INTRODUCTION

The role of zooplankton grazers in the development of monospecific toxic algal blooms has been mainly studied by examining the ability of grazers to select between toxic and non-toxic phytoplankton species, as well as the effect of toxins on the physiology of grazers. Zooplankton organisms are constrained by morphological features (body size and feeding appendages) to be either selective or generalist feeders (DeMott 1986, 1988, Gilbert 1990, Kirk & Gilbert 1992). The most selective-feeding organisms are able to recognize and avoid the consumption of toxic phytoplankton species (Huntley et al. 1986, DeMott 1989, Gilbert 1990, DeMott & Moxter 1991, Kirk & Gilbert 1992, Teegarden 1999, Guisande et al. 2002a), which would otherwise negatively affect their survival and/or reproduction (Ives 1987, Lampert 1987, Sykes & Huntley 1987, Gilwicz & Lampert 1990, Bagoien et al. 1996, Dutz 1998, Frangópulos et al. 2000, Guisande et al. 2002a). It has been suggested that behavioral avoidance of toxic phytoplankton by selective grazers has been devel-
oped by associative learning processes (Uye & Takamatsu 1990). That is, selective grazers would distinguish between markedly morphological and/or chemical features of phytoplankton species, and would then re-direct their grazing pressure towards non-toxic species (Guisande et al. 2002a). This may represent a competitive advantage for toxic phytoplankton species and a hypothetical mechanism that favors the onset of monospecific toxic phytoplankton blooms (Gilbert 1990, Guisande et al. 2002a).

The latter reasoning assumes that, before the bloom begins, the phytoplankton community consists of a mixture of toxic and non-toxic phytoplankton species, and that the population of each toxic species is composed of individuals with homogeneous toxic properties.

In this study, we suggest a more complex pre-bloom ecological scenario: toxic and non-toxic strains of the same species coexist within a population. This is more likely to occur in natural environments. Indeed, it has already been proposed for some cyanobacteria (Kirk & Gilbert 1992), has been documented for the genus Nodularia in the Baltic Sea (Laamanen et al. 2001), and may also be a consequence of ballast waters, which presents a global environmental problem to many species (Hallegraeff & Bolch 1992). In such a scenario, prior to the onset of a bloom, it would not be so easy for selective grazers to distinguish between toxic and non-toxic phytoplankton cells.

An attempt was made in this study to examine some aspects of the relationship between selective grazers and endotoxic phytoplankton in a simplified version of the scenario proposed above. To explore this relationship it was considered necessary to integrate different aspects involved in the feeding ecology of grazers. These were: optimal diet theory predictions about food selection; the toxin-dilution hypothesis as a feeding strategy to balance diets when toxic species are included; and the consequences of the feeding behavior on the fitness of grazers.

The calanoid copepod Acartia clausi is the most abundant mesozooplankton organism in the Galician Rias (NW coast of Spain). In its natural environment, toxic individuals of the dinoflagellate Alexandrium minutum are present, and they occasionally form small monospecific blooms (unpubl. data from the monitoring management program of the Galician Government). The genus Acartia and other calanoid copepods have been recorded as being relatively highly selective grazers (Wilson 1973, DeMott 1988, Kirk & Gilbert 1992, Guisande et al. 2002a).

The aims of this study were (1) to test whether Acartia clausi is able to distinguish between 2 strains of Alexandrium minutum with different toxicities. One strain was isolated from the same environment as the copepods and produces high levels of paralytic shellfish poisoning (PSP) toxins, whereas the other strain was isolated from Mallorca (Mediterranean Sea) and was low in toxicity; (2) to analyze the consequences of the resulting copepod feeding strategies by estimating food ingestion, copepod mortality, egg production and egg hatching, in order to distinguish the toxic effects on maintenance metabolism from those on reproductive fitness, and; (3) to discuss the role of feeding strategies of grazers within the proposed phytoplankton community scenario in the framework of evolutionary relationships between grazers and microalgae.

MATERIALS AND METHODS

Zooplankton collection. Zooplankton was collected by vertically integrated tows from a depth of 20 m to the surface, at a field station of 39 m depth located in the Galician Ría de Vigo, Spain (42° 13.3’N, 8° 47.7’W). Samples were then transported to the laboratory and maintained for several days with air bubbling in a 25 l container and, prior to the beginning of the experiments, were fed with the green algae Tetraselmis suecica and the dinoflagellate Heterocapsa triqueta. Strains of Alexandrium minutum. The toxic strain of Alexandrium minutum (AT) was isolated from the Galician Rías (northwestern Spain, Atlantic Ocean) and came from a long-established population cultured in the Instituto Español de Oceanografía (Vigo, Spain). The non-toxic strain of A. minutum (ANT) used in this study was isolated in Mallorca (eastern Spain, Mediterranean Sea) and also came from a long-established population cultured in the Instituto Español de Oceanografía (Mallorca, Spain). Both strains of A. minutum contain only gonyautoxins 1, 2, 3 and 4 (Franco et al. 1994). Cultures were maintained in K medium (ccmp.bigelow.org), but the AT strain was cultured without the addition of phosphate to the medium so as to achieve higher toxin production. A. minutum cultures grew with a maximum rate of approximately 0.25 d⁻¹ (ANT) and 0.15 d⁻¹ (AT). For a period of 2 mo, up to the beginning of the experiment, about 25% of the medium was renewed once a week, producing semi-continuous conditions.

Experimental design. There were 3 different experimental food treatments, with 160 replicates each: only AT, only ANT, and 50% mixed treatments with both AT and ANT. Each replicate consisted of 1 adult female Acartia clausi in a 25 ml beaker containing the experimental food suspensions. Alexandrium minutum cells stayed in suspension during feeding experiments because of their swimming behavior. For all experimental food concentrations, the abundance of A. minutum was approximately 500 cells ml⁻¹. The culture
medium was prepared with aged natural seawater (salinity of 33.6‰) filtered through GF/F Whatman filters. The experiment was run at 18°C under a 12 h:12 h light-dark cycle. The copepods were transferred to new food suspensions every day. Replicates were not taken into account if the copepods died. Cell size, cell carbon and nitrogen content, toxin content per cell, ingestion rates, egg production, egg hatching, and copepod mortality were measured on a daily basis. Copepod content per copepod was estimated every 2 d. The experiment was performed for 6 d.

**Cell size, volume and carbon content.** Cell size was measured daily in a flow cytometer (FACSCalibur, Beckton Dickinson), and scatter light-detector measurements were calibrated with a Size Calibration Standards Kit (Bangs Laboratories). Measurements were undertaken in triplicates with samples of 1000 to 3000 cells each. Biovolume was calculated by the most likely geometrical shape method (Edler 1979, Hillebrand 1999), with *Alexandrium minutum* cells considered to be spherical. Cell carbon content was measured daily for each strain in triplicate; algal cells were collected on pre-combusted 13 mm GF/F Whatman filters and analyzed in a Fisons EA 1108 CHN analyzer.

**Toxin analysis.** To estimate the cell toxin content of *Alexandrium minutum* on a daily basis, 3 samples of algal cells were collected on pre-combusted 13 mm GF/F Whatman filters, stored at −30°C in ultracentrifuge plastic tubes and lyophilized. A total of 500 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 and frozen twice. Finally, the extract was centrifuged twice at 4000 rpm for 10 min, after which 200 µl of the supernatant were carefully collected with a Hamilton syringe and stored at −30°C.

To analyze the evolution of the content of toxin in the copepod throughout the experiment, around 10 to 15 copepods were transferred from each experimental food treatment to filtered seawater every 2 d. To assure that the recently ingested toxins were excreted, copepods were transferred to distilled water after 2 to 3 h and immediately collected in 40 µl of distilled water. This experimental design allowed measurement of only those toxins accumulated by the copepods. Samples of copepods were stored at −30°C in ultracentrifuge plastic tubes and lyophilized. Acetic acid (125 µl, 0.05 M) was added to the lyophilized material, and then the steps described above were followed.

Analysis of the PSP-related toxins by high-performance liquid chromatography (HPLC) with fluorescence detection 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 quadruplicate injections of 30 µl of extracts (diluted with 0.05 M acetic acid, as necessary). Toxins from the National Research Council of Canada (Halifax) were used as toxin standards.

**Ingestion rates.** Daily ingestion rates of *Acartia clausi* were estimated for each experimental food treatment by counting cell concentration in 20 replicates from a pooled sample of all grazers, as well as in 9 control replicates and 3 initial replicates. The grazing period was 24 h. Samples were preserved with 4% formaldehyde and counted in a Sedgewick-Rafter chamber under an inverted microscope. In the mixed-strain food treatment, the percentage of abundance of each strain was estimated using a flow cytometer by means of a fluorescence labeling technique using a monoclonal antibody. Frost’s (1972) equations were used to calculate ingestion rates. Preference for each strain in the mixed treatment was calculated with Manly’s α index (Manly 1974):

\[
\alpha_i = \frac{\ln e_i}{\sum_{j=1}^{m} \ln e_j/n_j}
\]

where \(e\) is the number of prey at the end of the experiment, \(n\) is the number of prey at the beginning of the experiment and \(i, j\) are different preys. The value of \(\alpha\) changes in relation to changes in the abundance of resources.

**Antibody staining.** The monoclonal antibody used in this work, the 90.3, was generated from a mouse immunized with the AT strain (M. Carrera unpubl. data), following the method described by Köhler & Milstein (1975) and further modified by Galfré & Milstein (1981). The algal staining was performed by indirect immunofluorescence: algal cells were incubated with a Hamilton syringe and stored at −30°C.

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fluorescence measured at 530/30 and 670 nm were also useful in the discrimination between the 2 strains. All these parameters, measured by cytometer detectors, were introduced as independent variables in a discriminant analysis to achieve the best possible identification of strains. In each sample, between 1000 and 3000 cells were analyzed. For each daily ingestion experiment, we measured 7 replicates from beakers with copepods, 2 initial replicates and 3 control replicates. In order to have sufficient algal cells for the analysis, 2 replicates were placed together in a 50 ml centrifuge tube. They were then centrifuged and preserved with 1 ml of 4% formaldehyde for 2 d, and subsequently indirect immunofluorescence was performed (see above).

**Strain identification.** Discriminant analysis allowed the correct identification of most of the individuals for each strain in the mixed-strain food suspension (Table 1). The significance of the discriminant function was always <0.001. Small errors in classifying individuals (AT or ANT) were randomly distributed between the 2 strains. For all analyses performed, the total correlations of each variable (cytometer detector measurements) with the discriminant function used in the analysis were in the following order of importance: 670+ nm fluorescence > 585/42 nm fluorescence (antibody labeling) > side scatter light (cell size) > 530/30 nm fluorescence > forward scatter size (cell internal complexity).

**RESULTS**

**Cell size and toxicity**

Cell size was reasonably constant in both AT and ANT strains throughout the entire experimental period (Table 2). The AT strain was always larger than the ANT strain and there were approximately 2-fold differences in biovolume between the 2 strains (Table 2). However, the relationship between cell biovolume and carbon content was not straightforward (Table 2). As it was limited by phosphorous (see ‘Materials and methods’), the AT strain had lower growth rates than ANT; this produced an increase in cell size in relation to the ANT strain which grew without limitations. AT toxicity was enhanced to a considerable extent by phosphorus limitation.

**Egg production and hatching success.** For each experimental food concentration, the eggs produced by 15 copepods were gathered on a daily basis. Triplicates were maintained for 2 d at 18°C and then fixed with 4% formaldehyde. Subsequently, nauplii and unhatched eggs were counted.

<table>
<thead>
<tr>
<th>Day</th>
<th>Initial</th>
<th>Control</th>
<th>Grazers</th>
<th>Grazers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>88</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
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<td>6</td>
<td>94</td>
<td>97</td>
<td>97</td>
<td>97</td>
</tr>
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</table>

Table 1. *Alexandrium minutum*. Percentage of individuals of both strains correctly classified by cross validation in discriminant analysis

<table>
<thead>
<tr>
<th>Day</th>
<th>Strain</th>
<th>Size (µm)</th>
<th>Volume (µm³ 10⁻³)</th>
<th>Carbon (pg cell⁻¹) (fg STXeq)</th>
<th>Toxicity (fmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>8.6</td>
<td>2451</td>
<td>5.6</td>
</tr>
<tr>
<td>2</td>
<td>ANT</td>
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<td>0.1</td>
</tr>
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<td>3</td>
<td>AT</td>
<td>21.3</td>
<td>5.1</td>
<td>3416</td>
<td>7.7</td>
</tr>
<tr>
<td>4</td>
<td>ANT</td>
<td>17.7</td>
<td>2.9</td>
<td>31</td>
<td>0.1</td>
</tr>
<tr>
<td>5</td>
<td>AT</td>
<td>21.9</td>
<td>5.6</td>
<td>3664</td>
<td>8.4</td>
</tr>
<tr>
<td>6</td>
<td>ANT</td>
<td>18</td>
<td>3.1</td>
<td>55</td>
<td>0.1</td>
</tr>
<tr>
<td>7</td>
<td>AT</td>
<td>25</td>
<td>8.2</td>
<td>4590</td>
<td>10.5</td>
</tr>
<tr>
<td>8</td>
<td>ANT</td>
<td>18.3</td>
<td>3.2</td>
<td>61</td>
<td>0.1</td>
</tr>
<tr>
<td>9</td>
<td>AT</td>
<td>26.4</td>
<td>9.6</td>
<td>4219</td>
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</tr>
<tr>
<td>10</td>
<td>ANT</td>
<td>17.7</td>
<td>2.9</td>
<td>59</td>
<td>0.3</td>
</tr>
<tr>
<td>11</td>
<td>AT</td>
<td>26.6</td>
<td>9.9</td>
<td>4155</td>
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</tr>
<tr>
<td>12</td>
<td>ANT</td>
<td>17.2</td>
<td>2.7</td>
<td>60</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 2. *Alexandrium minutum*. Cell parameters measured during experiment. Toxicity is total gonyautoxin (GTX) per cell (fg STXeq: saxitoxin equivalents). AT: toxic strain; ANT: non-toxic strain
rrous limitation. The same strain cultured under nutrient replete conditions contained between 1.1 and 2.5 fmol toxin cell\(^{-1}\) (Frangopulos et al. 2000, Guisande et al. 2002a,b), which was considered to be at the low end of the toxicity range for *Alexandrium minutum* (Chang et al. 1997). PSP content in other *Alexandrium* species reaches values of 250 fmol cell\(^{-1}\) (*A. tamarense*, Frangopulos et al. 2004).

The ANT toxin content may have had no toxic effects or only weak toxic effects on our population of *Acartia clausi*, which had adapted to the presence of *Alexandrium minutum* in the environment. In an adapted population of *Acartia tonsa*, Colin & Dam (2002) did not find any effect at concentrations of 1.44 pg toxins cell\(^{-1}\) (approximately 3.5 fmol cell\(^{-1}\)), at approximately the same cell concentration as in our experiment. In contrast, Dutz (1998), Frangopulos et al. (2000) and Guisande et al. (2002a,b) observed negative effects on *A. clausi* reproduction when cell toxicities were 1.1 to 2.5 fmol cell\(^{-1}\).

### Ingestion experiments

Mean cell densities throughout the experiment were 404 ± 72 cells ml\(^{-1}\) in treatments with only ANT; 560 ± 84 cells ml\(^{-1}\) in treatment with only AT; and 927 ± 226 total cells ml\(^{-1}\) in the mixed-strain treatment with 538 ± 66 and 405 ± 89 cells ml\(^{-1}\) for AT and ANT, respectively. Within this range of cell concentrations, copepods are not expected to be limited by food (Huntley et al. 1986, DeMott 1988). Fig. 2 shows the total ingestion rates for each experimental condition during the 6 d of the experiment. Feeding pressure was higher in the food suspension with ANT than in the food suspension with AT (Fig. 2a). For each experimental condition, daily grazing rates increased for ANT and decreased for AT. The amount of food ingested in relation to the food available was higher in the single cultures with ANT and lower in cultures with AT, while medium values were recorded in the treatment with a mixture of both strains (Fig. 3).

This reduction in grazing rates in the AT treatment may be due to physiological incapacitation or rejection due to ‘bad taste’. Copepods in AT treatments looked unhealthy and exhibited erratic swimming behavior; in addition, as will be shown later, copepod mortality was higher in AT, which indicated that while rejection of toxic cells due to ‘bad taste’ could not be eliminated as a possibility, there was clear physiological incapacitation due to ingestion of toxins.

The maximum ingestion rates reported here (ANT treatment) are similar to those of Frangopulos et al. (2000) and Dutz (1998) for the same carbon concentration, with low toxicity strains of *Alexandrium minutum* and *A. lusitanicum*, respectively. Both authors stated that these values were lower than expected. Therefore, the ANT strain may also exhibit reduced ingestion rates to some extent, due either to ‘bad taste’ or cell toxicity.

![Fig. 2. *Acartia clausi*. Food ingested (mean ± SD, n = 20) in food treatments with (a) only one of each strain or (b) with mixed toxic (AT) and non-toxic (ANT) strains of *Alexandrium minutum*. •: AT strain; ⊙: ANT strain](image1)

![Fig. 3. *Acartia clausi*. Food ingested (mean, n = 20) as a function of *Alexandrium minutum* abundance. ■: ANT diet; ○: AT diet; □: ANT mixed diet; ⊙: AT mixed diet. SD of mean food ingested not shown, all lower than 0.6](image2)
Fig. 2b shows the results for the treatment with a mixture of both strains. There was an initial pattern (Days 1 to 3) that demonstrated preference for the AT strain. This could be expected if food was not limited and both strains were of the same nutritional quality, but AT was of a larger size. However, from Days 4 to 6, preference for the AT strain decreased to almost the same value as that for ANT (Day 4), and was lower than that for ANT on Day 5; however, there was again a slight preference for AT on Day 6. The \( \alpha \) calculations for Manly’s index of preference (Table 3) agreed with these observations, with \( \alpha \) values from Days 4 to 6 close to a non-selective pattern.

### Copepod toxin accumulation

Differences in toxin accumulation between experimental conditions are explained by the presence or absence of the AT strain (Fig. 4), and by total cells ingested. There were no differences in toxins accumulated by the copepods between AT and the mixed culture (Table 4), which corroborated that the number of cells of the toxic strain ingested by copepods was similar for both the single culture and the mixed cultures that contained the toxic strain.

Other studies on toxin accumulation by *Acartia clausi* fed on *Alexandrium minutum* (toxicity 1.1 to 2.5 fmol cell\(^{-1}\)) have reported values of 0 to 500 pg toxin copepod\(^{-1}\) after 1 d of feeding, and 1500 pg toxin copepod\(^{-1}\) after 6 d of feeding on mixed diets with non-toxic species (Frangopulos et al. 2000, Guisande et al. 2002a,b); negative effects were also observed for ingestion rates, egg production and egg hatching, but not for mortality.

#### Copepod mortality

Mortality was assumed to be representative of treatment effects after Day 1, so the data for this day were omitted from Fig. 5. As expected, toxic AT cells caused a higher rate of mortality than the ANT cells, as is shown in treatments with only 1 of the 2 strains (Fig. 5). In the mixed-strain food suspension, the pattern of daily mortality was intermediate to that of the other 2, and there was a small delay (Day 2) before the mortality in the mixed-strain food suspension exceeded that of ANT (Fig. 5a). However, in this mixed treatment, the accumulated toxin in the copepods was the same as for the AT strain on Days 1 and 3 (Fig. 4). This suggested a mechanism of toxin dilution. The significant decline in mortality in the AT treatment during the last 2 d of the experiment could be due to a reduction in food ingestion (Fig. 2a). The 3 patterns of mortality can be well summarized by accumulated mortality (Fig. 5b).

### Table 3. *Acartia clausi*. Food selection; Manly’s \( \alpha \) coefficients for strain selection in the mixed-strain treatment. AT: treatment with only AT; ANT: treatment with only ANT

<table>
<thead>
<tr>
<th>Day</th>
<th>( \alpha ) AT</th>
<th>( \alpha ) ANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.92</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>0.65</td>
<td>0.35</td>
</tr>
<tr>
<td>3</td>
<td>0.92</td>
<td>0.08</td>
</tr>
<tr>
<td>4</td>
<td>0.54</td>
<td>0.46</td>
</tr>
<tr>
<td>5</td>
<td>0.36</td>
<td>0.64</td>
</tr>
<tr>
<td>6</td>
<td>0.59</td>
<td>0.41</td>
</tr>
</tbody>
</table>

### Table 4. *Acartia clausi*. ANCOVA results using time as a covariate. AT: treatment with only AT; ANT: treatment with only ANT; Mx: treatment with a mixture of both strains; df: degrees of freedom

<table>
<thead>
<tr>
<th>Dependent variable: treatments compared</th>
<th>df Intersection Error Total</th>
<th>( F )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accumulated toxin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT–ANT</td>
<td>1  13  16</td>
<td>35.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AT–Mx</td>
<td>1  13  16</td>
<td>0.23</td>
<td>0.63</td>
</tr>
<tr>
<td>ANT–Mx</td>
<td>1  13  16</td>
<td>46.7</td>
<td>&lt;0.001</td>
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<tr>
<td>Egg production</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AT–ANT</td>
<td>1  21  24</td>
<td>298.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AT–Mx</td>
<td>1  21  24</td>
<td>168.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ANT–Mx</td>
<td>1  21  24</td>
<td>0.03</td>
<td>0.86</td>
</tr>
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<td>Egg hatching</td>
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<td></td>
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<tr>
<td>AT–ANT</td>
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<td>0.015</td>
</tr>
<tr>
<td>AT–Mx</td>
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</tr>
<tr>
<td>ANT–Mx</td>
<td>1  21  24</td>
<td>17.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
toxin in the mixed-strain treatment as in the single AT food suspension (Fig. 4, Table 4), copepods in the mixed-strain treatment showed an intermediate pattern of accumulated mortality and a delay prior to an increase in daily mortality. This clearly demonstrates a mechanism of toxin dilution.

Egg production and hatching success

Data from Day 1 were excluded from Fig. 6 and were not statistically analyzed, as it was assumed that they were not dependent on treatment effects but on recent nutritional life history in the field. Egg production is affected by total biomass ingested (Kiørboe et al. 1985). However, due to either the toxic effects of the AT strain or to reduced ingestion rates it was almost suppressed in the single diet with AT (Fig. 6). A toxic effect is more likely to explain the results we obtained when both strains were present, because egg production increased while total biomass ingestion was kept constant and AT ingestion decreased (Fig. 2). Although toxins accumulated in copepods were the same in the AT and mixed-strain diets, egg production values in the mixed-strain diet were the same as for the ANT diet (Fig. 6, Table 4). If reduced egg production was due to toxicity, this may be evidence of a toxin-dilution effect.

ANOVA (where food ingested was a covariate [log transformed]) showed that there were significant differences in egg production (log transformed) among treatments ($F_{2,8} = 14.5, p = 0.002$) (Fig. 7). These results
confirm that, in addition to reduced egg production due to reduced ingestion of food, there was an effect of toxins on egg production. However, when the AT treatment was excluded from the ANCOVA, there were not significant differences between ANT and the mixed diet (F_{1,5} = 0.52, p = 0.502), which supported the hypothesis of a dilution mechanism.

Highest egg production values (20 to 25 eggs female\(^{-1}\) d\(^{-1}\), ANT treatment) were lower than those reported by Kiørboe et al. (1985) for optimal conditions in *Acartia tonsa* (about 45 eggs female\(^{-1}\) d\(^{-1}\)). This difference does not affect our conclusions and may be accounted for by the fact that (1) ingestion rates of the ANT strain in the present study were lower than optimal values and (2) ingestion rates were negatively affected by the toxins ingested.

Egg hatching was also affected in the AT treatment (Fig. 6) and did not present a toxin-dilution effect in percentage terms (Fig. 6), with no statistical differences between the AT and mixed-strain treatments (Table 4). Hence, regardless of any other food source consumed, egg-hatching success is related to toxins accumulated by copepods.

**DISCUSSION**

Experiments with zooplankton to test optimal diet theory predictions have shown abilities to optimize diet composition by selecting among different food categories (DeMott 1986, 1988, 1989, 1995). Prey size seems to be the main criterion when the foods offered have the same nutritional quality (Wilson 1973, Vanderploeg et al. 1984, Vanderploeg & Paffenhofer 1985, Bogdan & Gilbert 1987, DeMott 1995). It has been demonstrated that differences in food quality can be detected between algal-flavored and unflavored microspheres, live algae and microspheres, live and dead algae, toxic and non-toxic algae (DeMott 1989), and even between algal cells in different growth phases (Cowles et al. 1988). These selective abilities were shown to be taxon-specific among zooplankton groups. Therefore, with predictions made by the optimal diet theory, selective behavior is dependent on total food concentration, which means that food selection appears to be stronger when the level of food is not restricted by limiting conditions (DeMott 1989, 1995).

The food selection behavior displayed (Fig. 2b, Table 3) showed an initial pattern of positive selection for the AT strain (Days 1 to 3). We hypothesize that this may be due to a size-selective behavior. Size-selective behavior is performed by scanning the particle size distribution in food suspensions, and is developed within the first 15 to 30 min of exposure to a particular experimental food treatment (Wilson 1973). The genus *Acartia* and other copepods are known to be proficient at size selection among particles with equivalent nutritional quality (Wilson 1973, Vanderploeg et al. 1984, Vanderploeg & Paffenhofer 1985, Bogdan & Gilbert 1987, DeMott 1995).

This initial size-selective pattern seemed to be disrupted from Day 4 to Day 6, when \(a\) values were close to the non-selective value of 0.5 (Table 2). Subsequently, grazing pressure was weakly redirected towards the non-toxic strain. We hypothesized that copepods were not able to effectively reject the toxic AT strain when mixed with the ANT strain because of difficulties in recognizing it. Fig. 2b shows that after Day 3, the decline in grazing pressure towards the AT strain was stronger than the parallel increase towards ANT. This led to \(a\) values close to a non-selective pattern (Table 3). In other words, copepods were no longer selecting cell size from Day 4 to 6, but this behavior was not adequate for an efficient rejection of the toxic strain. However, we should not reject the idea that the relatively low values of toxicity of our strain (see ‘Results’) may have influenced food selection, and it is possible that more toxic strains would have enhanced selective behavior of copepods against toxic cells.

The effects of PSP toxins on maintenance metabolism and physiology include reductions in swimming performance and survival rates and the inhibition of food ingestion (Ives 1987, Sykes & Huntley 1987, Bagoien et al. 1996, Colin & Dam 2002). All of these effects were observed in the present study (Figs. 2a, 3 & 5). It has been suggested that reduction in the rates of egg production is an indirect effect of reduced food intake (Colin & Dam 2002). This hypothesis cannot be rejected in our study, but our results confirmed that there is a negative effect on egg production due to toxin ingestion by copepods (Fig. 7).

We considered egg-hatching success to be our best estimate of reproductive physiology and individual fitness. For this parameter, there was also a negative effect related to presence of the AT strain (Fig. 6), which has been previously described in the literature (Dutz 1998, Frangopulos et al. 2000, Guisande et al. 2002a, 2002b). The pattern of hatching inhibition only appeared clearly after Day 3. This was probably due to a slow passage of the toxins and to accumulation in the gonads from the digestive system, affecting embryogenesis. Only 0.98% of the daily PSP toxins assimilated are allocated to the eggs (Guisande et al. 2002b).

The physiological effect of mixed diets which include non-toxic species has rarely been thoroughly examined (Turner et al. 2001, Colin & Dam 2002). In our experiments, for most of the parameters examined, a clear toxin-dilution effect was observed for the mixed-strain treatment for copepod mortality and egg production. In this treatment, with similar or even
higher amounts of the AT strain ingested and about the same level of toxin accumulated as in the AT treatment (Fig. 4), neither food ingestion nor egg production seemed to be affected by PSP toxins (Figs. 2b, 6 & 7). An additional toxin-dilution effect was demonstrated by mortality incurred from the mixed-strain treatment: higher mortality values were delayed until Day 3, and accumulated mortality was intermediate between those of the ANT and AT treatments. Our results are in agreement with those of Turner et al. (2001), who found that the consumption of alternative food items provided an amelioration of toxic effects.

Conversely, egg hatching did not exhibit such a toxin-dilution effect. Although overall fitness was enhanced with respect to the AT treatment, due to total egg production (Fig. 6), the percentage of eggs hatched was the same for both the AT treatment and the mixed-strain treatment (Fig. 6). These results for hatching success might indicate a dose-dependent effect.

Colin & Dam (2002) examined mixed diets by feeding a high-toxic strain of *Alexandrium minutum* and a non-toxic species (*Tetraselmis suecica*) to the copepod *Acartia tonsa*. They did not find any effect on hatching success, but found a dose-dependent effect that inhibited food ingestion and, indirectly, egg production. This meant that there was neither inhibition of embryonic development nor toxin-dilution effects. The presence of a different species as a non-toxic control in the study of Colin & Dam (2002) may make the interpretation of their results difficult, because of the possibility of unknown effects produced by this second species. In addition, their experimental period (3 d) was shorter than that of our study, and they only examined the results on the third day. Two days of acclimatization followed by sampling only on the third day seem insufficient to record clear patterns, especially for mortality and egg hatching. Major differences between the results from Colin & Dam (2002) and our study should, however, be species-specific relationships. The *Acartia tonsa* population in the former study was adapted to recurrent blooms of the toxic *Alexandrium minutum*, whereas our *Acartia clausi* population was adapted to the presence of this species but not to recurrent blooming events.

Feeding strategies to nullify the effects of toxic compounds have often been examined in invertebrates within the framework of the toxin-dilution and the non-additive hypotheses (Pennings et al. 1993, Bernays et al. 1994, Hagele & Rowell-Rahier 1999). Although reduction in growth is assumed to have negative consequences on fitness, considered here as maintenance physiology, we showed that the effects of toxins on maintenance physiology can be different to those on reproductive physiology (a better estimate of fitness). Indeed, in our study, the toxic effects were not dose-dependent for maintenance physiology (because of toxin-dilution), but they were for reproductive physiology (egg-hatching success).

If, prior to the onset of a bloom, a phytoplankton community consists of a set of toxic or potentially toxic and non-toxic species, each with genetically different strains, grazers such as *Acartia clausi* do not appear to have sufficient selective abilities to achieve 100% distinction between these strains if there are not strong morphological differences between them. At this point, prior to the onset of a bloom, grazers may not yet experience any effects from favoring toxic strains of the same species over non-toxic ones. However, at least with PSP toxins, any feeding strategy that does not avoid consumption of toxic cells may produce a reduction in the fitness of the grazers, because the effects on egg-hatching success are dose-dependent and cannot be compensated for by any toxin-dilution strategy. This suggests that in evolutionary terms, individuals would be favored if they performed any feeding strategy that avoided consumption of toxic cells. Yet, as our results indicated, this selective feeding behavior may be of greater importance after the beginning of a bloom of a toxic species, when toxic cells dominate the community and grazers can effectively reject them, as has been shown in previous studies (Huntley et al. 1986, DeMott 1989, Gilbert 1990, DeMott & Moxter 1991, Kirk & Gilbert 1992, Teegarden 1999, Guisande et al. 2002a).

It has been suggested that the evolution of endotoxicity can be explained by the ‘kin selection’ theory (Kirk & Gilbert 1992). Toxic individuals may be captured by grazers, which will recognize them as toxic and will then avoid the consumption of close relatives that are also toxic. Subsequently, the inclusive fitness of the initially grazed individuals is enhanced. As Kirk & Gilbert (1992) also pointed out, this strategy may be susceptible to take-over by cheaters. To some extent, our findings support this possibility, because grazers are not always able to efficiently select between toxic and non-toxic strains; however, we should also consider that our strain was not of the highest possible toxicity, and that use of more toxic strains could produce different findings. Efficient selection can only be made when toxic and non-toxic strains coexist in similar numbers before a bloom. However, the ‘kin selection’ explanation is still accurate for the early and late phases of a bloom, when only a toxic strain becomes dominant in the phytoplankton community.
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LITERATURE CITED


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