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Testing for toxic effects of prey on zooplankton using sole versus mixed diets

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Abstract

Negative effects of prey species on consumers could be due to deterrence, nutritional insufficiency, or toxicity of the prey. These effects can be discerned in experiments in which the suspect prey is offered to the consumers in a food mixture containing another prey item that is not toxic and in which the ingestion rates on the prey and the grazers' responses (e.g., egg production) are measured. We used this framework to determine whether several algae that have been reported to have harmful effects on grazers (*Prorocentrum minimum*, low- and high-toxin *Alexandrium* sp., *Heterosigma carterae, Thalassiosira rotula*, and *Phaeodactylum tricornutum*) are toxic to females of the calanoid copepod *Acartia tonsa.* Ingestion, egg production, and egg-hatching rates were measured for *A. tonsa* offered sole diets of the suspect alga and mixed diets containing the suspect alga and a control alga (the green flagellate *Tetraselmis* sp.) at an ecologically relevant concentration (250 μg C L^{-1}) and duration (3 d). With the exception of the *Alexandrium* strain with the high-toxin content (16.3 pgSaxitoxin [pgSTX] equivalents $cell^{-1}$), none of the diets studied can be considered toxic. The high-toxin *Alexandrium* reduced *A. tonsa*'s total ingestion rate, and thus egg production, as the proportion of *Alexandrium* increased in the diet. *A. tonsa* exhibited significantly reduced ingestion and egg production rates when feeding on sole food diets of *H. carterae* and *P. tricornutum* relative to the mixed food diets. However, a comparison of ingestion rates among the diet mixtures revealed that *H. carterae* and *P. tricornutum* acted as feeding deterrents when provided as sole foods. These results stress the importance of using mixed food diets when examining putative toxic effects of preys on consumers.

Reports of putatively harmful effects of phytoplankton on grazers ranging from feeding inhibition and physiological incapacitation to reductions in egg production and hatching rates are common (reviewed in Turner and Tester 1997). The broader ecological significance of these effects depends on whether they are severe enough to reduce secondary production or the grazers' impact on algal population growth.

Many studies dealing with harmful effects of phytoplankton on grazers have focused only on feeding activity, which has been found to be reduced by some phytoplankters (Ives 1987; Uye and Takamatsu 1990; Teegarden 1999). Other studies have focused on the effects on egg production and hatching rates without measuring ingestion rates (Ianora and Poulet 1993; Laabir et al. 1995; Uye 1996; Ban et al. 1997). These limitations hinder our ability to evaluate fully and predict the ecological effects of putatively harmful algae in natural systems.

The harmful nature of phytoplankters is typically assumed to be due to their toxicity. Determining phytoplankton toxicity is straightforward when the effects are lethal, which is typically not the case. Moreover, a negative effect of a phytoplankter on a grazer relative to some other diet—such as a reduction in egg production—is not a sufficient condition to deem that phytoplankter toxic or even harmful. This is because the negative effect could be due to a nutritional insufficiency of the diet in question. To address these concerns, Jónasdóttir et al. (1998) put forward an experimental approach to discern whether any given prey item is beneficial, nutritionally poor, or toxic to grazers (Fig. 1). Specifically, this approach was designed to examine effects on egg production and egg-hatching rates. In this approach, the suspect prey (the treatment) is offered to the grazer singly and in mixtures with a control prey. If the treatment is indeed toxic, then its effects could not be masked by, and in fact would detract from, the beneficial effects of the control prey. In Fig. 1A, a reference line is drawn connecting the rate values of the grazer feeding on 100% treatment and 100% control diets. If the treatment is less nutritious than the control, then the reference line has a positive slope. That is, in a mixed diet the performance of the grazer is entirely dependent on the proportion of the control in the diet. If the treatment is not toxic, but has no nutritional value, the rate process values should fall along the reference line as the proportion of the control increases because the concentration of the control also increases. If the treatment has some nutritional value, it will act as a supplement to the control. Hence, observations should fall above the reference line when the grazer is offered the mixed diet (area 1). However, if the treatment is toxic, its presence in the food mixture will detract from the value of the control, and observations for the mixed diet will fall below the reference line (area 2).

This scenario assumes that the grazer is offered a constant and limiting total food concentration and, more importantly, that there is no prey selection. Thus, it is essential that the ingestion rates of the prey items be measured. If the grazer consistently selects one prey over the other or if the grazer's

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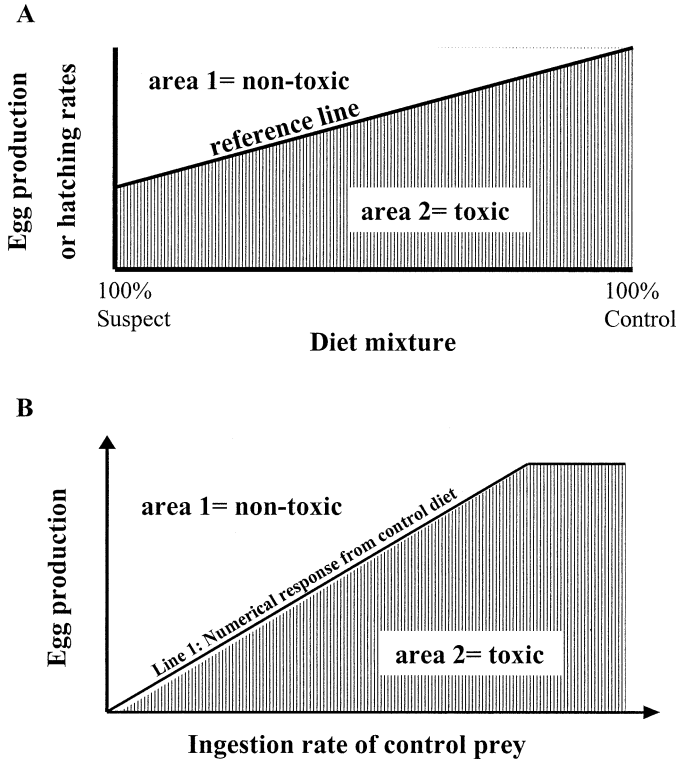

Fig. 1. Model for testing toxicity effects. (A) Schematic of possible egg production or hatching rates versus percent of a suspect toxic alga and control alga that make up the total carbon food concentration. Reference line is drawn connecting the rates at 100% suspect alga to 100% control alga. (B) Schematic of egg production rates of copepods feeding on varying concentrations of 100% control diet versus ingestion rate of control diet (line). For both A and B, if egg production or hatching rates from diets consisting of different mixtures of both suspect and control algae fall on reference line (or on numerical response line for part B) or in area 1, then the suspect diet is not toxic to the grazer. If the rates fall in area 2, then the suspect diet may be toxic to the grazer. Modified from Jónasdóttir et al. (1998).

selectivity switches as the relative proportion of prey changes, then the grazer's response (e.g., egg production) must be considered relative to the ingestion rate on the control prey (Fig. 1B). Hence, the grazer's functional and numerical responses on the control prey are first determined (Fig. 1B, line 1). The grazer's responses on the mixed and the control diets can be compared by plotting them against the ingestion rate of the control diet for each food concentration. Hence, if observations of the grazer's response based on the mixed diet fall on or above line 1, the suspect diet is not toxic. In contrast, toxic effects of a diet are evidenced by observations falling in area 2.

Since the Jónasdóttir et al. (1998) framework is designed to examine toxic diets that affect the grazer's egg production or hatching rates directly, toxic diets that reduce ingestion rates directly (thus egg production indirectly), such as incapacitating effects attributed to paralytic shellfish poisoning (PSP; Ives 1987), may not be identified. If such diets are toxic, their detrimental effects on ingestion should reduce egg production rates to levels below the reference line in Fig. 1 (area 2). However, egg production will be reduced in proportion to the reduction of ingestion; thus, these effects will not be identified by the test illustrated in Fig. 1B, which compares the grazer response to suspect diets based on ingestion rate of the control. Therefore, in order to identify incapacitating toxic effects, the reduced egg production rates observed in Fig. 1A should be accompanied by a decrease in the total ingestion as the concentration of the toxic food increases. Thus, changes in the total ingestion rates across the various mixtures must be examined to assess whether the copepods are physiologically incapacitated.

In the present study we used the framework of Jónasdóttir et al. (1998) to test whether several algae that have been previously reported to have negative effects are in fact toxic to the calanoid copepod *Acartia tonsa.* We examined the effects of two dinoflagellates (*Prorocentrum minimum*, low-, and high-toxin strains of *Alexandrium* sp.) and a flagellate (*Heterosigma carterae*) that have been known to produce dense regional harmful blooms (Tomas and Deason 1981; Uye and Takamatsu 1990; Anderson 1997), and two diatoms (*Thalassiosira rotula, Phaeodactylum tricornutum*) that have been reported as being harmful/toxic to copepods (Ianora and Poulet 1993; Shaw et al. 1995; Ban et al. 1997).

Materials and methods

Collection and culture of organisms—The five suspect algal species (treatments) examined in this study were *Heterosigma carterae* (isolated from Long Island Sound [LIS]), *Prorocentrum minimum* (isolated from the York River, Virginia), *Alexandrium* sp. (low-toxin strain was isolated from Casco Bay, Maine, toxicity = 1.44 pgSaxitoxin (pgSTX) equivalents cell^{-1} measured by the receptor binding assay method of Doucette et al. 1997 by S. Morton, NOAA, high-toxin strain was isolated from the Passamoquoddy Bay, Canada, toxicity = 16.3 pgSTX equivalents cell^{-1} measured by HPLC following Oshima et al. 1989), *Thalassiosira rotula* (isolated from the Mediterranean), and *Phaeodactylum tricornutum* (isolated from LIS). The control prey was the green flagellate *Tetraselmis* sp. (isolated from Narragansett Bay). Characteristics of the algae are summarized in Table 1. All diets used in the experiments were maintained in exponential growth phase by diluting the culture every 2 d with f/2 medium (Guillard 1975). *Acartia tonsa* was collected from Long Island Sound off Avery Point, Connecticut and was cultured in cohorts following the procedure of Feinberg and Dam (1998). The algal and copepod cultures were maintained at 20°C and 12 : 12 h light : dark regime. Copepods were reared on a mixed diet consisting of *Thalassiosira weissflogii, Isochrysis galbana,* and *Rhodomonas lens,* which was maintained at a near saturating concentration of 400–500 μg C L^{-1} by replenishing the amount every other day. The same temperature and light cycle were subsequently employed in the experimental incubations.

Experiments—Forty-eight hours prior to experimental incubations, mature and actively swimming adult female *A. tonsa* with intact appendages were isolated from their culture, sized, and kept in separate 1-liter beakers containing the control, the treatment, and mixtures of the control and

Table 1. Algal food species. Equivalent spherical diameter (μm), volume (μm^3), and carbon (μgC cell^{-1}) of algal cells at the time of each mixture experiment. Experimental concentration is the mean total concentration of the five diets in each experiment.

Experimental algal mix	Strain name	Mean equivalent spherical diameter (±SD) (μm)	Mean volume (μm^3)	Mean carbon (μgC cell^{-1})	Mean experimental concentration (±SD) (μgC L^{-1})
Heterosigma carterae	OL	11.86 ± 1.08	873.5	1.6E−04	253.2 ± 42.3
Tetraselmis sp.		7.3 ± 1.04	200.9	3.6E−05	
High-toxin *Alexandrium* sp.	NB-05	25.6 ± 1.45	8825.8	2.5E−03	222.6 ± 15.7
Tetraselmis sp.		7.3 ± 1.04	200.4	3.6E−05	
Low-toxin *Alexandrium* sp.	CB-307	19.6 ± 1.38	3918.4	1.1E−03	240.1 ± 23.0
Tetraselmis sp.		7.3 ± 1.04	201.6	3.6E−05	
Prorocentrum minimum	JA-98-01	11.8 ± 1.25	860.3	2.3E−04	247.8 ± 15.6
Tetraselmis sp.		7.3 ± 1.04	203.7	3.6E−05	
Thalassiosira rotula	CCMP 1647	20.4 ± 1.27	4412.6	2.6E−04	234.9 ± 13.3
Tetraselmis sp.		7.3 ± 1.03	200.9	3.6E−05	
Phaeodactylum tricornutum	PHAO	4.3 ± 1.07	41.9	4.6E−06	268.5 ± 39.8
Tetraselmis sp.		7.7 ± 1.05	235.3	4.2E−05	

treatments at the same concentrations as the experimental incubations. The food medium was refreshed after 24 h. Copepod mortality during the acclimation period was negligible.

For each of the five suspected harmful algae, experimental incubations consisted of nominally 100%, 75%, 50%, 25%, and 0% of the control. Food concentrations (Table 1) were sufficiently high to sustain ample egg production, but below typical saturation points for the numerical and functional responses of *A. tonsa* (Berggreen et al. 1988). The dilution medium for the experimental incubations was f/2 except in the case of *Heterosigma carterae.* Preliminary experiments with *H. carterae* yielded negative copepod ingestion rates. Consequently, to be able to measure ingestion rates we replaced nitrate in the f/2 medium with ammonium chloride for the *H. carterae* experiments to further stimulate plant growth in the control bottles (Keller et al. 1987). This was somewhat successful, although in two of the 100% *H. carterae* experimental incubations, ingestion rates were still negative. In these two cases ingestion rate was assumed to be zero.

Ingestion, egg production, and egg-hatching rates of *A. tonsa* for each diet were measured using triplicate 540-ml experimental bottles containing five to six copepods (four or five females and one male). Two control bottles (no copepods) were used to measure ingestion rates. Bottles were placed in a plankton wheel and rotated (end over end) at 1.3 rpm for 24 h. At the end of the incubation period, samples were taken for cell counts and the copepods and eggs were separated. Copepods were counted, sized, and examined for general condition. Eggs and nauplii were counted, placed into a Petri dish with 0.2-μm filtered seawater, and incubated at 20°C for 2 or 3 d in order to measure egg-hatching rates. Cell counts were performed using an Elzone® 280 particle counter, with the algal size distribution used to count cells determined from initial samples and kept constant for final treatment and control cell counts. It was possible to employ the particle counter in the mixed diet experiments because the mean size of the control cells did not overlap with that of the other diets (Table 1). Clearance rates and ingestion rates were calculated using equations from Frost (1972). To determine the carbon content of the diets (Table 1), aliquots from the control bottles were filtered onto combusted (500°C, 6 h) GF/F filter pads and dried for over 24 h. Carbon content was determined using Carlo Erba EA1108 elemental analyzer.

In the case of *Phaeodactylum tricornutum* and high-toxin *Alexandrium* sp. it was necessary to perform the experiment illustrated in Fig. 1B. Following the procedure detailed above, we measured the numerical response of *A. tonsa* from their egg production rates measured for eight concentrations of the control: 50.5, 89.6, 146.4, 153.8, 219.2, 308.7, 448.1, and 582.7 μg C L^{-1}. To compare this numerical response to the egg production rates of *A. tonsa* fed the mixed diets from Fig. 2, we replotted the *A. tonsa*'s egg production rates from Fig. 2 (for *Phaeodactylum tricornutum* and high-toxin *Alexandrium* sp.) against their ingestion rates of *Tetraselmis* sp. in those mixtures. These experiments were performed less than 48 h after the food mixture experiment.

Carbon-based gross growth efficiencies (GGEs) were calculated from the ratio of egg production to ingestion rates. This assumes that all copepod growth was manifested in egg production, which is a reasonable assumption since there was no significant increase (paired *t*-test, $P > 0.05$) in size of the females before and after the experiment.

Results

When female *Acartia tonsa* fed on monocultures of *Prorocentrum minimum, Thalassiosira rotula,* or low-toxin *Alexandrium* sp. (left side of Fig. 2), they did not exhibit reduced egg production rates relative to the control monoculture of *Tetraselmis* sp. (comparing 0% with 100% *Tetraselmis* sp.; Tukey–Kramer post hoc test, $P > 0.05$). In contrast, egg production rates with monocultures of high-toxin *Alexandrium* sp., *Heterosigma carterae,* and *Phaeo-*

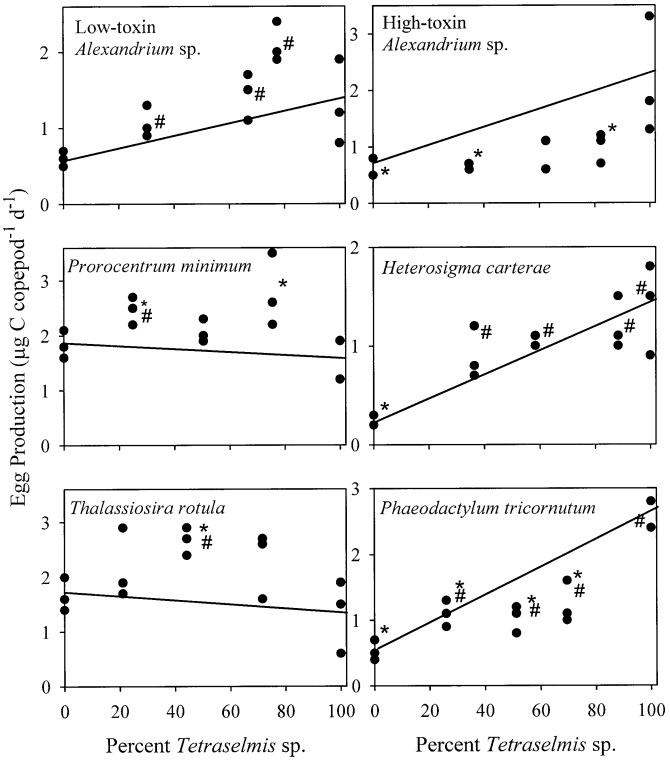

Fig. 2. Egg production rates of female *Acartia tonsa* versus the percent carbon of *Tetraselmis* sp. (control) in the diet. Mixture diets consist of control and the suspect alga that is indicated on each figure (0% control indicates 100% suspect alga). The line connecting the mean rates at 100% suspect alga and 100% *Tetraselmis* sp. is the reference line in Fig. 1A. Symbols indicate observations in the mixed diet were significantly different from the control (asterisk; 100% *Tetraselmis* sp.) or from 100% suspect diet (number sign; *t*-test, df = 4, $P < 0.05$).

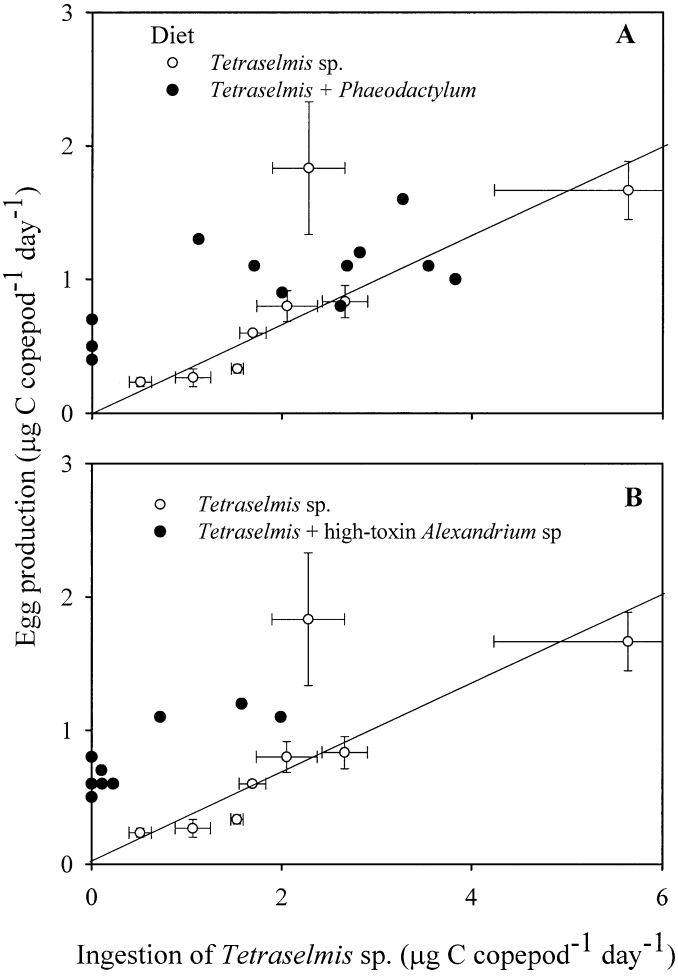

Fig. 3. Mean egg production rates of female *Acartia tonsa* feeding on varying concentrations of 100% control diet *Tetraselmis* sp. or feeding on various mixtures of (A) *Phaeodactylum tricornutum* and the control or (B) high-toxin *Alexandrium* and the control versus mean ingestion rates of the control. Regression line is for egg production versus ingestion by copepods fed 100% control diet. Error bars are standard error ($n = 3$). The regression line is based on all the observations for the control diet.

dactylum tricornutum were significantly lower than with the control (right side of Fig. 2; Tukey–Kramer post hoc test, $P < 0.05$).

Egg production was not significantly different from the control for food mixtures containing *H. carterae,* low-toxin *Alexandrium* sp., and most mixtures of *T. rotula* (comparing 20–80% mixtures with 100% *Tetraselmis*; Tukey–Kramer post hoc test, $P > 0.05$). Egg production was only greater than the control when the food mixture contained *P. minimum* (Fig. 2; Tukey–Kramer post hoc test, $P < 0.05$). Hence, only *P. minimum* served as a supplement to the control diet. In contrast, when *A. tonsa* fed on food mixtures containing the high-toxin *Alexandrium* sp. and *P. tricornutum,* observations of egg production rates fell below the reference line, which suggests a harmful effect of these two species.

At first glance, it would appear that *P. tricornutum* and high-toxin *Alexandrium* were toxic to *A. tonsa* according to the criteria illustrated in Fig. 1A. That is, in some instances egg production was below the reference line. Hence, we ran the type of experiment illustrated in Fig. 1B, in which the egg production rates for food mixtures containing *P. tricornutum* or high-toxin *Alexandrium* were compared to egg production rates based on ingestion of the control (Fig. 3). The great majority of observations for the mixed treatments of both diets (shown as filled symbols in Fig. 3) fell above the regression line derived from the control observations (shown as open symbols in Fig. 3). Accordingly, it became apparent that the presence of *P. tricornutum* or high-toxin *Alexandrium* in the diet did not reduce the egg production per amount of *Tetraselmis* sp. ingested (Fig. 3). However, *Alexandrium* contains saxitoxins, a suite of neurotoxins that may impair feeding (Ives 1987). This would then create an indirect toxic effect not evident in Fig. 3. A comparison of *A. tonsa*'s ingestion rates between unialgal and mixed diets revealed that their ingestion rates on diets containing high-toxin *Alexandrium,* sole or mixed, were significantly lower than on the control diet (Fig. 4; comparing 0–63% with 100% *Tetraselmis* sp.; Tukey–Kramer post hoc test, $P < 0.05$). This was not the case for *P. tricornutum* (Tukey–Kramer post hoc test, $P > 0.05$). Therefore, *P. tricornutum* does not appear to have toxic effects on *A. tonsa*'s ingestion.

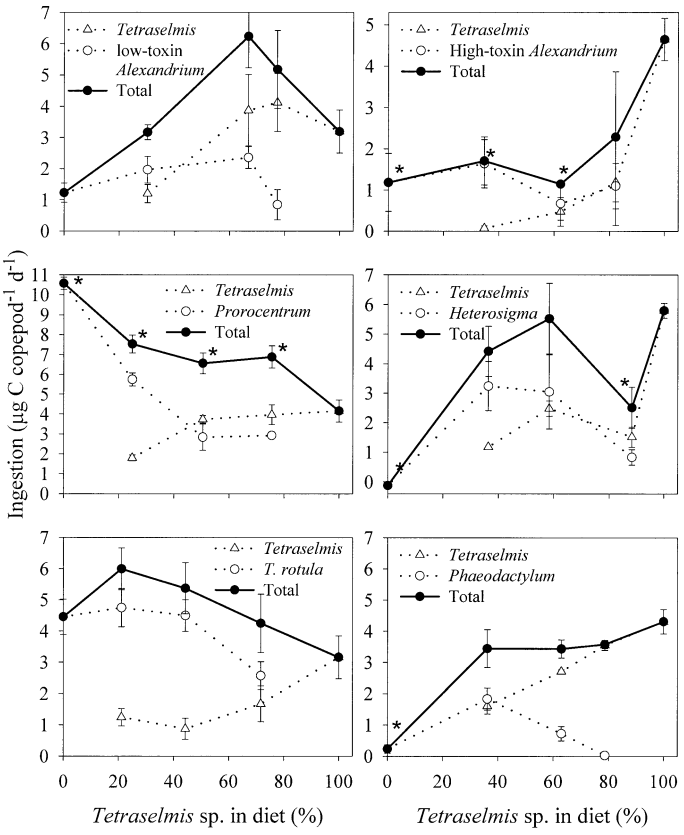

Fig. 4. Mean *Acartia tonsa* ingestion rates, total and ingestion rates of each component of the diet (open circles, suspect alga; open triangles, *Tetraselmis* sp.) versus the percent carbon of *Tetraselmis* sp. (control) in the diet. Suspect algal species are indicated in each figure legend. Symbols indicate observations that were significantly different from the control (asterisk; 100% *Tetraselmis* sp.). Error bars are standard error ($n = 3$).

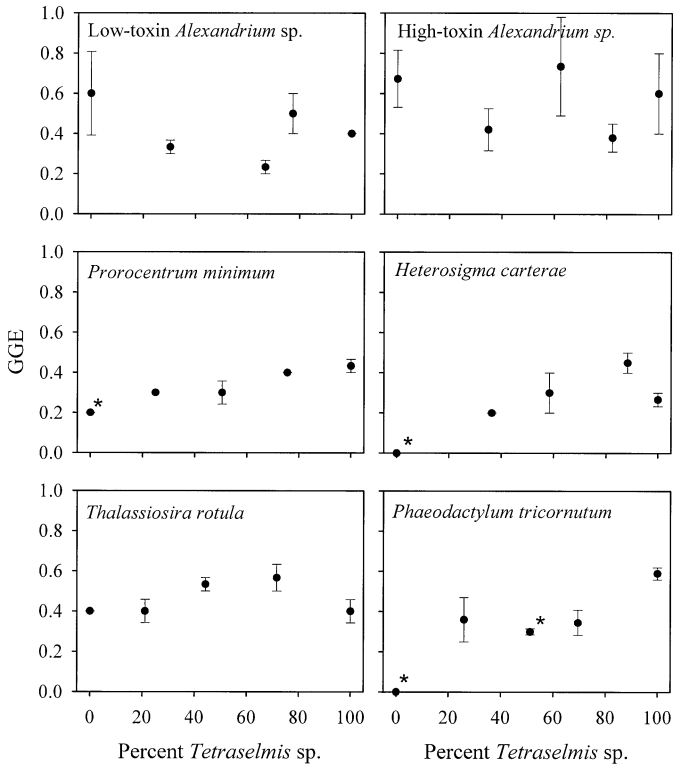

Fig. 5. Mean carbon gross growth efficiencies (GGEs) of *Acartia tonsa* versus percent carbon of *Tetraselmis* sp. (control) in the diet. Suspect algal species are indicated in each figure. Asterisks indicate efficiencies that were significantly different from the 100% control diet (Tukey–Kramer post hoc test, $P < 0.05$). Error bars represent standard error ($n = 3$).

However, diets containing high-toxin *Alexandrium* reduce *A. tonsa*'s ingestion rates, on both *Tetraselmis* and *Alexandrium* in the diets, enough to reduce the copepod's egg production below the reference line in Fig. 1.

The presence of low-toxin *Alexandrium* in the diet of *A. tonsa* females did not significantly reduce their total ingestion rates relative to the control (Fig. 4, Tukey–Kramer post hoc test, $P > 0.05$). Thus, it did not have the same effect as the higher toxin strain. The same result was obtained when the three other suspect algae were offered in food mixtures (*Prorocentrum*, *Heterosigma*, and *T. rotula* in Fig. 4; Tukey–Kramer post hoc test, $P > 0.05$). However, when *A. tonsa* fed on monocultures of *P. tricornutum* and *H. carterae*, the ingestion rate was indistinguishable from zero, which is consistent with the very low egg production rates obtained with these diets (Fig. 2). In contrast, monocultures of *P. minimum* increased ingestion rates relative to the control (Fig. 4). This is also consistent with the enhancement in egg production observed when *P. minimum* was offered in food mixtures to *A. tonsa* (Fig. 2).

The gross growth efficiency (GGE) of female *A. tonsa* fed monocultures of *H. carterae*, *P. minimum*, and *P. tricornutum* was lower than that of *A. tonsa* fed the control (comparing 0% with 100% *Tetraselmis* sp. in Fig. 5; Tukey–Kramer post hoc test, $P < 0.05$), which indeed suggests that these are not optimal diets. But, none of the suspect algae had an effect on GGE relative to the control when they were offered as food mixtures (comparing 20–80% with 100% *Tetraselmis* sp. in Fig. 5, Tukey–Kramer post hoc test, $P > 0.05$). Hence, there was no indication that the suspect algae reduced food quality when offered in food mixtures.

When *A. tonsa* fed on monocultures of *H. carterae*, egg-hatching rate was 46% ± 5% (SD), which was significantly lower than the control or the food mixtures (Fig. 6, Tukey–Kramer post hoc test, $P < 0.05$). The other four suspect algae did not significantly affect egg-hatching rates when offered in any of the sole food or mixture diets (single ANOVA, df = 4, $P > 0.2$, Fig. 6). In these cases, egg-hatching rates were relatively high, ranging from 80 to 100%.

Discussion

As far back as the 16th century, Paracelsus recognized that ''all substances are poisons; there is none that is not a poison. The right dose separates the poison and the remedy'' (Walker et al. 2001). Accordingly, the Jónasdóttir et al. (1998) framework assumes that a toxic effect is dose dependent, which in turn is given by the amount of toxin ingested. Therefore, in the context of the experiments presented here,

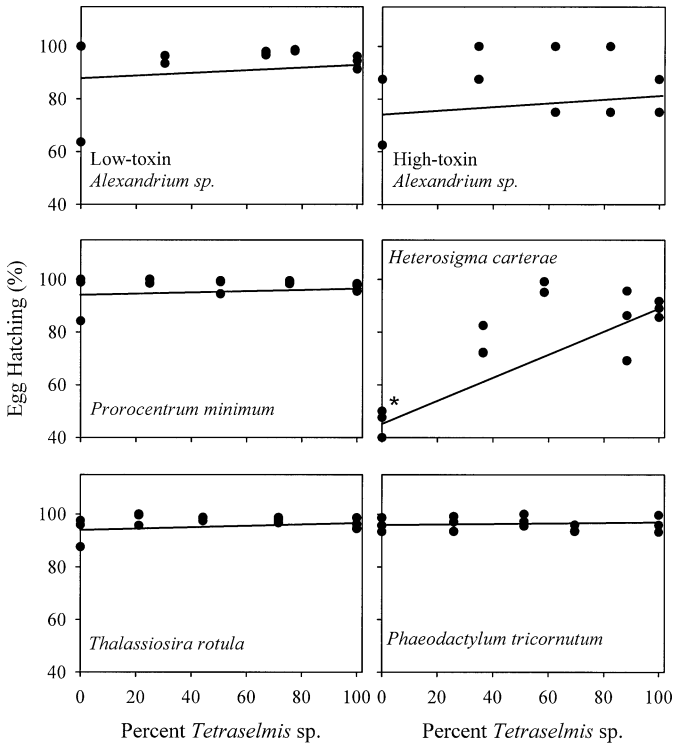

Fig. 6. Egg-hatching rates versus the percent carbon of *Tetraselmis* sp. (control) in the diet. The reference line connects the mean rates at 100% suspect alga and 100% *Tetraselmis* sp. Asterisks indicate observations that were significantly different from the 100% control diet (Tukey–Kramer post hoc test, $P < 0.05$).

toxic effects should depend on algal ingestion regardless of whether the alga was offered as a sole food or in a food mixture. Based on this premise, one must conclude that of the suspect algal species examined, only the highly toxic *Alexandrium* sp. strain is toxic to female *Acartia tonsa* at our experimental concentrations. Even though ingestion of high-toxin *Alexandrium* did not detract from the beneficial effects of the control diet on egg production and hatching (Figs. 2 and 6), it reduced *A. tonsa*'s total ingestion rates (Fig. 4), indirectly reducing egg production. *Heterosigma carterae* and *Phaeodactylum tricornutum* were the two other prey items that significantly reduced egg production or egg-hatching rates. However, in the case of *H. carterae* these reductions were observed only when the prey was given singly. In the case of *P. tricornutum*, egg production rates for the mixed diets were reduced below the reference line (Fig. 2). Egg production was not reduced relative to the ingestion of the control (Fig. 3), nor was the total ingestion rate reduced as the proportion of *P. tricornutum* in the diet increased (Fig. 4). Hence, neither one of these two prey items can be considered toxic to *A. tonsa.*

Other than a toxic effect, reductions in egg production could be due to nutritional deficiency (Schmidt and Jónasdóttir 1997) or to reductions in feeding (present study, Koski et al. 1999). Ingestion rates can provide information on both of these causes; however, they are often not measured or reported (e.g., Ianora and Poulet 1993; Laabir et al. 1995; Uye 1996; Ban et al. 1997). By comparing ingestion rates to egg production, we can estimate the amount of ingested carbon that is contributed to growth (GGE). This is an index of the nutritional quality of a diet. By this criterion, we did not observe significant differences in the nutritional quality of the diets (though we could not determine this for the monoalgal *H. carterae* and *P. tricornutum* diets since no measurable ingestion was observed) (Fig. 5). Therefore, the observed differences in egg production are most likely due to differences in feeding.

Other studies conducted to examine the effects of suspected toxic diets on copepods have reported reductions in egg production (Dinoflagellate diet, Dutz 1998; Haptophyte diet, Koski et al. 1999; Flagellate diet, Uye and Takamatsu 1990) and egg-hatching rates (Diatom diet, Ianora and Poulet 1993; Laabir et al. 1995; Uye 1996; Ban et al. 1997; Starr et al. 1999; Turner et al. 2001; Dinoflagellate diet, Frangópulos et al. 2000). In those studies that measured ingestion rates, it became evident that either a decrease or a cessation of feeding was the reason for the observed reduction in egg production. Similarly, we observed in our study no detectable ingestion of the two monoalgal diets, *H. carterae* and *P. tricornutum,* in which lower egg production rates were observed (0% *Tetraselmis* sp. in Fig. 3). Others found a similar copepod rejection of *P. tricornutum* and *H. carterae* (Tomas and Deason 1981; Van Alstyne 1986; Uye and Takamatsu 1990; Shaw et al. 1995). However, in our experiments, the presence of *Tetraselmis* sp. in the diet, even as a low percentage, stimulated ingestion and egg production by *A. tonsa.* Koski et al. (1999), in experiments with sole food and mixed diets, observed a similar pattern for the putatively toxic prymnesiophyte *Prymnesium patelliferum.*

A reduction in feeding can result from behavioral selection against a food item (acting as a feeding deterrent) (Van Alstyne 1986) or presumably by physiological incapacitation after ingestion (Ives 1987). In the latter case, the total ingestion should decrease as the concentration of the toxic food increases. Thus, changes in the total ingestion rates across the various mixtures must be examined to assess whether the copepods are physiologically incapacitated (Fig. 4). This was observed for the high-toxin *Alexandrium* sp. diets (Fig. 4), and it is likely that this strain of *Alexandrium* incapacitated *A. tonsa.* Ives (1987) found *Alexandrium tamarense* to incapacitate copepods. Likewise, *A. tonsa* has been found to be severely incapacitated by unialgal diets of toxic *A. tamarense,* but not by mixed diets containing the toxic alga (Teegarden 1999). We, however, observed reduced feeding on both sole and mixed diets containing the high-toxin *Alexandrium* strain. Differences among studies examining the effects of *Alexandrium* on *A. tonsa* could be due to variations in algal toxin content or in the resistance of different *A. tonsa* populations to the toxins (Colin and Dam in press). In our study, the low-toxin *Alexandrium* strain had no effect on *A. tonsa*'s ingestion or egg production. Thus, the low level of saxitoxins in the low-toxin *Alexandrium* diets, at 1.44 pgSTX equivalents cell^{-1}, was most likely below incapacitating levels. Again, this illustrates the importance of measuring ingestion rates in studies examining putatively toxic algae.

A more plausible explanation for the observed feeding patterns of *A. tonsa* feeding on *P. tricornutum* is behavioral

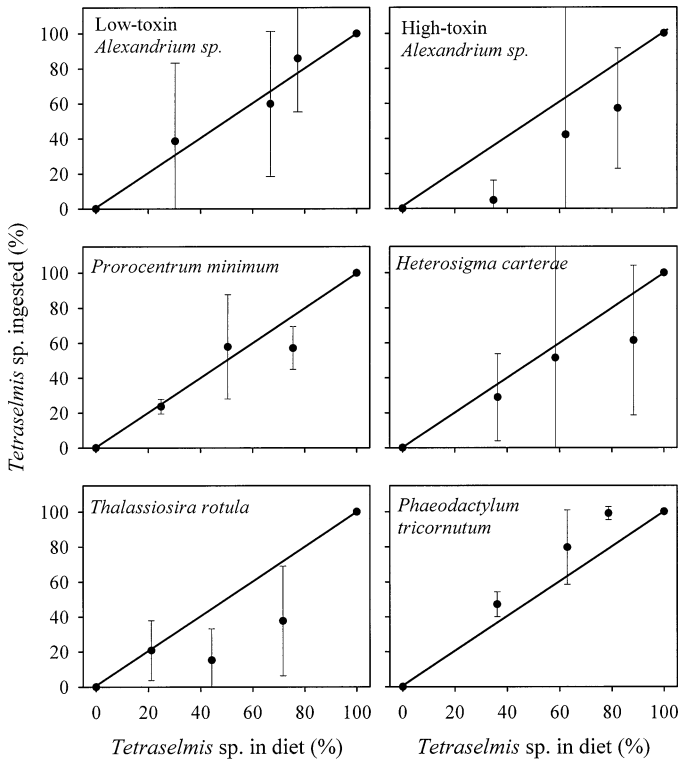

Fig. 7. Percent *Tetraselmis* sp. ingested (based on carbon) versus percent carbon of *Tetraselmis* sp. (control) in the diet. When ingestion rates fall along the 1:1 line there is no prey selection. Deviations above or below the 1:1 line indicate positive or negative selection, respectively. *A. tonsa* selected against *P. tricornutum* only (*see text*). Suspect algal species are indicated in each figure. Error bars represent 95% confidence intervals ($n = 3$).

prey selection. Apparent selection against *P. tricornutum* reduced *A. tonsa*'s egg production to a level that might lead one to conclude that *P. tricornutum* is harmful to *A. tonsa* (Ban et al. 1997). However, when one compares *A. tonsa*'s egg production versus the amount of *Tetraselmis* sp. ingested, it is clear that the addition of *P. tricornutum* to the *Tetraselmis* sp. diet does not decrease egg production (Fig. 3). Additionally, it did not decrease *A. tonsa*'s ingestion rate (Fig. 4). The discrepancy between Figs. 2 and 3 is most likely due to prey selection. A comparison of the proportion of *Tetraselmis* ingested for each treatment versus its proportion in the diet reveals that *A. tonsa* selected against *P. tricornutum* (ingestion rates fall above 1:1 line, Fig. 7). In fact, electivity indices (Ivlev 1961) indicated that *P. tricornutum* was the only alga negatively selected by *A. tonsa* when present as a major component (>50%) of the mixed diet. Except for *P. tricornutum* and *T. rotula* (which was selected for), *A. tonsa* did not feed preferentially on either prey item in the diet mixtures (Fig. 7).

Our study demonstrates that *P. tricornutum* and *H. carterae* act as feeding deterrents when provided as sole food sources. Thus, when mixed with another alga normal feeding rates resume. Others studies have observed the same pattern for feeding deterrents (Koski et al. 1999; Teegarden 1999). Any effects of these algae on grazers are dependent upon whether they are provided singly or as part of an assemblage. This could have implications on our understanding of what type of bloom conditions in nature can negatively affect zooplankton grazers. Specifically, it may be that these algal species reduce copepod fitness only under extreme monoprey bloom conditions.

Differences in hatching rates have been suggested to be due to the nutritional deficiency of a diet (Harrison 1990; Jónasdóttir and Kiørboe 1996; Jónasdóttir et al. 1998) or to the accumulation of toxins in the gonads of copepods (Miralto et al. 1999). If low hatching rates are the result of toxin accumulation, then hatching success should be directly related to the amount of the toxic alga ingested, regardless of whether such ingestion occurs from a single or mixed diet (Jónasdóttir et al. 1998). The mixed diet assay allows one to test whether reduced hatching success is due to toxin accumulation. Reduced hatching was observed in one treatment, the monoalgal diet of *H. carterae.* However, others have reported reduced hatching success at low egg production regardless of the diet (Jónasdóttir and Kiørboe 1996; Tang and Dam 2001). Moreover, because ingestion was negligible for that treatment, it is not possible to ascribe the low hatching success to the *H. carterae* diet. In the mixed diets, when ingestion rates were high on both *Tetraselmis* and *H. carterae,* high hatching success was observed (Figs. 2 and 4). Likewise, none of the other diets appeared to cause reduced hatching when given singly or as part of a food mixture. This may be a surprising result since other studies have found low hatching rates from monoalgal diets of *T. rotula* and *P. tricornutum* (Ianora and Poulet 1993; Ban et al. 1997) and from mixed diets containing *T. rotula* (Turner et al. 2001). In these studies, however, decreases in egg-hatching rates were evident only after prolonged exposure to the diets—a minimum of 4 d to several weeks. Our 3-d experiments were not designed to examine long-term effects, but they are clearly relevant to the length of time for which animals may encounter blooms in nature, since survival time of adult *Acartia* females in the wild is only a few days (reviewed by Peterson, in press).

In summary, only the high-toxin *Alexandrium* strain reduced *A. tonsa*'s ingestion and egg production rates when mixed with a control diet. None of the other species were observed to reduce hatching rates significantly when mixed with a control diet. Thus, only the high-toxin *Alexandrium* strain appears to be toxic to *A. tonsa* at the ecologically relevant exposure durations and concentrations of our experiments. Accordingly, it seems that *A. tonsa* possesses a high level of tolerance to several algal species known to cause acute physiological incapacitation or even death in shellfish grazers (Luckenbach et al. 1993; Wikfors and Smolowitz 1995; Bricelj and Shumway 1998). By examining ingestion along with egg production rates, we can begin to infer why reduced egg production was observed for two of the algal species (*Heterosigma carterae* and *Phaedoactylum tricornutum*) when given as single diets. Such egg production does not appear to be due to the nutritional quality of the food, but rather to total feeding reduction. However, these negative effects of monoalgal diets on *A. tonsa* were not observed when these algal species were mixed with even a small fraction of the control diet. Therefore, it is important that studies examining the toxic effect of a diet go beyond single food assays.

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