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Latitudinal Differentiation in the Effects of the Toxic Dinoflagellate Alexandrium spp. on the Feeding and Reproduction of Populations of the Copepod Acartia Hudsonica

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Latitudinal differentiation in the effects of the toxic dinoflagellate *Alexandrium* spp. on the feeding and reproduction of populations of the copepod *Acartia hudsonica*

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Abstract

Blooms of the dinoflagellate *Alexandrium* spp. increase in their frequency, toxicity and historical presence with increasing latitude from New Jersey (USA) to the Gaspé peninsula (Canada). Biogeographic variation in these blooms results in differential exposure of geographically separate copepod populations to toxic *Alexandrium*. We hypothesize that the ability of copepods to feed and reproduce on toxic *Alexandrium* should be higher in copepods from regions that are frequently exposed to toxic *Alexandrium* blooms. We tested this hypothesis with factorial common environment experiments in which female adults of the copepod *Acartia hudsonica* from five separate populations ranging from New Jersey to New Brunswick were fed toxic and non-toxic strains of *Alexandrium*, and the non-toxic flagellate *Tetraselmis* sp. Consistent with the hypothesis, when fed toxic *Alexandrium* we observed significantly higher ingestion and egg production rates in the copepods historically exposed to toxic *Alexandrium* blooms relative to copepods from regions in which *Alexandrium* is rare or absent. Such differences among copepod populations were not observed when copepods were fed non-toxic *Alexandrium* or *Tetraselmis* sp. These results were also supported by assays in which copepods from populations both historically exposed and naïve to toxic *Alexandrium* blooms were fed mixtures of toxic *Alexandrium* and *Tetraselmis* sp. Two-week long experiments demonstrated that when copepods from populations naïve to toxic *Alexandrium* were fed a toxic strain of *Alexandrium* they failed to acclimate, such that their ingestion rates remained low throughout the entire two-week period. The differences observed among populations suggest that local adaptation of populations of *A. hudsonica* from Massachusetts (USA) to New Brunswick (Canada) has occurred, such that some populations are resistant to toxic *Alexandrium*.

Keywords: *Acartia*; Adaptation; *Alexandrium*; Biogeography; Evolution; Feeding deterrence; Red tide algae; Toxin resistance; Zooplankton

1. Introduction

It has recently been demonstrated that the ecological relationship between some zooplankton grazers and harmful phytoplankton blooms is closely shaped by their evolutionary history (Hairston et al., 1999; Hairston et al., 2002). Freshwater studies examining the grazer–toxic algae relationship have shown that the populations of *Daphnia* from lakes where toxic cyanobacteria have bloomed for generations have evolved resistance to the toxic algae (Gilbert, 1990; Hairston et al., 1999; Hairston et al., 2002). The resistance to toxic algae has enabled the zooplankton to feed and grow at higher rates in the presence of the toxic cyanobacteria than *Daphnia* never exposed to the toxic algae.

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Harmful algal blooms (HABs) in marine environments are increasing worldwide (Hallegraeff, 1993). Since, these blooms only occur in the presence of relaxed grazing pressure, it is important to understand how HAB algae affect zooplankton grazing rates. However, if marine zooplankton populations are able to adapt to the toxic algae, then their relationship to the toxic algae must be examined in the context of the historical exposure of each population to the algae.

The biogeographic characteristics of the toxic dinoflagellate *Alexandrium* spp. (Balech, 1990) along the northeast coast of North America are ideal to examine whether historical exposure of marine zooplankton affects their ecological relationship with toxic algae. Biogeographic variation in the toxin content and bloom characteristics of the dinoflagellate throughout the Northeast coast of North America has been well documented (Cembella et al., 1988; Anderson et al., 1994; Anderson, 1997). In this region, *Alexandrium* has two morphospecies that have been linked to the production of paralytic shellfish poisoning (PSP) toxins (*A. tamarense* and *A. fundyense*). Blooms of highly–moderately toxic *Alexandrium fundyense* occur annually along the East Coast of Canada south to Massachusetts Bay. South of Massachusetts Bay there are isolated blooms of *A. fundyense* and *A. tamarense* on Cape Cod and the southeast New England coast in enclosed embayments and salt ponds. These southern blooms have lower toxicity and occur less regularly (Maranda et al., 1985; Cembella et al., 1988; Anderson et al., 1994; Anderson, 1997). From detailed surveys of cysts and motile planktonic cells along the New Jersey coast, it is known that *A. tamarense* occurs in one isolated embayment along the northern New Jersey coast where it has only rarely bloomed, but it is not toxic (Cohn et al., 1988; Mahoney et al., 1995; Anderson, 1997). In addition to higher toxicity levels, *Alexandrium* blooms in Canada and along the Maine coast have a longer history of occurrence, with the first reported PSP event attributed to *Alexandrium* sp. in 1889 in eastern Canada (Prakash et al., 1971). The introduction of *Alexandrium* to more southern waters is believed to have occurred during a large bloom event in 1972 (Anderson et al., 1994). Thus, there appears to be a latitudinal gradient in the historical occurrence, annual frequency and toxicity of *Alexandrium* blooms from north to south along the east coast of North America.

Biogeographic variation in the blooms results in differential exposure of geographically separate copepod populations to toxic *Alexandrium*. In this study, we examine the effects of the toxic dinoflagellate, *Alexandrium*, on copepod grazers from geographically separate populations. We hypothesized that copepod populations from regions which experience frequent and highly toxic blooms of *Alexandrium* exhibit enhanced fitness parameters when feeding on toxic *Alexandrium* compared to copepod populations from regions where the blooms rarely occur and are less toxic. We tested this hypothesis with the calanoid copepod, *Acartia hudsonica* (*clausi*), which occurs throughout the geographic range of *Alexandrium* and is the most abundant copepod in coastal waters during toxic blooms in the north (Teegarden et al., 2001).

2. Materials and methods

2.1. Collection and culturing of copepods and algae

Populations of *Acartia hudsonica* were collected from Passamaquoddy bay, NB, Canada, Casco bay, ME, Great Pond, MA, Mumford Cove, CT, and Great bay, NJ, using a 200 mm mesh plankton net. Upon collection, copepods were transported to the laboratory within 24 h. Cohorts of 1000–1500 individuals from each population were separated and cultured under identical conditions following Feinberg and Dam (1998). This method proved to be an efficient and gentle way to maintain separately, for over a year (~11 generations), the different copepod populations with densities of 500–1000 individuals per 20 l. One concern of maintaining cultures for long periods of time is that their small population sizes may cause genetic drift and decreased genetic variation within the populations. To sustain natural levels of genetic variation, we maintained high copepod densities within the cultures and refreshed the cultures with new individuals from the field each season when *A. hudsonica* was present in the water column.

The copepod cultures were maintained at 12–14 °C and 12/12 h light/dark (L/D) regime during rearing and experiments. The standard rearing diet consisted of a mixture of *Thalassiosira weissflogii*, *Isochrysis galbana*, and *Rhodomonas lens*, which was kept at a

concentration of 400–500 μg C l^{-1} by replenishment every other day. This concentration is near the saturation level of the functional and numerical response of *A. hudsonica*. All copepod populations were reared at the same temperature, light and food regimes for several generations to eliminate both maternal effects and environmental variance. This allowed us to attribute the observed differences among populations to genetic variance (Falconer, 1996). Copepods were cultured for 11 generations before the latitudinal experiment, eight generations before the toxicity experiment and five generations before the acclimation experiment.

Three strains of *Alexandrium* spp., two high-toxin strains and one low-toxin strain, were used for this study (Table 1). The toxic strain of *A. tamarense* was isolated from Casco bay, ME (CB-307 strain; ME *Alexandrium*) and a toxic strain of *A. fundyense* from the bay of Fundy, NB, Canada (NB-05 strain; NB *Alexandrium*). The non-toxic strain of *A. tamarense* was isolated from Mumford Cove, Connecticut (GTCN-16 strain; CT *Alexandrium*). The strain from the bay of Fundy was isolated in the laboratory from cysts. All other strains were obtained from various other laboratories. All cultures were grown in F/2 media (Guillard, 1975) at 14 °C with 12/12 h L/D cycle. The cultures were maintained in exponential growth for use in the experiments by replacing half of the cultured medium with fresh F/2 media each week.

Before each experiment, replicate aliquots of the *Alexandrium* culture to be used were collected for toxin extraction (Table 1). Toxins were extracted according to Anderson et al. (1994) and analyzed by HPLC using methods of Oshima et al. (1989) in our laboratory (our source for the STX standards was NRC, Halifax, Canada). Of the suite of saxitoxins present in *Alexandrium*, we quantified the most potent, saxitoxin (STX), neosaxitoxin (NEO), and gonyautoxins I–IV (GTX1–4). Analysis of this suite of toxins was adequate for this study, since toxin analyses were performed only to confirm that the toxic strains were indeed toxic and that the non-toxic strains were non-toxic. Additionally, the toxins that were not analyzed, the B and C saxitoxins and decarbamoyl saxitoxins, have been found to be the least potent of the toxins (Schatz, 1986; Indrasena and Gill, 1999). More importantly, toxicity levels are not central to this study, since our goal was to examine relative differences among copepods that were fed the same *Alexandrium* strain.

2.2. Latitudinal comparison experiments

Experimental conditions were identical to rearing conditions. A factorial design was used to compare the rate processes of the five *A. hudsonica* populations given four different diets: three strains of *Alexandrium* of varying toxicity (Table 1; isolated from bay of Fundy, NB; Casco bay, ME; Mumford Cove, CT) and the non-toxic flagellate *Tetraselmis* sp. Prior to the experiments, none of the copepods had been exposed to toxic *Alexandrium*. A period of 48 h before an experiment, 20–25 healthy adult *A. hudsonica* females

Table 1
Experimental diets. Means of the equivalent spherical diameter (ESD), carbon content and toxicity of each algal strain are shown at the time of the experiments. The *Alexandrium* strains are identified by the location of collection (CT-Mumford Cove, CT; ME-Casco bay, ME; and NB-bay of Fundy, NB) and the name of the strain

Experiment and diet	Strain name	ESD (μm)	Carbon (μg C per cell)	Toxicity (pg STX equivalent per cell)
Latitudinal comparison				
CT *Alexandrium* sp.	GTCN-16	26.0	2.6×10^{-3}	0.00
ME *Alexandrium* sp.	CB-307	21.5	1.5×10^{-3}	5.22
NB *Alexandrium* sp.	NB-05	26.6	2.8×10^{-3}	16.12
Tetraselmis sp.		8.5	5.7×10^{-5}	
Mixed diet				
ME *Alexandrium* sp.	CB-307	19.6	1.1×10^{-3}	4.93
Tetraselmis sp.		7.6	4.1×10^{-5}	
Acclimation				
NB *Alexandrium* sp.	NB-05	23.7	2.0×10^{-3}	9.25
Tetraselmis sp.		7.4	3.8×10^{-5}	

and 10 males were hand picked from the five copepod cultures and placed into separate 1000 ml beakers filled with 0.2 μm filtered seawater. The beakers were lightly aerated. After 24 h the seawater in each of the vessels was replaced. During this 48 h period, copepods were not fed. This ensured that egg production during the experiments reflected the effects of the experimental diet (Tester and Turner, 1990).

At the end of the starvation period, pairs of females were picked from each population beaker, sized by microscopy, and placed into eight separate 140 ml screw cap bottles containing 70 μg C l^{-1} of the experimental diet. The choice of food concentration was consistent with concentrations of *Alexandrium* that the copepods may encounter during natural *Alexandrium* blooms (Anderson et al., 1983; Watras et al., 1985). Triplicate control bottles contained the diet solution without copepods. The bottles were topped off with the diet solution, sealed to prevent the formation of air bubbles and placed on a plankton wheel, rotating at 1.3 rpm for 24 h. Initial water samples were taken and preserved in a 0.5% acid lugols solution for later cell counts.

After 24 h, samples were taken and preserved for cell counts. Algal concentrations for *Alexandrium* were determined from microscopic cell counts using the Utermöhl (1958) technique. Cell counts for *Tetraselmis* were performed using an Elzone® 280 particle counter, where the algal size distribution used to count cells was determined from initial samples and kept constant for final cell counts. Clearance and ingestion rates were calculated using equations from Frost (1972). To determine the carbon content of the diets (Table 1), aliquots from the grazing control bottles were filtered onto combusted (500 °C, 24 h) GF/F-filter pads and dried. Carbon content was determined using a Carlo-Erba EA1108 elemental analyzer.

Eggs and copepods were counted and examined for general condition and females were resized. The copepods were then kept for 24 h in petri dishes containing 0.2 μm filtered seawater. After this second incubation, females were checked and the newly laid eggs were counted and added to the egg count from the previous day. This allowed us to calculate gross growth efficiencies (GGE):

$$\mathrm{GGE} = \frac{\text{carbon growth}}{\text{carbon ingested}}. \quad (1)$$

The estimate of the GGE assumes that all of the copepod growth was manifested in egg production, which is a reasonable assumption, since there was no significant increase (unpaired *t*-test, $P > 0.01$) in size of the females before and after the experiment.

From each copepod population, we individually placed four replicates of eight eggs into 250 μl wells containing 0.2 μm filtered seawater. Hatching rates were determined according to Tang et al. (1998) over 4 days.

The factorial experiments were designed to allow for statistical comparison using a two-way ANOVA (location versus diet) (Sokal and Rohlf, 1981). In this design, we compared the response of the five copepod populations within and among each diet. Post hoc comparisons employed the Tukey–Kramer method.

2.3. Mixed diet experiment

To test whether *Alexandrium* is indeed toxic to *A. hudsonica*, we carried out experiments with mixed diets. We measured the ingestion and egg production rates of a northern (New Brunswick) and southern (Connecticut) copepod populations given different mixtures of toxic *Alexandrium* (Maine) and non-toxic *Tetraselmis* sp. based on the percent of carbon (100% *Alexandrium*/0% *Tetraselmis*, 75/25, 50/50, 25/75, 0/100). The experimental conditions, starvation and incubation period and methodology were the same as those of the experiment already outlined. The total concentration of each diet was 250 μg C l^{-1} which is limiting to the ingestion and growth of *A. hudsonica* and within the range of *Alexandrium* blooms. The toxic effects of *Alexandrium* can be determined by plotting the ingestion and egg production rates versus the percentage of *Tetraselmis* in the diet (Jónasdóttir et al., 1998; Colin and Dam, 2002). *Alexandrium* can be considered toxic to grazers if it is present in the mixed diet: (a) reduces the grazers' total ingestion rates; or (b) detracts from the beneficial effects of the control diet by reducing egg production in the food mixtures (Jónasdóttir et al., 1998; Colin and Dam, 2002). This latter point can be examined by drawing a reference line between the egg production at 100% *Alexandrium* toxic diet and 100% *Tetraselmis* diet. A detrimental effect is suggested when observations of egg production for the food mixtures fall below the reference line.

2.4. Acclimation experiment

Changes in copepod ingestion rates were monitored for 14 days (336 h) to determine if the southern *A. hudsonica* population from New Jersey was able to acclimate to the presence of toxic *Alexandrium* in its diet. Prior to each experiment, adult *A. hudsonica* were maintained in cultures under the standard rearing conditions with the standard diet. Then, 170 female copepods were transferred to a large batch (4 l) filled with a food solution of 50% toxic *Alexandrium* and 50% *Tetraselmis*, or a control solution of 100% *Tetraselmis* (representing the conditions at $T = 0$). The total food concentration was maintained at 250 $\mu g\,C\,l^{-1}$ in the mixture and control diets, a concentration that is typically limiting to the ingestion and growth of *A. hudsonica*. Copepod ingestion rates were determined from 24 h incubations using 540 ml bottles (three replicates for treatments and two replicates for controls). These were done at different times, T, ($T = 24$, 48, 72, 120, 192, and 336 h) with T representing the end of each incubation. Treatment bottles each contained seven females and three males, which had been removed from the large batch container. Environmental conditions were the same as the previously detailed experiments. Control and treatment bottles were kept on a plankton wheel during incubations. At the end of the incubation period, samples were taken for cell counts. Copepods were counted and examined for general condition and returned to the large batch. Cells were counted and ingestion rates measured as described earlier for the latitudinal comparison experiments.

3. Results

3.1. Latitudinal comparison experiment

To confirm the hypothesis that copepod fitness parameters when feeding on toxic *Alexandrium* are determined by the historical exposure of copepod populations to the dinoflagellate, two predictions must be met. First, copepod ingestion, egg production, egg hatching or survival rates should be lower in the populations naïve (southern) to toxic *Alexandrium* than the historically exposed populations (northern). Second, these specific differences between northern and southern copepod populations should not exist in the absence of toxic *Alexandrium* as a food source. Copepod egg production and ingestion rates when feeding on toxic versus non-toxic diets differed among the copepod populations and diets (Table 2). Post hoc analyses revealed that the relative egg production rates observed among the copepod populations were consistent with both of the above predictions (Fig. 1). When the copepods fed on the toxic *Alexandrium* strains from New Brunswick (Fig. 1A; NB *Alexandrium*; Tukey–Kramer, $P < 0.05$) and Maine (Fig. 1B; ME *Alexandrium*; Tukey–Kramer, $P < 0.05$; Fig. 1) there was a dramatic decrease, relative to the control diet, in the egg production rates of only the New Jersey and Connecticut copepod populations (Tukey–Kramer, $P < 0.05$). In contrast, no such decrease was observed with the non-toxic *Alexandrium* strain from Connecticut (Fig. 1C, CT *Alexandrium*, ANOVA, d.f. $= 4$, $P = 0.1$) or non-toxic *Tetraselmis* sp. (Fig. 1D; Tukey–Kramer, $P > 0.05$).

Table 2
The ANOVA table of ingestion and egg production rates of the five copepod populations (NJ, CT, MA, ME, NB) feeding on four diets (non-toxic *Tetraselmis* sp., non-toxic CT *Alexandrium*, medium-toxin ME *Alexandrium*, and higher-toxin NB *Alexandrium*)

Source	d.f.	*P*-value
Ingestion		
Copepod population	4	0.04
Algal diet	3	0.02
Interaction	12	<0.0001
Egg production		
Copepod population	4	<0.0001
Algal diet	3	<0.0001
Interaction	12	<0.0001

The ingestion rates of the copepods from New Jersey relative to the northern copepod populations were also consistent with both of the above predictions. When the copepods fed on the toxic *Alexandrium* strains from New Brunswick (Fig. 2A; NB *Alexandrium*; Tukey–Kramer, $P < 0.05$) and Maine (Fig. 2B; ME *Alexandrium*; Tukey–Kramer, $P < 0.05$; Fig. 1) there was a dramatic decrease in the ingestion rates of only the NJ copepod population (Tukey–Kramer, $P < 0.05$), while no such decrease was observed for the non-toxic diets (Fig. 2C and D; ANOVA, d.f. $= 4$, $P = 0.1$ for CT *Alexandrium*; Tukey–Kramer, $P > 0.05$ for *Tetraselmis* sp.). Unlike egg production, the

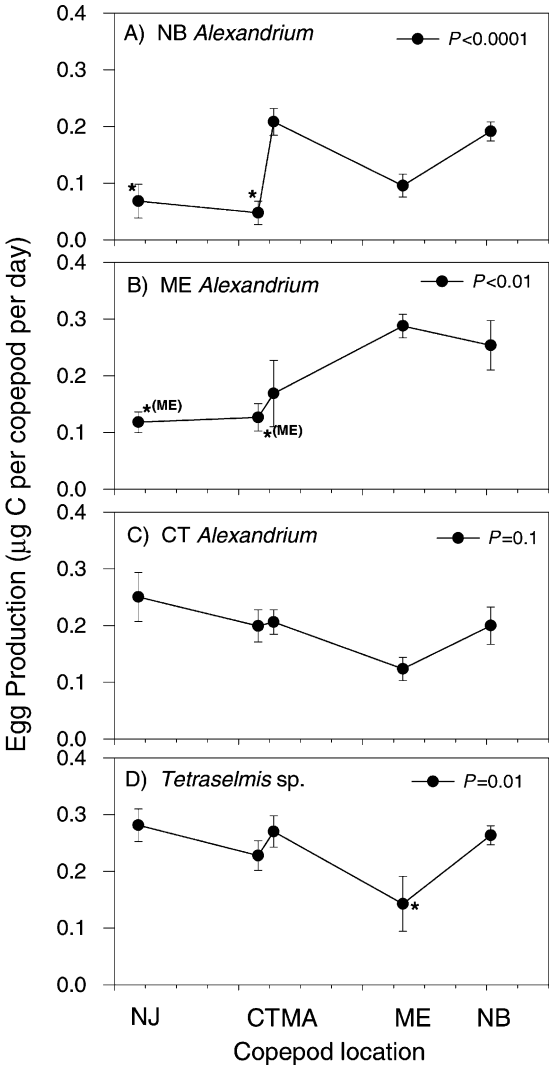

Fig. 1. Mean egg production rates of the five geographically distinct populations of *Acartia hudsonica*. The origin of each copepod population is identified by location (NB: New Brunswick, ME: Maine, MA: Massachusetts, CT: Connecticut, and NJ: New Jersey) and plotted relative to latitude (NB: 45°04′, ME: 43°39′, MA: 41°34′, CT: 41°19′, CT: 39°23′). Copepods were fed diets containing: (A) high-toxin NB *Alexandrium*, (B) medium-toxin ME *Alexandrium*, (C) non-toxic CT *Alexandrium*, and (D) non-toxic *Tetraselmis* sp; the mean and S.E. ($n = 8$) are shown; *P*-values for single ANOVA comparing differences among copepod populations are given within figures; asterisks indicate values significantly different from three northern copepod populations (MA, ME, NB) based upon Tukey–Kramer post hoc tests ($P < 0.05$, if value is only different from one population which is shown in parentheses); except for D, where asterisks indicate values were significantly different from all other populations.

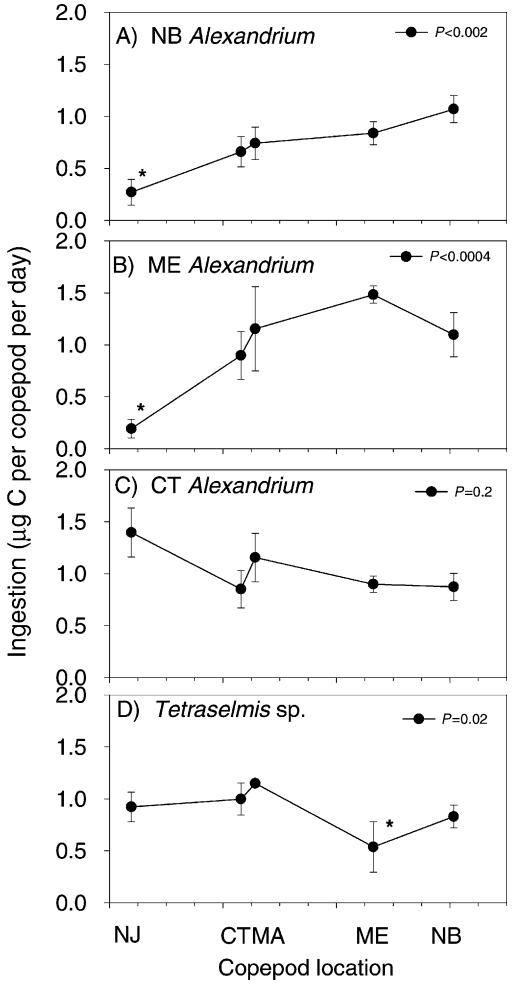

Fig. 2. The mean ingestion rates of the five geographically distinct populations of *Acartia hudsonica*. The origin of each copepod population is identified by location (NB, ME, MA, CT, and NJ refer to Fig. 1 for the full form of the abbreviations) and plotted relative to latitude. Copepods were fed diets containing: (A) high-toxin NB *Alexandrium*, (B) medium-toxin ME *Alexandrium*, (C) non-toxic CT *Alexandrium*, and (D) non-toxic *Tetraselmis* sp; the mean and S.E. ($n = 8$) are shown; *P*-values for single ANOVA comparing differences among copepod populations are given within figures; asterisks indicate values significantly different from all other copepod populations based upon Tukey–Kramer post hoc tests ($P < 0.05$).

ingestion of toxic *Alexandrium* strains by the CT copepods was not lower than the northern copepod populations (Fig. 2A and B; Tukey–Kramer, $P > 0.05$).

The toxic strains of *Alexandrium* did not have lethal effects on any of the individuals from the five copepod

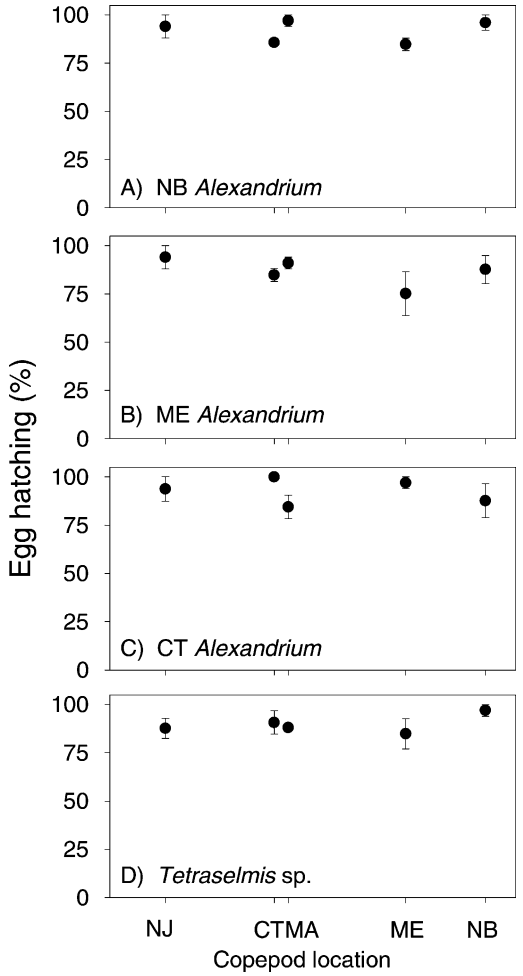

Fig. 3. The mean egg hatching rates of the five geographically distinct populations of *Acartia hudsonica*. The origin of each copepod population is identified by location (NB, ME, MA, CT, and NJ refer to Fig. 1 for the full form of the abbreviations) and plotted relative to latitude. Copepods were fed diets containing: (A) high-toxin NB *Alexandrium*, (B) medium-toxin ME *Alexandrium*, (C) non-toxic CT *Alexandrium*, and (D) non-toxic *Tetraselmis* sp; the mean and S.E. ($n = 4$) are shown.

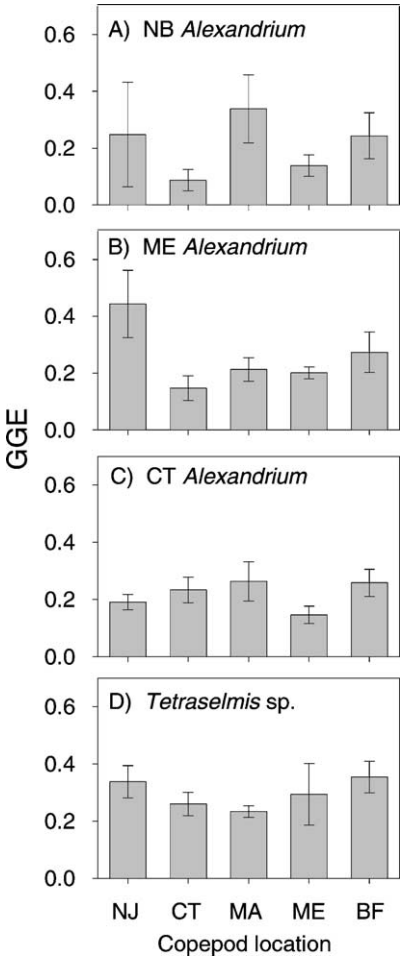

Fig. 4. The GGE of the five *Acartia hudsonica* populations fed different diets: (A) high-toxin NB *Alexandrium*, (B) medium-toxin ME *Alexandrium*, (C) non-toxic CT *Alexandrium*, and (D) non-toxic *Tetraselmis* sp.; the mean and S.E. ($n = 8$) are shown.

populations. Survival (not shown) was always high, averaging above 90% regardless of diet.

Egg hatching rates were high and did not significantly differ among diets or within diets among the copepod populations (Fig. 3; ANOVA for arcsine transformed data, $P > 0.05$).

The GGE ranged from 0.1 to 0.4 and typically varied independent of copepod populations and diets (Fig. 4). Only the copepods from Connecticut exhibited significantly lower GGEs when fed the NB *Alexandrium* strain (arcsine transformed Tukey–Kramer, $P < 0.05$).

Since, copepod survival, egg hatching or GGE were insensitive to toxic *Alexandrium*, the latitudinal effects of *Alexandrium* on copepod fitness are only manifest in their ability to feed and reproduce.

3.2. Mixed diet experiment

Since, the comparisons from the factorial experiment utilized sole food diets (i.e. 100% toxic

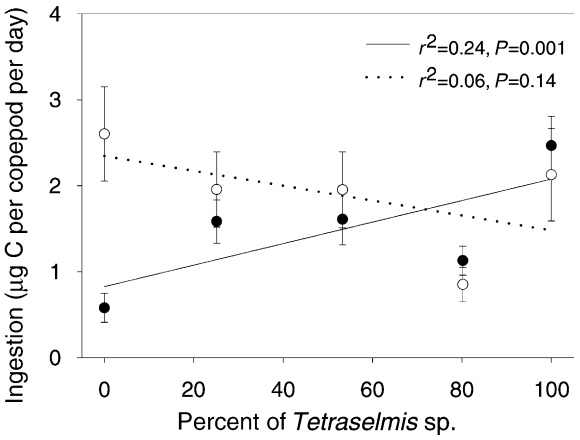

Fig. 5. The mean ingestion rates of NB (circles) and CT (filled circles) copepods vs. the carbon (%) of *Tetraselmis* sp. in the diet; mixture diets consist of *Tetraselmis* and ME *Alexandrium* (0% *Tetraselmis* sp. indicates 100% *Alexandrium*); linear regressions for all of the data from each population are shown (NB, dotted; CT, solid); error bars are S.E. ($n = 8$) (refer to Fig. 1 for the full form of the abbreviations).

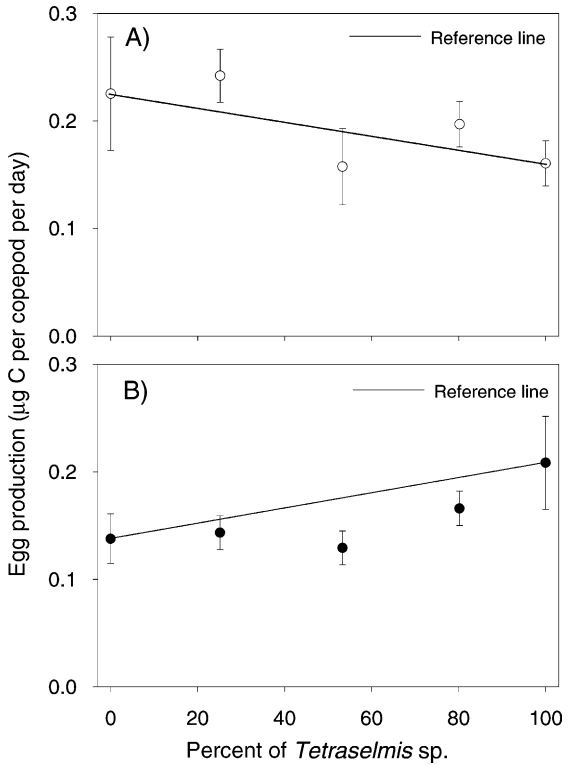

Fig. 6. The mean egg production rates of: (A) NB and (B) CT copepods vs. the carbon (%) of *Tetraselmis* sp. in the diet; mixture diets consist of *Tetraselmis* and ME *Alexandrium* (0% *Tetraselmis* sp. indicates 100% *Alexandrium*); the line connecting the mean rates at 100% *Alexandrium* and 100% *Tetraselmis* sp. is the reference line; error bars are S.E. ($n = 8$).

Alexandrium) we compared the ingestion and egg production rates of a southern (Connecticut) and northern (New Brunswick) copepod population fed different mixtures of toxic *Alexandrium* and non-toxic *Tetraselmis* sp. A two-way ANOVA indicated that while the effect was not significant between populations (d.f. $= 1$, $P = 0.07$) there was a significant interaction (population $\times$ diet, d.f. $= 4$, $P = 0.01$). Thus, the ingestion rates of populations were affected differently by increased amounts of *Alexandrium* in the diet. The ingestion rates of copepods from Connecticut decreased as the proportion of toxic *Alexandrium* in the diet increased (Fig. 5), whereas, the ingestion rates of the copepods from New Brunswick remained unaffected (Fig. 5). Additionally, the CT copepods produced fewer eggs than the NB copepods (two-way ANOVA, d.f. $= 1$, $P = 0.04$). However, within both the New Brunswick and the Connecticut populations there were no observable differences in egg production rates among diets (Fig. 6).

3.3. Acclimation experiment

Since, all of the experiments comparing the copepod populations utilized 24 h incubation periods, we measured the changes in copepod ingestion in the NJ copepod population over a 14-day period to determine if the copepods were able to physiologically acclimate to toxic *Alexandrium*. Ingestion rates of the New Jersey *Acartia hudsonica* fed a 50/50 mix of toxic NB *Alexandrium* and non-toxic *Tetraselmis* were significantly lower than their ingestion rates on a control diet of 100% *Tetraselmis* (Fig. 7, ANOVA, d.f. $= 1$, $P < 0.001$). Ingestion rates on the mixed toxic diet remained consistently low over the 14 days (Fig. 7, linear regression, $P = 0.3$). Thus, individuals from the New Jersey population were not able to mitigate the negative effects that toxic *Alexandrium* has on their ingestion rates. There was no significant difference (ANOVA, d.f. $= 1$, $P = 0.4$) in the ingestion rates of the two components of the mixed diet, *Tetraselmis* sp. and *Alexandrium*.

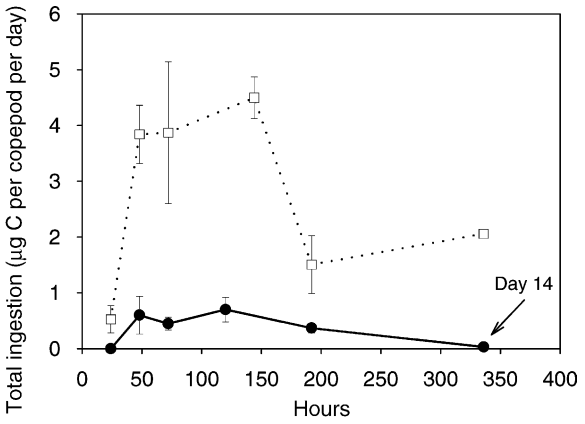

Fig. 7. The NJ copepod population mean ingestion rates on 50/50 mixed diet of toxic NB *Alexandrium* sp. and *Tetraselmis* sp. (filled circle) and on a sole diet of non-toxic *Tetraselmis* sp. (square) measured from 24 h incubations at different time intervals: times indicate the end times of the incubation periods; error bars are S.E. ($n = 3$) (refer to Fig. 1 for the full form of the abbreviation).

4. Discussion

4.1. Latitudinal differentiation

Biogeographic differences in populations of conspecifics often arise due to genetic differentiation among the populations. In order for genetic differentiation to occur in the populations with large dispersal capabilities, such as marine copepods, selective pressures must act on the populations (Bucklin and Marcus, 1985; Burton, 1986; Hilbish, 1996). Several studies in marine systems have identified genetically distinct populations of conspecifics, with high dispersal capabilities, which have locally adapted to selective pressures (e.g. copepods, Burton and Feldman, 1981; Burton, 1986; Bradley, 1986; killifish, Powers et al., 1986; softshelled clam, Bricelj et al., 2000, mussel, Koehn et al., 1980; Hilbish and Koehn, 1985, oligochaete, Klerks and Levinton, 1989). Phenotypic variation is due to genetic and environmental variances and their interactions (Falconer, 1996). Hence, genetic differences among populations of conspecifics are often determined by comparing phenotypes of individuals from different populations reared for generations in common environments (Lonsdale and Levinton, 1985; Schultz and Conover, 1997; Boersma et al., 1999). Because our comparisons of ingestion, egg production and hatching rates among geographically separate copepod populations were done after several generations of being reared under common environmental conditions to all populations, we can attribute any of the observed trait differences among the populations to genetic variation (Falconer, 1996; Conover and Schultz, 1995).

The observed differences in the *A. hudsonica* populations' tolerance to toxic *Alexandrium* sp. are consistent with local adaptation in some populations to the toxic dinoflagellate. The northern populations (MA, ME, NB), where *Alexandrium* blooms are most frequent and toxic, exhibited enhanced ingestion and egg production rates when given toxic *Alexandrium* relative to the two southern populations (Figs. 1A and B and 2A and B). These differences are congruous with the environmental "grain" exerted by *Alexandrium* (as seen in the frequency and toxicity of the blooms) among the geographic locations. However, the rate processes among the copepod populations did not differ when the copepods were fed the non-toxic *Alexandrium* strain (Figs 1C and D and 2C and D). These results support the idea that the MA, ME and NB copepod populations have evolved toxin resistance to *Alexandrium*.

While both the southern populations were less tolerant than the three northern populations, we observed tolerance differences between the two southern copepod populations (New Jersey and Connecticut) as well. The copepods from New Jersey could not tolerate either of the higher toxin (NB *Alexandrium*) or the lower toxin (ME *Alexandrium*) *Alexandrium* strains. However, the CT copepods did not exhibit reduced ingestion rates on either of the toxic strains (Fig. 2), although their egg production on the higher toxin strain was reduced (Fig. 1). These results might be related to differences in the presence of toxic *Alexandrium* in each region. The NJ copepods are from a region where toxic blooms of *Alexandrium* have not occurred (Cohn et al., 1988; Mahoney et al., 1995); thus, they would not have been exposed to toxic *Alexandrium*. Without an opportunity to adapt to the toxic dinoflagellate, we would expect the NJ copepods to have the lowest tolerance for *Alexandrium*. The ingestion rates of the NJ copepods were similar to rates of other *Acartia* spp. populations fed

monoalgal diets of toxic *Alexandrium* (Ives, 1985; Teegarden and Cembella, 1996; Teegarden, 1999). Ives (1985) collected copepods from regions where *Alexandrium* did not bloom. Teegarden and Cembella (1996) and Teegarden (1999) employed a copepod species, *A. tonsa*, whose seasonal cycle does not overlap with that of *Alexandrium* blooms. Hence, in these three studies the copepods were probably naïve to *Alexandrium* blooms. Likewise, all five copepod populations, in our study, ingested the non-toxic *Alexandrium* cells at the rates similar to other studies where *A. hudsonica* (clausi) was fed non-toxic to low-toxic *Alexandrium* spp. cells (Ives, 1985; Dutz, 1998).

The mixed tolerance that the CT copepods exhibited for toxic *Alexandrium* could also be related to their exposure history. Toxic *Alexandrium* has bloomed in the past in the region where the CT copepods were collected; but these blooms were much less frequent and much less toxic than the bloom in the northern regions (Anderson et al., 1994; Anderson, 1997). Additionally, we might expect some genetic exchange between the CT and MA populations due to their proximity. Both of these factors would contribute to the existence of some tolerant individuals in the CT copepod population. Higher genetic variability within the Connecticut population, relative to the New Jersey population, could explain the mixed tolerance we observed in the Connecticut population.

To investigate further the differences in the harmful effects of toxic *Alexandrium* on the CT copepod population versus a northern (New Brunswick) copepod population, we measured the differences in ingestion and egg production on diets containing different mixtures of ME *Alexandrium* and *Tetraselmis*. Despite using a strain with moderate toxin content (about 5 pg STX equivalents per cell, Table 1), we still observed that the northern NB and southern CT copepod populations were affected differently by the presence of toxic *Alexandrium* in their diet. The CT copepods' ingestion decreased as the amount of toxic *Alexandrium* increased in the mixed diets, whereas, the NB copepods' ingestion remained unchanged (Fig. 5). Likewise, these differences in ingestion translated into differences in egg production (Fig. 6). However, while it appears the CT copepod's egg production rates fell just below the reference line, the differences among diet mixtures were not significant. Thus, according to Jónasdóttir et al. (1998), the framework to the test for toxicity, these results show that at this low toxin content, *Alexandrium* did not have toxic effects on the CT copepods.

The three northern populations (MA, ME, NB) displayed similar ingestion and egg production rates when fed toxic *Alexandrium* diets (Figs. 1A and B and 2A and B). The MA copepods were collected from a region where *Alexandrium* has only bloomed occasionally and has modest toxin content. The ME and NB copepods were collected from regions where *Alexandrium* blooms are much more frequent, about once per year, and of high–moderate toxin content (Anderson et al., 1994; Anderson, 1997). This inconsistency between the exposure and resistance the three copepod populations to *Alexandrium* deserves further consideration. The existence of adapted individuals in a population must result by local selection or the immigration of individuals from already adapted populations. In the present case, this would be from the north (Crisp, 1978; Burton, 1986). If adaptation took place in each region, we might expect individuals to be adapted to the particular *Alexandrium* strain or level of toxicity common to that region. Thus, the copepods from MA would be less resistant to the higher toxin *Alexandrium* strains, since the blooms in MA are less toxic (Anderson et al., 1994). However, if there were high gene flow among the three copepod populations, then the populations would become less genetically distinct and the geographic range of the resistance to toxic *Alexandrium* could increase. Since, the resistance of the MA copepods to toxic *Alexandrium* was similar to the ME and NB copepod's tolerance, we could speculate that there is genetic exchange between the MA and northern populations. It is highly reasonable to expect individuals from the ME and NB population to mix due to the circulation in the Gulf of ME (Smith and Schwing, 1991; Anderson et al., 1994). However, Cape Cod has generally been found to be a barrier that restricts the gene flow between populations to its north and south (Schopf, 1979; Buss and Yund, 1989). Nevertheless, the MA population was collected from a site near the Cape Cod Canal, which could readily allow exchange among the populations. Much more work is needed to fully understand the mechanisms involved in making each copepod population resistant to toxic *Alexandrium*.

4.2. Toxic versus feeding deterrent effects

Both feeding deterrent and incapacitating effects of *Alexandrium* sp. have been hypothesized to reduce copepod feeding rates on *Alexandrium* (Huntley et al., 1986; Ives, 1987; Uye and Takamatsu, 1990; Turner et al., 1998). Several studies have found that copepods that ingested toxic *Alexandrium* often showed erratic behavior and reduced ingestion (Huntley et al., 1986; Ives, 1987; Uye and Takamatsu, 1990). Anecdotally, we observed similarly described erratic behavior. Our mixed diet experiments can be used to examine if the reduction in the ingestion rates of the southern populations were caused by physiological incapacitation or feeding deterrence. A feeding deterrence reduces ingestion rates when given as a sole food, but not when given in a mixed diet (Huntley et al., 1986; DeMott and Moxter, 1991; Turriff et al., 1995; Koski et al., 1999; Teegarden, 1999; Engstrom et al., 2000; Colin and Dam, 2002). In both experiments where *Alexandrium* was mixed with non-toxic *Tetraselmis*, the ingestion rates on both the toxic and non-toxic components of the diet decreased (Figs. 5 and 7). Furthermore, the copepods did not appear to select against toxic *Alexandrium*. Hence, the results of the present study we observed are not consistent with the feeding deterrence hypothesis.

4.3. Toxic Alexandrium as a selective force

The documented accounts of grazer adaptation to toxic *Alexandrium*, including this study and a study on adapted populations of softshell clams (Bricelj et al., 2000), suggest that toxic *Alexandrium* may exert selective pressure on its grazers. Such selection may be manifested by inducing physiological changes that may alter demographics of a population and by increasing grazer mortality (Travis, 1996). The results of the present study provide direct evidence that the presence of toxic *Alexandrium* has the potential to alter the demographics of a population by severely reducing the egg production of non-resistant individuals within the population. While we did not observe any mortality in copepods feeding on toxic *Alexandrium*, some toxic effects that are sub-lethal in laboratory settings, such as physiological incapacitation, may have lethal consequences under natural conditions by increased vulnerability to predation (Newman, 1995).

5. Conclusion

We have found that geographically separate copepod populations are affected differently by toxic *Alexandrium*. These differences are consistent with the hypothesis that some copepod populations have adapted to the presence of toxic *Alexandrium*. The present study may provide insight to help unravel the disparity among studies examining the grazer–toxic algal relationship (Turner et al., 1998). More importantly, this study demonstrates that historical exposure and evolved resistance of zooplankton populations are important determinants of whether some algae are harmful to zooplankton grazers. Hence, grazer adaptation will affect the fate of HABs and the fate of toxins in marine food webs. Understanding the evolution of grazer resistance is critical to understanding and predicting the effects of the spreading of HABs in marine systems.

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