Deleterious consequences of a red tide dinoflagellate *Cochlodinium polykrikoides* for the calanoid copepod *Acartia tonsa*

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ABSTRACT: The dinoflagellate *Cochlodinium polykrikoides* Margalef has formed dense blooms and caused severe fish kills on a global scale in recent decades. Survivorship, feeding, and fecundity of the copepod *Acartia tonsa* Dana fed *C. polykrikoides* were investigated to assess potential impacts of these harmful events on herbivores. Survivorship of female *A. tonsa* was significantly reduced with increasing *C. polykrikoides* concentrations from 900 to 4700 µg C l^{-1} (500 to 2600 cells ml^{-1}). Copepods suffered 100% mortality within 1.5 d at *C. polykrikoides* concentrations of 3300 and 4700 µg C l^{-1} (1800 and 2600 cells ml^{-1}), which are within the range of bloom densities of this alga. Stage-specific mortality of *A. tonsa* showed that copepod susceptibility to *C. polykrikoides* decreased with development. Two bioassay experiments suggested that copepod mortality was due to multiple harmful compounds produced by *C. polykrikoides.* Ingestion rates of *A. tonsa* fed *C. polykrikoides* were 25 to 60% lower than ingestion rates of non-toxic *Rhodomonas lens* Pascher and Ruttner when the food concentrations ranged from 150 to 1500 µg C l^{-1}. *C. polykrikoides* supported higher egg production rates of *A. tonsa* than *R. lens* at the low algal concentrations (18 to 180 µg C l^{-1}), while egg production rates of *A. tonsa* fed *C. polykrikoides* were significantly less than those fed *R. lens* when the concentrations increased from 360 to 1080 µg C l^{-1}. Egg hatching success of *A. tonsa* fed *C. polykrikoides* ranging from 90 to 1080 µg C l^{-1} was very low (20 to 43%) compared to the higher values when fed *R. lens* (83 to 100%). Egg sizes of *A. tonsa* fed *C. polykrikoides* were significantly lower than those fed *R. lens*. All of these deleterious consequences may lead to *A. tonsa* population collapses during *C. polykrikoides* blooms.

KEY WORDS: Harmful algae · Copepods · Survivorship · Feeding · Fecundity · *Cochlodinium polykrikoides* · *Acartia tonsa*

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INTRODUCTION

Harmful algal blooms (HABs) have increased in frequency, duration, and distribution in recent decades. Fish kills and accumulation of phycotoxins in shellfish with subsequent poisoning of humans have been well documented. However, studies of the interactions between harmful algae and their zooplankton grazers have been less common, and results are often controversial (Turner & Tester 1997, Turner 2006). These complex and inconsistent interactions are partly due to the wide variety of phycotoxins associated with >200

algal species from 20 genera (Landsberg 2002), substantial changes in toxicity levels of a single algal clone with culture age and nutrients (Granéli & Flynn 2006), and variations of grazers in terms of feeding patterns, binding sites of toxins, and structures of nervous systems (Turner & Tester 1997). Furthermore, phenotypic plasticity and rapid evolution of resistance to harmful algae can significantly shape the interactions between algae and herbivores (Hairston et al. 1999, Colin & Dam 2004).

Despite this complexity, zooplankton grazers are often considered to be adversely affected by harmful

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algae. Effects include impaired feeding, avoidance behavior, physiological dysfunction, depressed growth and reproduction, and reduced population fitness (Turner & Tester 1997, Landsberg 2002, Prince et al. 2006, Barreiro et al. 2007, Cohen et al. 2007, Flynn & Irigoien 2009). Reduced feeding rates of zooplankton may be due to behavioral rejection of harmful algae prior to ingestion or physiological incapacitation (Ives 1987). Inability to continue feeding may result in low growth and reproduction, eventually causing a decline in population abundance. Zooplankton grazing may impact the development and termination of HABs. However, many studies suggest that the top-down controls are limited due to poisoning of grazers by phycotoxins and/or their relatively low growth rate (reviewed by Turner & Tester 1997). Beyond directly feeding on harmful algae, zooplankton grazing may transport toxins along the food web and they may serve as vectors for higher trophic levels (Jester et al. 2009).

The unarmored, chain-forming, gyrodinioid dinoflagellate *Cochlodinium polykrikoides* Margalef has formed dense blooms and caused severe economic damage in Southeast Asia during the past 2 decades (Lee 2008). Recently, *C. polykrikoides* blooms have been documented in many coastal waters ranging throughout temperate, subtropical, and tropical latitudes in both Asia and North America (Anton et al. 2008, Curtiss et al. 2008, Gobler et al. 2008, Park et al. 2009). Cell densities during blooms usually range from 10^3 to 10^4 cells ml^{-1} (Anton et al. 2008, Curtiss et al. 2008, Gobler et al. 2008, Park et al. 2009). Bloom patches can achieve cell densities exceeding 10^5 cells ml^{-1} (Gobler et al. 2008). *C. polykrikoides* isolated in East Asia can be mixotrophic, feeding on small phytoplankton species (<11 μm) by engulfing the prey through the sulcus (Jeong et al. 2004). Strong diel vertical migration has been observed in field populations of *C. polykrikoides* (Park et al. 2001). *C. polykrikoides* appears to be resistant to attack by 6 algicidal bacteria (Imai & Kimura 2008), and, in turn, *C. polykrikoides* inhibits growth of the dinoflagellate *Akashiwo sanguinea* and caused morphologically abnormal cells (Yamasaki et al. 2007). All of these attributes likely provide *C. polykrikoides* with competitive advantages over other occurring microalgae and, at least partly, explain the mechanisms of *C. polykrikoides* bloom formation.

Although the emergence of *Cochlodinium polykrikoides* blooms and subsequent severe fish kills have been well recorded, the precise toxic mechanisms of this alga are still poorly understood. *C. polykrikoides* blooms occurring along the coast of Japan were reported to release water-soluble ichthyotoxic substances with characteristics of paralytic shellfish toxins (Onoue et al. 1985) and 3 toxin fractions: neurotoxic, hemolytic and hemagglutinative (Onoue & Nozawa 1989). In Korean isolates, *C. polykrikoides* has been reported to generate the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (Kim et al. 1999), which resulted in the inactivation of transport-related enzyme activities in fish gills, a drop in blood pO_2, and abnormal secretion of gill mucus (Kim et al. 2000). Interestingly, the production of reactive oxygen species (ROS) in 2 *C. polykrikoides* strains isolated in Japanese waters was very low compared to *Chattonella marina*, a species well known for ROS production. Fish kills by these 2 strains were related to biologically active metabolites, such as cytotoxic agents and mucus substances (Kim et al. 2002). Further, the harmful effects of *C. polykrikoides* from the US east coast waters to fish was caused by a labile, extracellular toxic principle produced by actively growing cells (Tang & Gobler 2009).

The interactions between *Cochlodinium polykrikoides* and zooplankton have been poorly studied. *C. polykrikoides* retarded metamorphosis of the Pacific oyster *Crassostrea gigas* from the trochophore stage to the D-shaped larval stage (Matsuyama et al. 2001). Egg production rates and egg viability of the copepod *Acartia omorii* were low when fed *C. polykrikoides* (Shin et al. 2003). In contrast, the planktonic ciliate *Strombidinopsis* sp. ingested *C. polykrikoides* and grew well (Jeong et al. 2008). In the present study, the deleterious effects of *C. polykrikoides* on survival, feeding, and fecundity of the copepod *A. tonsa* Dana were investigated to assess potential impacts of harmful blooms on lower trophic grazers.

MATERIALS AND METHODS

Collection and culture of organisms. The dinoflagellate *Cochlodinium polykrikoides* clone CP1 was isolated from Peconic Bay, Long Island, New York in 2006 (Gobler et al. 2008). 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 h light:10 h dark cycle (approximately 50 μmol photons m^{-2} s^{-1}). The cultures were maintained in exponential growth phase by biweekly dilution with f/2 medium. The length and width of >100 cells were measured under a compound microscope. The carbon contents of the 2 microalgae (Table 1) were estimated from their cell volumes (Stoecker et al. 1994).

The copepod *Acartia tonsa* was collected from Stony Brook Harbor, Long Island Sound, New York with a

Table 1. Characteristics of 2 algae used in the experiments. ESD: equivalent spherical diameter

Alga	Length (µm)	Width (µm)	ESD (µm)	C content (pg cell^{-1})
Cochlodinium polykrikoides	34 ± 4.7	27 ± 4.1	28.2	1816
Rhodomonas lens	11 ± 1.2	7.0 ± 1.0	7.97	39.5

202 µm mesh plankton net. The population was continuously cultured in 20 l tanks at a density of 20 to 50 ind. l^{-1}. The copepods were offered *Rhodomonas lens* at a carbon concentration of approximately 500 µg C l^{-1} and maintained at 20°C with a 12 h light:12 h dark cycle (approximately 1 µmol photons m^{-2} s^{-1}). The low irradiance level minimized the potential effects of light on copepods and algal growth during experiments. Half of the copepod culture medium was refreshed twice a week with 0.2 µm filtered seawater (FSW).

Survival experiments. A life table experiment was performed to compare survivorship of *Acartia tonsa* when fed *Cochlodinium polykrikoides* at 5 concentrations ranging from 900 to 4700 µg C l^{-1} (500 to 2600 cells ml^{-1}). Our experimental concentrations were within the range of *C. polykrikoides* densities observed in the field (Gobler et al. 2008). Copepod survivorship in FSW and 2 *Rhodomonas lens* solutions (900 and 2200 µg C l^{-1}) were used as the controls. Approximately 400 *A. tonsa* females were transferred into a 5 l plastic container and acclimated in 0.2 µm FSW for 24 h. For each treatment, 20 to 48 healthy females were transferred individually into 6-well tissue culture plates. Each well was filled with 13 ml of the food medium and 1 *A. tonsa*. The copepods were checked every 12 h until they all died. Approximately 80% food medium was refreshed daily. All experiments were conducted in a temperature-controlled incubator at 20°C with a 12 h light:12 h dark cycle (approximately 1 µmol photons m^{-2} s^{-1}).

An acute toxicity experiment was conducted to elucidate stage susceptibility of copepods to *Cochlodinium polykrikoides*. *Acartia tonsa* from the first and fourth naupliar stages (N1 and N4, respectively), the first and fourth copepodite stages (C1 and C4, respectively), adult females, and eggs were exposed to a series of *C. polykrikoides* solutions ranging from 0 to 4700 µg C l^{-1} (0 to 2600 cells ml^{-1}). Each treatment had 4 replicates. *A. tonsa* (n = 8 to 12) were individually held in tissue culture plates filled with *C. polykrikoides* solutions. After 24 h, the copepods were observed under a dissecting microscope.

The mode of harmful effects of *Cochlodinium polykrikoides* on copepods was explored using two 48 h bioassay experiments. Healthy *Acartia tonsa* females were exposed to either live culture, frozen and thawed culture, or culture filtrate (0.2 µm) of *C. polykrikoides*, or 0.2 µm FSW (control). The culture density was 2200 µg C l^{-1} (1200 cells ml^{-1}). The procedures were the same as for the acute toxicity experiment. Another experiment was designed to investigate whether the toxic reaction of copepods was dependent on physical contact with *C. polykrikoides* cells. The experiment was performed using cages made from polyethylene centrifuge tubes (50 ml) with sealed nylon-mesh bottoms. The mesh sizes were 100 and 5 µm for Treatments 1 and 2, respectively. Cages with 100 µm mesh would permit the passage of *C. polykrikoides* cells while the 5 µm mesh did not, which was verified by using the microscope. Each treatment had 4 replicates. Healthy females (n = 8 to 12) were transferred into each cage. The cages in Treatments 1 and 2 were immersed in a 4 l culture of *C. polykrikoides* at a concentration of 2200 µg C l^{-1} (1200 cells ml^{-1}). Cages with 5 µm mesh were also immersed in 4 l of 0.2 µm FSW (control). Copepod mortality was compared after 48 h.

Feeding experiments. Active adult copepods with intact appendages were transferred into 2 l beakers with 0.2 µm FSW for 24 h prior to the feeding experiments. Six food concentrations of *Cochlodinium polykrikoides* and *Rhodomonas lens* ranging from 150 to 1500 µg C l^{-1} were used to determine copepod ingestion rates. We used 3 or 4 replicates of 250 ml bottles for each experimental diet and concentration. The bottles without copepods were used as the controls. Ten active adult females were transferred into each bottle. The bottles were placed on a plankton wheel and rotated at 1 rpm for 24 h at 20°C with a 12 h light:12 h dark cycle (approximately 1 µmol photons m^{-2} s^{-1}). At the beginning and end of the experiment, samples for cell densities were taken. Algal densities were approximated by measuring *in vivo* chlorophyll fluorescence with a Turner AU-10 fluorometer. Actual cell densities were quantified on Lugol's iodine-preserved samples. *In vivo* fluorescence of *C. polykrikoides* and *R. lens* was significantly linearly related to algal concentration (regression coefficients: r = 0.997 and 0.999, respectively; p < 0.001 for both; authors' unpubl. data). The significant relationships between fluorescence and algal concentration provided a rapid and simple measurement to monitor algal concentration during this experiment. The ingestion rates (*I*) were calculated according to the equation described by Båmstedt et al. (2000):

$$I = \frac{V \times \ln \frac{C_t^{'}}{C_t}}{t \times n} \times \frac{C_0 + C_t}{2}$$

where C_0 and C_t are the food concentrations at the beginning and end of the experiment; C_t' is the final food concentration in the controls; V is the volume of the bottles; t is the duration of the experiment; and n is the number of copepods.

Egg production and egg hatching experiments. Egg production rates and hatching success were measured at algal concentrations of 18, 90, 180, 360, 540, and 1080 µg C l^{-1}. Approximately 300 healthy adult *Acartia tonsa* were transferred to each of 6 containers filled with 5 l of the appropriate diet suspension and acclimated for 24 h. Approximately 80% diet medium was refreshed daily. Two healthy female *A. tonsa* were then transferred from the container into a dish filled with 50 ml food solution. A 200 µm mesh was fixed above the bottom to minimize egg cannibalism. All eggs and nauplii were enumerated after a 24 h incubation. There were 7 replicates for each treatment. Eggs were placed individually in 1 ml wells of a multi-depression dish contained within a closed plastic box. Distilled water was added to the bottom of the box to reduce evaporation from the wells. Fresh FSW was added to the wells. Eggs were observed once a day for 2 to 3 d. The measurements in the *Cochlodinium polykrikoides* treatments ran for 10 d or until all copepods in the containers were dead. The measurements in *Rhodomonas lens* treatments only ran for 1 d.

Copepod egg sizes were measured when exposed to *Cochlodinium polykrikoides* and *Rhodomonas lens* at concentrations of 90, 180, 360, 540, and 720 µg C l^{-1} during the 10 d period. Approximately 600 healthy *Acartia tonsa* adults were transferred to each of 5 containers filled with 10 l of the appropriate diet suspension. Copepod eggs were collected by 60 µm mesh and 80% food solutions were refreshed every day. At least 15 eggs from a sample were measured under a compound microscope using 100× magnification to determine the mean egg diameter.

Statistical analyses. Survivorship curves were compared using the Gehan-Wilcoxon test (Pyke & Thompson 1986). Lethal median concentration (LC$_{50}$) was determined by applying a probit analysis. One-way ANOVAs followed by Tukey multiple comparison tests were used to compare means of different treatments in bioassay experiments. A 2-level nested ANOVA was used to test the effects of algal species and concentration on ingestion rates. A 2-way ANOVA was used to analyze the effects of algal species and concentration on egg production rates and hatching success. A 3-way ANOVA was used to analyze the effects of algal species, concentration, and exposure

time on egg sizes (Sokal & Rohlf 1995). All statistical analyses were conducted using the SPSS 16.0 statistical package.

RESULTS

Survival experiments

Life table experiments revealed that survivorship of female *Acartia tonsa* was significantly reduced when fed *Cochlodinium polykrikoides* compared to those starved or fed non-toxic *Rhodomonas lens* (Fig. 1, Table 2). Survivorship of female *A. tonsa* significantly decreased with increasing *C. polykrikoides* concentrations (Fig. 1, Table 2). Female *A. tonsa* experienced rapid mortality at high (3300 and 4700 µg C l^{-1}, or ~1800 and 2600 cells ml^{-1}) and intermediate

Table 2. *Acartia tonsa*. Gehan-Wilcoxon test results of survivorship curves in the life table experiment. Arrow indicates whether the survivorship curve indicated by the column header is greater (↑) or less (↓) than that indicated in the row header. *p < 0.05; **p < 0.01; ***p < 0.001; ns: not significant; CP: *Cochlodinium polykrikoides*; RL: *Rhodomonas lens*; FSW: 0.2 µm filtered seawater. Numbers indicate algal carbon concentrations (µg C l^{-1})

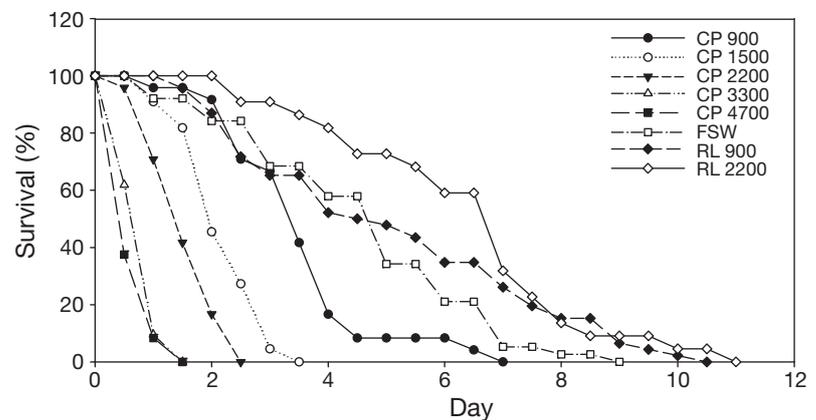|	CP 900	CP 1500	CP 2200	CP 3300	CP 4700	FSW	RL 900
CP 1500	***↑	–					
CP 2200	***↑	**↑	–				
CP 3300	***↑	***↑	***↑	–			
CP 4700	***↑	***↑	***↑	ns	–		
FSW	*↓	***↓	***↓	***↓	***↓	–	
RL 900	*↓	***↓	***↓	***↓	***↓	ns	–
RL 2200	***↓	***↓	***↓	***↓	***↓	**↓	ns

Fig. 1. *Acartia tonsa*. Survivorship when exposed to 5 *Cochlodinium polykrikoides* (CP) suspensions (900, 1500, 2200, 3300, and 4700 µg C l^{-1}), 2 *Rhodomonas lens* (RL) suspensions (900 and 2200 µg C l^{-1}), and 0.2 µm filtered seawater (FSW)
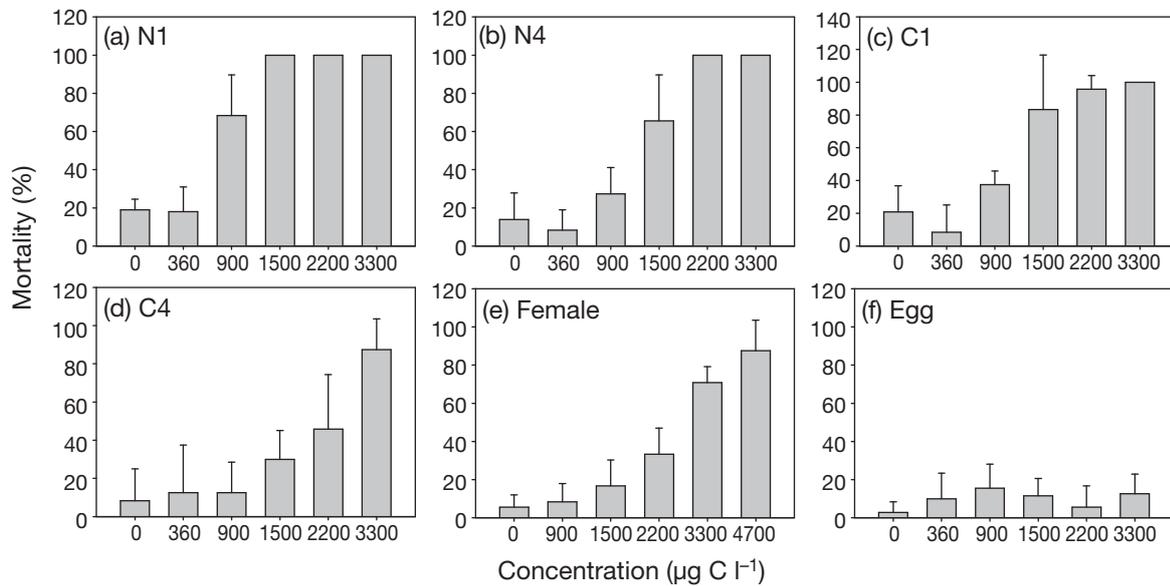
Fig. 2. *Acartia tonsa*. Stage-specific mortality (mean ± SD) when exposed to *Cochlodinium polykrikoides* for 24 h. (a) N1, the first naupliar stage; (b) N4, the fourth naupliar stage; (c) C1, the first copepodite stage; (d) C4, the fourth copepodite stage; (e) adult female; (f) egg

(1500 and 2200 µg C l^{-1}, or ~800 and 1200 cells ml^{-1}) concentrations of *C. polykrikoides*, with 100% of individuals expiring within 1.5 and 3.5 d, respectively (Fig. 1). Survivorship of female *A. tonsa* fed *C. polykrikoides* was moderately improved at the low concentration of 900 µg C l^{-1} (~500 cells ml^{-1}), with individuals surviving 7 d (Fig. 1). All of these survival times were significantly shorter than those in

Fig. 3. *Acartia tonsa*. The 24 h lethal median concentration (LC$_{50}$) and 95% CIs for 5 development stages when exposed to *Cochlodinium polykrikoides*. N1 and N4: first and fourth naupliar stages; C1 and C4: first and fourth copepodite stages, respectively

FSW and in the *R. lens* control treatments (Fig. 1, Table 2).

Mortality of *Acartia tonsa* from early nauplii to adult females significantly increased with increasing *Cochlodinium polykrikoides* concentrations after a 24 h exposure (p < 0.001 for all, 1-way ANOVA; Fig. 2). In contrast, egg hatching was not affected by *C. polykrikoides* (p > 0.05, 1-way ANOVA; Fig. 2). LC$_{50}$ values indicated that the susceptibility of *A. tonsa* to *C. polykrikoides* decreased with development, especially from early copepodite to adult stage (Fig. 3). Early nauplii of *A. tonsa* were approximately 4 times more sensitive to *C. polykrikoides* than adult females after a 24 h exposure, with LC$_{50}$s of 607 µg C l^{-1} (334 cells ml^{-1}; 95% CI: 399 to 877 µg C l^{-1} or 220 to 483 cells ml^{-1}) and 2511 µg C l^{-1} (1383 cells ml^{-1}; 95% CI: 1769 to 3602 µg C l^{-1} or 974 to 1983 cells ml^{-1}), respectively.

Mortality of *Acartia tonsa* exposed to the frozen and thawed *Cochlodinium polykrikoides* culture was significantly reduced to half of that observed when copepods were exposed to the live culture (Fig. 4), but was significantly higher than that in FSW (Table 3). Copepods in the 0.2 µm culture filtrate had significantly increased survivorship compared to those in the live culture, and their mortality did not significantly differ from that in FSW (Fig. 4, Table 3). Copepod mortality in the cages with 5 µm nylon mesh and immersed in *C. polykrikoides* live culture was significantly lower than that in the cages with 100 µm nylon mesh; however, it was significantly higher than that in FSW (Fig. 4, Table 3).
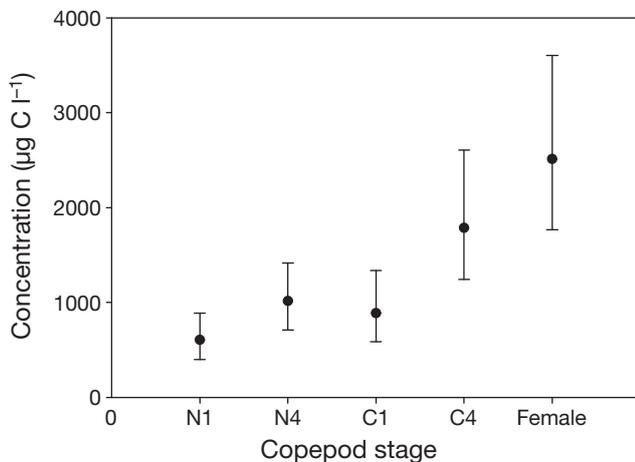
Feeding experiments

The ingestion rates of *Acartia tonsa* were significantly affected by algal species ($F_{1,29}$ = 10.2347, p < 0.01, 2-level nested ANOVA) and algal concentration ($F_{10,29}$ = 2.9841, p < 0.05, 2-level nested ANOVA). The ingestion rates of *A. tonsa* fed *Cochlodinium polykrikoides* were 25 to 60 % lower than ingestion rates of *Rhodomonas lens* (Fig. 5). The ingestion rates of *C. polykrikoides* and *R. lens* by *A. tonsa* significantly increased with increasing algal concentration ($F_{10,29}$ = 2.9841, p < 0.05, 2-level nested ANOVA; Fig. 5). The maximum daily ingestion rates were 3.15 and 6.18 µg C ind.$^{-1}$ d^{-1}, respectively (Fig. 5).

Egg production and egg hatching experiments

The 2-way ANOVA showed that egg production rates of *Acartia tonsa* after a 1 d exposure were signif-
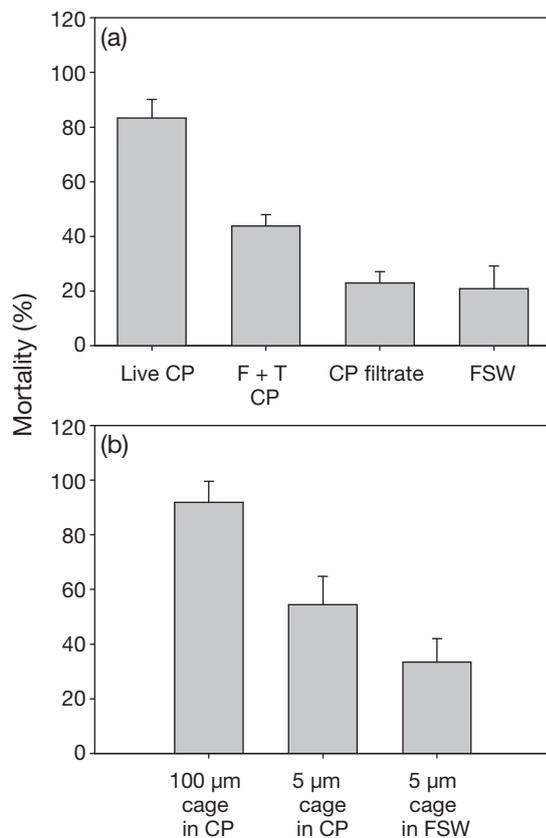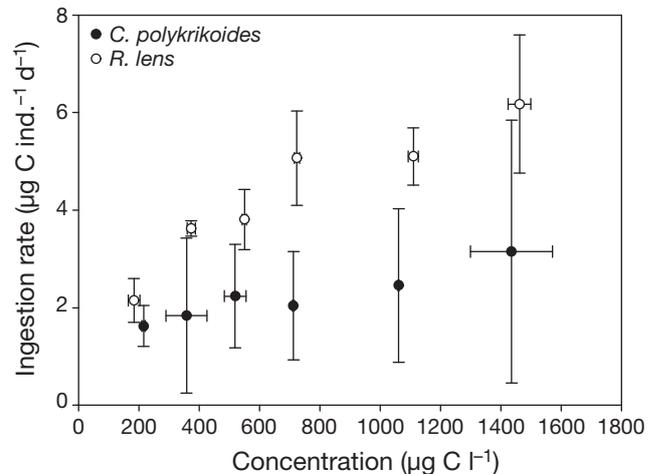

Fig. 5. *Acartia tonsa*. Ingestion rates (mean ± SD) when fed either *Cochlodinium polykrikoides* or *Rhodomonas lens*

icantly affected by algal species ($F_{1,60}$ = 13.9295, p < 0.001), algal concentration ($F_{5,60}$ = 8.0195, p < 0.001), and their interactions ($F_{5,60}$ = 13.8806, p < 0.001). Egg production rates of *A. tonsa* increased progressively with increasing *Rhodomonas lens* concentration (Fig. 6). In contrast, egg production rates of *A. tonsa* moderately increased with increasing *Cochlodinium polykrikoides* concentrations from 18 to 180 µg C l^{-1}, then were greatly reduced by the high concentrations of *C. polykrikoides* (360 to 1080 µg C l^{-1}; Fig. 6). *C. polykrikoides* supported higher egg production rates of *A. tonsa* than *R. lens* at low algal concentrations (18 to 180 µg C l^{-1}), while egg production rates of *A. tonsa* fed *C. polykrikoides* were greatly lower than those fed *R. lens* at high concentrations (360 to 1080 µg C l^{-1}; Fig. 6). The 2-way ANOVA showed that egg hatching success of *A. tonsa* was significantly affected by algal species ($F_{1,48}$ = 30.8405, p < 0.001), but not by algal concentration ($F_{51,48}$ = 2.2991, p = 0.06). Egg hatching rates of *A. tonsa* were very high (82 to 100 %) when fed *R. lens*, except the values at the lowest concentration (18 µg C l^{-1}; Fig. 6). Egg hatching success was very low, ranging from 20 to 43 % when fed *C. polykrikoides* (Fig. 6).

The 2-way ANOVA showed that exposure time did not significantly change egg production rates ($F_{26,155}$ = 1.5491, p = 0.055; Fig. 7) and hatching rates ($F_{26,142}$ = 1.3164, p = 0.165; Fig. 8) of *Acartia tonsa* when fed *Cochlodinium polykrikoides*. The moderate concentrations of *C. polykrikoides* (90 to 360 µg C l^{-1}) supported higher egg production rates of *A. tonsa* than the lowest concentration (18 µg C l^{-1}) and the higher concentrations (540 and 1080 µg C l^{-1}, Fig. 7). Egg production of *A. tonsa* when fed *C. polykrikoides* at 90 µg C l^{-1} persisted during the entire experiment (10 d). In contrast, egg production of *A. tonsa* only persisted for several

Fig. 4. *Acartia tonsa*. Bioassay experiments conducted for 48 h. Percent mortality (mean ± SD) when (a) exposed to live *Cochlodinium polykrikoides* (CP) culture, frozen and thawed (F + T) CP culture, CP culture filtrate (0.2 µm), or 0.2 µm filtered seawater (FSW); or (b) placed in cages covered with 5 or 100 µm nylon mesh and immersed in live CP culture or 0.2 µm FSW

Table 3. *Acartia tonsa*. Results of Tukey multiple comparison tests for mortality in 2 bioassay experiments. Healthy females were (a) exposed to live *Cochlodinium polykrikoides* (CP) culture, frozen and thawed (F + T) CP culture, CP culture filtrate (0.2 μm), or 0.2 μm filtered seawater (FSW); or (b) placed in cages covered with 5 or 100 μm nylon mesh and immersed in live CP culture or 0.2 μm FSW. *p < 0.05; **p < 0.01; ***p < 0.001; ns: not significant

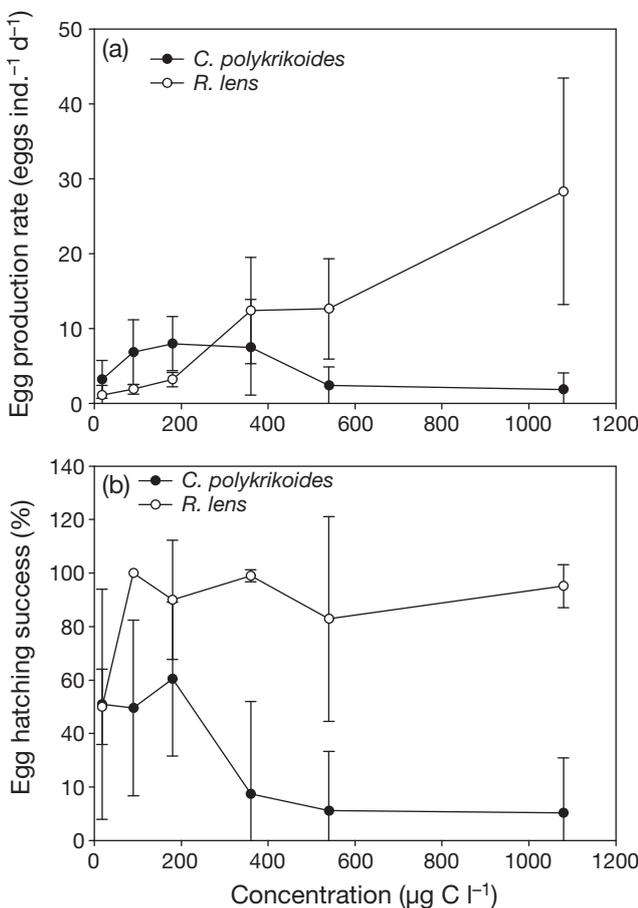| | (a) | | | | (b) | |
	Live CP	F + T CP	CP filtrate		100 μm cage in CP	5 μm cage in CP
F + T CP	***	–		5 μm cage in CP	***	–
CP filtrate	***	***	–	5 μm cage in FSW	***	*
FSW	***	***	ns			

Fig. 6. *Acartia tonsa*. (a) Egg production rates (mean ± SD) and (b) hatching success (mean ± SD) when fed either *Cochlodinium polykrikoides* or *Rhodomonas lens* for 1 d as a function of algal concentration

days at the lowest and 2 highest concentrations of *C. polykrikoides*. *C. polykrikoides* at 1080 μg C l⁻¹ reduced *A. tonsa* egg production to zero within 2 d (Fig. 7). The hatching success of *A. tonsa* eggs when fed *C. polykrikoides* at 18 and 90 μg C l⁻¹ was higher than other concentrations. *C. polykrikoides* at 1080 μg

C l⁻¹ reduced *A. tonsa* egg hatching success to zero within 1 d (Fig. 8).

The 3-way ANOVA showed that *Acartia tonsa* egg sizes were significantly affected by algal species ($F_{1,2370}$ = 89.337, p < 0.001), algal concentration ($F_{4,2370}$ = 7.273, p < 0.001), and exposure time ($F_{9,2370}$ = 2.35, p < 0.001; Fig. 9). Egg sizes of *A. tonsa* when fed *Cochlodinium polykrikoides* were lower than those fed *Rhodomonas lens* at each experimental concentration. The average egg sizes of *A. tonsa* when fed *C. polykrikoides* and *R. lens* for all concentrations were 76.40 and 77.60 μm, respectively. Egg sizes of *A. tonsa* when fed *C. polykrikoides* decreased from 77.30 to 75.96 μm with increasing concentrations from 90 to 720 μg C l⁻¹. In contrast, egg sizes of *A. tonsa* remained constant (77.34 to 77.89 μm) when fed non-toxic *R. lens* from 90 to 720 μg C l⁻¹. The trend of egg sizes over time when fed either *C. polykrikoides* or *R. lens* was not clear (Fig. 9).

DISCUSSION

The present study showed that the dinoflagellate *Cochlodinium polykrikoides* had comparable or more deleterious impacts on copepods compared to other well-known toxic dinoflagellates. *Acartia tonsa* incurred 100% mortality within 1.5 and 3.5 d, respectively, at high (3300 and 4700 μg C l⁻¹, ~1800 and 2600 cells ml⁻¹) and intermediate (1500 and 2200 μg C l⁻¹, ~800 and 1200 cells ml⁻¹) concentrations of *C. polykrikoides*. Approximately 50% of *Acartia clausi* died during a 7 d exposure to the toxic strain *Alexandrium minutum* at a density of 650 cells ml⁻¹ (Barreiro et al. 2007). The dinoflagellate *Karenia brevis* cultured at densities ranging from 1.8 × 10⁴ to 2.1 × 10⁴ cells ml⁻¹ led to approximately 80% mortality of *Acartia tonsa* over a period of 5 d (Prince et al. 2006). Cohen et al. (2007) reported that the *Karenia brevis* culture at a density of 1 × 10⁴ cells ml⁻¹ caused approximately 10% mortality in *Temora turbinata*, 3% mortality in *Acartia tonsa*, and 1% mortality in *Centropages typicus* after a 24 h exposure. *Karlodinium corsicum* (as *Gyrodinium corsicum*) at concentrations around 1500 μg C l⁻¹ killed approximately 50% of *Acartia grani* after 6 d and all copepods after 12 d (da Costa et al. 2005). During blooms lasting 1 to 2 mo in US east coast waters, typical densities of *C. polykrikoides* were >10³ cells ml⁻¹, and frequently 10⁴ cells ml⁻¹ (Gobler et al. 2008, Mulholland et al. 2009). Since lethal concentrations of *C. polykrikoides* for *A. tonsa* are lower than their densi-
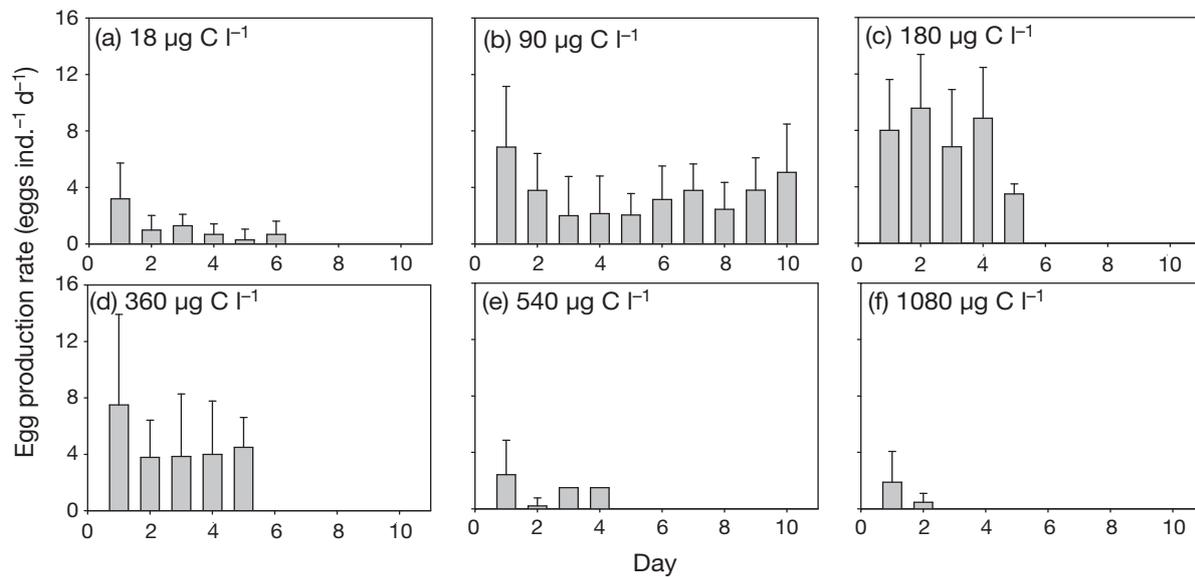
Fig. 7. *Acartia tonsa*. Egg production rates (mean ± SD) as a function of exposure time when fed *Cochlodinium polykrikoides* at concentrations of (a) 18, (b) 90, (c) 180, (d) 360, (e) 540, and (f) 1080 µg C l^{-1}
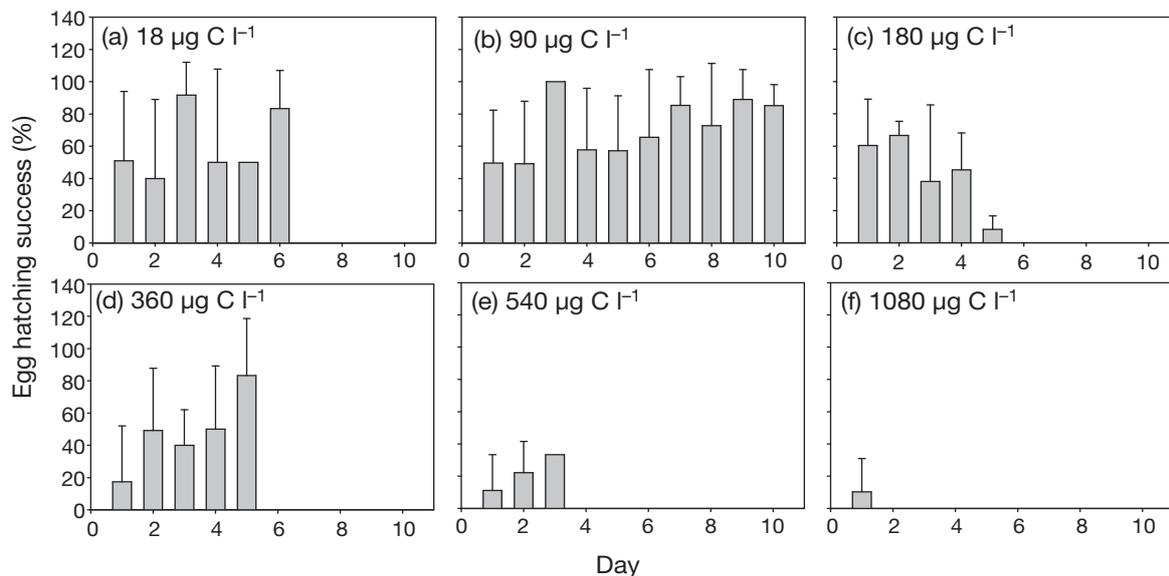

Fig. 8. *Acartia tonsa*. Egg hatching success (mean ± SD) as a function of exposure time when fed *Cochlodinium polykrikoides* at concentrations of (a) 18, (b) 90, (c) 180, (d) 360, (e) 540, and (f) 1080 µg C l^{-1}

ties during the blooms, exposure to high densities of toxic *C. polykrikoides* cells for such extended periods may cause substantial mortality within *A. tonsa* populations. The population dynamics of copepods are sensitive to variation in mortality, as a relatively small increase in female mortality can considerably change population growth by reducing recruitment. Even before blooms occur, the moderate densities of *C. polykrikoides* (~10^2 cells ml^{-1}) may lead to adverse effects on zooplankton, such as reduced feeding and fecundity. Thus, toxic blooms may reduce secondary

production and further lead to food restriction for consumers at higher trophic levels.

Extrapolating laboratory experiments to the natural environment can be complex. Rapid evolution of resistance may shape the interactions between zooplankton and toxic algae. Grazer populations that have experienced recurrent HABs can evolve local adaptations to toxic algae (Hairston et al. 1999, Colin & Dam 2004). An artificial selection experiment showed that copepods evolved resistance to toxic algae over only 2 to 5 generations (Colin & Dam 2004). The rapid evolution of
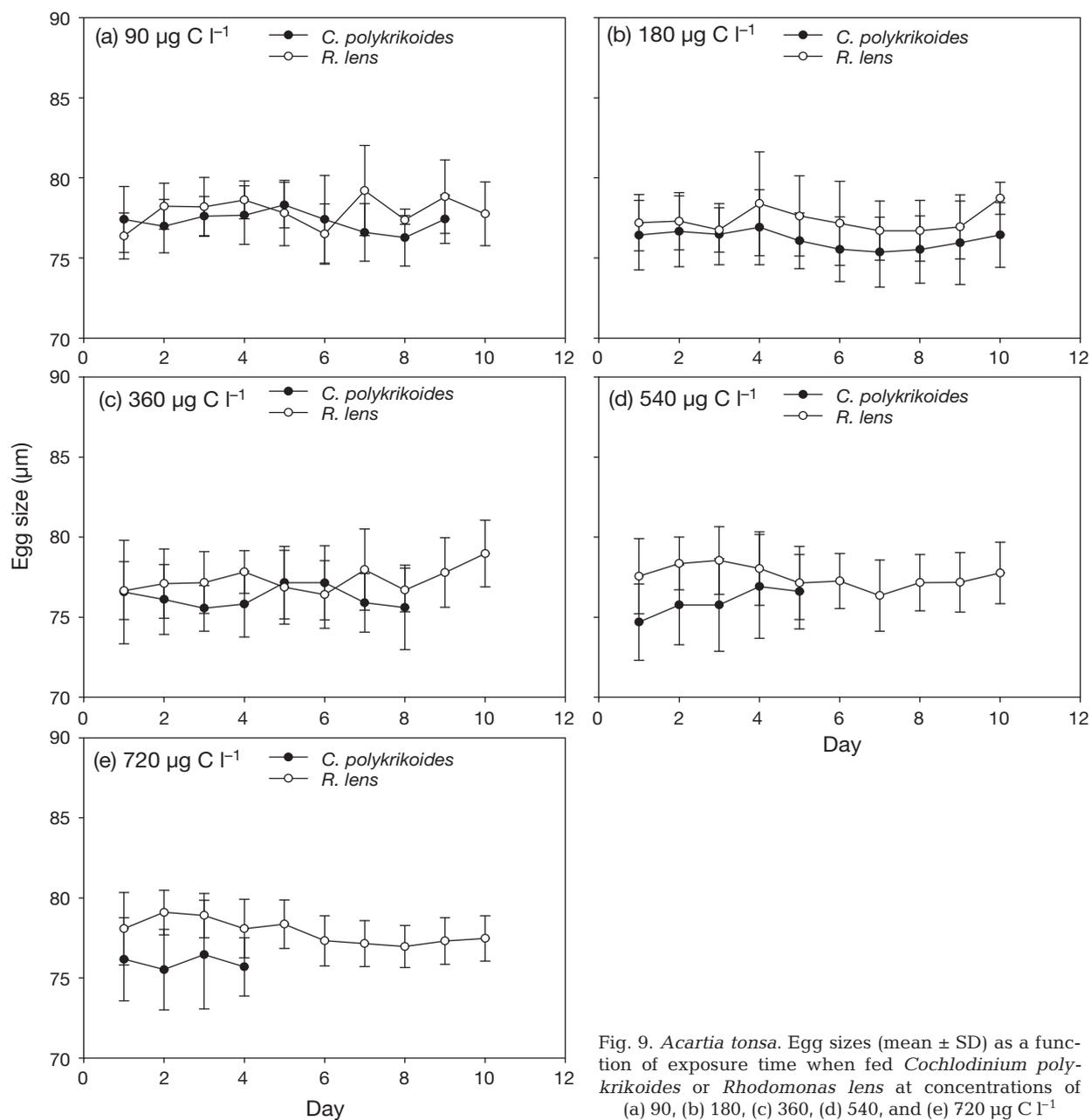
Fig. 9. *Acartia tonsa*. Egg sizes (mean ± SD) as a function of exposure time when fed *Cochlodinium polykrikoides* or *Rhodomonas lens* at concentrations of (a) 90, (b) 180, (c) 360, (d) 540, and (e) 720 µg C l⁻¹

resistance may be an important feedback mechanism to minimize the potential deleterious effects of toxic algae on zooplankton. In New York, *Cochlodinium polykrikoides* blooms only occur in eastern Long Island waters (Gobler et al. 2008). The copepod population used in the present study was collected from Stony Brook Harbor, Long Island Sound, where no *C. polykrikoides* blooms have been observed. Thus, the adverse consequences may be maximized assuming there is no zooplankton gene flow between bloom and non-bloom areas. Another potential factor is the complexity of plankton. Toxic algae rarely bloom in nature in the absence of other phytoplankters. Ingestion of *C.*

polykrikoides with other concurrent phytoplankters or heterotrophic prey may dilute potential adverse effects on copepods. Some zooplankton have the ability to actively select a non-toxic diet (Turner & Tester 1997). The adverse effects of *C. polykrikoides* may be reduced by the presence of other occurring microalgae (Tang & Gobler 2009).

Stage-specific effects of HABs on zooplankton have rarely been considered in prior studies of the interactions between harmful algae and zooplankton. The present results showed that the susceptibility of *Acartia tonsa* to *Cochlodinium polykrikoides* decreased with development. Early nauplii of *A. tonsa* were

4 times more sensitive to *C. polykrikoides* than adult females. These results are similar to the previous studies on stage-specific variations in sensitivity of copepods to toxic chemicals. The nauplii of *Tigriopus brevicornis* were 2 to 4 times more sensitive to 3 insecticides and 2 metals than the adults (Forget et al. 1998). The nauplii of *A. tonsa* were 28 times more sensitive to an organic pesticide (cypermethrin) than adults after 96 h of exposure (Medina et al. 2002). The greater sensitivity of copepod early life stages to toxic algae may be related to their relatively larger surface per unit volume, which may promote a greater diffusive flux of phycotoxins into the copepod body. Another possible explanation is that the later stages may have a greater ability to detoxify. Copepods may transfer toxins into fecal pellets and/or eggs, or eliminate them through excretion in dissolved form (Guisande et al. 2002). More developed metabolic systems in adults (Mauchline 1998) may improve detoxification abilities of copepods. Regardless, the studies on stage-specific effects of HABs on zooplankton may be necessary to understand their true impact on planktonic ecosystems. The investigation of all life stages also provides a more appropriate tool for predicting potential toxicity of harmful algae to copepod populations. Interestingly, live *C. polykrikoides* cells did not inhibit *A. tonsa* egg hatching. Tang & Dam (2001) reported a similar result, that marine diatom exudates did not have negative effects on *A. tonsa* egg hatching.

Mortality of *Acartia tonsa* exposed to the frozen and thawed *Cochlodinium polykrikoides* culture was significantly lower than that in the live culture. The freezing and thawing treatment destroyed *C. polykrikoides* cells (authors' pers. obs.). This result indicated that harmful effects were mainly dependent on the viability of *C. polykrikoides* cells. Similar results were observed on the lethal effects on fish by *C. polykrikoides* natural bloom waters (Gobler et al. 2008, Mulholland et al. 2009) and pure cultures (Tang & Gobler 2009). Copepod mortality exposed to the frozen and thawed *C. polykrikoides* culture was still significantly higher than that in FSW, which suggested that some harmful compound(s) remained after the freezing and thawing treatment. The extracellular secretion and continuous accumulation of polysaccharides in *C. polykrikoides* medium were considered to be one of the causes of fish kills (Kim et al. 2002). An extensive exocellular organic fibrillar matrix and a closely enclosing organic envelope surround the *C. polykrikoides* cells of the strain used in the present study (Gobler et al. 2008). The freezing and thawing treatment may not completely eliminate the harmful effect of such polysaccharides. Direct contact with those polysaccharides or other harmful compounds located on the *C. polykrikoides* cell surface may be responsible for the death of some

copepods in this treatment. Another possibility is that some harmful compounds in *C. polykrikoides* may be released when cells are broken. The results from our second bioassay support these explanations. The mortality of copepods in the cages with 5 µm nylon mesh and immersed in *C. polykrikoides* live culture was significantly lower than that in cages with 100 µm nylon mesh. The separation from harmful compounds in *C. polykrikoides* cells or on cell surfaces by the 5 µm nylon mesh may account for the improved survival of copepods. Yamasaki et al. (2007) observed that cell contact with *C. polykrikoides* inhibited the growth of another dinoflagellate *Akaskiwo sanguinea* and caused morphologically abnormal cells. This result indicated the presence of some harmful compounds located on the *C. polykrikoides* cell surface, but we still do not have evidence to exclude the possibility of the presence of harmful compounds in the cells. Interestingly, the freezing of *C. polykrikoides* culture was not toxic to juvenile fish (*Cypinodon variegates*) (Gobler et al. 2008, Tang & Gobler 2009). This dissimilarity is probably due to the differences in the physiology of these organisms, such as different binding sites and tolerance to harmful compounds. This harmful fraction may impact on lower trophic copepods, but not vertebrate fish.

Another harmful principle may be the dissolved, highly reactive, labile compounds released by live *Cochlodinium polykrikoides* cells. The complete lack of harmful effects of the 0.2 µm culture filtrate suggest that *C. polykrikoides* cells did not release water-soluble harmful compounds or that released compounds were very unstable. The second bioassay experiment supported the latter explanation. The mortality of copepods in the cages with 5 µm nylon mesh and immersed in *C. polykrikoides* live culture was higher than that in FSW. This result suggests that some water-soluble harmful compounds released by *C. polykrikoides* cells may pass through the 5 µm nylon mesh and affect copepods. Kim et al. (1999) reported that ROS generated from *C. polykrikoides* was responsible for oxidative damage leading to fish kills. Tang & Gobler (2009) also reported that the ichthyotoxicity of *C. polykrikoides* could be caused by non-hydrogen peroxide, highly reactive, labile compounds such as ROS-like chemicals. Thus, we propose that multiple harmful compounds produced by *C. polykrikoides* are responsible for their deleterious effects on copepods.

Cochlodinium polykrikoides significantly reduced ingestion rates of *Acartia tonsa* when compared to non-toxic *Rhodomonas lens*. Two possible mechanisms, behavioral rejection and physiological incapacitation, have been postulated to explain such reduced feeding due to harmful algae (Ives 1987). We did not directly test which mechanism was responsible for the

reduced feeding by *C. polykrikoides*. Higher mortality of *A. tonsa* when exposed to *C. polykrikoides* than in FSW suggested that poisoning rather than starvation was the main mechanism for copepod death. Therefore, physiological incapacitation may at least partially explain the reduced feeding of *A. tonsa* by *C. polykrikoides.* Our present experiments, however, did not directly rule out the possibility of feeding deterrents. Copepod feeding is shaped by prey size, motility, and quality (Berggreen et al. 1988, Hansen et al. 1994, Mauchline 1998). The equivalent spherical diameters (ESD) for *C. polykrikoides* and *R. lens* were 28.2 and 7.97 µm, respectively. The optimal particle size for feeding by *A. tonsa* females is 14.8 µm (Berggreen et al. 1988). Clearance rates of *A. tonsa* females were nearly equal when fed the flagellate *R. baltica* (ESD: 6.91 µm) and the dinoflagellate *Scripsiella faröense* (ESD: 19.0 µm; Berggreen et al. 1988). Thus, it is reasonable to assume that the effect of size difference in the present study was limited because the 2 algae used were very similar to *R. baltica* and *S. faröense* in size. Egg sizes of *A. tonsa* when fed *C. polykrikoides* were smaller than when fed *R. lens*. To our knowledge, this is the first report that toxic algae reduced copepod egg size. Cooney & Gehrs (1980) reported that there was a direct positive relationship between egg size and naupliar size in the calanoid copepod *Diaptomus clavipes*. Thus, copepod population fitness may be reduced by toxic algae, since larger nauplii usually have lower mortality rates or mature more rapidly than smaller nauplii (Mauchline 1998). We do not know the mechanism by which a *C. polykrikoides* diet yielded smaller eggs in *A. tonsa*. The ingestion rates of *A. tonsa* on *C. polykrikoides* were 25 to 60% lower than values on *R. lens*. The lack of adequate nutrition and/or physiological incapacitation would lead to impaired gametogenesis and spawning failure in copepods.

The present results clearly show that the red tide dinoflagellate *Cochlodinium polykrikoides* at our experimental concentrations caused deleterious consequences for the copepod *Acartia tonsa*. Is *C. polykrikoides* really a toxic alga? Harmful effects of algae on zooplankton can be explained by the absence of essential nutrients or the presence of toxins (Turner & Tester 1997, Colin & Dam 2002, Prince et al. 2006). One of major challenges in algae–grazer interactions is to separate potential toxic effects from nutritional inadequacy or deficiency. *C. polykrikoides* at high concentrations (≥ 900 µg C l^{-1} or 500 cells ml^{-1}) significantly reduced survivorship of female *A. tonsa* compared to those starved in FSW. The lethal effects suggest that *C. polykrikoides* was a toxic prey for *A. tonsa* at high concentrations. Recently, the mixed-diet approach has been developed to discern whether the suspect prey is beneficial, nutritionally inadequate, or toxic to grazers

(Colin & Dam 2002). We have also conducted mixed-diet experiments at 4 concentrations (100, 200, 600, and 1000 µg C l^{-1}) and 3 durations (1, 3, and 5 d). The results showed that harmful effects on *A. tonsa* at 1000 µg C l^{-1} were caused by *C. polykrikoides* toxicity (authors' unpubl. data). However, the nutritional value of *C. polykrikoides* was greater than or equal to the standard diet of *Rhodomonas lens* at 100 and 200 µg C l^{-1}. These results showed that the nutritional value of *C. polykrikoides* to *A. tonsa* ranged from beneficial to toxic with increasing cell density. The density-dependent nutritional value of this alga suggests that *C. polykrikoides* can be nutritious or toxic for *A. tonsa* depending on ambient concentrations.

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