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Prorocentrum minimum(clone Exuv) is nutritionally insufficient, but not toxic to the copepod *Acartia tonsa*

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Abstract

This study tested whether the dinoflagellate *Prorocentrum minimum* is nutritionally insufficient or toxic to the copepod *Acartia tonsa*. Experiments were carried out with adult female *A. tonsa* and the *P. minimum* clone Exuv, both isolated from Long Island Sound. Initially, the functional and numerical responses of *A. tonsa* feeding on exponentially growing *P. minimum* cells were characterized. These experiments revealed that *A. tonsa* readily ingested *P. minimum* cells, up to the equivalent of 200% of body carbon day^{-1}, but egg production was relatively low, with a maximum egg production rate of 22% of body carbon day^{-1}. Hence, the egg production efficiency (egg carbon produced versus cell carbon ingested) was low (10%). In a separate experiment, ingestion and egg production rates were measured as a function of food concentration with cells in different growth stages (early-exponential, late-exponential/early-stationary, and late-stationary growth phase) to simulate conditions during a bloom. There was no indication that cells in the stationary phase resulted in lower ingestion or egg production rates relative to actively growing cells. Egg hatching success remained high (>80%) and independent of the cell growth phase. In a third experiment specifically designed to test the hypothesis that *P. minimum* is toxic, ingestion, egg production and egg hatching success were measured when females were fed mixtures of *P. minimum* and the diatom *Thalassiosira weissflogii*, but in which total food concentration was held constant and the proportion of *P. minimum* in the mixed diet varied. *A. tonsa* readily ingested *P. minimum* when it was offered in the mixed diet, with no detrimental effects on egg production or egg hatching observed. Supplementing *P. minimum* with *T. weissflogii* increased both the egg production rate and the egg production efficiency. It is concluded that *P. minimum* is nutritionally insufficient, but not toxic to *A. tonsa*. Finally, it is estimated that in the field grazing by *A. tonsa* is approximately equivalent to 30% of the maximum daily growth rate of *P. minimum*. Hence, copepod grazing cannot be ignored in field and modeling studies of the population dynamics of *P. minimum*.

Keywords: *Acartia*; Copepod; Dinoflagellate; Ecotoxicology; Harmful algae; *Prorocentrum minimum*; Toxic effect; Zooplankton nutrition

1. Introduction

The autotrophic dinoflagellate *Prorocentrum minimum*, commonly found from estuarine to oceanic

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waters of the Northern Hemisphere, appears to be proliferating worldwide (Smayda, 1990). *P. minimum* often blooms in shellfish farming areas and can reach very high concentrations; for instance, bloom concentrations can reach $\sim 10^5$ mL^{-1} in the Chesapeake Bay, USA (http://www.dnr.state.md.us/bay/cblife/algae/dino/prorocentrum_minimum.html). Blooms of *P. minimum* have been associated with episodes of shellfish and human poisoning in Japan (Nakazima, 1965), Portugal (Silva, 1980) and Norway (Tangen, 1983). The toxicity of *P. minimum* seems to vary with strain. For instance, in Japan and Norway the symptoms of poisoning were those of venerupin shellfish poisoning, whereas those in Portugal were consistent with paralytic shellfish poisoning. A water-soluble toxin with neurotoxic activity was also isolated from four *P. minimum* clones from the Mediterranean, with the effects on mice being sublethal in exponentially growing cells and lethal in stationary growing cells (Grzebyk et al., 1997). Tests on frog heart muscle and Diptera larvae suggest that this neurotoxic activity is due to blocking of the sodium channel (Denardou-Queneherve et al., 1999).

Deleterious effects of *P. minimum* (clone Exuv isloated from Long Island Sound) have been observed on feeding larvae, spat and juveniles of the eastern oyster *Crassostrea virginica* from Long Island Sound (Wikfors and Smolowitz, 1995). Whether deleterious effects of *P. minimum* and other species in this genus on shellfish are due to cell toxicity, remains controversial (Wikfors, 2005). No harmful effects of *P. minimum* were observed on three species of ciliates from Long Island Sound (Rosetta and McManus, 2003), but the clone Exuv supported growth of the ciliates well while the clone JA 98-01 (isolated from a tributary of the Chesapeake Bay) did not (Rosetta and McManus, 2003).

Copepods are considered to be, among metazoan planktonic grazers, major consumers of dinoflagellates. Planktonic copepods are consequently an important vector for toxin transfer to higher trophic levels (Turner and Tester, 1997; Teegarden et al., 2003 and references therein). However, prey toxic effects are highly specific, depending on the consumer (Turner and Tester, 1997). Even among a species faced with the same food, there are strong population differences in susceptibility to toxic prey due to the evolution of resistance (Colin and Dam, 2002a, 2003). Hence, to properly interpret studies of toxic effects of prey on predators, experiments should be carried out with populations of predator and prey that come from the same environment.

It is not clear whether *P. minimum* is toxic to planktonic copepods. On the contrary, *P. minimum* is often used as a control diet in studies of the effect of different phytoplankton diets on copepod reproduction (Lacoste et al., 2001; Carotenuto et al., 2002; Ceballos and Ianora, 2003). In the present study, the effects of a clone (Exuv) of *P. minimum* collected from Long Island Sound were examined on feeding, reproduction and egg hatching of adult females of the abundant and ubiquitous copepod *Acartia tonsa*, also collected from Long Island Sound. First, the feeding and reproductive responses as a function of food concentration were examined. Then, in light of the work of Grzebyk et al. (1997) such response was followed as a function of the growth phase of the alga. Finally, because single-food assays are typically inadequate to discern prey toxic effects from deterrence or nutritional insufficiency effects (Jónasdóttir et al., 1998; Colin and Dam, 2002b), experiments using mixed diets experiments were conducted to determine whether *P. minimum* is toxic.

2. Methods

2.1. Collection and culture of organisms

The algal species used in this study, the dinoflagellate *P. minimum* (clone Exuv, isolated from Long Island Sound) and the diatom *Thalassiosira weissflogii,* were obtained from the National Marine Fisheries Service, Milford, CT. The cultures were maintained in a temperature-controlled incubator at 20 °C with a 12:12 light–dark cycle. Cultures were maintained in the exponential growth phase by dilution every 2 days with F/2 medium (Guillard, 1975). The mean characteristics of *P. minimum* were: equivalent spherical diameter (ESD) = 13 μm; volume = 1060 μm^3; and carbon content = 293 pg C cell^{-1}. Characteristics of *T. weissflogii* were ESD = 11.5 μm; volume = 800 μm^3; and carbon content = 88 pg C cell^{-1}.

Acartia tonsa were collected from the Long Island Sound off Groton, Connecticut, USA, with a 202 μm

mesh plankton net fitted with a solid cod end and immediately transported to the laboratory. Copepods were immediately isolated from the sample using a dissecting microscope and placed into 1 L beakers filled with 0.2 μm filtered seawater. Only mature and actively swimming adult females with intact appendages were used for the experiments. Copepods were transferred to the appropriate experimental food suspensions and acclimated for 48 h. The food medium was refreshed after 24 h. Copepod mortality during the acclimation period was negligible.

2.2. Functional and numerical response

In order to measure the functional (ingestion versus food concentration) and the numerical (egg production versus food concentration) response of *A. tonsa,* experiments were performed at concentrations of 39, 111, 168, 319 and 457 μg C L^{-1} of exponentially growing *P. minimum*. Ingestion and egg production rates and egg hatching success of adult female *A. tonsa* were measured using triplicate 540 mL experimental bottles containing 10 copepods (eight females and two males) and two control bottles with no copepods present. Before incubation, samples were taken from the food solutions for cell counts and the bottles were then 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 in each bottle and the copepods and eggs were separated. Copepods were counted, sized (prosome length) and examined for general condition. Eggs and nauplii from each bottle were counted, placed into a petri dish filled with 0.2 μm filtered seawater and incubated at 20 °C. The number of nauplii hatched was counted after 2 or 3 days. One set of egg production samples was lost before the eggs were counted. 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. Clearance rates and ingestion rates were calculated from total cell volume changes during the experiments using equations from Frost (1972). Negative ingestion rates were not included in the analysis of the data. To determine the carbon content of the food, aliquots from the control bottles were filtered onto combusted (500 °C, 6 h) GF/F filter pads and dried for over 24 h. Cell carbon content was determined using a Carlo Erba EA1108 elemental analyzer. Egg carbon content was assumed to be 45 ng (Kiørboe et al., 1985). Because body mass changes were not measured during the experiments (e.g., Durbin et al., 1983), egg production cannot be equated with total female growth. Hence, carbon-specific egg production efficiency (instead of gross-growth efficiency) was calculated from either the slope of the regression of ingestion rate versus egg production rate or from the ratio of these two variables.

2.3. Simulated bloom experiment

Ingestion, egg production and egg hatching success were examined at different times of a simulated *P. minimum* bloom to determine the effects of cell growth stage on adult female *A. tonsa*. Feeding, egg production and hatching success were measured using the same procedure as described in the previous section. The bloom was started on 30 June 1998 with an initial concentration of 14,423 cells mL^{-1}. The population density was measured every 3–4 days using the Elzone 280 Particle Counter. Copepod rate processes were measured three times throughout the bloom, during exponential (332,400 cells mL^{-1}), late-exponential/early-stationary (609,905 cells mL^{-1}) and late-stationary growth (854,382 cells mL^{-1}). These rate processes were measured at one food concentration during the exponential phase (234 μg C L^{-1}) and at two concentrations during the late-exponential/early-stationary (88 and 357 μg C L^{-1}) and late-stationary (91 and 306 μg C L^{-1}) phases.

2.4. Mixed diet experiments

Experiments with mixed diets consisting of different proportions of *P. minimum* and *T. weissflogii* were carried out to determine if *P. minimum* was toxic to adult female *A. tonsa* (Jónasdóttir et al., 1998; Colin and Dam, 2002b). This technique is based on the premise that growth is linearly dependent upon the percentage of good and poor foods in a mixed diet, i.e., the better diet is diluted by the poorer. If growth is lower than predicted by dilution, then toxicity of the poorer food is suggested. On the other hand, if growth is greater than predicted by dilution, then the good food complements the poor food's nutritional insuffi-

ciency. This method is particularly useful to discern sublethal toxic effects from nutritional effects of a food. The procedures for setting up the experimental and control treatments as well as for determining feeding, egg production and hatching success were the same as described earlier. In these experiments, the carbon fractions of *P. minimum* in the diet were nominally 100, 70, 50–60, 25–35 and 0%. The total carbon content for the diets was 275 μg L^{-1}. This concentration was sufficiently high to sustain ample egg production, but not beyond the typical saturation points for the numerical and functional responses of female *A. tonsa* (Kiørboe et al., 1985; Houde and Roman, 1987; Besiktepe and Dam, 2002). Changes in cell volume of the foods in the mixed diets were determined using the particle counter since the respective volume spectra for the different foods did not overlap.

3. Results

Female *A. tonsa* readily ingested cells of exponentially growing *P. minimum* (Fig. 1A). Ingestion rate increased with increasing cell concentration until about 300 μg C L^{-1}. Maximum ingestion rate was ~8 μg C day^{-1}, representing a daily carbon ratio of 200% of the typical body weight (~4 μg C) of a female *A. tonsa*. Egg production rate also increased with increasing food concentration similarly to ingestion rate (Fig. 1B). Egg production rates varied from 0.1 to 0.9 μg C $female^{-1}$ day^{-1}, corresponding to 3–20 eggs $female^{-1}$ day^{-1}. The maximum daily egg production rate was equivalent to 22% of female body carbon. The egg production rate was dependent on the ingestion rate (Fig. 2). The carbon-specific egg production efficiency was estimated, from the slope of the regression of egg production versus ingestion, to be 10%. That is, of the carbon ingested, 10% was allocated to growth in the form of egg production.

During the simulated bloom of *P. minimum*, the lag growth phase was evident during the first 10 days, the exponential growth phase approximately during days 10–20 and the stationary growth phase after 20 days (Fig. 3). The ingestion rate and egg production of *A. tonsa* females were measured during the exponential (day 15), during the late-exponential/early-stationary phase (day 20) and during the late-stationary phase

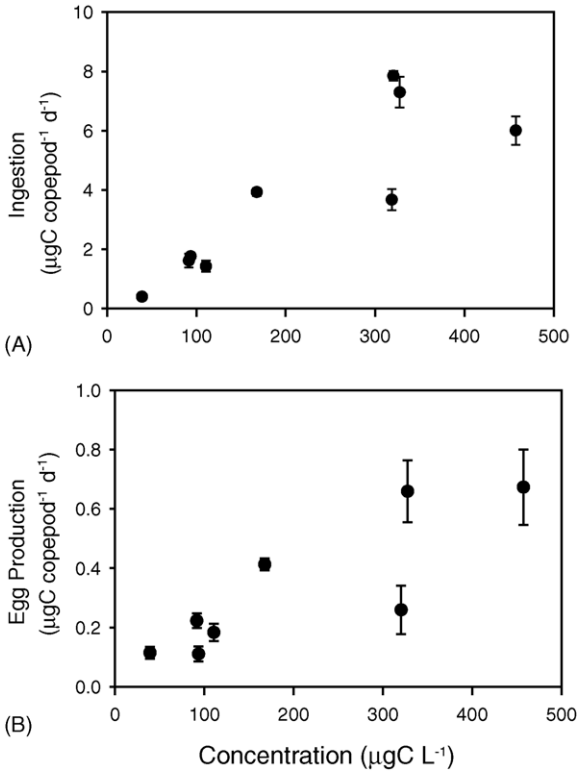

Fig. 1. Functional (A) and numerical response (B) of adult female *A. tonsa* fed exponentially growing cells of *P. minimum* (clone Exuv). Points represent the mean and the bars represent the standard error of triplicate measurements.

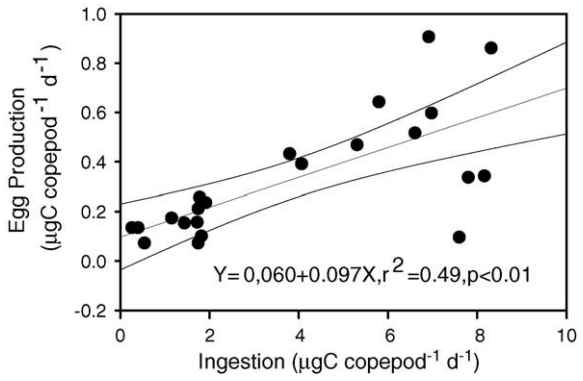

Fig. 2. Carbon-based ingestion rate vs. egg production rate of adult female *A. tonsa* fed exponentially growing cells of *P. minimum* (clone Exuv). Points represent individual observations. The slope of the regression equation represents the egg production efficiency.

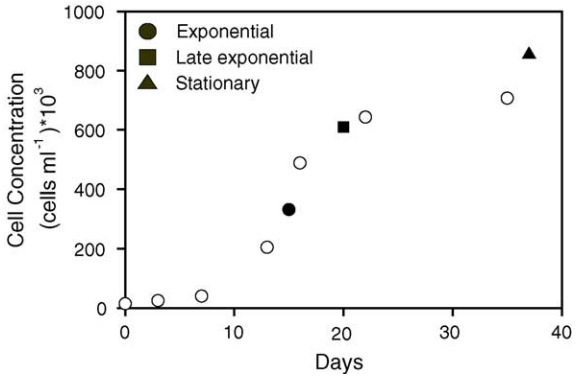

Fig. 3. Cell concentration of *P. minimum* (clone Exuv) vs. time during the simulated bloom experiment. The circles represent the times of cell sampling. The filled circle, square and triangle, represent the times (exponential, late-exponential/early-stationary and late-stationary, respectively) at which the ingestion, egg production and egg hatching success were measured.

(day 37). The ingestion rate increased with increasing food concentration during the simulated bloom (Fig. 4A). The ingestion rates for a given food concentration were similar to those observed in the experiments with exponentially growing cells (compare Figs. 1A and 4A). The maximum egg production rate of 0.5 μg C female^{-1} day^{-1} (Fig. 4B) was also similar to that observed in the experiments with exponentially growing cells (compare Figs. 1B and 4B). The interaction of growth phase and food concentration on ingestion and egg production was examined by dilution of the cultures in a given growth phase. Comparison of the ingestion rates at high and low food concentrations during the simulated bloom experiment indicates that cell growth phase had no effect on ingestion rate. That is, ingestion rate was the same at a given food concentration for exponentially growing and late-stationary growth cells (*t*-test, $p > 0.05$). The cell growth phase, on the other hand, did have an effect on egg production rate (Fig. 4B), with a dramatic decrease in egg production rate at high food concentrations during the late-exponential/early-stationary growth phase, despite a relatively high ingestion rate (*t*-test, $p < 0.05$). This is clearly seen in the relationship between ingestion and egg production for the simulated bloom study (Fig. 5). However, there was no indication that cells in late-stationary growth phase resulted in either lower ingestion or egg production rates (Fig. 4) or egg

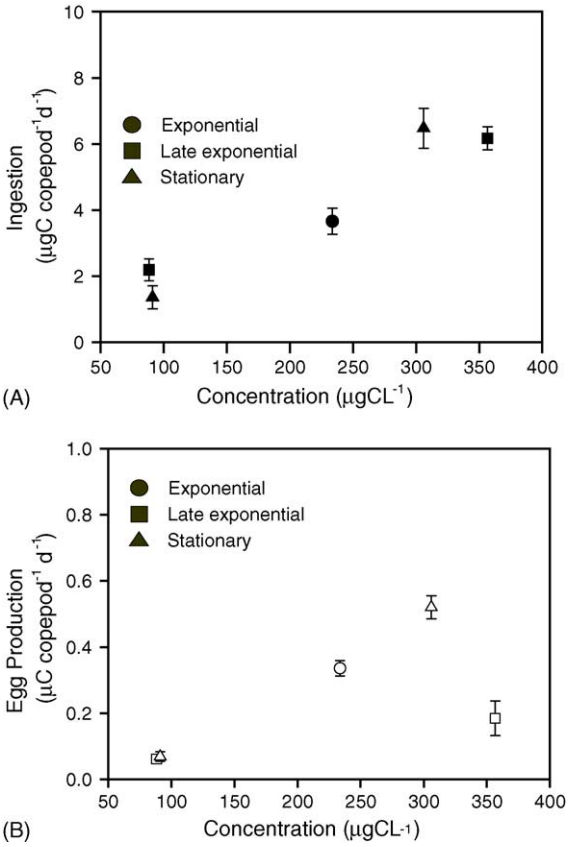

Fig. 4. Ingestion rate (A) and egg production rate (B) of adult female *A. tonsa* vs. *P. minimum* concentration during the simulated bloom study. Points represent the mean and the bars represent the standard error of triplicate measurements.

production efficiency (Fig. 5). Egg hatching success during the simulated bloom ranged from 77 to 100% and was independent of food concentration or cell growth phase (Fig. 6).

Results of the mixed diet experiment are illustrated in Fig. 7. The background for interpreting this experiment is outlined in Jónasdóttir et al. (1998) and Colin and Dam (2002b). Recall that the premise of this approach is that the effect caused by ingesting a toxic food cannot be masked or counteracted by the positive effect caused by ingesting a nontoxic food. In Fig. 7, a reference line (putatively a dilution line for the control food) is drawn connecting the measured egg production when the grazer feeds on 0% of the control food, *T. weissflogii* (=100% *P. minimum*, the

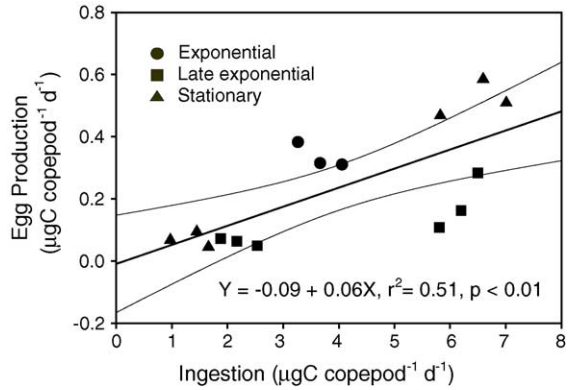

Fig. 5. Carbon-based ingestion rate vs. egg production rate of adult female *A. tonsa* during the simulated bloom of *P. minimum* (Exuv). Points represent individual observations. The slope of the regression equation represents the egg production efficiency.

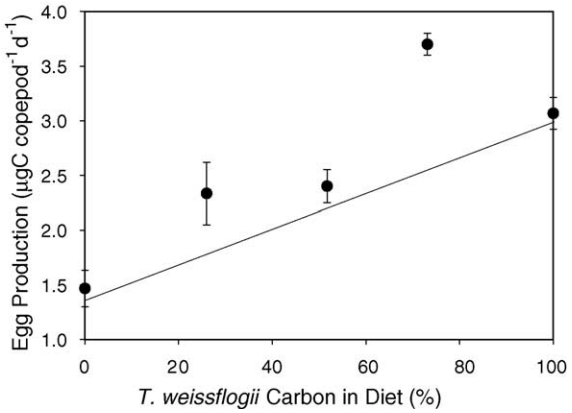

Fig. 7. Egg production of adult female *A. tonsa* in the mixed diet (*P. minimum* and *T. weissflogii*) experiment. Points represent the mean and the bars represent the standard error of triplicate measurements.

suspected, or test food) and 100% *T. weissflogii* (0%, *P. minimum*). If the observations fall along the reference line, the suspected food has no nutritional value. If observations fall above the reference line, the suspected food is nutritionally insufficient; that is, addition of the control food supplements egg production. When the suspected food is toxic, observations must fall below the reference line. This was not observed. However, unambiguous interpretation of Fig. 7 requires that there be no prey selection. This assumption was indeed confirmed in the experiments (figure not shown) since the slope of the fraction of the total ingestion represented by *P. minimum* (Y) versus the fraction of *P. minimum* in the diet (X) in the mixed diet experiments was not significantly different from 1 ($Y = -0.057 + 1.03X$, $r^2 = 0.90$, $n = 9$, $p < 10^{-4}$). The same exercise, of course, also applies to *T. weissflogii* ($Y = 2.47 + 1.03X$, $r^2 = 0.90$, $n = 9$, $p < 10^{-4}$).

Adult female *A. tonsa* showed a higher egg production rate when they feed exclusively on *T. weissflogii* than on *P. minimum* (Fig. 7, t-test, $p < 0.05$). When the two foods were offered in a mixture, observations of egg production fell above the reference line, indicating that the addition of *T. weissflogii* supplemented egg production. This

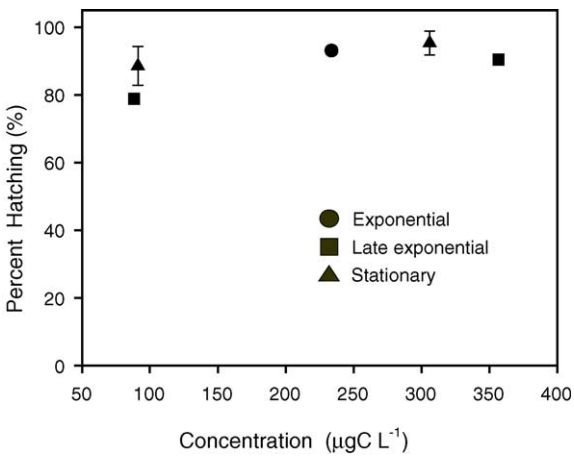

Fig. 6. Hatching success of *A. tonsa* eggs vs. food concentration during the simulated bloom of *P. minimum* (Exuv).

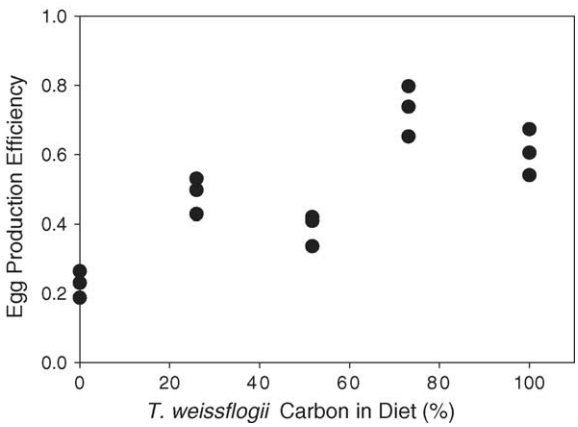

Fig. 8. Carbon-specific egg production efficiency (here calculated as the ratio of egg production to ingestion) of adult female *A. tonsa* in the mixed diet (*P. minimum* and *T. weissflogii*) experiment. Points represent individual observations.

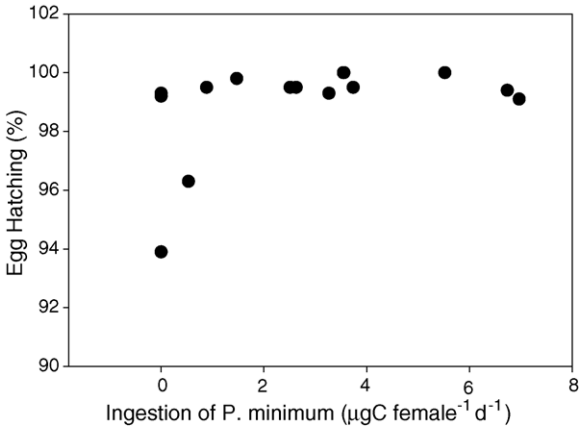

Fig. 9. Hatching success of *A. tonsa* eggs vs. ingestion of *P. minimum* (Exuv) by adult female *A. tonsa* in the in the mixed diet (*P. minimum* and *T. weissflogii*) experiment. Points represent individual observations.

inference was corroborated by examining the egg production efficiency (EPE) versus the fraction of *T. weissflogii* in the diet (Fig. 8). EPE was the lowest (∼20%) when *A. tonsa* fed exclusively on *P. minimum* and increased (∼0.5–0.7) as the fraction of *P. minimum* in the diet decreased. Finally, egg hatching success was not affected by *P. minimum*: egg hatching varied from 93 to 100% and was independent of the ingestion of *P. minimum* (Fig. 9).

4. Discussion

While it is almost universally accepted that the functional response of planktonic copepods can be described curvilinearly (Vanderploeg, 1994), such a relationship for a given copepod species is highly dependent on the prey type (Houde and Roman, 1987; Besiktepe and Dam, 2002). The results of the present study (Fig. 1A) were essentially similar to those reported by Besiktepe and Dam (2002) who carried out separate experiments on the functional response of adult female *A. tonsa* fed *P. minimum* (Exuv), with individual females incubated for 24 h in 144 mL bottles, and with food concentrations ranging from ∼25 to 800 µg C L^{-1}. The maximum ingestion rates observed in this study were also comparable to those reported by Besiktepe and Dam (2002) for the diatom *T. weissflogii*, and not much different than those for the scuticociliate *Uronema* sp. and the heterotrophic dinoflagellate *Oxyrris* sp. The results of the simulated bloom study (Fig. 4A) also revealed that the cell growth phase had little or no effect on ingestion rate. Hence, it appears that *A. tonsa* does not have any particular difficulty ingesting *P. minimum* cells.

Acartia tonsa is typically a very prolific copepod, with daily egg production rates that can readily exceed 100 eggs female^{-1} day^{-1} (e.g., McManus and Foster, 1998; Mauchline, 1998 and references therein). In the present study, however, when *A. tonsa* fed exclusively on *P. minimum*, egg production rates were modest, with the maximum observed rates ranging from 20 (Figs. 2) to 40 eggs female^{-1} day^{-1} (Fig. 7). These results suggest that *P. minimum* is not a sufficiently complete food source to support maximum egg production. This inference is further supported by the relatively low egg production efficiencies that ranged from 6 (Fig. 5) to 20% (Fig. 8), depending on the experiment, when the diet was solely *P. minimum.* Carbon egg production efficiency of *A. tonsa* is typically higher, >30% (e.g., Kiørboe et al., 1985; Dam et al., 1994) and the growth efficiency of copepods (typically assumed to equal egg production efficiency for adults) in general is about 27% on average (Straile, 1997). When *A. tonsa* fed on a mixed diet both egg production rate (Fig. 7) and egg production efficiency (Fig. 8) increased, indeed suggesting that *P. minimum* was not a sufficiently complete food source for egg production. The results of the mixed diet experiment also rule out the possibility that the relatively low egg production observed when *P. minimum* was the sole food was due to an artifact such as egg cannibalism.

Egg hatching success was uncorrelated to the quantity of *P. minimum* offered to *A. tonsa* or its cell growth phase (Fig. 6). Hatching success was also uncorrelated with the ingestion of *P. minimum* (Fig. 9). Furthermore, neither the pattern of low hatching success at low egg production observed by others (Jónasdóttir and Kiørboe, 1996; Tang and Dam, 2001; Dam and Lopes, 2003), nor a reduction in hatching success relative to other diets as observed for some diatoms (Ban et al., 1997) was evident in the present study. Hence, it appears that while *P. minimum* is not a good food for optimal egg production, it has no

insidious effect on the population recruitment of *A. tonsa*.

In contrast to nutritionally insufficient or unpalatable prey, toxic prey have true deleterious effects on predators, thereby reducing fitness. Toxic effects, unless they are lethal, can rarely be ascertained with single-prey experiments (Jónasdóttir et al., 1998; Colin and Dam, 2002b). A telling aspect of a toxic prey is that its effect cannot be masked by ingestion of alternative prey. In addition, a toxic effect is also dose dependent. The response of the predator to a food mixture containing the suspect prey and a control prey can separate nutritional and deterrence effects from toxic effects (Fig. 7). The production of toxins in some clones of *P. minimum* is unquestionable (Grzebyk et al., 1997; Denardou-Queneherve et al., 1999). Toxin production was observed to be greatest when the cells were in late-stationary growth phase (Grzebyk et al., 1997), with toxins appearing to act by blocking the sodium channel (Denardou-Queneherve et al., 1999). Hence, the expected effect of such toxins on the grazers would be to dramatically slow down ingestion rate (e.g., Colin and Dam, 2003). Because egg production of *A. tonsa* is tightly linked to recently ingested food (Kiørboe et al., 1985; Tester and Turner, 1990; McManus and Foster, 1998), then one would also expect to see a dramatic decrease in the egg production rate if *P. minimum* were toxic. This expectation was not met in the present study; that is, during the simulated bloom study ingestion rate was independent of cell growth phase (Fig. 4A) and egg production rate did not decrease when the cells were in the late-stationary phase (Fig. 4B). A puzzling decrease in egg production was noted when the cells were transitioning from exponential to stationary growth; however, such decrease was not due to a drop in the ingestion rate. Hence, it is impossible to ascribe the drop in egg production to a toxic effect of *P. minimum*. The same conclusion is reached from the experiments with the mixed food diet (Fig. 7) because no detrimental effects on egg production or egg hatching associated with the ingestion of *P. minimum* were observed. In separate experiments with mixed diets of *P. minimum* (clone JA 98-01) and the green flagellate *Tetraselmis* sp. (Colin and Dam, 2002b), and of *P. minimum* (clone Exuv) and the flagellate *Rhodomonas lens* carried out in this laboratory (data not shown), the same conclusion was reached.

Wikfors and Smolowitz (1995) reported negative effects of *P. minimum* on the oyster *C. virginica* and hypothesized that such effects were due to interference of feeding. Histological examination of adult female *A. tonsa* feeding on *P. minimum* (Exuv) in the present study revealed, on two of five separate occasions, decreased digestive gland and intestinal epithelial size and number, perhaps due to lack of food or poor food (Smolowitz, personal communication). Again, this assessment is consistent with the hypothesis that *P. minimum* is nutritionally insufficient to *A. tonsa*.

In conclusion, neither the results of the present study nor those of a previous study with another clone of *P. minimum* (Colin and Dam, 2002b) indicate that *P. minimum* is toxic to the copepod *A. tonsa*. Instead, all indications are that *P. minimum* is a nutritionally insufficient food source for *A. tonsa*. One limitation of the present study is that it is unknown whether *P. minimum* (Exuv) produces toxins. This uncertainty does not invalidate the present conclusion regarding toxic effects of this clone on *A. tonsa*, but it makes it impossible to ascertain whether the lack of toxic effects is related to the evolution of toxin resistance, as has been shown for *A. hudsonica* feeding on *Alexandrium fundyense* (Colin and Dam, 2002a, 2003). Because some clones of *P. minimum* from the French Mediterranean produce toxins, it would be instructive to carry out the kind of experiments shown here with *A. tonsa* from the Mediterranean feeding on the toxin producing *P. minimum* clones.

Experimental evidence suggests that microzooplankton grazing is an important loss term for *P. minimum* in Chesapeake Bay (Johnson et al., 2003). A first order estimate of the grazing impact of *A. tonsa* on *P. minimum* can be made. The growth rate of nutrient-saturated *P. minimum* is of the order $0.9\ \text{day}^{-1}$ (Lourenço et al., 2002). If one assumes that the abundance of *P. minimum* during blooms ranges from 10^3 to $10^5\ \text{mL}^{-1}$ and a cell carbon content of ∼293 pg (see methods), then the bloom concentrations of *P. minimum* would typically exceed the concentration at which ingestion rate of *A. tonsa* on *P. minimum* is saturated (Fig. 1). If one also assumes that the ingestion rate is saturated at a food concentration of $300\ \mu\text{g C L}^{-1}$ (Fig. 1), a clearance rate of $\sim 0.03\ \text{L female}^{-1}\ \text{day}^{-1}$ is derived. The maximum abundance of *A. tonsa* females in Long

Island Sound is 5 L^{-1} (Dam Guerrero, 1989). Hence, the expected maximum grazing rate of female *A. tonsa* during a *P. minimum* bloom would be ~0.15 day^{-1}. Because females account for roughly half of the population biomass of *A. tonsa* (Dam Guerrero, 1989), the total grazing rate could easily be double that of females (since the typical allometric effects on ingestion are not considered in this calculation). This would yield a total grazing impact of the order of 33% of the daily growth rate of *P. minimum*. This is not enough to keep *P. minimum* in check, but it does represent an important loss term. When one considers that the present exercise only applies to one copepod species, it is clear that copepod community grazing impact should be included in both field and modeling studies of the population dynamics of *P. minimum*.

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