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Repression of fecundity in the neritic copepod *Acartia clausi* exposed to the toxic dinoflagellate *Alexandrium lusitanicum*: relationship between feeding and egg production

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ABSTRACT: The effect of the saxitoxin-producing dinoflagellate *Alexandrium lusitanicum* on the reproductive success of the calanoid copepod *Acartia clausi* was examined in the laboratory. Experiments were carried out to investigate the functional response of feeding and fecundity of copepod females at increasing concentrations (200 to 1600 µg C l^{-1}) of either the toxic *A. lusitanicum* or the non-toxic *Rhodomonas baltica* as food sources. Additional experiments were performed to determine if prolonged exposure to *A. lusitanicum* affects copepod survival and fecundity. Results demonstrate that *A. clausi* fed on toxic cells at high rates without lethal effects and was able to produce eggs. Survival of females was similar with both diets. Depending on the food source, different functional responses were found. Feeding and fecundity of *A. clausi* on a diet of *R. baltica* followed simultaneously a typical satiation response. Fecundity was high and attained maximal rates of 32 to 36 eggs $female^{-1}$ d^{-1}. In contrast, functional responses of ingestion and fecundity by *A. clausi* fed on *A. lusitanicum* were not closely associated. Whereas feeding rates increased linearly with increasing food concentrations, egg production was limited and stayed constant at 16 to 20 eggs $female^{-1}$ d^{-1} over the range of food concentrations offered. The comparison of calculated gross growth efficiencies for females feeding on both algae indicated an inefficient utilization of ingested toxic food. High feeding rates on toxic *A. lusitanicum* suggest that saxitoxins do not act as allelopathic chemicals against grazing in *A. clausi*. Nevertheless, fecundity was adversely affected. It is suggested that ingested toxins probably interfere with digestive processes or cause an enhanced energy expenditure due to detoxification because copepods could cope with toxic algae. As a result, less energy is available and this might explain the reduced fecundity in females.

KEY WORDS: Copepods · Egg production · Toxic dinoflagellates

INTRODUCTION

Dinoflagellate species of the genus *Alexandrium* are responsible for the occurrence of paralytic shellfish poisoning (PSP) in a variety of hydrographical regions ranging from temperate to tropical areas (Hallegraeff 1995). More than 20 saxitoxin derivatives that differ in structure and toxicity have been identified as the causative toxins for PSP (Oshima 1995). Their specific significance as compounds of the intermediary metabolism or as secondary metabolites is less understood and several functions have been suggested. Saxitoxins may be important cellular constituents involved in growth and replication (Anderson & Cheng 1988), may function as N-storage compounds (Plumley 1997) or may act as pheromones (Wyatt & Jenkinson 1997). Additionally, the production of allelopathic chemicals by dinoflagellates as a potential predator defense mechanism has received great attention because reduced or inhibited grazing due to poisoning could be an important factor in harmful bloom dynamics (Fiedler 1982, Huntley et al. 1986, Smayda 1997).

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Recently, Shaw et al. (1997) showed that soluble saxitoxins were perceived by copepods and suggested that they may act as feeding deterrents. However, evidence that saxitoxins or other phycotoxins act as antipredator compounds is less conclusive because feeding interactions between toxic algae and copepods are highly variable and a feeding reduction occurred not exclusively in copepods exposed to algae known to produce phycotoxins (reviewed in Turner & Tester 1997). For instance, toxic strains of *Alexandrium* were rejected by copepods (Huntley et al. 1986, Turriff et al. 1995) or ingested at lower rates in response to increasing toxicity (Ives 1985, 1987). On the other hand, Teegarden & Cembella (1996) found similar high feeding rates in 2 copepod species exposed to toxic and non-toxic *Alexandrium* strains. Regardless of the level of feeding on toxic algae, the accumulation of saxitoxins in zooplankton upon exposure to toxic algae and the importance of transfer of toxins by zooplankton in the food web are well documented (White 1981, Turriff et al. 1995, Teegarden & Cembella 1996).

Apart from variable grazing interactions between *Alexandrium* spp. and zooplankton, toxic strains may have significant impacts on their consumers, as shown by high mortality rates of fish larvae (Gosselin et al. 1989, Robineau et al. 1991). Although enhanced mortality has been demonstrated for *Euterpina acutifrons* (Bagøien et al. 1996), copepods appear to be less sensitive or even unaffected (Ives 1987, Turriff et al. 1995, Teegarden & Cembella 1996). However, the potential effects of feeding on toxic algae or of toxin accumulation on copepod fecundity have received little attention. A single report by Gill & Harris (1987) demonstrated that *Alexandrium tamarense* did not support egg production in 2 species of copepods. Most other knowledge of the influence of toxic algae on fecundity is based on non-PSP-producing algae. In experiments performed with monocultures of ichthyotoxic Prymnesiophyceae, Raphidophyceae and Dinophyceae, egg production of several copepods was significantly reduced (Nielsen et al. 1990, Uye & Takamatsu 1990, Nejstgaard & Solberg 1996, Turner et al. 1998). From these investigations it appears that the depression of egg production is strongly correlated with a reduction in ingestion rates, which suggests that the level of feeding on *Alexandrium* spp. could be a primary factor determining copepod fecundity when exposed to dinoflagellates producing saxitoxins.

The specific objective of the present study was to investigate the effects of the toxic dinoflagellate *Alexandrium lusitanicum* on the reproductive success of *Acartia clausi*, a common calanoid copepod of the North Sea (Fransz et al. 1991). Experiments were carried out with focus on the functional response of feeding and fecundity of females in relation to increasing concentrations of toxic *A. lusitanicum* and the non-toxic cryptophycean *Rhodomonas baltica*. Results of experiments conducted to investigate influences of prolonged exposure to toxic algae on egg production and survival are also reported.

MATERIAL AND METHODS

Phytoplankton culture. *Alexandrium lusitanicum*, clone BAH-ME 91, was obtained from the Biologische Anstalt Helgoland located at List/Sylt (Germany). Cultures were kept in 1 to 3 l flasks containing TL-Medium (Larsen et al. 1994) in seawater of 30‰ salinity and exposed to a 16 h light:8 h dark irradiance cycle of 85 µE m^{-2} s^{-1}. Algae were maintained at 15°C in a temperature controlled walk-in chamber. Culture conditions for *Rhodomonas baltica* were similar except for an irradiance of 120 µE m^{-2} s^{-1}. The carbon and nitrogen content of both species was determined from subsamples of exponentially growing cultures, filtered on precombusted GF/C filters at low pressure, dried at 60°C and combusted in a Carlo Erba Nitrogen Analyzer Model 1500. Cell carbon and nitrogen were calculated by counts of triplicate subsamples on a Coulter Multisizer II model equipped with a 100 µm orifice. Because both species differ in size and carbon and nitrogen content (Table 1), experimental food concentrations were based on cell carbon concentration in order to facilitate comparison between algae. Only exponentially growing cultures were used in experiments. Variations in the toxicity of several *Alexandrium* strains occur during growth in batch cultures, with higher toxicities observed in the exponential growth phase (Boyer et al. 1987, Anderson et al. 1990). For this reason *A. lusitanicum* batch cultures were grown to a concentration of approximately 2000 cells

Table 1. Equivalent spherical diameter (ESD) measured by a Coulter Counter, carbon and nitrogen content and weight ratio of carbon to nitrogen (C:N) of algae used in experiments (mean ± SD; n = number of C/N determinations)

Species	ESD (µm)	C $cell^{-1}$ (pg)	N $cell^{-1}$ (pg)	C:N	n
Alexandrium lusitanicum	19.36	1005.1 ± 18.8	205.1 ± 1.85	4.9 ± 0.1	4
Rhodomonas baltica	6.62	52.9 ± 1.8	11.5 ± 0.4	4.6 ± 0.1	5

ml^{-1} in order to minimize possible effects of varying toxicities on *Acartia clausi*. At this growth stage, toxin content of *A. lusitanicum* was determined by HPLC to vary between 1.25 and 1.56 pg $saxitoxin_{eq.}$ $cell^{-1}$ (Dutz et al. unpubl.). The toxin profile was dominated by the gonyautoxins GTX 1, 4 (74%) followed by GTX 2, 3 (24%); only gonyautoxins were recorded.

Copepods. Females used in experiments were taken from a culture maintained at the institute to provide a continuous supply of organisms. *Acartia clausi* cultures were initiated from copepods collected in the German Bight near Helgoland in March 1996. Culture conditions were the same as for the algae except that copepods were kept at an irradiance of 3 to 11 µE m^{-2} s^{-1}. The culture was maintained in 1350 ml glass bottles fixed onto a plankton wheel. Copepods were fed a diet of *Rhodomonas baltica* at satiation levels. The culture was well established before starting the experiments and several generations were obtained by incubating freshly produced eggs. Experiments were performed between October 1996 and March 1997.

Functional response to increasing concentrations. Experiments were conducted to determine the feeding and egg production response of *Acartia clausi* to concentrations of both algae ranging from 200 to 1600 µg C l^{-1}. Food suspensions for estimations of feeding rates were prepared by diluting batch cultures with appropriate amounts of algal growth medium to prevent nutrient limitation of algal growth during incubation.

Adult females for feeding experiments were selected from the continuous culture and pipetted into 625 ml Duran bottles containing the food suspensions. Following a 24 h acclimatization period, females were transferred into new bottles containing the same algal suspension as before and experiments were run for additional 24 h. Bottles were carefully closed to prevent any air bubbles and placed onto a plankton wheel (40 cm diameter, 1.5 rpm) to maintain uniform cell distributions. For each food concentration, 3 experimental bottles containing 10 females each and 3 control bottles without copepods were run simultanously. Cell concentrations were measured from triplicate 100 ml subsamples preserved in Lugol's solution before and after the experimental procedure by counts on a Coulter Counter. In the case of *Rhodomonas baltica*, subsamples were immediately counted because fixation artifacts occurred as described by Klein Breteler (1985). However, repeated tests showed that counts of living and fixed cells were constant for 1 h. Rates of ingestion were calculated by applying formulas given by Frost (1972). Total particle reduction in experimental bottles decreased from on average 25 to 7% and 10 to 5% with increasing food concentration of *Alexandrium lusitanicum* and *R. baltica*, respectively.

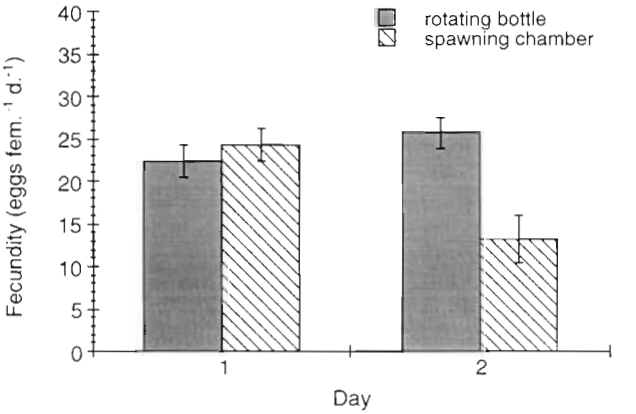

Fig. 1. *Acartia clausi*. Daily egg production of females obtained by 2 incubation methods. Females were fed *Rhodomonas baltica* at 600 µg C l^{-1}. Vertical bars indicate standard error of the mean

Egg production rates of females fed each phytoplankton species were examined by incubating 5 females in each of 3 experimental bottles. The experimental procedure was the same as in the ingestion experiments. In a previous experiment differences in egg production rates obtained by 2 methods were tested. Five females were incubated in each of 3 rotating 625 ml screw-cap bottles or 3 commonly used spawning chambers (volume of spawning chamber = 700 ml) equipped with a bottom of 100 µm mesh gauze in order to prevent copepods feeding on their eggs (Fig. 1). Results revealed no significant differences for the first day (2-tailed *t*-test, $t_s = -0.85$, Sokal & Rohlf 1995). Instead, after the second day of incubation the number of eggs produced in the spawning chamber was lower than in the bottle treatment due to sedimentation of food particles at the bottom of the chamber. Additionally, death of females was observed in spawning chambers because they were trapped between the meshes. Therefore it was decided to incubate females in rotating bottles as a standard procedure. After an acclimatization period of 24 h, experiments were run for 24 h. Females were removed from the bottles. Eggs laid were concentrated on 20 µm mesh gauze, transferred to petri dishes and counted under a dissecting microscope at 66× magnification. Empty egg shells were included in the counts, but they rarely contributed more than 2% of the total egg number.

Upon termination of feeding and egg production experiments, females were anaesthetized with MS 222 and their prosome length was measured under an inverted microscope at 100× magnification. After recovery in filtered seawater, groups of 30 to 40 females of known length were pooled on a precombusted GF/C filter (500°C, 4 h) and dried at 60°C. The carbon and nitrogen content was determined and

length-weight factors for females of 5.6 ± 0.52 µg C mm^{-1} and of 1.4 ± 0.13 µg N mm^{-1} (C:N = 4.1 ± 0.05, mean weight = 4.8 ± 0.58 µg C $female^{-1}$, n = 7) were established in order to calculate the gross growth efficiency of egg production of *Acartia clausi* feeding on both algae.

Long-term egg production and survival. Two experimental setups were designed to examine the effect of long-term exposure of copepods to toxic *Alexandrium lusitanicum*. First, an experiment was performed to test if *A. lusitanicum* affects survival of *Acartia clausi*. Females, sorted out of a cohort of copepods that had freshly moulted, were incubated in 625 ml screw-cap bottles containing food concentrations at satiation levels of 1200 µg C l^{-1} of either *A. lusitanicum* or *Rhodomonas baltica*. Five females were added to each of triplicate experimental bottles. Every 3 to 4 d, food suspensions were exchanged and restored to initial concentrations. Alternatively, females were transferred to new bottles to prevent accumulation of eggs. Copepods were checked for physiological condition daily and assigned to 1 of 3 catagories (sensu Teegarden & Cembella 1996): healthy, impaired or dead. If necessary, dead females were removed and the experiment continued until the last copepod died.

In a second experiment, egg production was monitored over a period of 9 d to investigate if prolonged exposure of females to toxic *Alexandrium lusitanicum* resulted in changes in egg production rates compared to short-term experiments. Five females were incubated in triplicate experimental bottles at 1000 µg C l^{-1} of each algae. Every day females were pipetted into new bottles containing the identical food suspensions. Their physiological condition and egg production were checked as described above. After 6 d, food conditions were switched and females kept in *A. lusitanicum* were transferred to bottles containing *R. baltica* as food and vice versa. Incubation continued for 3 more days.

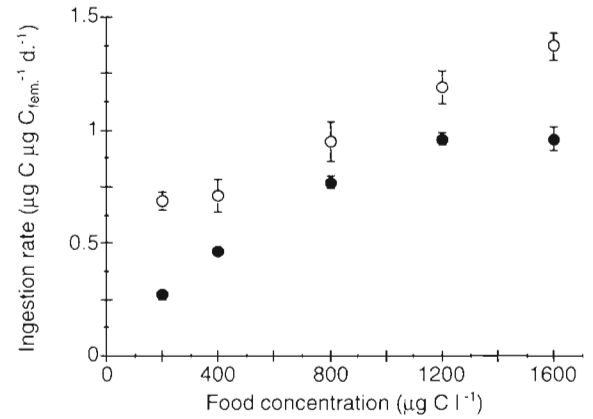

Fig. 2. *Acartia clausi*. Ingestion rates of females as a function of food carbon concentration of either *Alexandrium lusitanicum* (○) or *Rhodomonas baltica* (●). Vertical bars indicate standard error of the mean

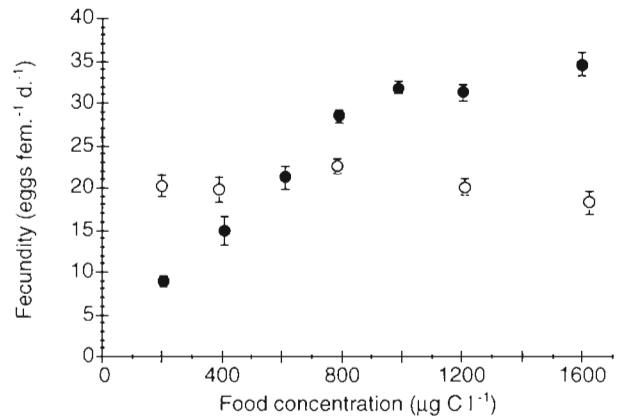

Fig. 3. *Acartia clausi*. Daily egg production of females as a function of food carbon concentration of either *Alexandrium lusitanicum* (○) or *Rhodomonas baltica* (●). Vertical bars indicate standard error of the mean

RESULTS

Functional response to increasing concentrations

Feeding rates of *Acartia clausi* observed in response to increasing food concentrations of *Alexandrium lusitanicum* and *Rhodomonas baltica* revealed that females actively preyed upon *A. lusitanicum* in unialgal treatments and did not avoid ingestion of toxic cells. Both algae were ingested at rates proportional to their abundance (Fig. 2). However, the functional response to increasing particle concentrations differed between the food treatments. For females feeding on *R. baltica*, ingestion rate satiated at food concentrations of 1200 µg C l^{-1}. Maximum daily ingestion was 0.96 µg C µg C_{female}^{-1} d^{-1} and similar at 1200 and 1600 µg C l^{-1}. In contrast, ingestion rates of females feeding on *A. lusitanicum* increased progressively with increasing amounts of food and did not appear to satiate over the range of concentrations examined. Generally, *A. clausi* preyed on *A. lusitanicum* at higher rates than on *R. baltica*. A maximum ingestion of 1.37 µg C µg C_{female}^{-1} d^{-1} was attained at 1600 µg C l^{-1}. Thus, maximal ingestion rates exceeded those observed with *R. baltica* as food by more than one third.

Egg production by *Acartia clausi* fed on *Rhodomonas baltica* exhibited a functional response to increasing food concentrations similar to that observed in feeding experiments (Fig. 3). Mean egg production rates satiated at 1000 µg C l^{-1} when 32 eggs $female^{-1}$ d^{-1} were produced and remained constant with further in-

Table 2. *Acartia clausi*. Carbon and nitrogen budget for copepods feeding on *Rhodomonas baltica* and *Alexandrium lusitanicum*. GGE: gross growth efficiency of egg production. See text for further explanations

Species	Food conc. ($\mu g\ C\ l^{-1}$)	Ingestion (I) ($\mu g\ C\ \mu g^{-1}\ C\ d^{-1}$)	($\mu g\ N\ \mu g^{-1}\ N\ d^{-1}$)	Egg production (E) ($\mu g\ C\ \mu g^{-1}\ C\ d^{-1}$)	($\mu g\ N\ \mu g^{-1}\ N\ d^{-1}$)	GGE = E/I C	N
Rhodomonas baltica	200	0.27	0.24	0.07	0.06	0.27	0.24
	400	0.46	0.41	0.12	0.10	0.26	0.24
	800	0.77	0.69	0.22	0.18	0.28	0.26
	1200	0.96	0.86	0.25	0.21	0.27	0.24
	1600	0.96	0.86	0.26	0.21	0.27	0.25
Alexandrium lusitanicum	200	0.69	0.58	0.16	0.13	0.23	0.22
	400	0.71	0.59	0.15	0.12	0.21	0.21
	800	0.95	0.79	0.17	0.14	0.18	0.18
	1200	1.19	1.00	0.16	0.13	0.14	0.13
	1600	1.37	1.15	0.14	0.12	0.10	0.10

creases in food concentration. Results obtained in experiments with *Alexandrium lusitanicum* as a food organism demonstrate that females were able to produce eggs on a diet of toxic algae. Egg production rates of less than 1 egg d^{-1} by females incubated without food under similar conditions (results not shown) indicate that fecundity depended on ingested *A. lusitanicum* and not on stored energy. However, in contrast to the functional response shown by females exposed to *R. baltica*, daily egg production did not increase in response to increasing amounts of *A. lusitanicum* offered. Eggs were produced at similar rates of 16 to 24 eggs $female^{-1}\ d^{-1}$ and were not significantly different between treatments (single classification ANOVA, $F_{4,10} = 2.72$, $p > 0.05$, Sokal & Rohlf 1995).

Weight-specific egg production rates in terms of carbon and nitrogen were calculated from mean rates obtained in experiments in order to estimate gross growth efficiencies of egg production of *Acartia clausi* exposed to varying concentrations of both algae (Table 2). Mean numbers of eggs produced were converted to carbon applying an average egg weight of 0.036 µg C given for *A. clausi* by Kiørboe & Sabatini (1995). Nitrogen was calculated assuming a C:N ratio of 5.05 as determined for *Acartia tonsa* eggs (Kiørboe et al. 1985). Since *Alexandrium lusitanicum* and *Rhodomonas baltica* had corresponding C:N ratios (Table 1), the relationship of ingestion rates calculated in terms of carbon and nitrogen is similar for both algae.

In experiments performed with *Rhodomonas baltica* as food, specific ingestion rates of carbon and nitrogen varied in a manner similar to specific egg production rates. Thus, females of *Acartia clausi* converted ingested food into eggs at nearly constant efficiencies of 0.26 to 0.28 for carbon and 0.24 to 0.26 for nitrogen. In contrast, gross growth efficiencies of females exposed to *Alexandrium lusitanicum* decreased with increasing food concentration from 0.23 for carbon and 0.22 for nitrogen at 200 µg C l^{-1} to 0.10 for both carbon and nitrogen at 1600 µg C l^{-1}. Because egg production remained constant over the range of concentrations examined, females of *A. clausi* were apparently unable to convert the amount of food ingested in excess of the rate observed at 200 µg C l^{-1} into eggs resulting in a linear decrease of the gross growth efficiency (Fig. 4). The regression of efficiencies in dependence of food concentration yields statistically significant results for carbon (ANOVA, $F_{1,3} = 308$, $p < 0.001$, Sokal & Rohlf 1995) and nitrogen (data not shown). Even at low food concentrations, females did not attain the efficiency observed with *R. baltica* despite a higher number of eggs produced at 200 and 400 µg C l^{-1}. Apparently, *A. lusitanicum* did not favor high egg production in *A. clausi*.

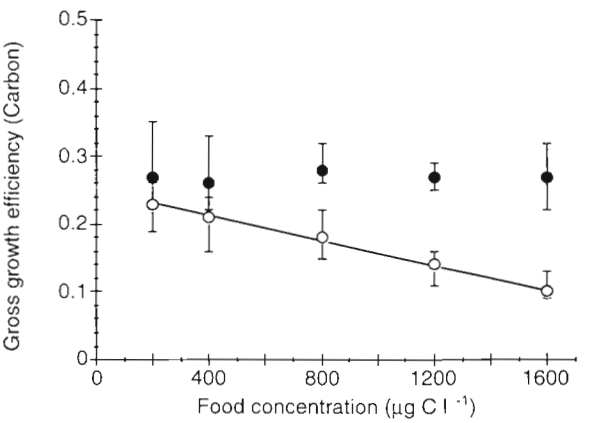

Fig. 4. *Acartia clausi*. Relationship between calculated gross growth efficiencies in terms of carbon and food carbon concentrations for females feeding on either *Alexandrium lusitanicum* (○) or *Rhodomonas baltica* (●). Vertical bars indicate maximum and minimum estimates. (Regression is: $b = -9.5e^{-0.5a}$, $a = 0.25$, $r^2 = 0.79$)

Long-term experiments

Results of the effects of prolonged exposure on the fecundity of *Acartia clausi* revealed that females were able to sustain egg production on a diet of toxic *Alexandrium lusitanicum* for several days (Fig. 5). However, the lower reproductive response observed in females exposed to *A. lusitanicum* resembled that obtained in short-term experiments and persisted during the 6 following days of incubation. Daily fecundity of females on a diet of toxic *A. lusitanicum* was on average 30% lower than that on non-toxic *Rhodomonas baltica*. Initial egg production rates by females feeding on *R. baltica* increased from 32 to 38 eggs female^{-1} d^{-1} and stayed constant with further incubation. A similar pattern of daily fecundity occurred in copepods kept in *A. lusitanicum* suspensions, but mean number of eggs produced increased more slowly from initially 22 eggs female^{-1} d^{-1} to 29 eggs female^{-1} d^{-1} on the fifth day, indicating that an equilibrium was achieved with delay. Upon exchange of the diet following Day 6 of incubation, daily fecundity of females acclimated on *R. baltica* declined rapidly to 26 eggs female^{-1} d^{-1} on Day 8, a rate that is representative of females reared on *A. lusitanicum* in the first part of the experiment. Conversely, females acclimatized to *A. lusitanicum* were not able to increase their egg production when fed with *R. baltica* at satiating levels and produced eggs at more or less constant rates of 24 to 29 eggs female^{-1} d^{-1} on 3 consecutive days. These results suggest that toxic *A. lusitanicum* affect egg production of *A. clausi* beyond direct exposure and recovery may take longer than the 3 d of observations.

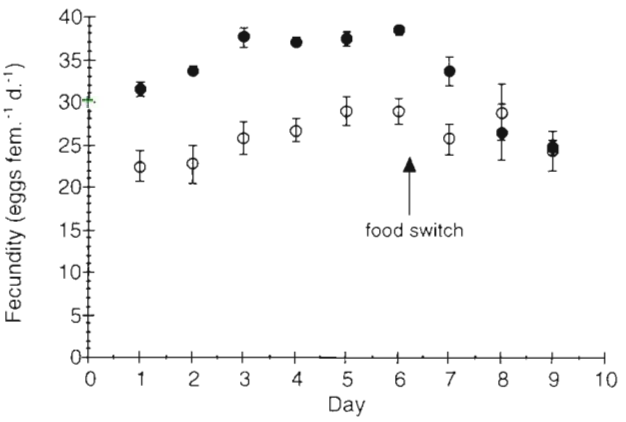

Fig. 5. *Acartia clausi*. Daily variations in fecundity of females fed either *Alexandrium lusitanicum* (O) or *Rhodomonas baltica* (●) at satiation levels. On Day 6 of the incubation, food type was exchanged between treatments. For clearness symbols continue. Vertical bars indicate standard error of the mean

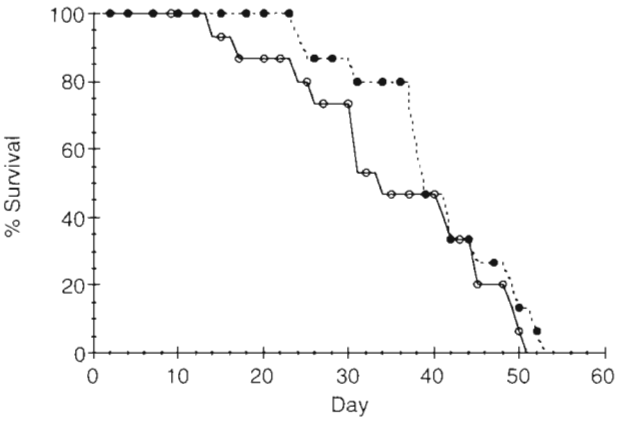

Fig. 6. *Acartia clausi*. Survival curves of females fed either *Alexandrium lusitanicum* (O) or *Rhodomonas baltica* (●) at satiation levels. Not all data points are shown

Survival of female *Acartia clausi* fed both algae at satiation concentrations was generally high during the initial 2 wk of incubation (Fig. 6). In experiments performed with a diet of *Alexandrium lusitanicum* the first female died on Day 14, followed by a steady decrease in cumulative survival until the last female died on Day 51. Survival of copepods feeding on *Rhodomonas baltica* appeared to be higher during the initial phase of the experiment. No mortality occurred during the first 3 wk. Cumulative survival began to decrease slowly from 100% on Day 24 to 80% on Day 38 followed by a sharp increase in mortality. Median survival time of 39 d of females feeding on *R. baltica* did not differ significanty from median survival of 35 d shown by females reared on *A. lusitanicum* (Mann-Whitney *U*-test, 2-tailed, U_s = 111, Sokal & Rohlf 1995).

Changes observed in the physiological condition of females exposed to *Alexandrium lusitanicum* followed the same temporal pattern in both the survival and the long-term egg production experiment and were therefore summarized (Fig 7). Results shown for the first 48 h of incubation were also representative for observations made in short-term feeding and egg production experiments. Death of females was rare; most of the females appeared healthy subsequent to the acclimatization period. Impaired females contributed one third of total females after 24 h. Typically, they were lethargic and displayed a reduced pipette avoidance behaviour as described for *Acartia hudsonica* by Ives (1985). Examination of these females under the dissecting microscope revealed an unusual trembling of the first antennae and uncoordinated appendage movements that may indicate a loss of neuromuscular control due to accumulated toxins. However, a constant feature observed throughout the experiments was that impairment of copepods decreased with fur-

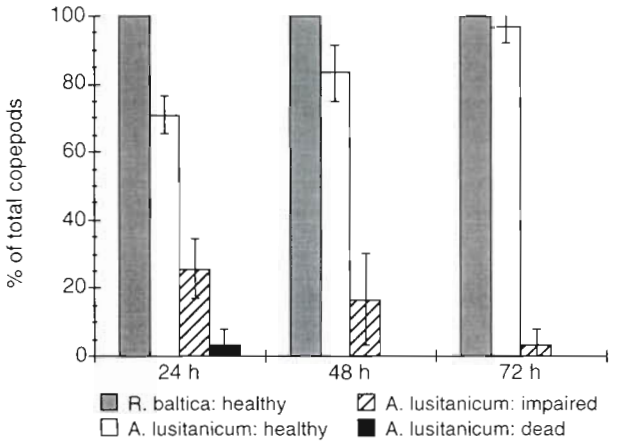

Fig. 7. *Acartia clausi*. Physiological condition of females feeding on either *A. lusitanicum* or *R. baltica* for 24, 48 and 72 h at satiation levels. Vertical bars indicate standard error of the mean

ther incubation to 4% of total copepods after 3 d of exposure to *A. lusitanicum*. Upon further incubation all of the copepods appeared healthy as observed in experiments conducted with *Rhodomonas baltica* as food, but impairment occurred irregularly from time to time (data not shown).

DISCUSSION

The present study reveals that *Acartia clausi* fed on *Alexandrium lusitanicum* without lethal implications. Ingestion of toxic cells by females was accompanied by short-term impairment, but adverse effects ceased on further incubation and did not result in reduced survival in comparison to high quality food. Ingestion rates obtained in feeding experiments were generally high and similar to rates reported for certain species of *Acartia* feeding on non-toxic dinoflagellates (Ives 1985, Stoecker & Sanders 1985, Kleppel & Burkart 1995). Several other studies have been conducted to understand feeding responses of copepods to toxic *Alexandrium* spp. Results have demonstrated not only behavioural rejection of algae or reduced grazing but also high ingestion rates (Ives 1985, Huntley et al. 1986, Turriff et al. 1995, Teegarden & Cembella 1996) which questions the potential significance of saxitoxins in controlling grazer interactions between toxic *Alexandrium* spp. and copepods.

It has been suggested that behavioural rejection prior to ingestion or an adverse physiological reaction to ingested phycotoxins accounts for the observed suppression of feeding rates (Huntley et al. 1986, Ives 1987, Turriff et al. 1995). In most of these investigations conclusions have been derived from short-term incubation experiments. For instance, Ives (1985, 1987) conducted experiments of 4 to 8 h without prior acclimatization of copepods to toxic strains of *Alexandrium tamarense*. From observations of copepod paralysis and decreasing ingestion rates with increasing toxicity of strains he concluded that copepods suffered from progressive physiological incapacitation which interfered with coordinated appendage movements and cell capture abilities. Similar adverse effects of *A. lusitanicum* on *Acartia clausi* were recorded in the present study. However, the observed recovery on further incubation strongly suggests that females were able to cope with ingested toxins. Therefore, the incubation time in experiments performed by Ives (1985, 1987) may have been too short for copepods to become accustomed to toxins. This in turn would lead to the observed reduction in feeding rates. Conversely, in other short-term experiments interspecific differences were found in feeding responses to toxic *Alexandrium* spp., with high and unaffected feeding rates in one species in comparison to the other (Huntley et al. 1986, Teegarden & Cembella 1996). The differences have been attributed to a diel feeding rhythm or species-specific rejection behaviour. Alternatively, these results may indicate that different abilities of species to cope with ingested toxins account for variable initial feeding responses observed in copepods. The present results suggest that short-term experiments should be interpreted with caution with respect to feeding behaviour.

Physiological implications of toxins could be of minor influence in determining feeding interactions because the significance of saxitoxins as antipredator compounds is doubtful. The present results provide no evidence for a reduced feeding on toxic cells (Huntley et al. 1986, Turriff et al. 1995). High feeding rates on *Alexandrium lusitanicum* by *Acartia clausi* generally support the conclusion of Teegarden & Cembella (1996) that saxitoxins are not crucial in determining feeding interactions between toxic *Alexandrium* spp. and copepods. Additionally, studies demonstrating behavioural rejection of *Alexandrium* spp. found no convincing evidence that saxitoxins were responsible for the observed feeding inhibition and hypothesized that other bioactive compounds induce avoidance behaviour in copepods (Huntley et al. 1986, Turriff et al. 1995). Similarly, in a recent study enhanced mortality observed in *Euterpina acutifrons* exposed to toxic *A. minutum* has been supposed to be related to toxins other than PSP that account for haemolytic activity (Bagøien et al. 1996). Because several non-toxic dinoflagellates were rejected by copepods as well (see Turner & Tester 1997), one might speculate that they share a common allelopathic chemical with some *Alexandrium* species or, alternatively, that distinct

compounds act as antipredator mechanisms. As most of the studies investigating feeding responses of copepods used different strains of *Alexandrium*, the role of saxitoxins or other compounds in guiding species-specific interactions and the ability of copepods to cope with them remain unresolved.

In addition to toxicity or other bioactive chemical compounds, Turner & Tester (1997) suggested that a lack of co-evolutionary experience in dealing with toxins might also be responsible for observed rejection of toxic cells by copepods. The present results demonstrate that this is not necessarily the case. Although no toxic blooms of *Alexandrium lusitanicum* or other PSP-producing dinoflagellates have been recorded from the German Bight, females of *Acartia clausi* ingested *A. lusitanicum* and apparently were able to cope with toxins without any prior experience. Interestingly, only some females were adversely affected subsequent to ingestion of toxic cells, indicating that intraspecific differences also exist in copepods.

The divergent responses in feeding and fecundity of females in relation to increasing food quantities of both algae afford further insights into the interaction of toxic *Alexandrium* and *Acartia*. The limited fecundity on a diet of *Alexandrium lusitanicum*, contrasting with high and constantly increasing ingestion rates over the range of concentrations considered, indicates that the reduced egg production is associated with ingestion of toxic cells. A reduced fecundity in response to feeding on *Alexandrium tamarense* was also demonstrated for *Calanus helgolandicus* by Gill & Harris (1987). Thus, results contrast with previous investigations on the impact of algae producing toxins other than saxitoxin, which revealed that suppressed fecundity resulted from feeding avoidance of these algae (Nielsen et al. 1990, Uye & Takamatsu 1990, Nejstgaard & Solberg 1996, Turner et al. 1998).

Apart from several environmental factors such as temperature or salinity that influence feeding or fecundity in copepods (Uye 1981, Stearns et al. 1989) and have been kept constant in the present study, the coherence of feeding and fecundity depends largely on food concentration and food quality. Algal species as well as their size, shape and nutritional composition are considered as important attributes of food quality that determine functional responses of copepods (Libourel Houde & Roman 1987, Støttrup & Jensen 1990, Jónasdóttir 1994). Size and shape likely influenced results obtained in the present study because food sources differed greatly in size and particle capture efficiency of *Acartia* spp. increases with increasing particle size up to an optimum food diameter (Nival & Nival 1976, Berggreen et al. 1988, Stóttrup & Jensen 1990). *Alexandrium lusitanicum* fits well into the optimum particle size of 15 to 50 µm reported for *Acartia clausi* (Nival & Nival 1976). Therefore, enhanced capture efficiency for *A. lusitanicum* explains the higher feeding rates and also higher fecundity at low food concentrations in contrast to the smaller *Rhodomonas baltica*. However, this cannot account for the observed divergent responses in feeding and fecundity observed on a diet of *A. lusitanicum*. Instead, estimated gross growth efficiencies indicate that even at low food concentrations the ingested amount of *A. lusitanicum* is less efficiently converted into eggs than ingested *R. baltica*. Thus, *A. lusitanicum* may be less nutritious for copepods.

Several studies have emphasized the importance of food biochemical composition in copepod nutrition (see Ianora 1998 for review). Variable algal nitrogen contents generated different responses in egg production rates of copepods (Checkley 1980, Kiørboe 1989), but likely did not contribute to lower efficiencies because the amount of nitrogen ingested by *Acartia clausi* feeding on *Alexandrium lusitanicum* was higher than on *Rhodomonas baltica*. Although some specific nutrients or trace substances required for high reproductive success may be lacking in *A. lusitanicum*, the observed non-satiated feeding on this alga suggest that an alternative mechanism could be responsible for the divergent responses. Previous studies on the influence of food nutritional composition on copepod feeding demonstrated that copepods increase their feeding effort in response to decreasing nutritional quality of food particles (Paffenhöfer & Van Sant 1985, Libourel Houde & Roman 1987). In this context, the non-satiated feeding on *A. lusitanicum* observed in the present experiments suggests that females obtained less nutrients or energy from the ingested amount of food than that needed to meet nutritional requirements for high egg production. As pointed out by Paffenhöfer & Van Sant (1985), the actual amount of nitrogen assimilated by copepods rather than nitrogen availability should govern satiation. As indicated by the high nitrogen content, the nutritional quality of *A. lusitanicum* is not expected to be poor. Thus, a nutritional shortage could result from the interference of ingested toxins with digestive or assimilatory processes. Alternatively, an enhanced energy expenditure of females caused by ingested or assimilated toxins could account for energetic deficiencies.

Assimilation depends largely on digestibility of food particles, digestive enzyme activities and assimilative capabilities of the copepod. A lower digestibility of the cellulose cell wall of *Alexandrium lusitanicum* likely did not influence digestive processes because Harvey et al. (1987) observed high assimilation in *Calanus helgolandicus* feeding on a non-toxic dinoflagellate. Additionally, several dinoflagellates have been reported to support egg production as high as that obtained from diatoms and flagellates (Ianora & Poulet

1993, Jónasdóttir 1994), indicating a high digestibility of dinoflagellates. Preconditioning and trophic history also affect a wide range of functions related to copepod nutrition, such as feeding selectivity, feeding rate and physiological responses (Donaghay 1988). Digestive enzyme activity in *Acartia* spp. has been shown to require a minimum of 2 d to acclimatize to changes in food ration and food type (e.g. Mayzaud et al. 1992). Accordingly, fecundity responds relatively quickly in *Acartia* spp. and achieves an equilibrium with ingested food in 1 to 3 d (Kiørboe et al. 1985, Tester & Turner 1990). A similar response in egg production was observed at the beginning of the long-term experiment in females feeding on *Rhodomonas baltica* and, with delay, on *A. lusitanicum*. The persistence of reduced fecundity for several days on a diet of the toxic dinoflagellate, however, provides no evidence that low initial egg production rates were caused by affected digestive enzyme activities or assimilatory capabilities due to rearing of *Acartia clausi* on *R. baltica* prior to the experiments. Thus, neither low digestibility nor feeding history contributed to the observed nutritional deficiencies of *A. clausi*. The results obtained upon dietary exchange from *A. lusitanicum* to *R. baltica* further demonstrate that fecundity was affected adversely beyond direct exposure to the toxic alga. If a low nutritional content or low digestibility of *A. lusitanicum* had been responsible for reduced fecundity, then egg production should increase subsequently with the transfer to *R. baltica*, at least on Day 9 of incubation. Thus, ingested and accumulated toxins probably account for the nutritional deficiencies in females.

Saxitoxins are known to block sodium channels in cell membranes which are important for maintaining cell resting potential, generating electrical membrane signals or controlling hormonally triggered or metabolic processes (Strichartz & Castle 1990). Therefore they could interfere with the control of enzyme secretion or active uptake of digested food compounds and consequently reduce the nutritional gain from ingested food. Alternatively, internal sequestering or detoxification of ingested toxins which may be an adaption of copepods (White 1981, Ives 1987) could cause an enhanced energy expenditure. Recovery of the physiological impairment after 3 d of incubation and the survival rates unaffected by *Alexandrium lusitanicum* indicate that females were able to cope with ingested toxins. If detoxification or internal sequestering accounts for lack of persistent adverse effects, then the processes to accomplish them could be energetically expensive and contribute to enhanced nutritional demands in females. Direct effects of saxitoxins on gonad maturation are also conceivable, but are unlikely to cause nutritional deficiencies. Therefore it is concluded that the reduced fecundity observed in *Acartia clausi* primarily resulted from the interference of saxitoxins with digestive processes or energetic expenditure due to detoxification, or both mechanisms, which diminish the nutritional gain from ingested food. Consequently, females seem to compensate for this deficiency by increasing their feeding effort, as indicated by non-satiated feeding.

The present study further demonstrated that females of *Acartia clausi* were unable to convert the amount of food ingested in excess of the rate observed at 200 µg C l^{-1} into eggs. These results suggest that a critical concentration of the amount of toxins ingested or accumulated exists upon which no further nutrients or energy could be attained to support egg production. Interestingly, this corresponds well with satiation responses in toxin accumulation observed for copepods feeding on increasing concentrations of toxic *Alexandrium* spp. (Gosselin et al. 1989, Robineau et al. 1991). However, the mechanism that caused reduced fecundity in *A. clausi* as well as toxin dynamics in copepods are poorly understood and need further investigations in which respiration and egestion are monitored in addition to food uptake and secondary production.

Previous reports on zooplankton grazing interactions with toxic *Alexandrium* spp. unaffected demonstrate a wide variation of responses ranging from avoidance to unaffected food uptake (Turner & Tester 1997). However, the factors controlling variable feeding responses are largely unknown and remain uninvestigated. The high grazing rates of *Acartia clausi* on toxic *Alexandrium lusitanicum* observed in the present study suggest that saxitoxins do not act as allelochemical compounds against zooplankton grazing. Nevertheless, feeding on toxic *Alexandrium* spp. unaffected has important consequences. First, it emphasizes the high significance of copepods as toxin vectors in the marine food web because toxin accumulation is well demonstrated (e.g. White 1981). Second, ingestion of toxic food depresses fecundity of copepods, because there is a less efficient utilization of ingested food. The proposed mechanisms of toxin action on fecundity in copepods suggest that not only egg production but also growth of copepods may be reduced by phytoplankton that produce saxitoxins. Additionally, the utilization of non-toxic food ingested together with toxic algae may also be adversely affected, leading to a trophic mismatch and reduced secondary production.

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