandrium* spp.: effect on a natural plankton community and on algal monocultures. *Aquat. Microb. Ecol*., 35: 45-56.
Garcés, E., M. Masó, J. Camp. – 1999. A recurrent and localized dinoflagellate bloom in a Mediterranean beach. *J. Plankton Res*., 21: 2373-2391.
Gentien, P., G. Arzul, and F. Toularastel. – 1991. Modes of action of the toxic principle of *Gyrodinium aureolum*. *Proccedings of Symposim on marine biotoxins*, Paris, 30-31 January 1991. Actes du Colloque sur les Biotoxins Marines, pp. 83-86.
Guisande, C., M. Frangópulos, I. Maneiro, A.R. Vergara and I. Riveiro. – 2002. Ecological advantages of toxin production by the dinoflagellate *Alexandrium minutum* under phosphorous limitation. *Mar. Ecol. Prog. Ser*., 225: 169-178.
Johnson, M.D., M. Rome and D.K. Stoecker. – 2003. Microzooplankton grazing on *Prorocentrum minimum* and *Karlodinium micrum* in Chesapeake Bay. *Limnol. Oceanogr*., 48: 238-248.
Olivos, A. – 2000. *Dissolved inorganic nutrients in littoral waters of the Catalan Sea*. PhD. Thesis, 134 pp. University of Barcelona.
Smayda T.J. – 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton bloom in the sea. *Limnol. Oceanogr*., 42: 1137-1153.
Suikkanen, S., G.O. Fistarol, and E. Granéli. – 2004. Allelopathic effects of the Baltic *Cyanobacteria Nodularia spumigena, Aphanizomenon flos-aquae* and *Anabaena Lemmermannii* on algal monocultures. *J. Exp. Mar. Biol. Ecol*., 308: 85-101.
Teegarden G.J. – 1999 Copepod grazing selection and particle discrimination on the basis of PSP toxin content. *Mar. Ecol. Prog. Ser.,* 181: 163-176.
Turner, J.T. and D.M. Anderson. – 1983. Zooplankton grazing during dinoflagellate blooms in Cape Cod embayment with observations of predation upon tintinnids by copepods. *PSZN I: Mar. Ecol*., 4: 358-374.
Turner, J.A. and P.A.Tester. – 1997. Toxic marine phytoplanktpn, zooplankton grazers and pelagic food webs. *Limnol. Oceanogr*., 42: 1203-1214.
Uye, S. – 1986. Impact of copepod grazing on the red tide flagellate *Chatonella antiqua*. *Mar Biol*., 95: 35-43.
Vaqué, D., J. Felipe and M. Alcaraz. – 2003. Effects of a toxic dinoflagellate on planktonic predators: repressed feeding and production rates on meso-and microzooplankton. *Abstracts of EUROHAB meeting*. Amsterdam March 2003.
Vila, M., J. Camp, E. Garcés, M. Masó and M. Delgado. – 2001. High resolution spatio-temporal detection of potentially harmful dinoflagellates in confined waters of the NW Mediterranean. *J. Plankton Res*., 23: 497-514.
Vila, M. and M. Masó. – 2005. Phytoplankton functional groups and harmful algal species in antropogenically impacted waters of the NW Mediterranean Sea. *Sci. Mar*., 69: 31-45.
Watras, C.J., V.C. Garcon, R.J. Olson, S.W. Chisholm and D.M. Anderson. – 1985. The effect of zooplankton on estuarine blooms of the toxic dinoflagellate *Gonyaulax tamarensis*. *J. Plankton Res*., 7: 891-908.

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