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Feeding behavior and development of *Acartia tonsa* nauplii on the brown tide alga *Aureococcus anophagefferens*

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Toxic attributes of the brown tide alga Aureococcus anophagefferens affect the ability of benthic and protistan grazers to control blooms. Yet, little is known regarding the effect of A. anophagefferens on a dominant component of the microzooplankton community, copepod nauplii. This study describes the grazer–prey relationship between nauplii of the calanoid copepod Acartia tonsa and A. anophagefferens. Four laboratory experiments using varying proportions of A. anophagefferens (2–4 μm) and a control alga, Isochrysis galbana (4–6 μm), were conducted to test the effects of A. anophagefferens isolate 1708 during exponential and stationary growth phases and A. anophagefferens isolate 1850 (exponential phase only) on naupliar grazing and development. A fifth experiment compared the effects of A. anophagefferens with an equal-sized control alga, Micromonas pusilla (1–3 μm). Isolate 1708 (exponential or stationary) as a single food item did not suppress naupliar ingestion rates (ng C nauplius^{-1} day^{-1}) when compared to I. galbana. No ingestion was detected on isolate 1850 when offered alone, suggesting that this isolate may be more harmful to nauplii. Overall, nauplii selectively grazed on I. galbana over A. anophagefferens in mixed diets, but size-selection could not be ruled out as selective feeding was not apparent in mixtures with M. pusilla. Both isolates of A. anophagefferens delayed naupliar development. Our results indicate that Acartia tonsa nauplii can graze on A. anophagefferens, and can potentially help suppress brown tides. However, the efficacy of grazing control by copepods will vary with availability of alternate food sources and toxicity of the A. anophagefferens strain(s) comprising the population.

INTRODUCTION

Blooms of the brown tide alga, *Aureococcus anophagefferens* Hargraves and Sieburth (Pelagophyceae), have recurred in many US mid-Atlantic coastal ecosystems since 1985. These blooms resulted in detrimental effects on the Long Island shellfish and other bay inhabitants, including eel grass, during the 1980s and into the 1990s (reviewed in Bricelj and Lonsdale, 1997). The geographical extent of the blooms spread south into other mid-Atlantic states during the 1990s (Gobler *et al.*, 2005). Since the inception of these harmful algal blooms, progress has been made toward understanding the dynamics of brown tides, particularly with regard to the alga's nutritional requirements in relation to cellular growth (Gobler *et al.*, 2005). Trophic interactions of the alga with microzooplankton, particularly heterotrophic protists, have also been examined. While microzooplankton are generally effective consumers of picoplanktonic alga, the grazing rates of some of these species have been shown to be depressed when feeding on *A. anophagefferens* cells relative to other picoplanktonic algae (Boissonneault-Cellineri *et al.*, 2001; Gobler *et al.*, 2002; Deonarine *et al.*, 2006).

Population growth rates of zooplankton have been found to be negatively impacted by *A. anophagefferens*.

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One field study demonstrated that the net growth rate of ciliates was negatively correlated with brown tide abundance (Lonsdale *et al.*, 1996). In the laboratory, *Strombidium* sp., a common ciliate in Long Island bays, did not grow on a unialgal diet of *A. anophagefferens*. In contrast with these results the brown tide alga was an adequate food source for three other ciliate species (Caron *et al.*, 2004). The inconsistency in these findings may be due to a number of factors, including the strain of *A. anophagefferens* used (i.e. CCMP 1708 and CCMP 1784) (Caron *et al.*, 2004). Bricelj *et al.* (2001) found that CCMP 1784, isolated in 1986, did not inhibit feeding by blue mussels, *Mytilus edulis*, while two more recent isolates did cause feeding inhibition (CCMP 1707 and 1708, collected in 1995).

Although a negative effect of *A. anophagefferens* on some consumers is apparent, the exact mechanism is not clear, as a precise chemical toxin associated with brown tide cells has never been identified. This effect of *A. anophagefferens* may manifest itself as suppression in grazer ingestion and/or development. The outer exopolymer layer of *A. anophagefferens* and the related pelagophyte *Aureoumbra lagunensis* may affect ciliary movement in ciliates and bivalves due to the "sticky" nature of this layer (Sieburth *et al.*, 1988; Liu and Buskey, 2000a). Reduced grazing rates in the presence of *A. anophagefferens* may also be a consequence of the production of a dopamine-like compound found in this layer which can affect the feeding ability of some bivalve species (Gainey and Shumway, 1991).

Little work has been done regarding the effects of *A. anophagefferens* on copepod feeding and development. Lonsdale *et al.* (1996) demonstrated that the survival of copepodites (*Acartia hudsonica*) and nauplii (*Coullana canadensis*) was poor when fed exclusively the brown tide alga (CCMP 1784 at 5×10^5 cells mL^{-1}), while the presence of the alga along with alternate phytoplankton had no detectable effects on copepod survivorship. Selective feeding is a common behavior of zooplankton and might explain these results. Copepods are known to actively select or reject certain food items. *Acartia tonsa* adults exhibited a strong avoidance reaction to the toxic dinoflagellate *Alexandrium* (Teegarden, 1999). Copepods also have been shown to select against poorer quality food items. For example, *A. tonsa* fed *Thalassiosira weissflogii* demonstrated a selective preference for faster-growing, high quality (i.e. high total protein and/or nitrogen) cells over slower growing, low quality cells (Cowles *et al.*, 1988).

We examined the ability of nauplii of the calanoid copepod, *Acartia tonsa* Dana to ingest *A. anophagefferens*, and to determine if nauplii experience negative effects when exposed to bloom conditions. *Acartia tonsa* increases in abundance in Long Island embayments in late spring around the time that *A. anophagefferens* blooms initiate. It is a dominant zooplankton species throughout the summer (Turner, 1982; Lonsdale *et al.*, 1996). Nauplii of some copepods are capable of ingesting nano-, pico- and even bacterioplankton (Allan *et al.*, 1977; Turner and Tester, 1992) and are thus potential grazers of *A. anophagefferens*. Although experiments using intact microzooplankton assemblages suggest that copepod nauplii may be capable of grazing *A. anophagefferens* (Lonsdale *et al.*, 1996), grazing rates have not been quantified. Our objectives in this study were to assess the effects of *A. anophagefferens* on naupliar grazing and development rates in relationship to (i) algal strain; (ii) algal growth phase and (iii) the availability of alternate food.

METHODS

Experimental design

A total of five experiments were conducted to examine the impacts of *A. anophagefferens* on copepod nauplii grazing and development (Table I). Naupliar feeding behavior on *A. anophagefferens* was evaluated by measuring (i) ingestion rates and (ii) selective feeding. Development was quantified by measuring percent nauplii that molted into the copepodite stage. Differential effects of *A. anophagefferens* strain (1708 versus 1850) and growth phase (1708 exponential versus stationary) on nauplii were investigated and compared to that of a control alga. The control alga for experiments 1 through 4 was *Isochrysis galbana*, an alga with greater biovolume than *A. anophagefferens*. For experiment 5, *Micromonas pusilla* was used as a control to test for selectivity on same-size prey items.

Algal cultures

Cultures of *A. anophagefferens* (CCMP 1708 and 1850), *I. galbana* (Prymnesiophyceae) (ISO) and *M. pusilla* (Prasinophyceae) (CCMP 490) were cultured at 22°C and exposed to a 14:10 h light:dark cycle at a light intensity of 83 μmol photons m^{-2} s^{-1}. *Aureococcus anophagefferens* was grown in a modified f/2 medium which contained selenite (10^{-8} M), citric acid instead of EDTA as a chelator and elevated Fe concentrations (10^{-6} M) (Cosper *et al.*, 1987, 1993). Strain 1708 has been shown to have deleterious effects on some bivalves (Bricelj *et al.*, 2001). The noxious attributes of CCMP 1850 have never been investigated. *Isochrysis galbana* and *M. pusilla* were cultured in unmodified f/2 medium (Guillard and Ryther, 1962). All media were prepared using 0.22 μm

Table I: Summary of experiments

Experiment	Aa strain	Growth phase	Control alga	Treatment
1	CCMP 1708	Exponential	100% ISO	50:50 Aa:ISO, 100% Aa 20:80 Aa:ISO, 80:20 Aa:ISO
2	CCMP 1708	Exponential	100% ISO	50:50 Aa:ISO, 100% Aa 20:80 Aa:ISO, 80:20 Aa:ISO
3	CCMP 1708	Stationary	100% ISO	50:50 Aa:ISO, 100% Aa 20:80 Aa:ISO, 80:20 Aa:ISO
4	CCMP 1850	Exponential	100% ISO	50:50 Aa:ISO, 100% Aa 20:80 Aa:ISO, 80:20 Aa:ISO
5	CCMP 1708	Exponential	100% Mp	50:50 Aa:ISO, 100% Aa 20:80 Aa:ISO, 80:20 Aa:ISO

Five total experiments were run comparing naupliar grazing of the copepod *Acartia tonsa* on 100% *Aureococcus anophagefferens* (Aa), 100% of a control alga and 20:80, 50:50, 80:20 mixtures Aa:ISO (by biovolume) of the two food types. *Isochrysis galbana* (ISO) was the control alga for experiments 1 through 4. *Micromonas pusilla* (Mp) was the control alga used in experiment 5.

filtered seawater collected from Stony Brook Harbor (salinity 27 ppt). Both *I. galbana* and *M. pusilla* were harvested during exponential growth phase for all experiments, while *A. anophagefferens* was harvested during exponential (both strains 1708 and 1850) or stationary phase (strain 1708 only), depending on the experiment. *Aureococcus anophagefferens* cells reached stationary phase 2 to 2.5 weeks after inoculation (data not presented). Exponentially growing cells were harvested within 1 week of inoculation. Stationary phase cells were collected no earlier than 2 weeks after inoculation.

Copepod cultures

Acartia tonsa nauplii were collected from Stony Brook Harbor in July 2004 using a 64 μm mesh plankton net, sorted using a dissecting microscope and placed into 1 or 2 L glass flasks for culturing. Stony Brook Harbor has no history of brown tides and thus copepods collected from this site are presumed naïve to brown tide. During all experimental incubations, copepod cultures were maintained at 22°C and exposed to a 14:10 h light:dark cycle. Natural seawater from Stony Brook Harbor (salinity 27 ppt) was filtered through 0.45 μm filters and used for copepod cultures. Copepods were fed every other day with a 25:25:50 (by culture volume) mixture of *I. galbana*, *T. pseudonana* (Cosinodiscophyceae) (3H), and *Rhodomonas lens* (Cryptophyceae) (CCMP 739), respectively. The latter two algal species were cultured in f/2 medium in the manner described above. *Acartia tonsa* adults can be cannibalistic when prey abundance is low; therefore, high algal densities were maintained in all flasks.

Grazing by nauplii on *A. anophagefferens*

Treatments consisted of 100% *A. anophagefferens* (Aa), 100% of a control alga and 20:80, 50:50, 80:20% mixtures of Aa:control. These percentages were based on carbon content. A total carbon concentration equivalent to 500 000 cells mL^{-1} of *A. anophagefferens* was used in all treatments (648 ng C mL^{-1} assuming 1.296 pg C $cell^{-1}$) (after Menden-Deuer and Lessard, 2000), representing a moderate brown tide (Gobler *et al.*, 2005). An equivalent carbon concentration for the control alga *I. galbana* corresponded to ~70 000 cells mL^{-1} (Bricelj *et al.*, 2001). Experiments were conducted in two parts: (i) 100% Aa, 50:50 Aa:ISO and 100% ISO were performed simultaneously and (ii) 20:80 Aa:ISO, 80:20 Aa:ISO and another 100% ISO treatment were conducted separately. Nauplii used in parts 1 and 2 were not taken from the same cohort.

Each treatment included three replicate acid-washed jars (70 mL glass amber) with 20 nauplii (stages NIV–NVI) and three control jars without nauplii. Final naupliar densities, 280 nauplii L^{-1}, fell within the range of densities measured in Long Island bays (Lonsdale *et al.*, 1996). To minimize the addition of culture water into mixtures, nauplii were rinsed twice with treatment water prior to the final transfer into experimental jars. An equivalent amount of water from the final naupliar transfer dish was added to control jars. Nitrate and phosphate were added to each treatment to achieve a concentration of 100 and 10 μM, respectively, to minimize any effects naupliar excretion may have had on algal growth. All jars were topped off with treatment water (final volume 71.5 mL) and closed tightly to minimize air bubbles and turbulence, which can affect normal feeding behavior (Saiz and Kiørboe, 1995). Jars were then rotated on a plankton wheel at 0.5 rev min^{-1} for a 24 h in the dark at 22°C to maintain suspension of the non-motile *A. anophagefferens* cells. Bottles were kept in the dark to limit algal growth during the incubation period. Clearance rates achieved by nauplii were such that a sufficient density of algae remained available over the course of the experiment without the addition of more food.

Triplicate 4.5 mL samples were collected for the determination of initial cell densities. A single sample was collected from each jar after the 24 h incubation period to determine final cell density. All samples were preserved in 1% gluteraldehyde (final concentration) and refrigerated until processing. Cell counts were conducted by compound light microscopy using a Neubauer haemocytometer. Total cell counts ranged between 100 and 300 cells per sample. Visual distinction between the two different cell types made it possible to measure grazing on individual algal species in mixtures in addition to total grazing. Samples containing *A. anophagefferens* were passed through a syringe (21 G 1″ needle by Becton Dickinson) prior to counting to disaggregate any cell clumps present. This process did not lyse or alter cell densities (data not presented). Clearance and ingestion rates were determined by comparing initial and final cell counts after Frost (Frost, 1972). Negative clearance and grazing rates due to higher final cell densities in experimental treatments were recorded as zero.

Two electivity indices were calculated and compared to determine if nauplii displayed selective preference or avoidance for either algal type in mixtures: (i) Ivlev's original index and (ii) Jacob's Index, a variation of Ivlev's, which takes the relative abundance of the prey items into account (Ivlev, 1961; Jacobs, 1974). Indices range from −1 (selective avoidance) to 1 (preference). A value of zero indicates no selective behavior. Calculations were based on algal carbon content. To evaluate whether *A. anophagefferens* is a "toxic" food source, we used an experimental design similar to that of Colin and Dam (Colin and Dam, 2002), whereby a reference line is drawn between the total ingestion rate of a food source known to support growth (control alga) and the potentially noxious alga in question. Ingestion rates on various mixtures of phytoplankton should fall on the reference line if *A. anophagefferens* is not toxic. If ingestion rates (±1 SE) for the mixtures fall above the reference line, the two algal species together have a supplementary effect. If ingestion rates (±1 SE) for the mixtures fall below the reference line, *A. anophagefferens* is deemed "toxic" as it would be suppressing total algal consumption.

Naupliar development

The effects of *A. anophagefferens* on copepod development were examined by determining the success with which nauplii from the experiments underwent metamorphosis to copepodites. All naupliar stages used (NIV–NVI) had potential to molt into the copepodite stage after 48 h given that nauplii can melt from one stage to the next within 24 h when provided an adequate food source (J. Smith, personal observation). Due to the small percentage (roughly 10%) of nauplii molting into copepodites after the first 24 h of incubation in experiments 1 and 2, experiments were evaluated for longer time intervals ranging from 48 to 120 h. Experimental jars were removed from the plankton wheel and the contents emptied into glass dishes and observed using a dissecting microscope. Those individuals retrieved were classified as either "nauplius" or "copepodite" depending on whether or not molting had occurred. All contents were then returned to their corresponding jars and placed back on the plankton wheel.

Feeding selection based on cell size

Experiment 5 was designed to determine whether selective feeding behavior in the previous experiments was due to differences in cell size of *A. anophagefferens* (2–4 μm) and *I. galbana* (4–6 μm) or if selection was the result of physical or chemical differences. In this experiment, *M. pusilla* was used as the control alga since its size range overlaps with that of *A. anophagefferens* (∼1–3 μm). Grazing and developmental experiments were conducted in the same manner described above.

Cell densities for this experiment were determined using a Becton Dickinson FACSCalibur flow cytometer because visual differentiation between preserved cells was not possible due to the similarities in size and shape of *A. anophagefferens* and *M. pusilla* cells. Samples were taken to the Stony Brook Hospital Flow Cytometry lab and processed live, within 1 h after experiments were sampled. Distinction of cell types in mixtures was made using unique pigment fluorescence signatures in FL3 (>670 nm) versus FL4 (661 ± 16 nm) dot plots. Cell densities were obtained by adding 0.1 mL of Fluoresbrite yellow–green microspheres (6 μm) from Polysciences, Inc. to 5 mL samples from all jars at a final concentration of 230 000–240 000 spheres mL^{-1} (exact concentrations were determined for each experimental date). Cell densities were calculated by comparing a set number of events (5000) for a known abundance of microspheres to the number of algal events.

Statistical analysis

Data for the grazing and developmental experiments were analyzed using a one-way ANOVA ($\alpha = 0.05$). Bartlett's test for equal variances was first run to test the assumption of equal variances. An arcsine transformation was necessary prior to analyses because data for the developmental portion of the experiments were represented as percentages. If the ANOVA indicated a

significant effect of diet, Tukey's one-way multiple comparisons was then used to analyze differences between individual treatments within a given experiment. The error rate for Tukey's comparison was set at 0.05. Electivity indices were compared using the Wilcoxon signed-rank test (Sokal and Rohlf, 1981).

RESULTS

Copepod responses to CCMP isolate 1708 in exponential growth phase

Grazing experiments

There were no significant differences in mean ingestion rates in both experiment 1 (100% ISO, 50:50 Aa:ISO and 100% Aa) and experiment 2, which replicated the treatments of experiment 1, where *Acartia tonsa* nauplii were provided with *A. anophagefferens* as a sole food source (Fig. 1a–e; ANOVA, $P > 0.05$). However, ingestion rates on *A. anophagefferens* (578 and 247 ng C nauplius^{-1} h^{-1} for experiments 1 and 2, respectively) were approximately two times greater than in treatments when *I. galbana* was provided as the single food source (Figs. 1a–d). Mean ingestion rates for mixed diets were below the reference line for naupliar ingestion on all but one occasion (Fig. 1a and b). Four of six mixed diets had positive electivity indices for *I. galbana* and negative indices for *A. anophagefferens* (Table II).

Developmental experiments

On average, 84% of nauplii were found and examined for molting. There were no significant differences in percent change to copepodites between treatments in either experiment 1 or 2 over the first 24 h period (Fig. 2a–d). There was a significant difference in developmental success among diet treatments in experiment 1 after 48 h of incubation ($P < 0.05$); nauplii fed *I. galbana* alone had a higher percentage change to copepodite than those where the diet consisted of 50:50 Aa:ISO (Tukey's one-way multiple comparison; Fig. 2a). Naupliar percent change to copepodite in the 100% *I. galbana* and 20:80 Aa:ISO diets were significantly different from the treatment containing 80:20 Aa:ISO ($P < 0.01$; Tukey's one-way multiple comparison; Fig. 2c). There were no significant results in experiment 2, which was only conducted for 24 h (Fig. 2d).

In general, experiments using younger (NIV or NV) naupliar stages were easier to interpret since the percent change to copepodite could be monitored between multiple days instead of having the bulk of the metamorphosis occur over the first 24 h. There were no significant differences in the percent metamorphosis across treatments in any experiment over the first 24 h.

Copepod responses to CCMP isolate 1708 in stationary growth phase

Grazing experiments

There were no significant differences among treatments in ingestion rates in experiment 3 which was designed to compare the ingestion of stationary phase *A. anophagefferens* (1708) to *I. galbana* (Fig. 3b and c). The mean ingestion rate on *A. anophagefferens* alone (100%) was approximately twice the ingestion rate on an *I. galbana* diet (averaged over the two parts of experiment 3), which was similar to results obtained in experiments 1 and 2 (Fig. 3a). The diet mixture 80:20 Aa:ISO had the lowest mean ingestion rate and the greatest electivity indices (Table II). The diet consisting of 20% stationary cells of 1708 had a positive electivity index for *A. anophagefferens* cells (Table II). The mixed diets of 50:50 and 80:20 Aa:ISO had negative indices for the brown tide alga and positive indices for *I. galbana* (Table II).

Developmental experiments

There were no significant differences in the percent change to copepodite between treatments after 24 or 48 h during experiment 3 (Fig. 4a and b). However, a significantly greater percent of *Acartia tonsa* nauplii developed into copepodites after 72 h of incubation when fed 100% *I. galbana* and 20:80 Aa:ISO compared to the 80:20 Aa:ISO treatment (ANOVA, $P < 0.01$; Tukey's multiple comparison; Fig. 4b).

Copepod responses to strain CCMP 1850 in exponential growth phase

Grazing experiment

No significant differences in ingestion rates occurred in experiment 4 (Fig. 5a–c). Unlike the results using strain 1708, no grazing was detected in bottles when *A. anophagefferens* clone 1850 was the only food source (Fig. 5b). Jacobs electivity indices (absolute value) for nauplii fed diets of 20:80 and 80:20 Aa:ISO in this experiment were four times greater than for the 50:50 Aa:ISO diet (experiment 4, Table II). A positive electivity index for *I. galbana* was measured in all mixed diets (Table II; see also Fig. 5b and c).

Developmental experiments

The percent change to copepodite in the 100% *I. galbana* treatment was significantly greater than in the

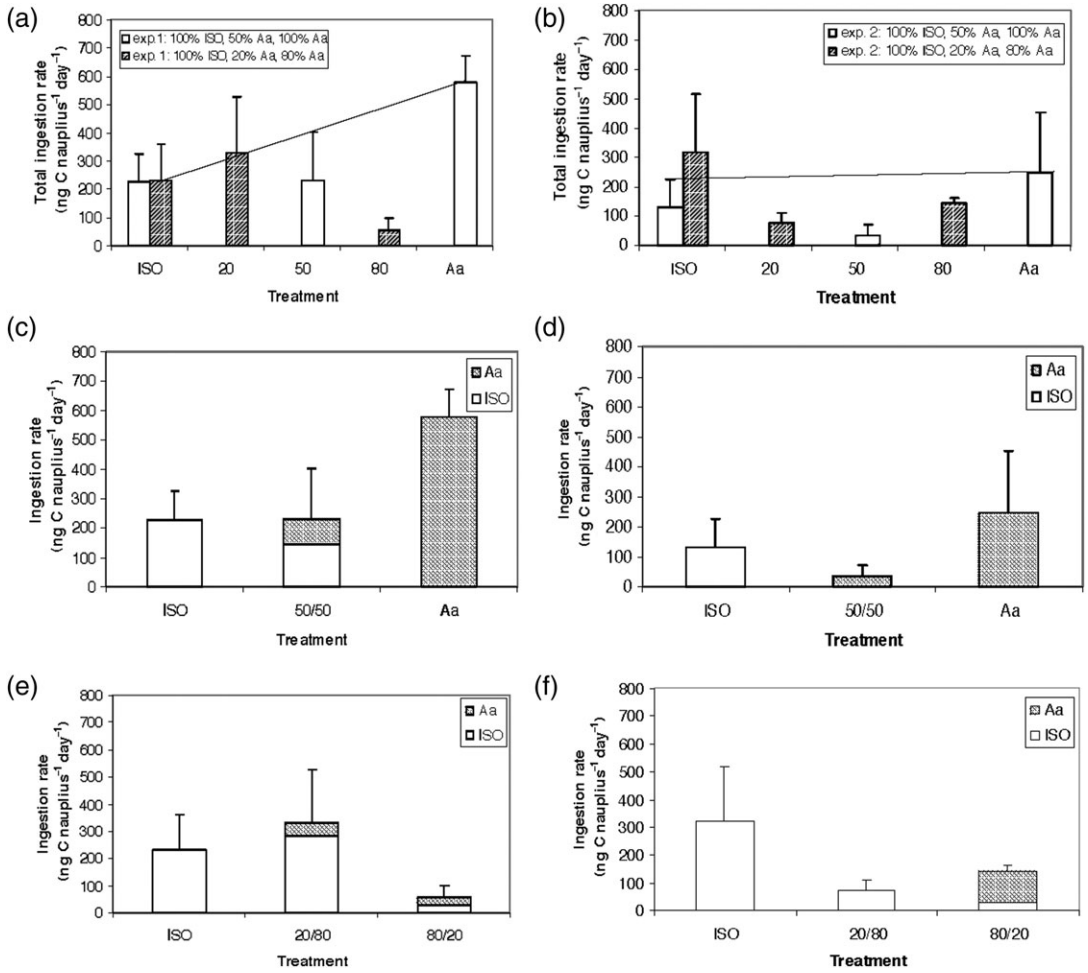

Fig. 1. Naupliar ingestion rates on mixed diets containing different proportions of exponential growth phase strain 1708 *Aureococcus anophagefferens* (Aa) and *Isochrysis galbana* (ISO). (**a** and **b**) Total ingestion for experiments 1 (a) and 2 (b) (part 1: 100% ISO, 50:50 Aa:ISO, 100% Aa; part 2: 100% ISO, 20:80 Aa:ISO, 80:20 Aa:ISO). Reference line drawn connects ingestion rate on 100% ISO (average from parts 1 and 2) and ingestion rate on 100% Aa. (**c** and **d**) Proportion of Aa and ISO ingested during experiments 1 (c) and 2 (d) (Part 1: 100% ISO, 50:50 Aa:ISO, 100% Aa). (**e** and **f**) Proportion Aa and ISO ingested during experiments 1 (e) and 2 (f) (Part 2: 100% ISO , 20:80 Aa:ISO, 80:20 Aa:ISO).

100% *A. anophagefferens* treatment (ANOVA, $P < 0.05$; Tukey's multiple comparison; Fig. 6a) in the first part of experiment 4. In part two of the experiment, there was an even greater difference in metamorphic success, with a 60% change to copepodite in the 100% *I. galbana* treatment and about a 40% change in mixed treatments (20:80, 80:20 Aa:ISO; Fig. 6b) than in the 48 and 72 h time intervals. However, there were no statistically significant differences among diets.

Feeding selection based on cell size

Grazing experiments

No significant differences in ingestion rates between *A. anophagefferens* clone 1708 and *M. pusilla* were observed in experiment 5 (Fig. 7a–c). The 50:50 Aa:ISO diet resulted in electivity indices close to zero (Table II Fig. 7b and c). When fed either a diet of 20:80 and 80:20 Aa:ISO, nauplii displayed positive electivity indices for the more abundant alga.

Developmental experiments

Although metamorphic success was typically lower in mixed treatments, these differences were not significant (Fig. 8a and b). The greatest percent change from nauplii to copepodites was seen after the first 24 h for all treatments. Additional molting to copepodite was not apparent in any treatment after the first 24 h interval.

Results across experiments

The pooled mean (± 1 SD) clearance rate of *A. tonsa* nauplii feeding on *A. anophagefferens* was 7.7 (± 7.1) μL

Table II: Electivity indices for Acartia tonsa nauplii grazing on mixtures of Aureococcus anophagefferens and a control alga, Isochrysis galbana or Micromonas pusilla

Isolate	Experiment	Treatment	Algal type	Ivlev's index	Jacob's index	+/−
CCMP 1708 (exp.)	1	20:80	Aa	−0.293	−0.361	−
			ISO	0.077	0.361	+
	2	20:80	Aa	−1	−1	−
			ISO	0.178	1	+
	1	50:50	Aa	0.252	1	+
			ISO	−1	−1	−
	2	50:50	Aa	−0.265	−0.51	−
			ISO	0.283	0.51	+
	1	80:20	Aa	−0.172	−0.437	−
			ISO	0.286	0.437	+
	2	80:20	Aa	0.071	0.268	+
			ISO	−0.201	−0.268	−
CCMP 1708 (stat.)	3	20:80	Aa	0.17	0.25	+
			ISO	−0.084	−0.25	−
	3	50:50	Aa	−0.15	−0.303	−
			ISO	0.161	0.303	+
	3	80:20	Aa	−0.879	−0.975	−
			ISO	0.674	0.975	+
CCMP 1850 (exp.)	4	20:80	Aa	−0.58	−0.658	−
			ISO	0.126	0.658	+
	4	50:50	Aa	−0.06	−0.159	−
			ISO	0.1	0.159	+
	4	80:20	Aa	−0.197	−0.653	−
			ISO	0.522	0.653	+
CCMP 1708 (exp.)	5	20:80	Aa	−1	−1	−
			Mp	0.103	1	+
	5	50:50	Aa	−0.025	−0.052	0
			Mp	0.027	0.052	0
	5	80:20	Aa	0.111	0.7	+
			Mp	−0.634	−0.7	−

Isochrysis galbana (ISO) was the control for experiments 1 through 4. *Micromonas pusilla* (Mp) was used as a control alga used in experiment 5 since its size range overlaps that of *A. anophagefferens,* to test whether selection in experiments 1 to 4 may have been the consequence of size selection. Both Ivlev's and Jacob's indices showed that nauplii significantly select against *A. anophagefferens* when in mixtures ($P < 0.05$; Wilcoxin signed-rank test).

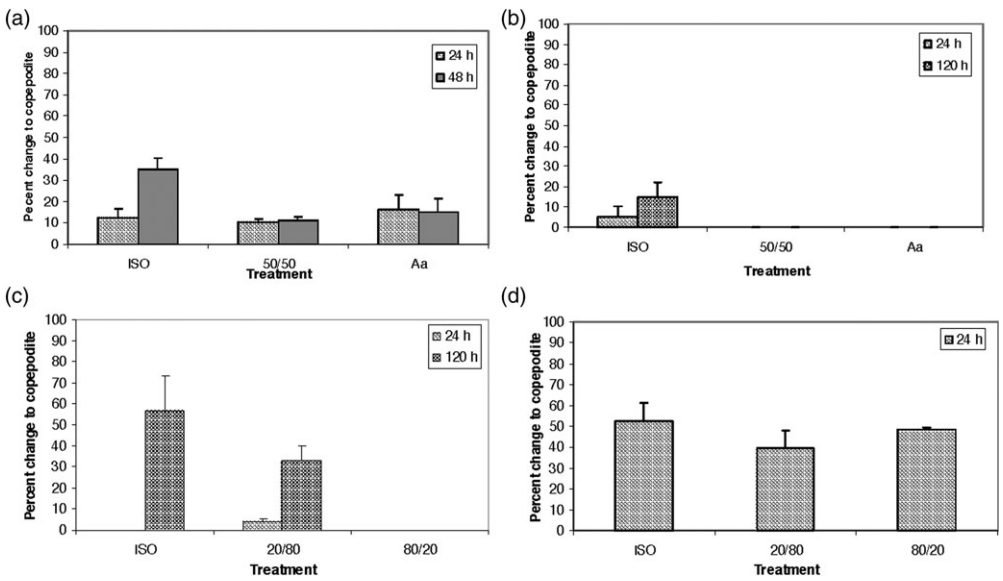

Fig. 2. Percent *Acartia tonsa* nauplii that molted to the copepodite stage when fed diets containing different proportions of exponential growth phase strain 1708 *Aureococcus anophagefferens* (Aa) and Isochrysis galbana (ISO). (**a**) Experiment 1, part 1 (100% ISO, 50:50 Aa:ISO, 100% Aa). At 48 h, development on ISO>50:50 Aa:ISO diet ($P < 0.05$; Tukey's multiple comparison). (**b**) Experiment 2, part 1 (100% ISO, 50:50 Aa:ISO, 100% Aa). (**c**) Experiment 1, part 2 (100% ISO, 20:80 Aa:ISO, 80:20 Aa:ISO). After 120 h, development on ISO and 20:80 Aa:ISO > 80:20 Aa: ISO ($P > 0.01$; Tukey's multiple comparison). (**d**) Experiment 2, part 2 (100% ISO, 20:80, Aa:ISO, 80:20 Aa:ISO).

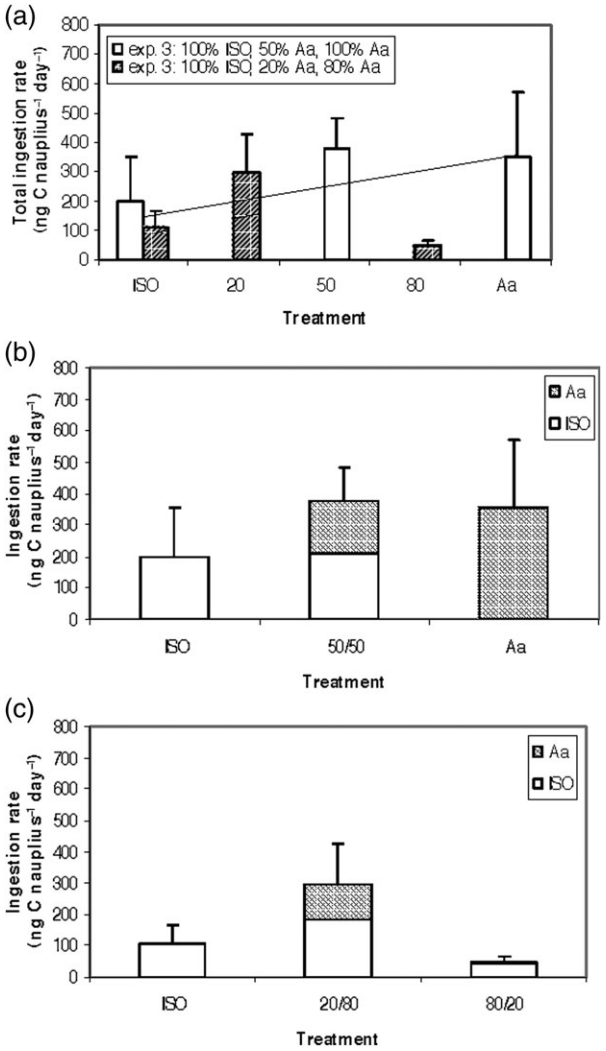

Fig. 3. Naupliar ingestion on mixed diets containing different proportions of stationary growth phase strain 1708 *Aureococcus anophagefferens* (Aa) and *Isochrysis galbana* (ISO). (**a**) Total ingestion rates for experiment 3 (part 1: 100% ISO, 50:50 Aa:ISO, 100% Aa; part 2: 100% ISO, 20:80 Aa:ISO, 80:20 Aa:ISO). Reference line drawn connects ingestion rate on 100% ISO (average from parts 1 and 2) and ingestion rate on 100% Aa. (**b**) Proportion of Aa and ISO ingested during experiment 3, part 1 (100% ISO, 50:50 Aa:ISO, 100% Aa). (**c**) Proportion Aa and ISO ingested during experiment 3, part 2 (100% ISO, 20:80 Aa:ISO, 80:20 Aa:ISO).

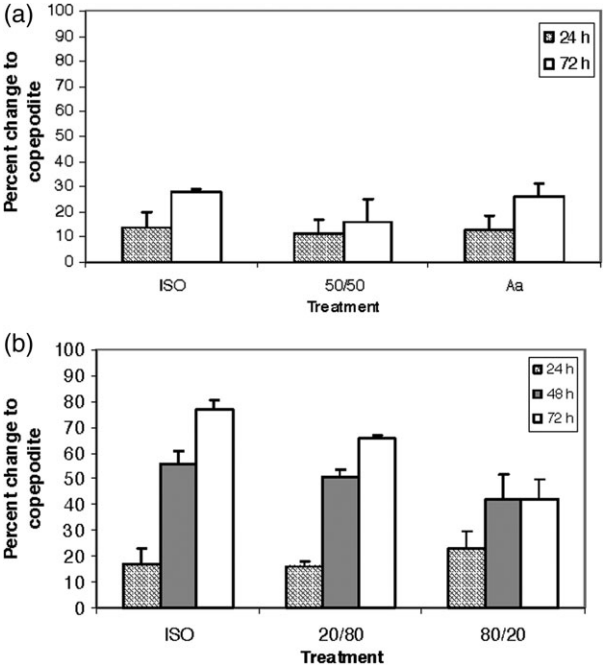

Fig. 4. Percent *Acartia tonsa* nauplii that molted to the copepodite stage when fed diets containing different proportions of stationary phase strain 1708 *Aureococcus anophagefferens* (Aa) and *Isochrysis galbana* (ISO). (**a**) Experiment 3, part 1 (ISO, 50:50 Aa:ISO, 100% Aa). (**b**) Experiment 3, part 2 (ISO, 20:80 Aa:ISO, 80:20 Aa:ISO). After 72 h, development on ISO and 20:80 Aa:ISO > 80:20 Aa:ISO ($P < 0.01$; Tukey's multiple comparison).

nauplius $^{-1}$ h^{-1}, whereas nauplii cleared on average 13.7 ($\pm$7.8) μL nauplius $^{-1}$ h $^{-1}$ on *I. galbana* and 5.4 ($\pm$5.3) μL nauplius^{-1} h $^{-1}$ on *M. pusilla*. The overall carbon ingestion rate of nauplii fed a diet of 100% *A. anophagefferens* was 292.9 ng C nauplius^{-1} day^{-1} (Table III), 230.8 ng C nauplius^{-1} day^{-1} on *I. galbana* and 186.9 ng C nauplius^{-1} day^{-1} on *M. pusilla*. Mean ingestion rates of nauplii fed mixed diets were lower overall than when nauplii were provided a single algal type (Table IV). Overall, *A. tonsa* nauplii significantly preferred *I. galbana* and avoided *A. anophagefferens* (Wilcoxon signed-rank test, $P < 0.05$; Table II). In summary, *A. tonsa* nauplii grew better on the larger-celled control alga *I. galbana* than in treatments with either *A. anophagefferens* or *M. pusilla*, even though grazing was, at times, higher on *A. anophagefferens* (Table V).

DISCUSSION

The effects of *A. anophagefferens* on naupliar grazing

Food selection by nauplii

Overall, both electivity indices indicate that *Acartia tonsa* nauplii exhibited a feeding preference for *I. galbana* in mixtures with *A. anophagefferens*. *Acartia tonsa* adults have been noted to select food items based on the chemical content of algal cells (Cowles *et al.*, 1988; Teegarden, 1999). However, selective feeding behavior based on cell size could not be ruled out as a factor causing the apparent selection, since *I. galbana* is a larger alga than *A. anophagefferens*. The size range for the control alga *M. pusilla*, used in experiment 5, is similar to *A. anophagefferens*. In

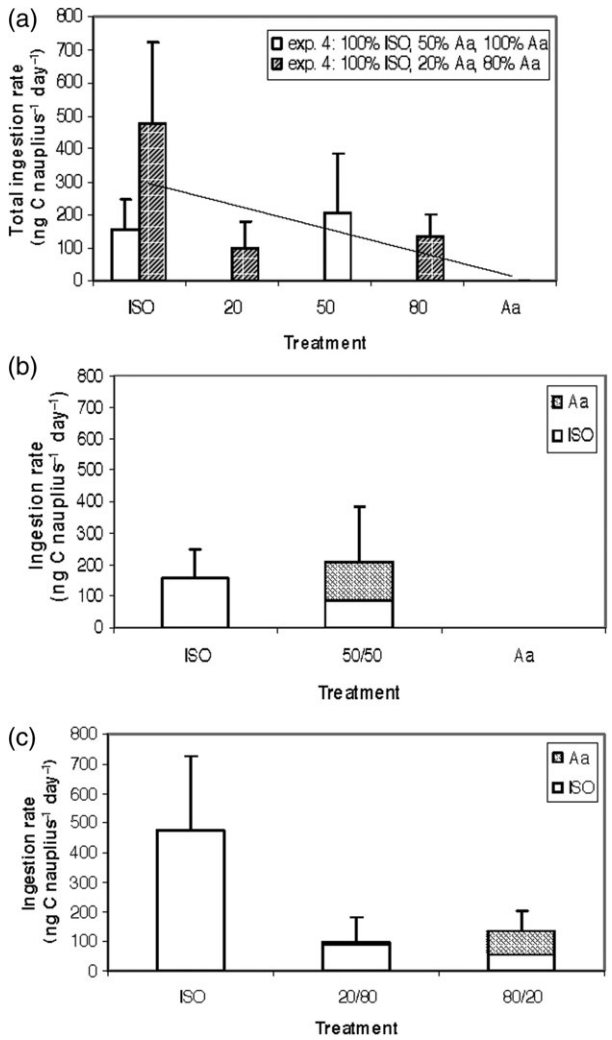

Fig. 5. Naupliar ingestion rates on mixed diets containing different proportions of exponential growth phase strain 1850 *Aureococcus anophagefferens* (Aa) and *Isochrysis galbana* (ISO). (**a**) Total ingestion rates for experiment 4 (part 1: 100% ISO, 50:50 Aa:ISO, 100% Aa; part 2: 100% ISO, 20:80 Aa:ISO, 80:20 Aa:ISO). Reference line drawn connects ingestion rate on 100% ISO (average from parts 1 and 2 100%) and ingestion rates on 100% Aa. (**b**) Proportion of Aa and ISO ingested during experiment 4, part 1 (100% ISO, 50:50 Aa:ISO, 100% Aa). (**c**) Proportion Aa and ISO ingested during experiment 4, part 2 (100% ISO, 20:80 Aa:ISO, 80:20 Aa:ISO).

the 50:50 Aa (1708 exponential):Mp mixture, electivity indices were close to zero, indicating no selective feeding by nauplii in this treatment. Additionally, there was no notable preference in the 20:80 and 80:20 Aa:ISO treatment mixtures in experiment 5 where nauplii selectively grazed on the algal species with the greatest biomass. Thus, the selective feeding behavior demonstrated in diet mixtures with *I. galbana* and CCMP 1708 could reflect naupliar selection for larger cells rather than an avoidance reaction to the physical or chemical attributes of *A. anophagefferens* cells.

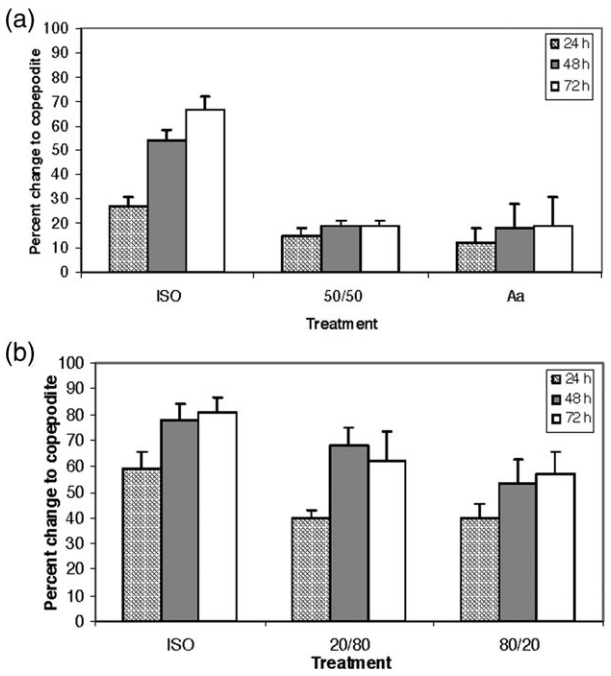

Fig. 6. Percent *Acartia tonsa* nauplii that molted to the copepodite stage when fed diets containing different proportions of exponential growth phase strain 1850 *Aureococcus anophagefferens* (Aa) and *Isochrysis galbana* (ISO). (**a**) Experiment 4, part 1 (100% ISO, 50:50 Aa:ISO, 100% Aa). (**b**) Experiment 4, part 2 (100% ISO, 20:80 Aa:ISO, 80:20 Aa:ISO). After 72 h, development on ISO>Aa ($P<0.05$; Tukey's multiple comparison).

Naupliar grazing on A. anophagefferens isolate 1708 in exponential growth phase

Experiments using exponentially growing cells of isolate 1708 indicated that naupliar ingestion rates were not suppressed on a diet of only *A. anophagefferens*, as naupliar ingestion rates on 100% *A. anophagefferens* (strain 1708) were not significantly different from those on 100% *I. galbana* and 100% *M. pusilla* control diets. *Aureococcus anophagefferens* has been found to be a poor food source for some copepods (Lonsdale *et al.*, 1996); therefore, high naupliar ingestion rates on the alga could indicate a compensatory response to an inadequate food source. Such a response has been noted in ciliates; unusually high ingestion rates by *Strombidium* sp. were measured on a diet of 98% *A. anophagefferens* cells, but there was negligible population growth (Mehran, 1996; Caron *et al.*, 2004). In mixtures of *I. galbana* and *A. anophagefferens* using isolate 1708 in exponential growth phase (experiments 1 and 2), mean ingestion rates of *Acartia tonsa* nauplii for all mixtures were lower than the reference line connecting ingestion rates on the single-food diets, indicating that *A. anophagefferens* may have toxic effects on *Acartia tonsa* nauplii in mixtures (after Colin and Dam, 2002).

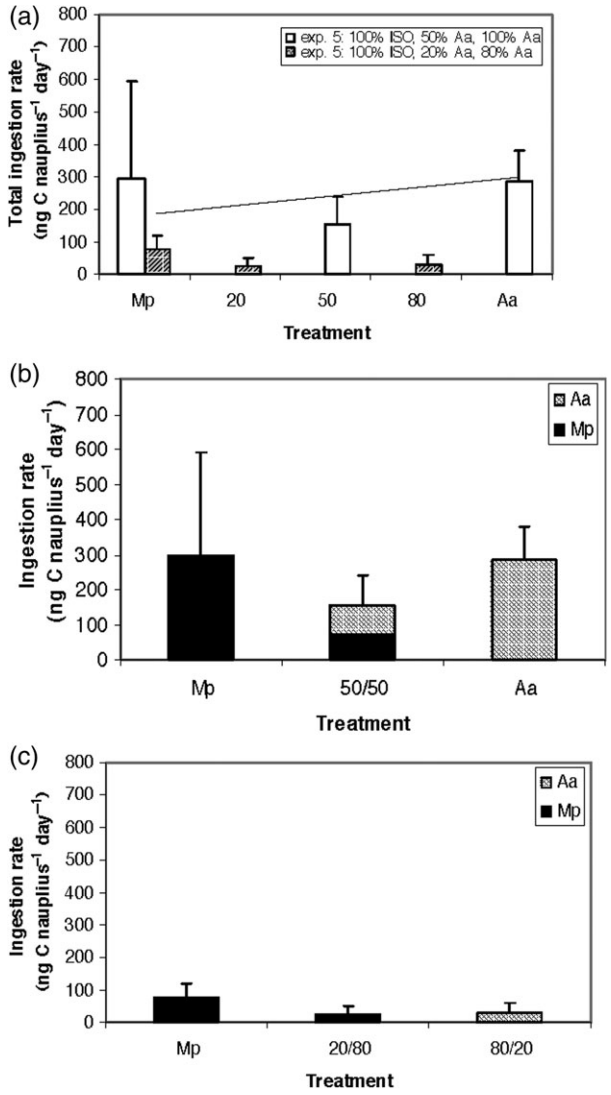

Fig. 7. Naupliar ingestion rates on mixed diets containing different proportions of exponential phase strain 1708 *Aureococcus anophagefferens* (Aa) and *Micromonas pusilla* (Mp). (**a**) Total ingestion rates for experiment 5 (part 1: 100% Mp, 50:50 Aa:ISO, 100% Aa; part 2: 100% Mp, 20:80 Aa:ISO, 80:20 Aa:ISO). Reference line drawn connects ingestion rate on 100% Mp (average from parts 1 and 2) and ingestion rate on 100% Aa. (**b**) Proportion of Aa and Mp ingested during experiment 5, part 1 (100% Mp, 50:50 Aa:ISO, 100% Aa). (**c**) Proportion Aa and Mp ingested during experiment 5, part 2 (100% Mp, 20:80 Aa:ISO, 80:20 Aa:ISO).

Conversely, lower naupliar ingestion rates on mixed diets may reflect increased handling time due to selective feeding behavior. A trade-off between handling time and food preference was measured in *A. tonsa* adults that were offered mixed diets containing a high quality (higher N content) food item (Cowles *et al.*, 1988). Therefore, the design used does not appear to be an ideal test for actual toxicity in the case of *A. anophagefferens*.

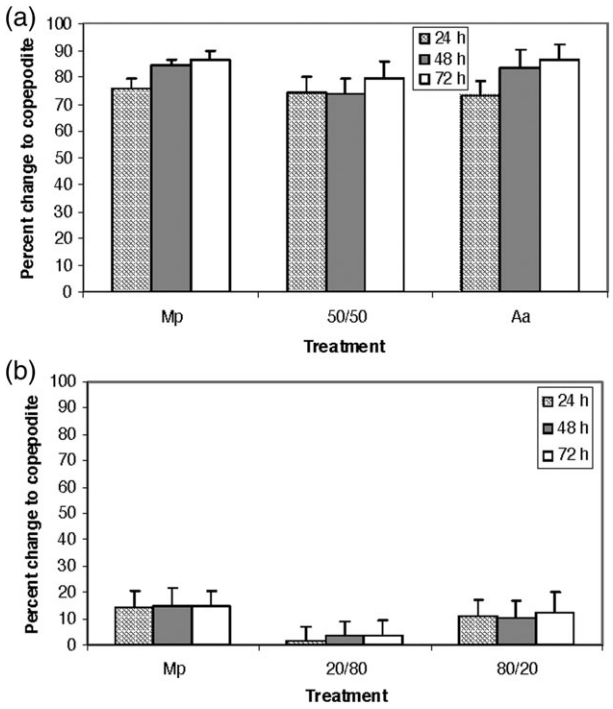

Fig. 8. Percent *Acartia tonsa* nauplii that molted to the copepodite stage in diets containing exponential growth phase strain 1708 *Aureococcus anophagefferens* (Aa) and *Micromonas pusilla* (Mp). (**a**) Experiment 5, part 1 (100% Mp, 50:50 Aa:ISO, 100% Aa). (**b**) Experiment 5, part 2 (100% Mp, 20:80 Aa:ISO, 80:20 Aa:ISO).

Naupliar grazing on A. anophagefferens isolate 1708 in stationary growth phase

There were no significant differences in naupliar ingestion on *A. anophagefferens* isolate 1708 in stationary phase, control or mixed treatments. This is surprising given that (i) copepods may ingest stationary phase cells at slower rates than exponential phase cells (Cowles *et al.*, 1988); (ii) *A. anophagefferens* isolate 1708 cells in stationary growth phase are significantly more toxic than exponentially growing cells to juvenile mussels (*Mytilus edulis*) and hard clams (*Mercenaria mercenaria*) (Bricelj *et al.*, 2001), as well as hard clam larvae (Padilla *et al.*, 2006); (iii) the sticky outer polysaccharide layer of stationary phase *A. anophagefferens* cells was found to cause feeding cessation in shellfish (Bricelj *et al.*, 2001); and (iv) in the related species *Aureombra lagunensis*, the amount of extracellular polysaccharide secretions was higher in algal cultures in the stationary or declining growth phase compared to the exponential growth phase and caused feeding reductions in ciliates (Liu and Buskey, 2000b). There was noticeable clumping in our stationary phase *A. anophagefferens* cultures in contrast to our exponentially growing cultures, which was most likely caused by sticky extracellular secretions. Yet despite reduced feeding measured in other organisms,

Table III: Mean clearance rates (μL nauplius^{-1} h^{-1}) pooled from all experimental treatments

Experiment	Treatment	ISO	Aa	Mp
1	100%	14.7	18.3	
1	50/50	17.6	5.5	
1	100%	16		
1	20/80	23	9.9	
1	80/20	9.5	3.1	
2	100%	8.8	3.2	
2	50/50	0	11.1	
2	100%	23		
2	20/80	6.9	0	
2	80/20	8.9	14.1	
3	100%	12.5	12.4	
3	50/50	27.9	15.4	
3	100%	6.2		
3	20/80	13.4	24.8	
3	80/20	8.6	0.1	
4	100%	11.3	0	
4	50/50	11.5	10.5	
4	100%	31.3		
4	20/80	6.3	1.3	
4	80/20	16.7	3.6	
5	100%		12.8	13.8
5	50/50		6.1	6.6
5	100%			4.4
5	20/80		0	1.9
5	80/20		2.6	0.5
	Averages	13.7	7.7	5.4
	SD	7.8	7.1	5.3

Pooled mean and standard deviation are included for each *Isochrysis galbana* (ISO), *Aureococcus anophagefferens* (Aa) and *Micromonas pusilla* (Mp).

Table IV: Total mean ingestion rates (ng C nauplius^{-1} day^{-1}) (n=3) of Acartia tonsa nauplii on Isochrysis galbana (ISO), Aureococcus anophagefferens (Aa) and mixed diets (proportions Aa:ISO) and total naupliar ingestion rates for Micromona pusilla (Mp), A. anophagefferens and mixed proportions of Mp:Aa. Pooled mean and standard deviation are also included.

Experiment	ISO	20/80	50/50	80/20	Aa
1	228.4		232.9		577.6
1	231.9	329.8		55	
2	130.3		35.9		247
2	319.1	74.2		142	
3	196.4		377		352.1
3	108.5	295.5		46.7	
4	155		206.6		0
4	476.8	95.5		131.4	
Mean	230.8	198.7	213.1	93.8	294.2
SD	112	132.5	139.9	49.9	
Experiment	Mp	20/80	50/50	80/20	Aa
5	296.4		153.3		287.8
5	77.4	25.6		30.9	
Mean	186.9	25.6	153.3	30.9	287.8
SD	154.9				

stationary phase cells did not appear to hinder naupliar ingestion in our experiments.

There are several possible explanations as to why naupliar ingestion rates were not suppressed by stationary phase cells. Since cells in stationary phase were more clumped, the particle diameter may have effectively been enlarged, and nauplii may be more efficient at consuming aggregates since they do select larger prey items (discussed above). Also, differences in naupliar feeding mechanics, as compared to shellfish and protozoa, may cause nauplii to be less susceptible to the physical nature (i.e. stickiness) of stationary phase cells. Whereas ciliates rely on ciliary movements for feeding, nauplii obtain food particles through the feeding currents created by their appendages. Therefore, naupliar ingestion may not be suppressed to the same degree as ciliary feeding.

Naupliar grazing on A. anophagefferens isolate 1850 in exponential growth phase

There was no grazing on *A. anophagefferens* isolate 1850 when provided as the only food source in experiment 4. Nauplii consumed *I. galbana* in all other treatments in the experiment, suggesting that nauplii were in good health and had the potential to graze on the brown tide alga. Ingestion rates in mixtures (20:80 and 80:20 Aa:ISO) within this same experiment were greatly reduced (though not significant) (Fig. 7). It is possible that lower ingestion rates in these mixed treatments were due to size selection, cellular toxicity, or a combination of the two. The lack of grazing in jars in the 100% *A. anophagefferens* treatment demonstrates that isolate CCMP 1850 displays a more toxic effect on nauplii than CCMP 1708. This could be related to the number of years each of these strains has been in culture (CCMP 1708 isolated in 1995; 1850 isolated in 1998), as Bricelj *et al.* (2001) demonstrated that older isolates of *A. anophagefferens* in culture became less noxious over time compared to more recently isolated clones.

Metamorphosis of *Acartia tonsa* nauplii

Naupliar development on A. anophagefferens and the control alga I. galbana

Naupliar development was clearly depressed by the presence of *A. anophagefferens* cells in exponential phase growth in mixed diets with *I. galbana* (experiments 1 and 2), which could indicate that *A. anophagefferens* is a nutritionally deficient food source for nauplii. Survival of

Table V: Summary of results for experiments on naupliar grazing and growth on the harmful alga Aureococcus anophagefferens (Aa) strain 1708 (exponential versus stationary) and strain 1850 comparing larger and same-size control algae Isochrysis galbana (ISO) and Micromonas pusilla (Mp), respectively, in the copepod Acartia tonsa

	ISO	Aa (1708) exponential	Aa (1708) stationary	Aa (1850)	Mp	Conclusion
Grazing[a]	100% ISO ≤ 100% Aa 1708; 100% ISO > Aa 1850; ISO was the preferred food item in mixed-diet treatments overall	100% ISO ≤ 100% Aa exponential =100% Aa stationary	100% ISO < 100% Aa exponential =100% Aa stationary	100% ISO > 100% Aa 1850 (zero grazing measured when 100%, grazing low when in mixed-diets); 100% Aa 1708 > 100% Aa 1850	Mp=Aa	Prey size has an effect on *Acortia tonsa* naupliar grazing rates; larger cells are consumed more efficiently and/or preferred. Although strain 1708 did not appear to hinder grazing, strain 1850 may cause inhibition
Growth	ISO>Aa=Mp	ISO>Aa exponential=Aa stationary	ISO>Aa exponential=Aa stationary	1708=1850 when 100%	Mp=Aa	*A. anophagefferens* and *M. pusilla* are either lower quality food items than *I. galbana* or nauplii have a lower energetic efficiency for these small cells

[a]Results for grazing experiments were non-significant. Results shown represent trends only.

nauplii of the harpacticoid copepod *C. canadensis*, however, was depressed only when nauplii were fed 100% *A. anophagefferens*, as the presence of alternate food (ambient plankton or *T. pseudonana*) ameliorated the negative effects of the brown tide alga in the copepod culture medium (Lonsdale *et al.*, 1996). Similarly, growth rates of the ciliate *Strombidium* sp. were not suppressed by mixtures containing less than 80% *A. anophagefferens* (isolate 1708) (Caron *et al.*, 2004).

Naupliar development on A. anophagefferens and the control alga M. pusilla

Nauplii did not undergo metamorphosis as readily on the control alga *M. pusilla* (experiment 5) as it did when *I. galbana* was used as the control alga. There was virtually no change to copepodite beyond the first 24 h interval. Although *M. pusilla* is not known to be toxic to copepods, it does not appear to be a nutritious food source for them. These findings suggest that the type of alternate food available determines how the brown tide alga will affect nauplii, whether by influencing selective feeding or nutrient deficiencies.

The role of Acartia tonsa nauplii in brown tide dynamics

Copepod nauplii often dominate the micrometazoan (64–202 μm) component of plankton assemblages in estuaries. In 1991, with the exception of one date, nauplii were the most abundant micrometazoans in samples taken throughout the summer in both Great South Bay and the Peconic Bay estuary, NY, USA ($n = 10$ observations) (Lonsdale *et al.*, 1996). Copepod nauplii were also the most abundant micrometazoans in Quantuck Bay, NY, USA in 2003 and 2004 (non-brown tide years in NY, USA), and were dominant in Chincoteague Bay, MD, USA throughout the summer of 2004 (a brown tide year in MD, USA) (Deonarine *et al.*, 2006). We have shown that naupliar ingestion rates on *A. anophagefferens*, CCMP isolate 1708, can be high. Therefore, in cases of dense blooms of *A. anophagefferens*, it is possible that naupliar grazing pressure can contribute to bloom decline, at least if cells in nature resemble isolate 1708. However, if larger food items are available, as often occurs in natural assemblages (Deonarine *et al*, 2006), *Acartia tonsa* nauplii may selectively avoid smaller food items such as *A. anophagefferens*. Due to this selective behavior, it is unlikely that nauplii are strong controls on bloom proliferation when other large algae are still readily available.

CONCLUSION

Our work provides insight on the grazer–prey relationship between *Acartia tonsa* nauplii and the harmful brown tide alga, *A. anophagefferens*. Nauplii exhibit a selective preference for the larger food item, *I. galbana*, when it was presented in mixtures with *A. anophagefferens* cells. However, when *A. anophagefferens* strain CCMP 1708 was the only available food, *Acortia tonsa* nauplii exhibited ingestion rates equal to or higher (though non-significant) than ingestion rates on *I. galbana* alone. Thus, these nauplii are more likely to play a role in

bloom demise than to act as a control during initial bloom proliferation. The potential for copepod nauplii to place grazing pressure on *A. anophagefferens* during blooms, when cell densities are high, may be affected by cellular toxicity of the particular strain in nature. Differences in the ability of nauplii to successfully graze on *A. anophagefferens* were seen when different strains were used; as grazing was not detected using CCMP 1850 as the only food source. Both strains resulted in reduced development, supporting the idea that this alga is a poor food type for copepod nauplii.

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