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Short-term and long-term effects of the toxic dinoflagellate *Alexandrium minutum* on the copepod *Acartia clausi*

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ABSTRACT: Several experiments were performed to determine the effects of cell toxin concentration, composition and toxicity of *Alexandrium minutum* on ingestion rate, egg production, hatching success and naupliar fitness of the copepod *Acartia clausi*. A combination of *A. minutum* and non-toxic algae (*Prorocentrum micans*, *Tetraselmis suecica* and *Isochrysis galbana*) was used as food. Copepods ingested a higher amount of *A. minutum* cells as the concentration of these toxic dinoflagellates increased, and also in response to decreasing total food concentration available for the copepods. A positive relationship was obtained between *A. minutum* cells ingested by copepods and total toxin concentration per copepod. Hatching success and naupliar production were lower when copepods ingested a higher amount of toxic dinoflagellates. This negative effect could has been due to the accumulated toxins in the egg and copepod tissues, and was higher when *A. minutum* had a higher cell concentration of GTX1. Finally, the results obtained from nauplii incubated with *T. suecica* and *I. galbana* showed that nauplii hatched from females fed non-toxic food (*T. suecica* and *I. galbana*) reached copepodite stage earlier than those nauplii hatched from females fed with a combined of toxic (*A. minutum*) and non-toxic (*T. suecica* and *I. galbana*) food.

KEY WORDS: Dinoflagellate · Copepod · Toxins · PSP · Reproduction

INTRODUCTION

It is well known that copepods can be negatively affected by toxic marine dinoflagellates (Turner & Tester 1997). Although enhanced mortality due to direct ingestion of toxic algae is known to occur in copepods (Carlsson et al. 1995, Bagøien et al. 1996), evidence of such mortality is limited. More frequently, the effects of toxins on copepods may be sublethal, such as reductions in food intake, food assimilation or fecundity (Gill & Harris 1987, Uye & Takamatsu 1990, Dutz 1998, Teegarden 1999).

Copepods may exhibit reduced feeding and/or fecundity after ingestion of toxic phytoplankton either due to behavioural rejection prior to ingestion or physiological impairment due to ingested toxins. Earlier studies supported a physiological basis for the observed suppression of feeding rate (Ives 1985, 1987, Huntley et al. 1986), whereas later studies have concluded that discriminatory feeding selection is important in the grazing response of copepods to toxic dinoflagellates, either by trial-and-error consumption of harmful phytoplankton that allow grazers to learn which species should be avoided (Uye & Takamatsu 1990), or by behavioural rejection prior to ingestion (Teegarden 1999). Other studies have however shown that phycotoxins are not crucial in determining feeding interactions between toxic dinoflagellates and copepods (Gill & Harris 1987, Teegarden & Cembella 1996, Dutz 1998). The reduced fecundity observed in copepods exposed to toxic dinoflagellates has been

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also explained either as a result of feeding avoidance (Uye & Takamatsu 1990, Nejstgaard & Solberg 1996) or physiological impairment (Gill & Harris 1987, Dutz 1998).

Dutz (1998) showed in *Acartia clausi* fed *Alexandrium lusitanicum* that gross growth-efficiency of copepods can also be negatively affected through the ingestion of toxic dinoflagellates. He explained this negative effect of ingested toxins as being due to either negative effects on food assimilation or an enhanced energy expenditure of females for detoxification purposes.

Finally, Turner et al. (1998) suggested that marine phycotoxins might also negatively affect future generations of copepods: offspring might be indirectly affected by toxins ingested by their parents. It has been suggested that hatching success in copepods may be negatively influenced by toxic dinoflagellates ingested by females (Nejstgaard & Solberg 1996, Turner et al. 1998), although the results obtained in both studies showed no significant differences between treatments.

Much of the variation observed in the effects of phycotoxins on copepod feeding and/or reproduction is probably related to the considerable variation in cellular toxin potency, composition and concentration (Turner & Tester 1997). Intracellular levels of toxins can vary within a single algal clone (Chang et al. 1997), depending upon culture conditions (Boyer et al. 1987, Flynn et al. 1996, Parkhill & Cembella 1999) or the presence of toxic intracellular bacteria (Gallacher et al. 1997). An additional problem is that the differing responses observed in copepod species to specific combinations of toxic and non-toxic prey phytoplankters (Turriff et al. 1995, Teegarden & Cembella 1996, Teegarden 1999), are likely to lead to differing grazing patterns (Turner & Tester 1997).

Many aspects of the interaction between toxic dinoflagellates and copepods therefore still remain unclear, especially the effects on copepods of cell toxin concentration, composition and toxicity, the effects of a different combination of toxic and non-toxic algal species available to the copepods and the effects on the offspring of toxin ingested by the parents. We therefore carried out a study to determine the effects of a different combination of toxic and non-toxic algal species on ingestion rate, egg production, hatching success and naupliar fitness of the copepod *Acartia clausi*. The toxic dinoflagellate *Alexandrium minutum* was used because it often co-occurs with *A. clausi* in Ría de Vigo (Spain).

MATERIALS AND METHODS

Zooplankton collection. Zooplankton were collected by vertically integrated tows at a field station 39 m deep, located in the Ría de Vigo, Spain (42° 13.3′ N, 8° 47.7′ W), an area that has experienced blooms of toxic and non-toxic *Alexandrium* spp. (Centro de Calidad del Medio Marino de la Xunta de Galicia, Vilaxoan, monitoring reports). Samples were transported to the laboratory within 2 h of collection, and adult *Acartia clausi* were sorted out for the experiments.

Algal species. The algal species used as food and their diameter and carbon and nitrogen content per cell is shown in Table 1. Filtering efficiency is a function of the diameter of the algal species, for *Acartia clausi* feeding on *Alexandrium minutum*, *Prorocentrum micans* and *Tetraselmis suecica* it is 100%, but only <10% on *Isochrysis galbana* (Donaghay & Small 1979). The strain of *A. minutum* (A1 IV) used in this study was isolated from the Galician rias and came from a long-established population cultured in the Instituto Español de Oceanografía (Vigo). This toxic strain only contains Gonyautoxins 1, 2, 3 and 4 (GTX1 to 4) (Franco et al. 1994). Copepods were fed algae at exponential phase because toxin composition is constant in exponential growth-phase cultures (Franco et al. 1994, Parkhill & Cembella 1999). The carbon and nitrogen contents, of the phytoplankton species were determined from subsamples of exponentially growing cultures, filtered on pre-combusted GF/F filters at low pressure, dried at 70°C, and combusted in a Fisons EA-1108. Cell carbon and nitrogen were calculated by counts of 15 replicate subsamples on an inverted microscope. Sulphanilamide was used as the standard.

Experimental design. Two experiments were performed. In Expt I, abundance of *Alexandrium minutum* varied while abundance of *Tetraselmis suecica* and *Isochrysis galbana* was kept constant. In Expt II, abundance of *A. minutum* was kept constant and abundance of *Prorocentrum micans*, *T. suecica* and *I. galbana* varied between the different experimental treat-

Table 1. Algal food species. Cell diameter measured with inverted microscope (*Alexandrium minutum* and *Prorocentrum micans*) and equivalent spherical diameter (*Tetraselmis suecica* and *Isochrysis galbana*) measured by Coulter counter, and carbon and nitrogen content of algal species used in experiments (means ± SD, n = number of determinations)

Species	n	Cell diam. (µm)	n	C cell^{-1} (pg)	N cell^{-1} (pg)
Alexandrium minutum	55	27.8 ± 2.4	21	445.4 ± 12.8	127.5 ± 4.1
Prorocentrum micans	50	38.3 ± 1.6	21	1061.3 ± 25.4	241.2 ± 5.1
Tetraselmis suecica		8.5 ± 2.4	12	99.9 ± 2.8	9.6 ± 0.4
Isochrysis galbana		3.7 ± 1.5	12	11.1 ± 1.2	2.0 ± 0.2

Table 2. Initial carbon concentrations (C_i) and average carbon concentrations (C_x) of algal species on day grazing experiment was run (means ± SE, µg C ml^{-1})

Experimental conc.	*Alexandrium minutum*		*Prorocentrum micans*		*Tetraselmis suecica*		*Isochrysis galbana*		Total	
	C_i	C_x	C_i	C_x	C_i	C_x	C_i	C_x	C_i	C_x
Expt I										
A	0.51 ± 0.01	0.48 ± 0.01	0.0	0.0	3.04 ± 0.11	2.97 ± 0.06	0.88 ± 0.03	0.84 ± 0.01	4.43 ± 0.11	4.29 ± 0.06
B	0.28 ± 0.01	0.24 ± 0.01	0.0	0.0	2.29 ± 0.11	2.35 ± 0.06	0.65 ± 0.01	0.74 ± 0.03	3.25 ± 0.11	3.32 ± 0.06
C	0.12 ± 0.01	0.10 ± 0.01	0.0	0.0	1.99 ± 0.09	2.30 ± 0.11	0.68 ± 0.02	0.71 ± 0.03	2.82 ± 0.09	3.11 ± 0.12
D	0.02 ± 0.01	0.02 ± 0.01	0.0	0.0	1.89 ± 0.06	1.78 ± 0.05	0.67 ± 0.01	0.56 ± 0.04	2.60 ± 0.06	2.36 ± 0.06
Expt II										
A	0.64 ± 0.01	0.70 ± 0.01	1.05 ± 0.03	1.06 ± 0.01	4.32 ± 0.13	4.70 ± 0.06	1.24 ± 0.06	1.24 ± 0.02	7.26 ± 0.15	7.69 ± 0.07
B	0.50 ± 0.02	0.54 ± 0.01	0.0	0.0	2.52 ± 0.05	2.70 ± 0.04	0.88 ± 0.02	0.89 ± 0.01	3.90 ± 0.06	4.13 ± 0.04
C	0.0	0.0	1.10 ± 0.09	0.91 ± 0.01	2.80 ± 0.14	2.55 ± 0.05	1.03 ± 0.02	0.84 ± 0.01	4.93 ± 0.17	4.30 ± 0.05
D	0.53 ± 0.03	0.55 ± 0.01	0.0	0.0	0.92 ± 0.10	1.02 ± 0.01	0.33 ± 0.02	0.37 ± 0.01	1.78 ± 0.10	1.94 ± 0.02
E	0.53 ± 0.04	0.52 ± 0.01	0.0	0.0	0.55 ± 0.03	0.64 ± 0.01	0.15 ± 0.01	0.19 ± 0.01	1.23 ± 0.05	1.35 ± 0.02

ments. Table 2 shows the initial (C_i) and average (C_x) cell concentrations obtained in each experiment on the day the grazing experiment was run (6th day of the experiment). The carbon concentration in each experiment ensured that the potential for selection was not affected by possible food limitation which might constrain grazers to consume less palatable prey (Teegarden 1999).

Egg production and hatching estimation. From the sample collected in the field, between 35 and 40 adult females of *Acartia clausi* were sorted and transferred to individual 25 ml beakers containing some of the experimental food concentrations. Copepods were kept with constant shaking (60 rpm) at 15°C under a 12:12 h light:dark cycle. Each day the copepods were transferred to fresh phytoplankton suspensions at the experimental concentration. In both experiments, copepod mortality ranged between 1 and 3% d^{-1} in all experimental food concentrations, with the exception of Concentration D in Expt II, which was 8% d^{-1}. After 3 d acclimatisation, eggs were collected daily and counted for an additional 3 d. The eggs daily produced by 7 to 10 females were pooled, so there were 3 replicates of each experimental food concentration. From each replicate, 150 eggs produced by females every day were incubated for a further 48 h before fixation and counting of hatched nauplii and the remaining unhatched eggs. The remaining eggs were used to estimated egg toxin-concentration. Egg production, naupliar production and hatching success were estimated as the mean of the values obtained during the 3 d of the experiment.

Grazing estimation. Grazing trials were performed on the 6th day of the experiment. Between 13 and 15 replicates with 1 copepod in each 25 ml beaker were used for each experimental food concentration. Seven replicate control containers and initial containers without copepods were prepared at the same time. Initial container samples were preserved immediately at the start of the experiment. Grazing experiments were run for 16 h in the same temperature, light and shaking conditions given above. Copepods were checked at the end of the experiment, and the samples were preserved with 4% formaldehyde for phytoplankton cell-counting with an inverted microscope. Frost's (1972) equations were used to calculate C_x in control containers and copepod ingestion rates in experimental containers.

Offspring fitness. Nauplii hatched from eggs produced on the 6th day of the experiment by females fed non-toxic food (Food Concentration C of Expt II: Table 2), and nauplii hatched from females fed *Alexandrium minutum* and non-toxic food (Food Concentration B of Expt II: Table 2) were grown at low (approx. 0.6 µg C ml^{-1} *Tetraselmis suecica* and 0.2 µg C ml^{-1} *Isochrysis galbana*, 0.8 µg C ml^{-1} total) and high (approx. 1 µg C ml^{-1} *T. suecica* and 0.4 µg C ml^{-1} *I. galbana*, 1.4 µg C ml^{-1} total) food concentrations. For each group of nauplii and experimental concentration, 6 replicates containing 30 nauplii in 30 ml petri dishes of experimental cell mixtures were prepared. Nauplii were transferred every 2 d to fresh phytoplankton, and the number of nauplii still alive was recorded. The experiment was run until some of the nauplii reached Copepodite Stage I, and the remaining nauplii were used to estimate naupliar body length.

Toxin analyses. To estimate daily cell toxin variation of *Alexandrium minutum* in both experiments, algal cells were collected daily on pre-combusted 13 mm GF/F Whatman filters and stored at –80°C in ultracentrifuge plastic tubes and lyophilised. Four hundred µl of 0.05 M acetic acid was added to the lyophilised material and the sample was homogenised 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 at 4000 rpm for 10 m twice, after which 200 µl of the supernatant was carefully collected with a Hamilton syringe, and stored at –20°C. Egg and copepod toxin contents were only analysed on copepods from the experimental food concentrations of Expt II. On the 6th day of the experiment, 20 copepods were transferred from each experimental food concentration to distilled water, and were immediately isolated with a known volume of distilled water (no higher than 40 µl). Eggs collected daily from the 3rd to the 6th day were transferred from each experimental food concentration to distilled water and were immediately isolated with a known volume of distilled water (no higher than 150 µl). Eggs from the same experimental food concentration were always grouped in the same sample; the final number of eggs in the samples ranged between 566 and 1090. Samples of both copepods and eggs were stored at –80°C in ultracentrifuge plastic tubes and lyophilised; 125 µl of acetic acid 0.05 M was added to the lyophilised material followed by the same steps as above.

Analysis of the paralytic shellfish poisoning (PSP) 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 quatruple injections of 20 µl of extracts (diluted with 0.05 M acetic acid, as necessary). Chromatographic profiles of copepods were determined by only a single injection of 35 µl of the extracts. Toxins from the National Research Council of Canada (Halifax) were used as toxin 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 individuals 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).

RESULTS

Table 3 shows the specific toxic composition, total toxin per cell and total cell toxicity of *Alexandrium minutum* in Expts I and II. The strain of *A. minutum* used in this study is at the low end of the toxicity range observed for this species (Chang et al. 1997). There were no significant differences in toxin composition, total toxin per cell and total cell toxicity of *A. minutum* over the course of the experiments (ANOVA, $F_{5,11} < 3.2$, $p > 0.05$), which is consistent with the numerous reports of constant toxin composition in exponential phase cultures (Franco et al. 1994, Parkhill & Cembella 1999). In both experiments, *A. minutum* had a very similar toxic composition. There were no significant differences in the amount of GTX2, GTX3 and GTX4 per cell, total toxin per cell and total cell toxicity between the 2 experiments (ANOVA, $F_{1,5} < 2.8$, $p > 0.1$), but there were significant differences in the concentration of GTX1 per cell (ANOVA, $F_{1,5} = 50.8$, $p < 0.001$).

The ingestion rates of *Acartia clausi* in response to increasing food concentrations of *Alexandrium minutum*, in mixed concentrations of toxic and non-toxic algae, showed that copepods preyed actively on toxic dinoflagellates, and no signs of reduced ingestion or satiation by the toxic cells were observed (Fig. 1, slope differed from zero, $F_{1,57} = 113.9$, $r^2 = 0.67$, $p < 0.001$). However, when the abundance of *A. minutum* remained constant, *A. clausi* ingested higher amounts of *A. minutum* at lower total food concentrations (Fig. 2; slope differed from zero, $F_{1,56} = 103.9$, $r^2 = 0.65$, $p < 0.001$). Using data from both experiments, a stepwise regression showed that ingestion rate of *A. clausi* on *A. minutum* (I, in µg C copepod^{-1} d^{-1}) was significantly related to *A. minutum* abundance (A, in µg C ml^{-1}) and the abundance of non-toxic (Nt) phytoplankton species (in µg C ml^{-1}); $I = 4.152 + 16.689\ A - 1.817\ Nt$, $F_{2,114} = 276.8$, $r^2 = 0.83$, $p < 0.001$).

The HPLC analyses of concentrated extracts from copepods exposed to *Alexandrium minutum* revealed the presence of gonyautoxins in the copepod and egg tissues (GTX1, GTX2, GTX3 and GTX4), but saxitoxin, neosaxitoxin and decarbamoyl analogues were absent. Fig. 3 shows that as copepods ingested a higher

Table 3. *Alexandrium minutum.* Specific toxin composition of gonyautoxins (GTX1–4, mean ± SE, fmol cell^{-1}), total toxin per cell (combined GTX1, GTX2, GTX3 and GTX4, mean ± SE, fmol cell^{-1}) and total cell toxicity (mean ± SE, fg STXeq cell^{-1}) of alga used as food Expts I and II (STXeq = saxitoxin equivalents). Data presented in order of decreasing toxin content

Experiment	GTX4	GTX1	GTX3	GTX2	Total toxin	Cell toxicity
Expt I	0.612 ± 0.049	0.146 ± 0.018	0.017 ± 0.003	0.010 ± 0.002	0.785 ± 0.055	345.2 ± 24.7
Expt II	0.654 ± 0.053	0.074 ± 0.006	0.014 ± 0.004	0.007 ± 0.001	0.704 ± 0.069	300.9 ± 29.3

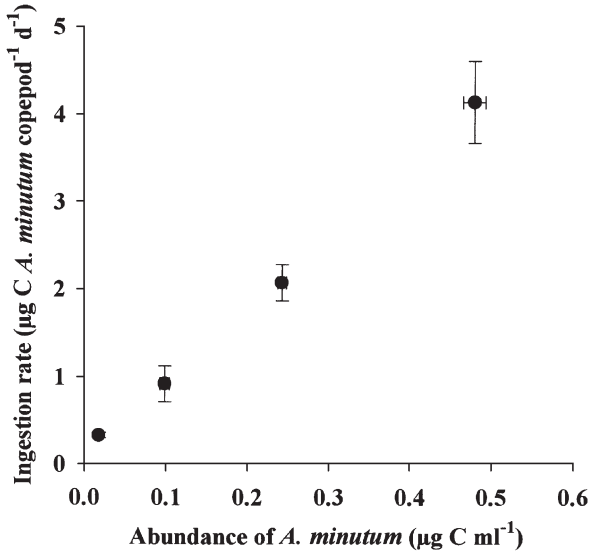

Fig. 1. *Acartia clausi.* Ingestion rates of *Alexandrium minutum* as a function of *A. minutum* abundance. Data are means ± SE

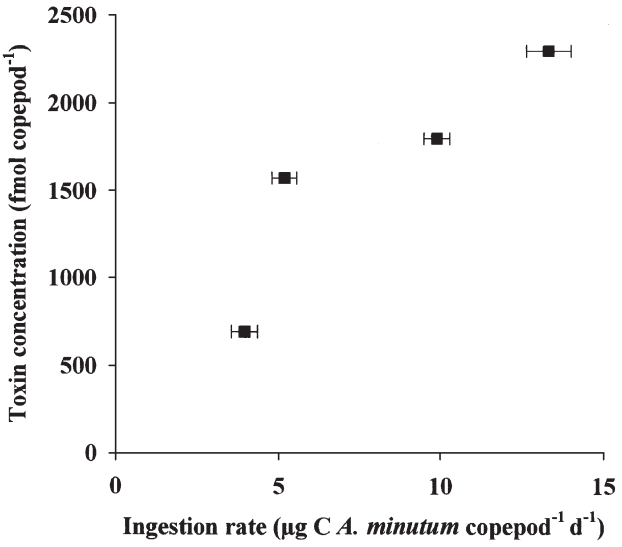

Fig. 3. *Acartia clausi.* Total toxic concentration (gonyautoxins, GTX1–4) in tissues of copepod as a function of ingestion rate of *Alexandrium minutum*. Data are means ± 1 SE

amount of *A. minutum*, the toxin concentration in the copepods increased (slope different from zero, $F_{1,56}$ = 107.4, r^2 = 0.66, p < 0.001).

There was a significant relationship between daily egg production (Fig. 4a, slope differed from zero, $F_{1,22}$ = 9.8, r^2 = 0.31, p = 0.005) and daily naupliar production (Fig. 4b, slope differed from zero, $F_{1,22}$ = 16.9, r^2 = 0.44, p < 0.001) and total food concentration in Expts I and II. There were no significant differences in neither egg production (ANCOVA, $F_{1,4}$ = 1.86, p > 0.2) or naupliar production (ANCOVA, $F_{1,4}$ = 0.29, p > 0.5) between experiments.

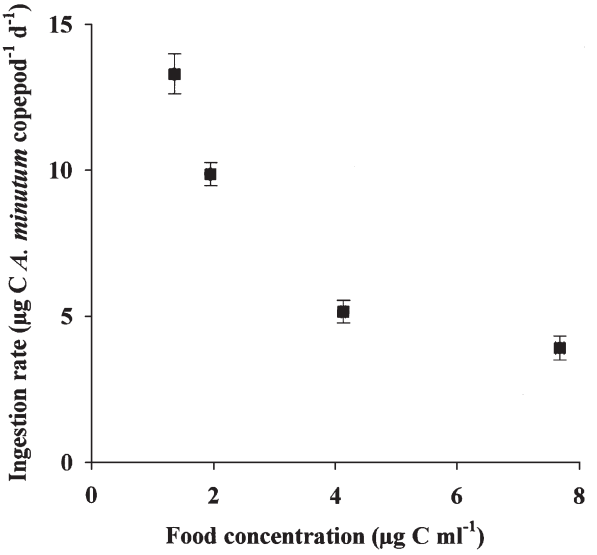

Fig. 2. *Acartia clausi.* Ingestion rates of *Alexandrium minutum* as a function of total food-carbon concentration. (Data are means ± SE)

Table 4 shows daily egg production and daily naupliar production of *Acartia clausi* in experimental food concentrations with similar carbon concentrations. There were no significant differences between egg production of females fed toxic and non-toxic algae at a similar food concentration (ANOVA, $F_{1,7}$ = 3.6, p = 0.099), but there were differences in naupliar production (ANOVA, $F_{1,7}$ = 9.7, p = 0.017).

Hatching success was not related to food concentration, but appeared to be negatively influenced by the amount of *Alexandrium minutum* ingested by the copepods (Fig. 5). In both experiments hatching success was related to ingestion rate of *A. minutum* (for Expt I, slope differed from zero, $F_{1,10}$ = 12.8, r^2 = 0.56, p = 0.005; for Expt II, slope differed from zero, $F_{1,13}$ = 46.8, r^2 = 0.78, p < 0.001), and there were also significant differences between slopes of the regressions obtained in experiments (ANCOVA, $F_{1,5}$ = 19.7, p = 0.006). Hatching success was also significantly related to the

Table 4. *Acartia clausi.* Mean ± SE daily egg production (eggs female^{-1} d^{-1}) and daily naupliar production (nauplii female^{-1} d^{-1}) in Food Concentrations B and C of Expt II, and Food Concentration A of Expt I

Experimental conc.	Egg production	Naupliar production
Expt I		
A	32.1 ± 4.0	24.2 ± 3.6
Expt II		
B	28.7 ± 1.9	22.8 ± 1.5
C	38.8 ± 4.2	34.2 ± 4.0

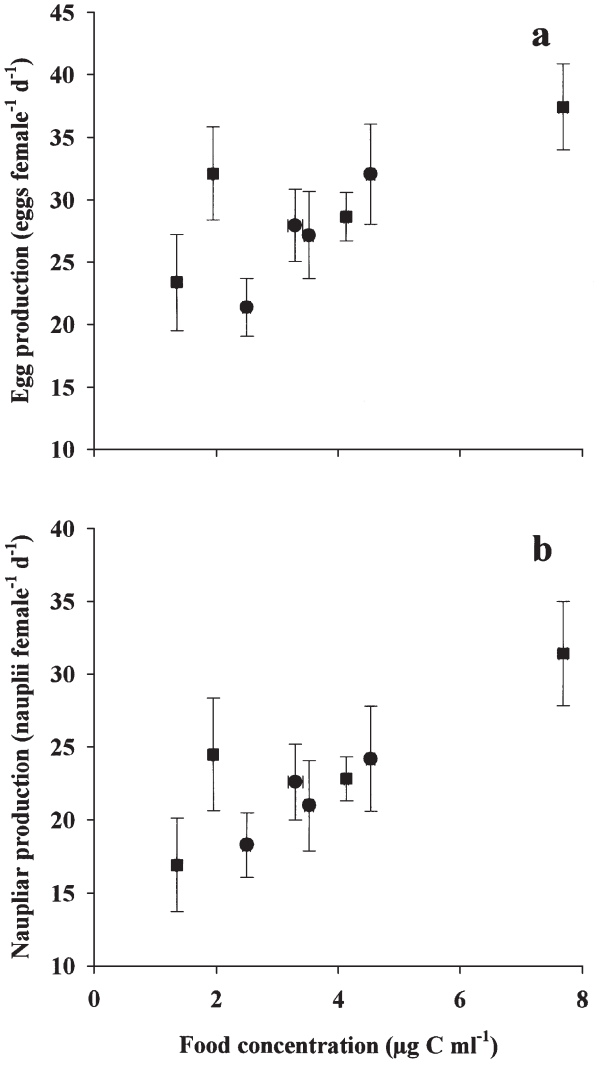

Fig. 4. *Acartia clausi.* (a) Daily egg production and (b) daily naupliar production of copepod as a function of total food-carbon concentration. (●) Expt I; (■) Expt II. Data are means ± 1 SE

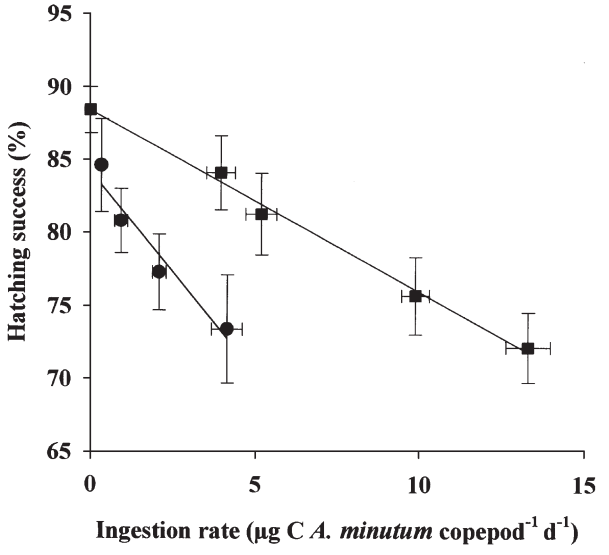

Fig. 5. *Acartia clausi.* Relationship between hatching success and ingestion rate of *Alexandrium minutum.* (●) Expt I; (■) Expt II. Data are means ± 1 SE

toxin ingested by the copepods (Fig. 6a, slope differed from zero, $F_{1,13}$ = 35.4, p < 0.001), and to egg toxin concentration (Fig. 6b, slope differed from zero, $F_{1,7}$ = 125.8, r^2 = 0.95, p < 0.001). Because of the low number of eggs collected it was not possible to detect toxins in the eggs from Food Concentrations D and E (Expt II). Therefore, although the relationship between hatching success and egg toxin concentration is significant, as only 2 toxic treatments were available, this relationship should be interpreted with caution.

The results of the offspring fitness experiment showed naupliar survival to be low at both low and high food concentrations (Fig. 7), and the values were within the range of naupliar survival observed for copepods in culture (Lopez 1991). Naupliar survival appeared to be higher during the initial phase of the experiment on nauplii hatched from females fed non-toxic food than on nauplii hatched from females fed toxic food, both at low and high food concentrations (Fig. 7). Nevertheless, on Day 12 naupliar survival (Table 5) was not significantly different for nauplii hatched from females fed non-toxic food than for those hatched from females fed toxic food at both high (ANOVA, $F_{1,10}$ = 1.8, p = 0.202) and low (ANOVA, $F_{1,8}$ = 0.3, p = 0.588) food concentrations. At Day 12, there were no significant differences in naupliar body length at the lowest food concentration (ANOVA, $F_{1,13}$ = 1.7, p = 0.212), but there were significant differences at the highest food concentration (ANOVA, $F_{1,56}$ = 7.8, p = 0.007). This is probably the reason why at the highest food concentration, nauplii hatched from females fed non-toxic food reached copepodite stage earlier than nauplii hatched from females fed toxic food (Table 5). However, because of the high naupliar mortality, the number of data may have been too low to estimate possible differences in development rate.

DISCUSSION

Our results showed that *Acartia clausi* fed on the toxic strain of *Alexandrium minutum* used in this study (Fig. 1). The relationship between ingestion rate and *A. minutum* abundance in the feeding experiments was similar to those reported for *Acartia clausi* feeding on a low-toxicity strain of *Alexandrium lusitanicum* (1.56 pg STXeq cell^{-1}, Dutz 1998). Moreover, grazing

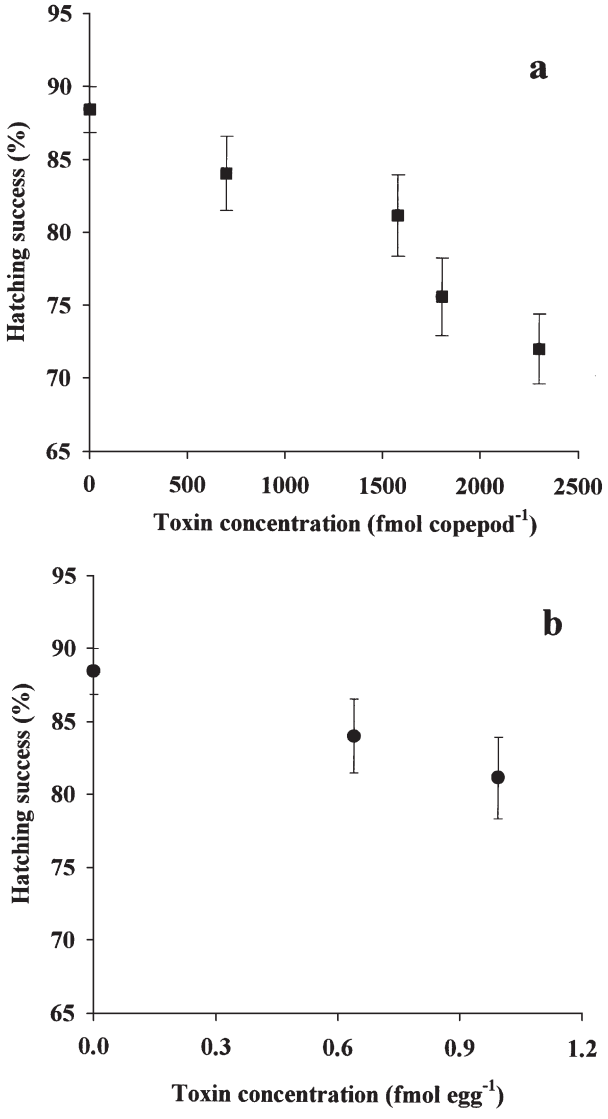

Fig. 6. *Acartia clausi.* Relationship between hatching success (means ± 1 SE) and total toxic concentration (gonyautoxins, GTX1–4) in tissues of (a) copepods and (b) eggs

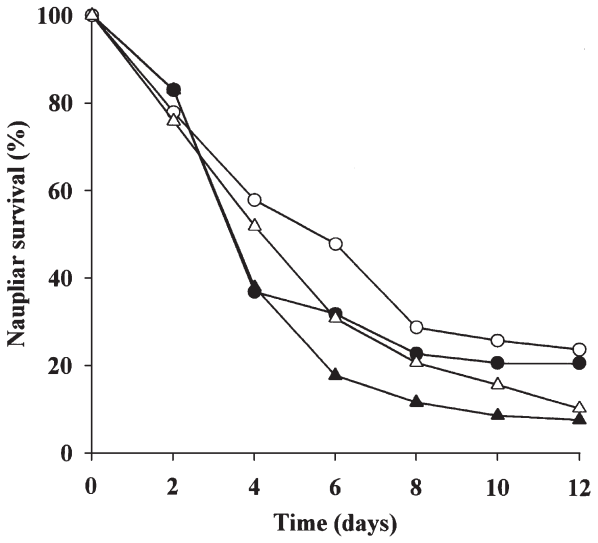

Fig. 7. *Acartia clausi.* Survival curves of nauplii hatched from females fed toxic (Food Concentration B of Expt II, filled symbols) and non-toxic (Food Concentration C of Expt II, open symbols) food. Nauplii were fed 2 different food concentrations; (1) mixture of *Tetraselmis suecica* and *Isochrysis galbana*: approx. 0.6 µg C ml^{-1} *T. suecica* and 0.2 µg C ml^{-1} *I.* galbana, 0.8 µg C ml^{-1} total (triangles); (2) approx. 1 µg C ml^{-1} *T. suecica* and 0.4 µg C ml^{-1} *I. galbana*, 1.4 µg C ml^{-1} total (circles). All SEs for means of naupliar survival were <5.6

pressure on *A. minutum* was higher in response to reduced food concentration of non-toxic species (Fig. 2), probably in order to maintain basic daily rations.

This study also showed non-satiated feeding of *Acartia clausi* on *Alexandrium minutum*, similar to the observation of Dutz (1998) in *Acartia clausi* feeding also on a low toxic strain of *Alexandrium lusitanicum*. Dutz suggested that this non-satiated feeding could be because the ratio energy-obtained to biomass-ingested is low, perhaps due to the interference of ingested toxins with the assimilatory processes and/or an enhanced energy expenditure of the females necessary to cope with the ingested toxins.

The high ingestion rates observed could be explained by the low-toxicity of the strain used in this study. Therefore, as mentioned by Teegarden (1999), non-toxic or low-toxicity dinoflagellates species are highly susceptible to grazing losses. Although previous studies have shown copepod fecundity to be negatively affected by the ingestion of toxic dinoflagellates (Gill & Harris 1987, Dutz 1998, Turner et al. 1998), our results are in agreement with those studies that found toxic

Table 5. *Acartia clausi.* Mean ± SE naupliar body length (µm), naupliar survival (%) and percent of copepodites of 12 d old that hatched from females fed toxic (Food Concentration B of Expt II: Table 2), and non-toxic (Food Concentration C of Expt II: Table 2) food, and cultured at 2 different experimental food concentrations

Naupliar food conc.	Female food conc.	Naupliar size	Naupliar survival	% copepodite
1.4 µg C ml^{-1}	Non-toxic	236.5 ± 5.8	23.9 ± 2.3	12.6 ± 9.2
	Toxic	219.2 ± 3.0	20.6 ± 1.2	0
0.8 µg C ml^{-1}	Non-toxic	220.0 ± 6.1	10.7 ± 4.4	0
	Toxic	210.0 ± 6.9	8.0 ± 2.3	0

algae to have no influence on egg production (Turner et al. 1997). However, the present study revealed that low-toxicity strains of dinoflagellates can also control grazing pressure. Hatching success fell as copepods ingested higher amounts of *Alexandrium minutum* cells (Fig. 5), perhaps because of the high amount of toxins ingested by the copepods, with especially high concentrations in the eggs (Fig. 6). This is not surprising because it is known that the chemical composition of freshly spawned copepod eggs is sensitive to the maternal diet (Guisande & Harris 1995, Guisande et al. 1999, Laabir et al. 1999). For this reason, reduced hatching success in copepods has been associated with nutritional inadequacy (Ianora et al. 1992, Ianora & Poulet 1993, Guisande et al. 1999, Laabir et al. 1999) or inhibitors (see Ban et al. 1997). Moreover, this study has also shown that nauplii hatched from females fed non-toxic food reached the copepodite stage earlier than nauplii hatched from females fed toxic food. This means a delay to attainment of first reproduction, and hence a reduced net reproductive rate of the next generation through ingestion of toxic cells by parental females.

In addition to decreasing hatching success with increasing toxin ingestion by the copepods, hatching success was lower in Expt I than in Expt II. The only difference between both experiments was the concentration of GTX1 present in *Alexandrium minutum* cells. This could be explained by the fact that GTX1 is the most toxic of the toxins in the PSP group (Oshima 1995). However, despite the differences observed in GTX1 cell concentrations between both experiments, there were no significant differences in total cell toxicity of *A. minutum* between experiments. As it is not known how gonyautoxins affect egg development, it is not clear whether the differences in hatching success observed between experiments arose from changes in the specific toxicity of the cells, or, was due to other factors such as differences in consumption on alternative non-toxic foods.

Dinoflagellate toxin production is often assumed to have evolved as a deterent to grazers (Turner et al. 1998). In the copepod *Tigriopus californicus*, Shaw et al. (1997) showed that a mixture of soluble PSP toxins could act as feeding deterrents. Many studies, including ours, have however shown that toxic dinoflagellates are ingested by copepods (Turner et al. 1998), which casts doubt on the advantage of toxin production if the dinoflagellates cannot repel all possible grazers. Teegarden (1999) concluded that the presence of toxins in a clone of *Alexandrium fundyense* conferred the definite advantage of reducing that dinoflagellate's palatability or desirability compared to a non-toxic *Alexandrium* species. This does not cause the dinoflagellate to be avoided or to be selected against by all grazer species. It confirms that grazing interaction between copepods and toxic dinoflagellates depends on the specific combination between copepod and toxic phytoplankton species (Teegarden & Cembella 1996, Teegarden 1999).

This study, however, showed that the loss of cells to grazers can be fairly well compensated by negative effects on grazer offspring-fitness. As predicted by Turner et al. (1998), toxic dinoflagellates can clearly adversely affect future generations of copepods. In the case of *Acartia clausi*, the ingestion of toxic cells by the copepod is accompanied by short-term (reduced hatching success) and long-term (reduced naupliar growth rate) impairments. According to the kin-selection theory, an individual can increase its gene representation in future generations by helping close relatives, who share copies of its genes which are identical by descent. As reproduction in *Alexandrium minutum* occurs mainly by simple cell division, future effects on grazers after a cell has already been consumed is of evolutionary significance. However, it is necessary to point out that Teegarden (1999) showed the tolerance to paralytic shellfish poisoning (PSP) toxins to be species-specific. In his study, *Acartia tonsa* showed a strong aversion to PSP toxins, whereas *Eurytemora herdmani* was tolerant of PSP toxin consumption. Therefore, it is possible that some copepod species are able to cope with ingested toxins and hence do not suffer the negative effects on offspring fitness observed in *A. clausi*.

To produce a high amount of toxins is probably energetically expensive, but the advantages are a possible discriminatory feeding behaviour against the toxic dinoflagellates and, hence, a possible increased grazing pressure on alternate non-toxic competitors of dinoflagellates. Low toxin production is energetically cheaper, and has sublethal effects on copepods if toxic cells are ingested by them. However, it implies a loss of cells, a long-term profit and, finally, it can also be beneficial for potential competitors of dinoflagellates. This possible trade-off between high and low toxin production could be of importance to the interaction between copepods and toxic dinoflagellates.

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