

Density-dependent nutritional value of the dinoflagellate *Cochlodinium polykrikoides* to the copepod *Acartia tonsa*

Xiaodong Jiang,* Darcy J. Lonsdale, and Christopher J. Gobler

School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York

Abstract

The nutritional value of the dinoflagellate *Cochlodinium polykrikoides* to the copepod *Acartia tonsa* was evaluated over a range of ecologically relevant cell densities. Based on egg production rate, egg hatching success, and naupliar recruitment rate of *A. tonsa*, mixed-diet experiments indicated *C. polykrikoides* was nutritionally insufficient or had no nutritional value to *A. tonsa* at 600 $\mu\text{g C L}^{-1}$ (330 cells mL^{-1}), and was toxic at 1000 $\mu\text{g C L}^{-1}$ (550 cells mL^{-1}) when compared with the nontoxic flagellate *Rhodomonas lens*. However, the nutritional value of *C. polykrikoides* to *A. tonsa* at 100 and 200 $\mu\text{g C L}^{-1}$ (55 and 110 cells mL^{-1}) was greater than or equal to that of *R. lens*. The density-dependent nutritional value of *C. polykrikoides* to *A. tonsa* was also demonstrated in the long-term survival experiments. Survivorship of *A. tonsa* fed *C. polykrikoides* was lower than those fed *R. lens* at 900 and 1800 $\mu\text{g C L}^{-1}$. In contrast, *C. polykrikoides* supported higher survivorship of *A. tonsa* than *R. lens* at 180 and 540 $\mu\text{g C L}^{-1}$. The nutritional value of *C. polykrikoides* to *A. tonsa* decreased from beneficial to deleterious with increasing cell density. A putatively “harmful” alga is not always deleterious to grazers, and its ecological effects may be distinctly different during bloom and non-bloom periods.

One long-standing ecological question in aquatic sciences is why a major fraction of dense phytoplankton blooms in aquatic environments, generally dominated by diatoms or dinoflagellates, is ungrazed and sinks out of the euphotic zone (Litchman and Klausmeier 2008). The low grazing pressure on these blooms has been attributed to the inability of herbivore populations, mainly copepods, to take advantage of the blooms due to the latter’s long development time, ranging from weeks to months (Mauchline 1998), relative to fast algal reproductive rates. An alternative explanation is that predation, especially cannibalism, constrains the cohort size of copepods (Ohman and Hirche 2001). On the other hand, the nutrition hypothesis argues that not all algae are good food sources due to nutritional inadequacies, morphological defenses, and/or chemical defenses. These traits are known to depress herbivore feeding and negatively affect herbivore fitness (Miralto et al. 1999; Prince et al. 2006). The nutritional value of algae is usually considered species specific and varies greatly in terms of digestion resistance, biochemical composition, and toxin production (Sterner and Schulz 1998). Some genera of phytoplankton such as *Rhodomonas*, *Chlamydomonas*, and *Scenedesmus* are typically considered as high-quality food sources for zooplankton (Sterner and Schulz 1998; Koski et al. 2008). Many biochemical components in algae, including certain vitamins, amino acids, and fatty acids, are nutritionally important for zooplankton success (Jónasdóttir 1994). Element imbalances can reduce phytoplankton quality and limit zooplankton growth (Litchman and Klausmeier 2008). Incomplete digestion, possibly due to thickened cell walls or increased extracellular mucilage, can also contribute to the low food quality of some algae (Sterner and Schulz 1998). When algae produce toxins, grazers are often deleteriously

affected due to impaired feeding, physiological dysfunction, depressed growth and reproduction, and reduced population fitness (Landsberg 2002; Prince et al. 2006). Therefore, the negative effects of algae on zooplankton may be explained by both the absence of essential nutrients and the presence of toxins. A major challenge in understanding the nutritional ecology of zooplankton is separating potential toxic effects of prey from their nutritional inadequacy (Colin and Dam 2002).

The mixed-diet technique has been developed to discern whether a given phytoplankton species is beneficial, nutritionally inadequate, or toxic to grazers (Jónasdóttir et al. 1998). This approach is based on the premise that grazer responses, such as clearance rate, egg production rate, and egg hatching success, are linearly related to the proportion of good and poor prey in a mixed diet. Grazers are offered sole diets of the suspected prey (the treatment), a well-known good prey (the control), and mixed diets. A reference line is drawn connecting the responses of the grazer feeding on the 100% suspect and 100% control prey. If the responses of the grazer with the suspected prey are higher than or similar to values with the control prey, the suspect prey is likely to be a nutritionally beneficial food. If the responses of the grazer fed the suspected prey are lower than values with the control prey, deleterious effects due to either toxicity or nutritional insufficiency are suggested. If the responses of the grazers with mixed diets fall along the reference line, the suspect prey has no nutritional value because the responses of the grazers are entirely determined by the dilution of the control prey. If the grazer responses fed mixed diets fall above the reference line, the suspect prey has some nutritional value. And, if the values of the grazer with mixed diets fall below the reference line, the suspect prey is toxic because it detracts from the beneficial effects of the control prey.

Using mixed-diet experiments, Colin and Dam (2002) investigated whether several algae that had been previously

* Corresponding author: xiajiang@ic.sunysb.edu

reported to have harmful effects on grazers were in fact toxic to the copepod *Acartia tonsa* Dana. The experiments performed at a concentration of $250 \mu\text{g C L}^{-1}$ indicated only a highly toxic *Alexandrium* sp. strain was toxic to female *A. tonsa* and other algae (low-toxicity *Alexandrium* sp. strain, *Heterosigma carterae*, *Thalassiosira rotula*, and *Phaeodactylum tricorutum*) could not be considered toxic (Colin and Dam 2002). The red tide dinoflagellate *Karenia brevis* is usually considered to be toxic, but mixed-diet experiments at a single food concentration showed it was only nutritionally inadequate for *A. tonsa* (Prince et al. 2006; Speckmann et al. 2006). Although the diatoms *P. tricorutum* and *T. rotula* produce polyunsaturated aldehydes, mixed-diet experiments at $240 \mu\text{g C L}^{-1}$ showed that *P. tricorutum* did not have any effects on the copepod *Temora longicornis* and *Thalassiosira rotula* had a beneficial effect (Koski et al. 2008). Variability between results of mixed-diet experiments and previous reports may not only reflect differences among copepod species, but also imply that using a single food concentration in experiments does not adequately reflect the nutritional value of an alga to zooplankton.

The dinoflagellate *Cochlodinium polykrikoides* Margalef has formed dense blooms and caused severe fish kills in Southeast Asia and North America during the past two decades (Gobler et al. 2008). *C. polykrikoides* negatively affects marine algae (Tang and Gobler in press), copepods (Jiang et al. 2009), shellfish, and fish (Tang and Gobler 2009). However, the precise mode of toxicity in this species has not been completely resolved. The production of reactive oxygen species is likely the main mode of toxicity to marine organisms (Kim et al. 1999; Tang and Gobler 2009). Cytotoxic agents and mucus substances may also contribute to the deleterious effects of *C. polykrikoides* (Kim et al. 2002).

In this study we used the mixed-diet approach to determine whether *C. polykrikoides* was beneficial, nutritionally insufficient, or toxic to the calanoid copepod *Acartia tonsa*. *A. tonsa* is an abundant species in many neritic and estuarine environments. The copepod is common in U.S. estuaries where *C. polykrikoides* blooms occur, and is capable of consuming *C. polykrikoides* (Jiang et al. 2009). Based on egg production rate, egg hatching success, and naupliar recruitment rate of *A. tonsa*, mixed-diet experiments showed that the nutritional value of *C. polykrikoides* ranged from beneficial to deleterious with increasing cell density. Long-term survival experiments also supported this conclusion. This density-dependent nutritional quality provides new insights into the ecological effects of putatively harmful algae and their bloom dynamics, and may explain some aspects of plant-herbivore interactions, in general.

Methods

Collection and culture of organisms—The dinoflagellate *C. polykrikoides* clone CP1 was isolated from Peconic Bay, Long Island, New York, U.S.A., in 2006. The flagellate *Rhodomonas lens* Pascher and Ruttner (CCMP 739) was obtained from the Provasoli-Guillard National Center for

Culture of Marine Phytoplankton. The cultures were maintained in a temperature-controlled incubator at 20°C with a 14:10 light:dark (LD) cycle ($\sim 50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). The cultures were maintained in exponential growth phase by biweekly dilution with autoclaved f/2 medium prepared with $0.2\text{-}\mu\text{m}$ -filtered seawater (FSW, salinity 30). The carbon contents of *C. polykrikoides* and *R. lens* were 1816 and $39.5 \text{ pg cell}^{-1}$, respectively (Jiang et al. 2009).

The copepod *A. tonsa* was collected from Stony Brook Harbor, New York, with a $202\text{-}\mu\text{m}$ -mesh plankton net. The population was continuously maintained in 20-liter tanks at 20°C with a 12:12 LD regime. Copepods were offered *R. lens* at a near-saturating concentration of $500 \mu\text{g C L}^{-1}$ (Mauchline 1998) every day. Half of the seawater in the copepod culture was replaced with fresh FSW twice a week.

Mixed-diet experiments—The experiments were performed at four concentrations of total algal carbon: 100, 200, 600, and $1000 \mu\text{g C L}^{-1}$. The corresponding densities of *C. polykrikoides* were 55, 110, 330, and $550 \text{ cells mL}^{-1}$, which represented their densities from the initiation to the development of blooms in the natural environment (Gobler et al. 2008). For each concentration, the carbon fractions of *C. polykrikoides* in diets were nominally 100%, 75%, 50%, 25%, and 0%. Each experimental algal suspension was prepared by diluting algal cultures at the concentration of $\sim 1800 \mu\text{g C L}^{-1}$ ($1000 \text{ cells mL}^{-1}$ for *C. polykrikoides* and $45,600 \text{ cells mL}^{-1}$ for *R. lens*) with FSW. On day 0, 150 *A. tonsa* adults were isolated from culture and kept in a 2-liter beaker containing sole diets of *C. polykrikoides* or *R. lens*, or mixed diets. Approximately 80% of the algal suspension was changed daily. Although *C. polykrikoides* has been reported as a mixotrophic alga when fed picoplankton (Jeong et al. 2004), our initial study with 50% *C. polykrikoides* and 50% *R. lens* at $600 \mu\text{g C L}^{-1}$ showed that *C. polykrikoides* did not feed on *R. lens* because the ratio of two species did not significantly change after 24 h (paired two-sample *t*-test, $t = 1.4887$, $df = 3$, $p = 0.2333$, authors' unpubl. data). Another feeding experiment of *A. tonsa* with 50% *C. polykrikoides* and 50% *R. lens* at $600 \mu\text{g C L}^{-1}$ showed that *A. tonsa* did not selectively feed on *R. lens* (paired two-sample *t*-test, $t = 1.4910$, $df = 3$, $p = 0.2327$, authors' unpubl. data).

Copepod performances were assessed by three functional responses: egg production rate, egg hatching success, and naupliar recruitment rate. Egg production rate (eggs $\text{female}^{-1} \text{ d}^{-1}$) and egg hatching success (%) of *A. tonsa* for each treatment were measured on days 1, 3, and 5. The experiments at $1000 \mu\text{g C L}^{-1}$ did not persist beyond 3 d due to the massive mortality of *A. tonsa* in the 100% *C. polykrikoides* treatment. Two healthy females were transferred into five to seven replicated glass dishes filled with 50 mL of algal suspension. A $202\text{-}\mu\text{m}$ mesh was fixed above the bottom to minimize egg cannibalism. All eggs and nauplii were quantified after 24 h. Eggs were then incubated in 1-mL wells of a multi-depression dish filled with FSW. The dishes were contained within a closed plastic box with distilled water added to the bottom of the box to minimize evaporation from the wells. Eggs were

examined daily for 2 to 3 d. All experiments in this study were performed at 20°C with a 12:12 LD cycle. The irradiance level was $\sim 1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ to minimize the potential effects of light on copepods and algal growth during experiments. Naupliar recruitment rate (nauplii female⁻¹ d⁻¹) was calculated by multiplication of egg production rate and the proportion of hatched eggs.

Survival experiment—A 10-d experiment was carried out to compare survivorship of *A. tonsa* when fed *C. polykrikoides* and *R. lens* at four carbon concentrations ranging from 180 to 1800 $\mu\text{g C L}^{-1}$ (100 to 1000 cells mL⁻¹ for *C. polykrikoides*). Approximately 300 *A. tonsa* females were transferred into a 2-liter beaker and acclimated in FSW for 24 h. For each treatment 16–44 healthy females were transferred individually into 15-mL wells of six-well tissue culture plates. Each well was filled with one female and 13 mL of the experimental algal suspension. The copepods were examined and 80% of the algal suspension was refreshed daily.

Statistical analyses—Egg production rate, egg hatching success, and naupliar recruitment rate on five diet treatments were compared by one-way analysis of variance (ANOVA) for each carbon concentration and exposure time, respectively. Multiple comparisons among the fractions were made using the Tukey post hoc test for equal sample sizes or the Gabriel post hoc test for slightly unequal sample sizes. The original data were transformed to meet the assumptions of ANOVA when necessary. A linear regression line and 95% confidence limits were set for the responses of copepods fed 100% *C. polykrikoides* and 100% *R. lens* when their means were significantly different. The linear regression line was treated as the reference line in mixed-diet experiments. *t*-tests were used to compare differences between the predicted means from the reference line and the observed means on each mixed diet. The overall difference between the observed data on three mixed diets and the reference line was compared using Fisher's procedure of combining probabilities from three independent *t*-tests. Survivorship curves of copepods when fed two algae were compared using the Gehan–Wilcoxon test. Statistical analyses were conducted using SPSS 16.0 statistical package.

Results

When *A. tonsa* females were fed 100% *C. polykrikoides* at 100 $\mu\text{g C L}^{-1}$, egg production rates on days 1, 3, 5 and naupliar recruitment rates on days 1, 3 were significantly higher than the control *R. lens* (Fig. 1, one-way ANOVA with post hoc tests, $p < 0.05$ for all). Similarly, copepod responses when fed 100% *C. polykrikoides* at 200 $\mu\text{g C L}^{-1}$ were significantly improved (e.g., egg production rates on days 3, 5 and naupliar recruitment rate on day 3; Fig. 2, one-way ANOVA with post hoc tests, $p < 0.05$ for all). In contrast, all responses of *A. tonsa* when fed 100% *C. polykrikoides* at 600 $\mu\text{g C L}^{-1}$ were significantly reduced compared with the controls (Fig. 3, one-way ANOVA with post hoc tests, $p < 0.05$ for all) except egg production rate

and naupliar recruitment rate on day 1. The overall egg production rates of *A. tonsa* when fed mixed diets on day 3, egg hatching success on day 5, and naupliar recruitment rates on days 3, 5 were significantly above the reference lines connecting the two monoculture diets at 600 $\mu\text{g C L}^{-1}$ (Fig. 3, Fisher's procedure of combining probabilities, $\text{df} = 6$, $p < 0.05$ for all), while the overall egg production rate on day 5 and egg hatching success on days 1, 3 were not (Fisher's procedure of combining probabilities, $\text{df} = 6$, $p > 0.05$). Furthermore, all egg production and naupliar recruitment rates with mixed diets at 1000 $\mu\text{g C L}^{-1}$ were significantly below the reference lines (Fig. 4, Fisher's procedure of combining probabilities, $\text{df} = 6$, $p < 0.01$ for all), except for egg hatching success on day 1 which was significantly above the reference line (Fisher's procedure of combining probabilities, $\text{df} = 6$, $p < 0.01$).

The nutritional value of the dinoflagellate *C. polykrikoides* to the copepod *A. tonsa* decreased from beneficial to deleterious with increasing *C. polykrikoides* concentration (Table 1). The nutritional value of *C. polykrikoides* was more beneficial than or equal to *R. lens* at 100 and 200 $\mu\text{g C L}^{-1}$. In contrast, *C. polykrikoides* was nutritionally inadequate or had no nutritional value to *A. tonsa* relative to *R. lens* at 600 $\mu\text{g C L}^{-1}$. The nutritional value of *C. polykrikoides* to *A. tonsa* became toxic at 1000 $\mu\text{g C L}^{-1}$.

Survivorship of *A. tonsa* females when fed *C. polykrikoides* was significantly higher than *R. lens* at 180 $\mu\text{g C L}^{-1}$ (Fig. 5; Gehan–Wilcoxon test, $\text{df} = 1$, $p < 0.05$) and 540 $\mu\text{g C L}^{-1}$ (Fig. 5; Gehan–Wilcoxon test, $\text{df} = 1$, $p < 0.01$). In contrast, survivorship of *A. tonsa* when fed *C. polykrikoides* was significantly lower than *R. lens* at 900 $\mu\text{g C L}^{-1}$ (Fig. 5; Gehan–Wilcoxon test, $\text{df} = 1$, $p < 0.05$) and 1800 $\mu\text{g C L}^{-1}$ (Fig. 5; Gehan–Wilcoxon test, $\text{df} = 1$, $p < 0.001$).

Discussion

Ecological significance of density-dependent nutritional value—Our results showed *C. polykrikoides* had variable nutritional effects on the copepod *A. tonsa* over concentrations ranging from 100 to 1000 $\mu\text{g C L}^{-1}$. Contrary to expectation, *C. polykrikoides*, which has been reported as a harmful red-tide alga (Gobler et al. 2008; Tang and Gobler 2009), was more beneficial to *A. tonsa* than the flagellate *R. lens* at low concentrations. Harmful algae are typically considered universally deleterious to target organisms (Landsberg 2002), even though harmful effects often vary with growth stage, inorganic nutrients, organic matter, temperature, salinity, light, and grazers (Granéli and Flynn 2006). Our results, however, clearly showed that *C. polykrikoides* was a nutritious alga for grazers at low densities, which challenges the traditional view on harmful algae. On the other hand, *C. polykrikoides* was toxic to *A. tonsa* at the highest concentration of 1000 $\mu\text{g C L}^{-1}$ (550 cells mL⁻¹). Jiang et al. (2009) found that survivorship of *A. tonsa* females was significantly reduced when fed *C. polykrikoides* monocultures at high concentrations ($\geq 900 \mu\text{g C L}^{-1}$, 500 cells mL⁻¹) compared to copepods starved in FSW. These results along with our current findings using mixed diets indicate the deleterious mode of

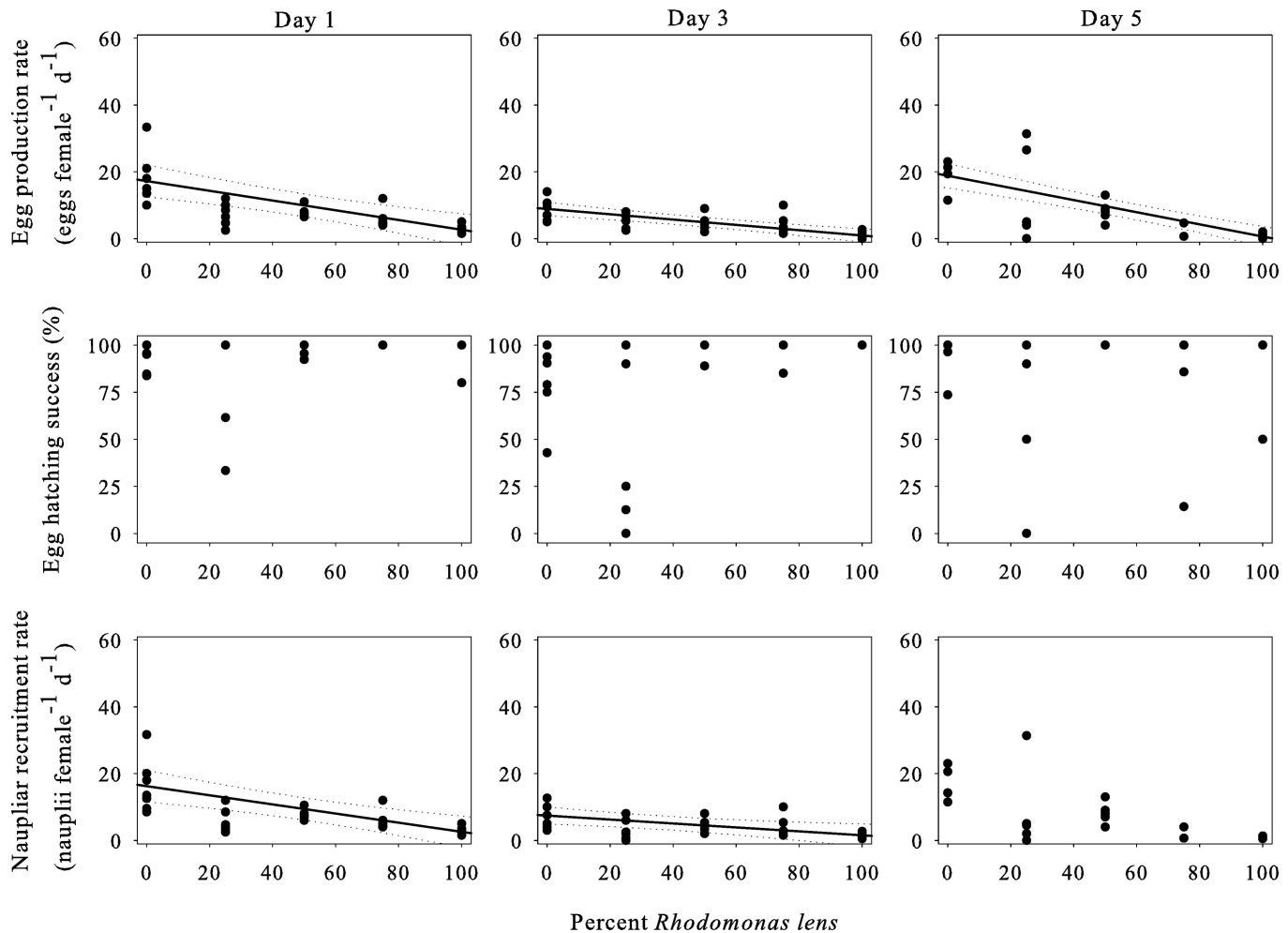


Fig. 1. Performances of *Acartia tonsa* vs. the percent carbon of *Rhodomonas lens* at the total carbon concentration of $100 \mu\text{g C L}^{-1}$. The linear regression line (solid line) and 95% confidence limits (dotted line) are set for the performance with 0% and 100% *R. lens* when they are significantly different.

this alga is related to cellular toxicity rather than nutritional insufficiency. There is no doubt that *C. polykrikoides* is highly deleterious to a variety of marine organisms at high densities, but its effects on ecosystems at low densities are likely different from those observed at high densities.

This study demonstrates that the ecological effects of putatively harmful algae in natural systems can be density dependent. Typical densities of *C. polykrikoides* in U.S. eastern coast waters during blooms have been $> 10^3$ cells mL^{-1} , frequently exceeding 10^4 cells mL^{-1} , with bloom events persisting for ~ 1 month during late summer (Gobler et al. 2008). Harmful effects of *C. polykrikoides* on copepods may only occur at high densities during blooms. In contrast, the alga may serve as a good nutritional resource and support copepod production when its cell densities are low. Such density-dependent nutritional value may shed light on the controversy regarding the interaction between diatoms and copepods (Miralto et al. 1999). Diatoms, which were traditionally considered an ideal food source for copepods, have been reported to cause impaired recruitment of copepods, especially when fed high

concentrations of diatoms (Miralto et al. 1999). The nutritional inadequacy hypothesis argues that reduced egg production or hatching of copepods fed diatoms was due to the deficiency in some mineral or lipid (Jones and Flynn 2005). The toxicity hypothesis states that the negative effects on copepods were specifically related to the production of polyunsaturated aldehydes by diatoms (Miralto et al. 1999). One important but often overlooked factor, cell density, may contribute to the diatom–copepod controversy. Most laboratory experiments and some field observations (Miralto et al. 1999) showing harmful effects on zooplankton were conducted at high diatom concentrations. In the context of our results, we hypothesize that a density-dependent nutritional value of diatoms may account for the observed discrepancies of diatom–copepod interactions.

Density-dependent nutritional quality of algae may provide some insights into the formation of monospecific *C. polykrikoides* blooms. The maximum growth rate of *C. polykrikoides* is $\sim 0.4 \text{ d}^{-1}$ (Kim et al. 2004), which is comparable to some dinoflagellates but slower than most diatoms and flagellates (Smayda 1997). Hence, killing

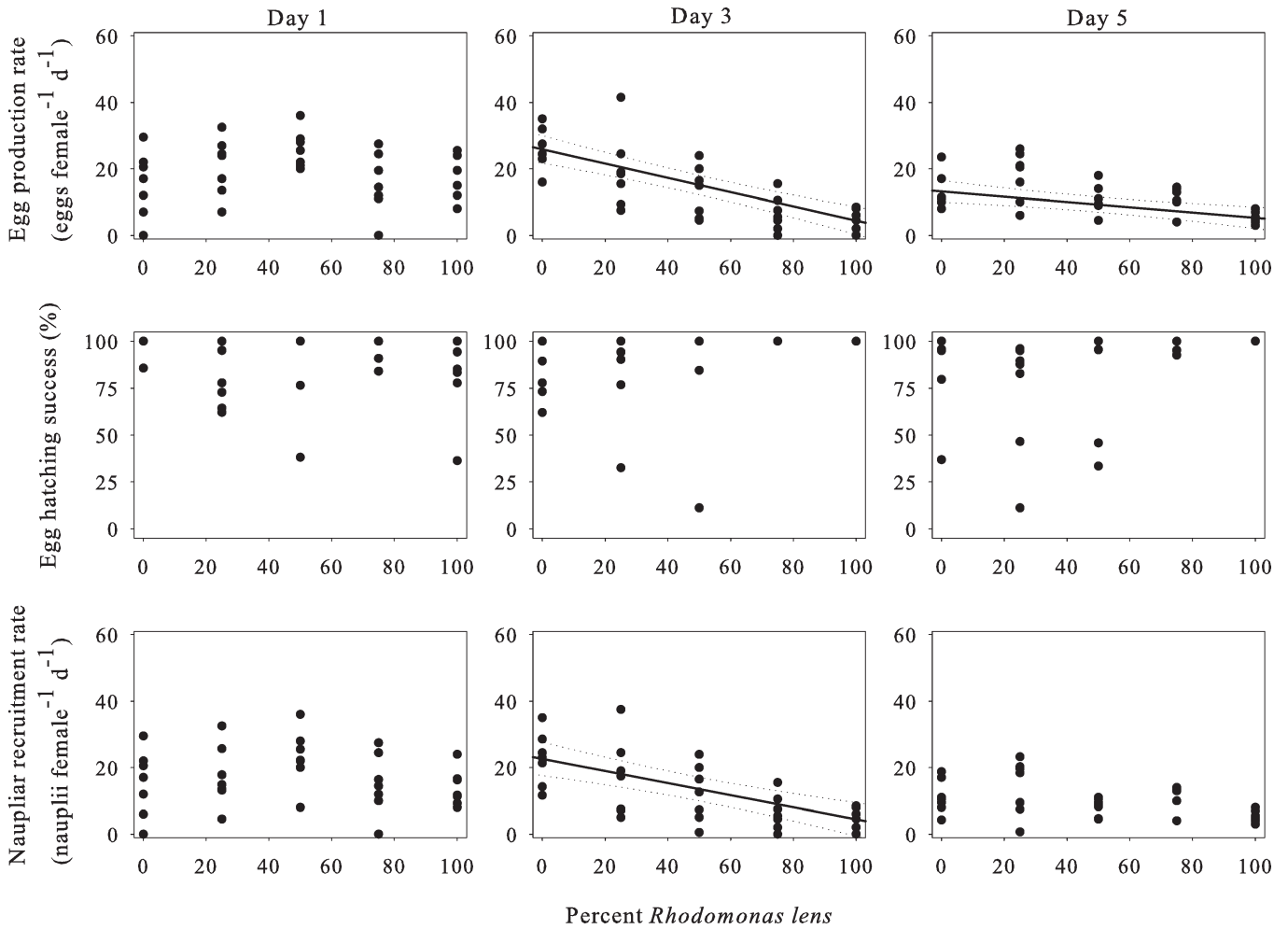


Fig. 2. Performances of *Acartia tonsa* vs. the percent carbon of *Rhodomonas lens* at the total carbon concentration of $200 \mu\text{g C L}^{-1}$. Regression line and 95% confidence limits as Fig. 1.

zooplankton during early stages of bloom development would be a dangerous strategy for *C. polykrikoides* because this would facilitate the dominance of fast-growing competitors within the algal community (Flynn 2008). Although supporting grazers at low densities of *C. polykrikoides* would depress its populations, grazers also control the population size of fast-growing algae. Several attributes of *C. polykrikoides*, such as mixotrophy (Jeong et al. 2004), allelopathy (Tang and Gobler in press), and resistance to algicidal bacteria (Imai and Kimura 2008), may elevate its population density and facilitate bloom formation. Once a bloom population with high cell densities is established, *C. polykrikoides* gains advantages with competing algae and subsequent killing grazers would benefit *C. polykrikoides*, particularly because its allopathic effects on competing algae are also maximal under elevated cell densities (Tang and Gobler in press). Thus, the harmful effects of *C. polykrikoides* to grazers do not contribute to the bloom initiation, but become increasingly important as blooms develop and likely contribute toward bloom maintenance as its nutritional value switches from beneficial to deleterious with increasing cell density. In addition to the support from our empirical study, the competition–

predation hypothesis is also consistent with model simulations of algal blooms, which indicate that the ability of an alga to kill a generalist zooplankton predator can only be considered advantageous when the alga has strong competitive advantages with regard to substrate affinity and/or maximum growth rates (Flynn 2008). Testing the competition–predation hypothesis, in combination with some important factors in trophic interactions (e.g., grazing deterrence, nutrient regeneration by zooplankton) and other traditional hypotheses (e.g., nutrient-uptake adaptations, allelopathy, and turbulence effects, Smayda 1997; Flynn 2008), will enable us to better understand bloom formation of slow-growing dinoflagellates, such as *C. polykrikoides*.

Proposing the competition–predation hypothesis puts forward a new question regarding how *C. polykrikoides* cells at low densities avoid being completely decimated by grazers before they gain a window of opportunity for bloom formation. Our preliminary observations suggest that *C. polykrikoides* cells can detect the presence of grazers and increase cell chain length (authors' unpubl. data). Chain formation in *C. polykrikoides* could be an effective defense by creating predator–prey size mismatch. Addi-

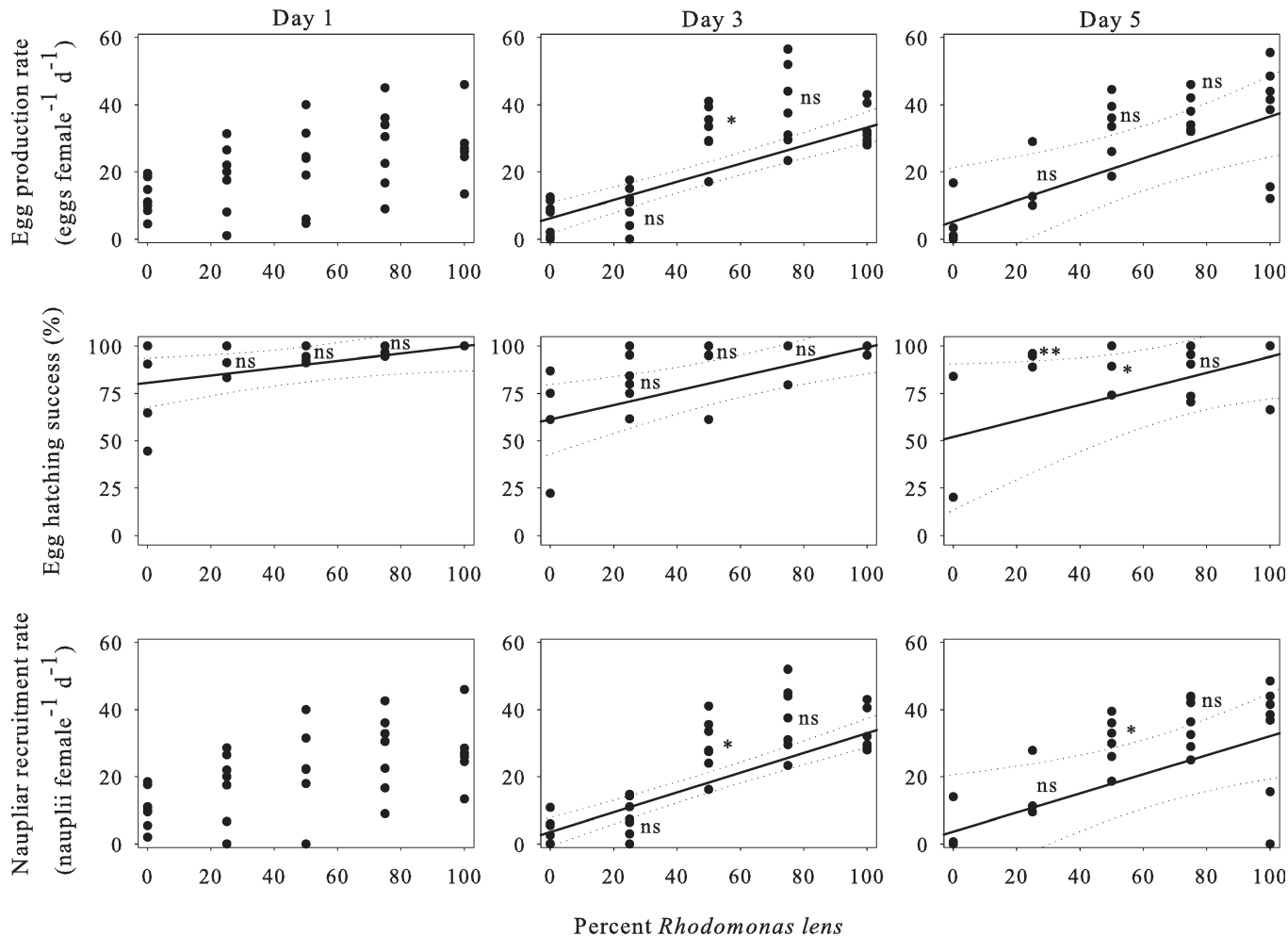


Fig. 3. Performances of *Acartia tonsa* vs. the percent carbon of *Rhodomonas lens* at the total carbon concentration of $600 \mu\text{g C L}^{-1}$. Regression line and 95% confidence limits as Fig. 1; Significant differences between the observed means on mixed diets and the predicted means from the regression line are indicated by asterisks (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) and ns (not significant).

tionally, because the swimming speeds of the dinoflagellates *Gymnodinium catenatum* and *Alexandrium affine* increased by 1.5 times from single cells to chains of four cells (Fraga et al. 1989), chain formation in *C. polykrikoides* could increase motility and subsequent escape ability. Thus, induced chain formation by grazers may help *C. polykrikoides* to avoid grazing even when their nutritional value is beneficial to zooplankton at low densities.

Possible mechanisms of density-dependent nutritional value—The mechanism(s) of density-dependent nutritional quality of *C. polykrikoides* is not clear. The nutritional value of phytoplankton indicated by zooplankton performances is an overall balance between positive factors (e.g., nutritional compounds such as fatty acids) and negative factors (e.g., toxins). Production of fatty acids and toxins (harmful compounds) by microalgae is greatly variable, even on a daily or hourly scale (Sterner and Schulz 1998; Granéli and Flynn 2006). Toxin production in some dinoflagellates is positively related to cell density (Granéli and Flynn 2006). *C. polykrikoides* cells should have had equal nutritional value at the beginning of the experiments

because they were diluted from the same culture at approximately $1000 \text{ cells mL}^{-1}$. After the dilutions, *C. polykrikoides* cells may respond to density changes and thus production of fatty acids and harmful compounds may change with cell densities and/or growth rate, although this likelihood is small given the slow growth of this species and the short duration between transfers (24 h). A more plausible explanation is that zooplankton responses to harmful compounds are dose dependent. *C. polykrikoides* would not have deleterious effects on *A. tonsa* when cell concentrations are below a threshold value. We hypothesize that the amount of nutritional components in *C. polykrikoides* probably exceeds *R. lens* and thus zooplankton perform better when fed *C. polykrikoides* at low concentrations. Jónasdóttir (1994) reported that egg production of the copepods *A. tonsa* and *Acartia hudsonica* was positively correlated with some specific fatty acids (20:5 (n-3), 22:6 (n-3), and 18:0). The relative concentrations of fatty acids 20:5 (n-3) and 18:0 to total fatty acids were 17.5% and 2.1% in *C. polykrikoides* (Dorantes-Aranda et al. 2009), which were higher than those in *R. lens* (Jónasdóttir 1994). These two fatty acids may

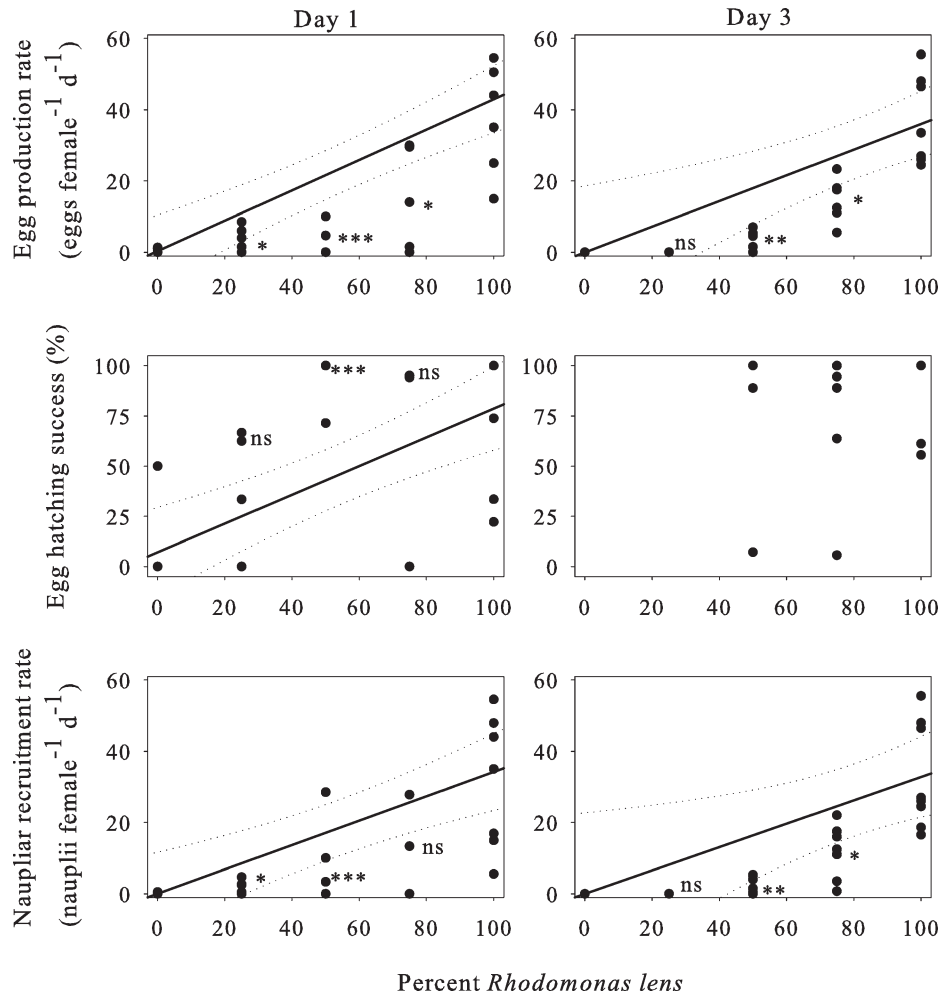


Fig. 4. Performances of *Acartia tonsa* vs. the percent carbon of *Rhodomonas lens* at the total carbon concentration of 1000 $\mu\text{g C L}^{-1}$. Regression line and 95% confidence limits as Fig. 1; statistical symbols as Fig. 3.

Table 1. Nutritional value of *Cochlodinium polykrikoides* to *Acartia tonsa* inferred from the mixed-diet experiments with *Rhodomonas lens*. EPR, egg production rate; EHS, egg hatching success; NRR, naupliar recruitment rate; ++, more beneficial; +, equally beneficial; -, nutritionally insufficient; --, no nutrition; ---, toxic.

Concentration ($\mu\text{g C L}^{-1}$)	Time (d)	EPR	EHS	NRR
100	1	++	+	++
	3	++	+	++
	5	++	+	+
200	1	+	+	+
	3	++	+	++
	5	++	+	+
600	1	+	--	+
	3	-	--	-
	5	--	-	-
1000	1	---	-	---
	3	---	-	---

contribute to the high nutritional value of *C. polykrikoides* for *A. tonsa* at lower cell densities. On the other hand, deleterious effects on zooplankton occur when *C. polykrikoides* concentration exceeds a critical level and further increase with increasing cell concentrations. The potential modes of toxicity in *C. polykrikoides* include the production of reactive oxygen species (Kim et al. 1999; Tang and Gobler 2009) and the production of mucus polysaccharides (Kim et al. 2002), both of which are extracellular and would increase in total toxicity with increasing cell densities. Hence, the nutritional value of some algae, such as *C. polykrikoides*, which is inherently nutritious but also produces harmful compounds, may frequently range from beneficial to deleterious with increasing cell density. Given the known variations in production of fatty acids, toxins, or harmful compounds among algal clones and species (Granéli and Flynn 2006), one must take care in extrapolating prior chemical composition data of algae to the present study. Investigations of production of these

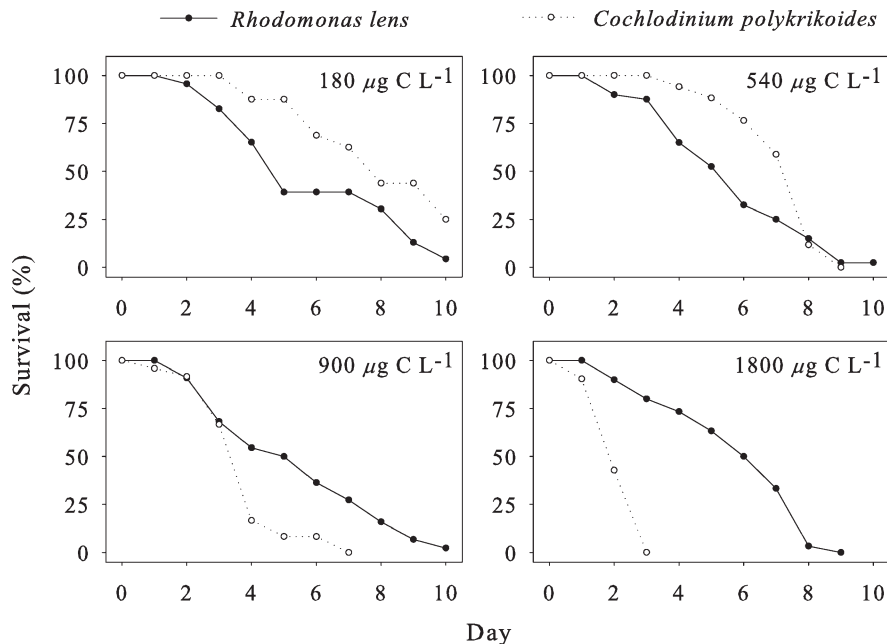


Fig. 5. Survivorship of *Acartia tonsa* when exposed to either *Cochlodinium polykrikoides* or *Rhodomonas lens* at four concentrations.

compounds by both *C. polykrikoides* and other algae at different cell densities with respect to the physiological responses of copepods are expected to provide more insight regarding the mechanisms influencing the density-dependent nutritional value of phytoplankton.

Some zooplankton exposed to recurrent harmful algal blooms can rapidly evolve and adapt to toxic algae (Colin and Dam 2004). The copepods used in this study came from Stony Brook Harbor, Long Island Sound, where no *C. polykrikoides* blooms have been observed. Thus, it is unlikely that these copepods have evolved resistance to any putative *C. polykrikoides* toxins. Some copepods compensate for low food quality by increasing the quantity of food consumed (Mauchline 1998). Although ingestion rates ($\mu\text{g C copepod}^{-1} \text{d}^{-1}$) of *A. tonsa* on *C. polykrikoides* were reduced 40–60% relative to *R. lens* when food concentrations increased from 350 to 1500 $\mu\text{g C L}^{-1}$, there was no significant difference in ingestion rates between the two diets at $\sim 200 \mu\text{g C L}^{-1}$ (Jiang et al. 2009). Thus, the high food quality of *C. polykrikoides* to *A. tonsa* relative to *R. lens* at low concentrations was not due to higher ingestion rates.

Interpreting mixed-diet experiments—Our findings regarding the density-dependent nutritional value of phytoplankton suggests that algal food quality should be assessed using multiple concentrations. Previous studies using mixed-diet experiments (Colin and Dam 2002; Prince et al. 2006; Speckmann et al. 2006) were performed only at a single food concentration. The results showed that several toxic algae were nutritionally insufficient for copepods (Colin and Dam 2002; Prince et al. 2006; Speckmann et al. 2006). However, similar experiments performed under a wide range of environmentally realistic prey densities may

alter this conclusion. Usually, a food-limiting concentration is used in mixed-diet experiments (Jónasdóttir et al. 1998; Colin and Dam 2002), which is appropriate when toxins are intracellular and grazer performances are influenced by how much of the toxin they ingest. Within this context, the experimental concentrations for algae with intracellular toxins must be below the feeding saturation point. However, the modes of toxicity for many harmful algae depend on extracellular toxins, exudates, or cell surface contact (Landsberg 2002). In these cases, the toxic reactions of grazers are influenced by the concentration of the toxic algae in the environment and not by how many toxic algae they ingest. Thus, the experimental abundances of these algae would not be limited by the feeding saturation point. As discussed above, the toxic modes of *C. polykrikoides* are extracellular reactions (C. Kim et al. 1999; D. Kim et al. 2002; Tang and Gobler 2009). Even in the case of intracellular toxins, if toxin production is density dependent (Granéli and Flynn 2006), the concentrations above the feeding saturation point should also be examined. Toxin amounts ingested by grazers could differ, even if ingestion rates were constant.

Our results indicated that egg hatching success was a less sensitive indicator of the nutritional condition of copepods than egg production rates in mixed-diet experiments. Egg production rates of *A. tonsa* fed *C. polykrikoides* were higher than when fed *R. lens* at the concentration of 100 $\mu\text{g C L}^{-1}$, while the differences in hatching success were not significant. Also, the toxic effects of *C. polykrikoides* at 1000 $\mu\text{g C L}^{-1}$ were more evident for egg production rates than egg hatching success. Furthermore, egg hatching success of *A. tonsa* was more variable than egg production rates in our experiments. Some extremely low values of egg hatching were observed when copepods were fed mixed

diets. In some cases, egg hatching success was not statistically reliable because low egg production did not provide adequate sample numbers for hatching experiments. Alternatively, events, such as unfertilized eggs, may influence hatching results. Copepod behavioral changes, including prey switching when feeding on mixed diets (Mauchline 1998), may also contribute to higher variation in the responses of *A. tonsa* compared to monospecific diets.

The size, motility, and quality of prey all influence copepod feeding (Berggreen et al. 1988; Mauchline 1998). The equivalent spherical diameters (ESDs) of *C. polykrikoides* and *R. lens* are 28.2 and 7.97 μm (Jiang et al. 2009). The optimal particle size for feeding by *A. tonsa* females is about 15 μm (Berggreen et al. 1988). Clearance rates of *A. tonsa* females were nearly equal when fed on the flagellate *Rhodomonas baltica* (ESD: 6.91 μm) and the dinoflagellate *Scropsiella farøense* (ESD: 19.0 μm ; Berggreen et al. 1988). Thus, the effects of prey size on feeding of *A. tonsa* should have been minimal because the two algae used in the present study were very similar in size to *R. baltica* and *S. farøense* (Berggreen et al. 1988). Although copepods may actively select for particular prey (Mauchline 1998), we did not observe significant prey selection by *A. tonsa* when fed 50% *C. polykrikoides* and 50% *R. lens* at 600 $\mu\text{g C L}^{-1}$ (authors' unpubl. data). Given that prey selection was only examined within this treatment, care should be taken when extrapolating this result to all treatments.

Our results may challenge the traditional view that harmful algae are chronically deleterious to ecosystems. The nutritional value of the red tide dinoflagellate *C. polykrikoides* to the copepod *A. tonsa* ranged from beneficial to deleterious with increasing cell densities. Therefore, the ecological roles of *C. polykrikoides* during bloom and non-bloom periods may be distinctly different. Density-dependent nutritional quality also suggests that supporting grazers may benefit slow-growing *C. polykrikoides* at low densities because grazers may keep fast-growing algae in check. Once *C. polykrikoides* gains a competitive advantage at high concentrations, its effect on grazers may then switch to deleterious, which leads to monospecific blooms. Testing our results under field conditions is expected to bring more insights into the complexity of such planktonic interactions.

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