



Toxicity and nutritional inadequacy of *Karenia brevis*: synergistic mechanisms disrupt top-down grazer control

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ABSTRACT: Zooplankton grazers are capable of influencing food-web dynamics by exerting top-down control over phytoplankton prey populations. Certain toxic or unpalatable algal species have evolved mechanisms to disrupt grazer control, thereby facilitating the formation of massive, monospecific blooms. The harmful algal bloom (HAB)-forming dinoflagellate *Karenia brevis* has been associated with lethal and sublethal effects on zooplankton that may offer both direct and indirect support of bloom formation and maintenance. Reductions in copepod grazing on *K. brevis* have been attributed to acute physiological incapacitation and nutritional inadequacy. To evaluate the potential toxicity or nutritional inadequacy of *K. brevis*, food removal and egg production experiments were conducted using the copepod *Acartia tonsa* and *K. brevis* strains CCMP 2228, Wilson, and SP-1, characterized using liquid chromatography-mass spectrometry (LC-MS) as having high, low, and no brevetoxin levels, respectively. Variable grazing rates were found in experiments involving mixtures of toxic CCMP 2228 and Wilson strains. However, in experiments with toxic CCMP 2228 and non-toxic SP-1 strains, *A. tonsa* grazed SP-1 at significantly higher rates than the toxic alternative. Additionally, *A. tonsa* experienced significantly greater mortality when exposed to toxic *K. brevis* strains, particularly after prolonged exposure. Egg production rates of copepods fed toxic *K. brevis* strains were similar to those of starved copepods, while those of copepods fed non-toxic SP-1 and the nutritious *Rhodomonas salina* were significantly higher. Analysis indicates that *K. brevis* impacts grazer populations via multiple synergistic mechanisms: (1) decreased ingestion rates, (2) decreased egg production, and (3) increased mortality of copepods through a combination of toxicity and nutritional inadequacy.

KEY WORDS: Copepod grazing · Harmful algae · Toxic dinoflagellate · Chemical deterrent · *Karenia brevis* · Brevetoxin

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INTRODUCTION

As the primary grazers of oceanic phytoplankton, zooplankton are known to often regulate phytoplankton populations and may exert a top-down control on the abundance and composition of phytoplankton communities (Watras 1985, Banse 1992, Granéli et al. 1993). Many phytoplankton species that form harmful algal blooms (HABs) have evolved chemical, physical, or morphological defenses such as toxins (Huntley et al. 1986, Ives 1987, Waggett et

al. 2008), extracellular polymeric substances (EPS) (Liu & Buskey 2000), or spines (Nielsen 1991) that make them less palatable to zooplankton grazers and thereby disrupt the stable state of food web dynamics (Irigoiien et al. 2005). The release of HAB species from top-down, grazer-mediated control can lead to a positive feedback interaction supporting bloom formation and proliferation (Sunda et al. 2006 and references therein).

The toxic, red-tide-producing dinoflagellate *Karenia brevis* is commonly found in the Gulf of Mexico

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(GOM) and the United States South Atlantic Bight (US SAB) (Tester et al. 1993, Tester & Steidinger 1997, Magana et al. 2003). Throughout the GOM, *K. brevis* is routinely present at low levels (Geesey & Tester 1993); however, massive, nearly monospecific blooms of *K. brevis* form on an almost annual basis along the western Florida coast (Tester & Steidinger 1997, Stumpf et al. 1998). These blooms occur at concentrations often exceeding 10^5 cells l^{-1} . They can cover areas 10 to 1000 km² and may persist for months at a time (Steidinger & Joyce 1973). *K. brevis* is typically an oceanic species existing at background concentrations <1000 cells l^{-1} in offshore environments and has adapted to low nutrient, oligotrophic environments by efficiently utilizing all sources of nitrogen and phosphorous (Baden & Mende 1979, Bronk et al. 2004, Vargo et al. 2008).

Karenia brevis produces a suite of cyclic polyether compounds known as brevetoxins (PbTx) (Shimizu et al. 1986, Baden et al. 2005). Brevetoxins are lipid-soluble, depolarizing agents that bind to and subsequently open voltage-sensitive sodium channels (VSSC) causing an uncontrolled influx of sodium ions into the cell (Poli et al. 1986, Trainer & Baden 1999). Significant environmental and human health consequences have been linked to the actions of brevetoxins. In humans, consumption of brevetoxin-laden shellfish results in the disruption of normal neurological processes, causing the illness clinically described as neurotoxic shellfish poisoning (NSP). Symptoms include acute gastrointestinal distress and neurological disorders (Steidinger & Baden 1984, Baden et al. 1995). Aerosolized brevetoxins are also responsible for triggering respiratory distress in humans and marine mammals, and through various routes of exposure, they are the cause of mass mortality events of fish, seabirds, and marine mammals (Gunter et al. 1948, Forrester et al. 1977, Flewelling et al. 2005).

Numerous brevetoxins have been isolated from natural bloom events and laboratory cultures. Of these brevetoxins, PbTx-1 and PbTx-2 are suspected to be the parent molecules of 13 other known brevetoxin derivatives and, as such, are the most potent of the characterized toxins (Roszell et al. 1990, Baden et al. 2005). PbTx-1, -2, and -3 are the dominant toxins measured during *Karenia brevis* bloom events. PbTx-2 is the most abundant intra-cellular brevetoxin, and therefore dominates during bloom situations with viable *K. brevis* populations and in laboratory cultures during logarithmic growth (Roszell et al. 1990). PbTx-3, a likely derivative of PbTx-2, is the dominant extra-cellular brevetoxin observed during blooms and prevails during later phases of a bloom

and in declining phases of culture growth as *K. brevis* cells lyse and degrade (Roszell et al. 1990, Pierce et al. 2008). Both intra- and extra-cellular brevetoxins contribute to the toxin accumulation in shellfish and are linked to negative human and environmental health effects (Roberts et al. 1979, Pierce et al. 2006). Each brevetoxin exhibits a unique set of activities, interacting differently with the VSSC to produce a specific toxicity.

Brevetoxins are not the only bioactive compounds produced by *Karenia brevis*. *K. brevis* also manufactures multiple brevetoxin antagonists known collectively as brevenals (Bourdelaïs et al. 2004, 2005, Baden et al. 2005). Brevenals are fused-ring polyether compounds, shorter than brevetoxins, that counteract the effects of brevetoxins (Bourdelaïs et al. 2004, Baden et al. 2005). A second potential antagonist, brevesin, has recently been characterized as an inhibitor of PbTx-3 binding to VSSC (Satake et al. 2009). In addition, recent evidence suggests that *K. brevis* produces hemolytic toxins, compounds that lyse red blood cells, which may contribute to the overall toxicity of bloom events (Neely & Campbell 2006, Tatters et al. 2009).

Within the plankton community, *Karenia brevis* has been reported to have negative impacts on both algal competitors and grazers. Competition experiments have revealed that *K. brevis* may be capable of allelopathy, since interactions with live cells and cellular extracts suppress the growth of multiple phytoplankton species (Freeberg et al. 1979, Kubanek et al. 2005). Additionally, competition experiments have demonstrated that *K. brevis* is avoided by most grazers. Multiple copepod species have been found to discriminate against *K. brevis* based on their toxin content, and lower reproductive and ingestion rates have been found for copepods fed diets dominated by *K. brevis* (Huntley et al. 1986, Speckmann et al. 2006, Breier & Buskey 2007).

Although brevetoxin has not been implicated as the causative agent in *Karenia brevis* allelopathy, it is possible that brevetoxin is responsible for deterring grazers. Conflicting evidence currently exists within the literature regarding the role of brevetoxins as grazing deterrents. While some research suggests that copepods avoid consuming *K. brevis* due to its nutritional inadequacy (Collumb & Buskey 2004, Speckmann et al. 2006, Breier & Buskey 2007), other reports offer support that toxins are the cause of this avoidance (Huntley et al. 1986, Cohen et al. 2007). Recent studies offer convincing evidence that *K. brevis* is a low quality food for copepods, particularly *Acartia tonsa*, one of the dominant

zooplankters found within blooms of *K. brevis* (Lester et al. 2008).

The current research was undertaken to answer the following question: Do copepods avoid *Karenia brevis* because it is nutritionally inadequate or because brevetoxins exert lethal and/or sublethal effects on grazers? To address this question, the calanoid copepod *Acartia tonsa* was exposed to monoalgal and mixed algal diets of *K. brevis* strains differing in toxicity using the model framework of Jonásdóttir et al. (1998). Preliminary toxin analysis of several *K. brevis* strains identified *K. brevis* CCMP 2228 as a toxic strain possessing high levels of brevetoxins (PbTx-1, -2, -3) and therefore served as our 'suspect' diet. Control diets consisted of a less toxic *K. brevis* strain Wilson (producing low levels of brevetoxins), the non-toxic *K. brevis* strain SP-1 (no quantifiable levels of brevetoxins), and an alternative good food, *Rhodomonas salina*. All strains were screened for hemolytic activity and were negative for hemolysis of red blood cells. Because *K. brevis* strain SP-1 contained no detectable traces of brevetoxin and tested negative for hemolytic activity, it will be referred to as 'non-toxic' in relation to the following experiments. Food removal experiments were first conducted to assess the short-term (24 h) effects of *K. brevis* strains ranging from highly toxic (CCMP 2228) to non-toxic (SP-1) on survivorship and grazing of copepods. Follow-up egg production experiments allowed us to evaluate the potential toxic or nutritional effects of *K. brevis* on copepod populations over a 48 h period.

MATERIALS AND METHODS

Experimental organisms

Karenia brevis (C. C. Davis) G. Hansen & Ø. Moestrup, 2000 strains 2228 (Provasoli-Guillard National Center for Culture of Marine Phytoplankton; CCMP), Wilson and SP-1 (isolated by S. Pargee and provided by E. Buskey, University of Texas Marine Science Institute, Port Aransas, TX, USA) were cultured in L1 media (Guillard & Hargraves 1993) at a salinity of 36 and a temperature of 23°C under fluorescent lights with a 14 h light:10 h dark cycle and 102 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR). The cryptophyte *Rhodomonas salina* CCMP 1319 was maintained under the same conditions using f/2 media (Guillard & Rhyther 1962). Cell densities and average cell diameters were monitored daily using a Beckman Multisizer 3 Coul-

ter Counter interfaced with a PC running Multisizer 3 ver. 3.51 software. Experiments were run with cells harvested during the exponential phase of their growth cycle. Seawater for cultures and experiments was collected from waters offshore North Carolina, USA, recognized as Gulf Stream waters from physical properties.

The calanoid copepod *Acartia tonsa* Dana, 1849 was used as the model grazer in food removal and egg production experiments with monoalgal and mixed algal diets of *Karenia brevis*. On the day of experimental set-up, a 102 μm mesh, 0.5 m diameter plankton net was suspended from the National Oceanic and Atmospheric Administration (NOAA) dock on Pivers Island, Beaufort, NC, USA (34° 41.8' N, 76° 40.2' W) during an outgoing tide for up to 45 min. Contents of the cod end were diluted with ambient seawater and returned to the laboratory. Adult copepods were isolated from the plankton under a dissecting microscope and thoroughly examined to ensure individuals appeared healthy and free of external parasites. Groups of 20 (food removal experiments) or 12 (egg production experiments) *A. tonsa* were held within individual 25 ml plastic cups filled with 10 ml sterile-filtered seawater (36) for ~1 h until added to experimental bottles (500 ml).

Biochemical analyses

Prior to each experiment, 3 subsamples of the selected diets (*Karenia brevis* strain(s) and/or *Rhodomonas salina*) were filtered through pre-combusted Pall Type A/E 13 mm filters and frozen for future analysis of particulate C and N. The day before analysis, filters were fumed overnight with concentrated HCl to remove inorganic carbon. Filters were then dried for 1 h at 100°C and analyzed on a Costech Elemental Combustion System (ECS) 4010 interfaced with a PC running Elemental Analysis Software (EAS).

Brevetoxin analysis was performed using liquid chromatography-mass spectrometry (LC-MS). Liquid-liquid extractions of brevetoxins were performed on the date of the experiment by mixing 40 ml of a *Karenia brevis* culture at a 1:1 ratio with ethyl acetate. The solution was sonicated with a Branson Sonifier 250 equipped with a microtip to disrupt the cells. Extraction was repeated 2 additional times, and the 3 resulting extracts were collected and combined. The extract was washed by adding an equivalent amount of Milli-Q water to desalt the solution. The ethyl acetate layer was then collected, and the vol-

ume was reduced to dryness using a Yamato RE500 Rotary Evaporator and then redissolved in 0.5 ml acetonitrile. Samples were then run on a LC1100 coupled to a Thermo Finnigan TSQ Quantum LC-MS system. The instrument was run in multiple-response monitor mode to identify and quantify brevetoxins PbTx-1, PbTx-2, and PbTx-3 in units of pg cell⁻¹. The presence or absence of brevenal was also recorded; however, we were unable to obtain a brevenal standard at the time of the experiments to quantify the amount per cell.

Food removal experiments

Three sets of feeding experiments were conducted using different *Karenia brevis* prey mixtures, including CCMP 2228:Wilson at high and medium cell densities, and CCMP 2228:SP-1 at high cell densities. These classifications were based on the categorization scheme used by Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute (FWRI) where medium *K. brevis* concentrations range from 10⁵ to 10⁶ cells l⁻¹ and high concentrations are >10⁶ cells l⁻¹. In each experiment, copepods were exposed to 6 treatment diets: a 'no food' treatment and 5 monoalgal or mixed algal diets. Treatment bottles contained both *K. brevis* cells and 20 *Acartia tonsa* females, and control bottles contained *K. brevis* cells only. In the 'no food' treatment, *A. tonsa* adults were added to bottles containing only 0.22 µm filtered seawater. Each experiment was run in triplicate with 3 bottles per treatment (n = 15 treatment bottles) and control (n = 15 control bottles). Monoalgal diets consisted of *K. brevis* cells from only one of the cultures (CCMP 2228, Wilson, or SP-1). Three mixed algal diets were created by adding cultures of *K. brevis* strains to 500 ml polycarbonate bottles in ratios of 75% 2228:25% Wilson, 50% 2228:50% Wilson, and 25% 2228:75% Wilson for the CCMP 2228:Wilson experiments, or 75% 2228:25% SP-1, 50% 2228:50% SP-1, and 25% 2228:75% SP-1 for the CCMP 2228:SP-1 experiment. Contents of the bottles were then diluted with 0.22 µm filtered seawater inoculated with L1 nutrients at a salinity of 36 to a density of 1.4 × 10⁶ cells l⁻¹ for CCMP 2228:Wilson (High), 3.5 × 10⁵ cells l⁻¹ for CCMP 2228:Wilson (Medium), and 1.35 × 10⁶ cells l⁻¹ for CCMP 2228:SP-1 (High). All bottles were filled to a volume of 400 ml. Experimental bottles were then mixed via slow, gentle inversion several times, and 10 ml subsamples were taken from each bottle to perform live cell counts using a Coulter Counter. Following sampling,

20 *A. tonsa* adult females were added to each of the 15 treatment bottles containing one of the diets and the 3 'no food' treatment bottles. Ten ml 0.22 µm filtered seawater was added to each of the control bottles to maintain equivalent cell densities among treatment and control bottles. Experimental bottles were maintained at 23°C on a 14 h light:10 h dark cycle with 102 µmol quanta m⁻² s⁻¹ PAR for 17 h.

Following this incubation period, experimental bottles were once again mixed via gentle inversion, and 10 ml subsamples were taken from each bottle to perform live cell counts using a Coulter Counter as soon as the last sample had been collected. Subsamples were examined under a dissecting microscope, and, if present, copepods were removed with a pipette and placed in a petri dish with filtered seawater. Contents of treatment bottles were then gently poured over a 150 µm mesh sieve to collect copepods. The bottle was rinsed 3 times to collect any remaining *Acartia tonsa* on the sieve, and the retained copepods were gently rinsed into the appropriate petri dish. Total surviving and dead copepods were enumerated under a dissecting microscope. Clearance and ingestion rates were calculated using the equations of Frost (1972). Results are reported in terms of both clearance rates, *F*, and ingestion rates, *I*, which are the product of clearance rates and the average cell concentration in the treatment bottle.

Egg production experiments

To assess the quality of *Karenia brevis* monoalgal and mixed algal diets, 3 egg production experiments were performed using the following prey mixtures: CCMP 2228:Wilson, CCMP 2228:SP-1, and CCMP 2228:*Rhodomonas salina*. In the 2 *K. brevis* prey mixture experiments (CCMP 2228:Wilson and CCMP 2228:SP-1), the same experimental design as the food removal experiments was used, with copepods exposed to 6 treatment diets; however, in all egg production experiments only 12 adult *Acartia tonsa* (10 female, 2 male) were used per treatment bottle. Once again, experiments were run in triplicate, and the same monoalgal and mixed algal diet ratios were used as treatments. The cryptophyte *R. salina* is a known nutritious dietary choice when rearing copepods. Therefore, the egg production experiment involving toxic *K. brevis* CCMP 2228 and *R. salina* served as a control experiment to assess the maximum egg and fecal pellet production of the local wild population of *A. tonsa*. In this experiment, only 4 treatment diets were used: a 'no food' treatment, 2

monoalgal diets consisting of either 100% *K. brevis* CCMP 2228 or 100% *R. salina*, and a 50% *K. brevis* CCMP 2228:50% *R. salina* mixed algal diet. In all 3 egg production experiments, contents of the treatment bottles were diluted with 0.22 μm filtered seawater inoculated with L1 nutrients to $\sim 1.5 \text{ mg C l}^{-1}$ ($\sim 2.0 \times 10^6 \text{ cells l}^{-1}$ for *K. brevis* strains and $\sim 1.1 \times 10^8 \text{ cells l}^{-1}$ for *R. salina*). The calculated average carbon concentrations of the *K. brevis* strains were similar: SP-1: 0.738 ng cell^{-1} , Wilson: 0.795 ng cell^{-1} , CCMP 2228: 0.790 ng cell^{-1} while *R. salina* had a calculated carbon content of only 0.033 ng cell^{-1} . Ten ml subsamples were collected from gently mixed experimental bottles (final volume, 400 ml) prior to adding copepods. Subsamples were used to perform live cell counts using a Coulter Counter. After adding copepods and seawater, experimental bottles were incubated at 23°C on a 14 h light:10 h dark cycle with 102 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ PAR for 24 h.

After 24 h, a 10 ml subsample was taken from each bottle for cell counts via the Coulter Counter. Contents of the experimental bottles were then filtered through nested 153 and 40 μm mesh sieves. Copepods were retained on the 153 μm mesh sieve, rinsed into a Petri dish, and examined under a dissecting microscope. The survivorship of the copepods was assessed and all remaining live copepods were returned to their original bottle. Eggs and fecal pellets were captured by the 40 μm sieve and discarded. After 48 h, contents of the bottles were again filtered through nested 153 and 40 μm mesh sieves, and a 10 ml subsample was taken from each bottle for live cell counts with the Coulter Counter. Copepods were counted under a dissecting microscope and mortality assessed. Eggs and fecal pellets retained on the 40 μm mesh were rinsed into 25 ml scintillation vials with 0.22 μm filtered seawater at a salinity of 36. Collected eggs and fecal pellets were preserved with 1% acid Lugol's iodine 24 h after collection to allow nauplii to hatch and counted within 1 wk. Grazing rates were calculated as described in 'Food removal experiments'.

Statistical analysis

Student's *t*-test was used to compare the carbon:nitrogen ratios of *Karenia brevis* strains and *Rhodomonas salina* during the food removal and egg production experiments.

Normality and equal variance tests were run on measured survival, clearance, and ingestion rates of *Acartia tonsa* as well as their egg and fecal pellet production rates. Data passing these tests were analyzed using 1-way factorial analysis of variance (ANOVA) and post hoc Tukey's honestly significant differences (HSD) tests for multiple comparisons.

RESULTS

Biochemical analyses

Elemental analysis of the strains revealed a high degree of variability among the strains and between the experiments; however, all strains were nitrogen replete and contained $>1 \text{ mole N l}^{-1}$. During one food removal experiment (CCMP 2228:Wilson [Medium]) and 2 egg production experiments (CCMP 2228:Wilson and CCMP 2228:SP-1), no significant differences were found in the C:N ratios of the *Karenia brevis* strains used (Table 1). In the remaining experiments, C:N ratios of the diets were significantly different. C:N ratios ranged from 3.3 ± 0.7 (mean \pm SD, $n = 3$) to 7.4 ± 0.2 among the experiments (Table 1).

Brevetoxin analysis was performed using LC-MS and results confirmed that toxin content varied both among and within the strains. *Karenia brevis* CCMP 2228 was determined to be highly toxic with levels of PbTx-2 ranging from 11.67 ± 2.28 to 73.20 $\text{pg PbTx-2 cell}^{-1}$ during food removal and egg production experiments (Table 2). PbTx-1 and PbTx-3 were also present in CCMP 2228 during all experiments, but in lower quantities. The *K. brevis* Wilson strain had much lower levels of PbTx-2 and PbTx-3 than CCMP

Table 1. *Karenia brevis* and *Rhodomonas salina*. Summary of the carbon:nitrogen ratios of *K. brevis* strains (CCMP 2228, Wilson and SP-1) during the 2228:Wilson, 2228:SP-1, and 2228:*R. salina* food removal and egg production experiments. C:N ratios represent mean (SD); $n = 3$ for each experiment and strain. Probability values (*p*) calculated using Student's *t*-test where $n = 3$ and $df = 4$. **Bold** values are significant at the $\alpha < 0.05$ level

Experiment	Strain	Food removal			Egg production		
		C:N	<i>t</i>	<i>p</i>	C:N	<i>t</i>	<i>p</i>
2228:Wilson (Medium)	2228	5.2(0.3)	0.94	0.402			
	Wilson	5.0(0.2)					
2228:Wilson (High)	2228	5.3(0.2)	-6.10	0.004	4.2(0.3)	-2.14	0.100
	Wilson	6.5(0.3)			5.3(0.8)		
2228:SP-1	2228	7.4(0.2)	5.54	0.005	3.9(0.3)	1.71	0.163
	SP-1	5.9(0.4)			3.3(0.7)		
2228: <i>R. salina</i>	2228				7.2(0.1)	29.28	<0.001
	<i>R. salina</i>				5.1(0.1)		

Table 2. *Karenia brevis*. Summary of the toxin content of *K. brevis* strains during the 2228:Wilson and 2228:SP-1 food removal and egg production experiments. Values represent mean (SD); n = 3 except the 2228:Wilson (High) food removal experiment where n = 1. NQ: non-quantifiable; -: no toxins detected; +: present, yet not quantified; NA: not applicable

Experiment type Diet	Strain	PbTx-1 (pg cell ⁻¹)	PbTx-2 (pg cell ⁻¹)	PbTx-3 (pg cell ⁻¹)	Brevetoxin
Food removal					
2228:Wilson (High)	2228	9.28	73.20	6.78	+
	Wilson	NQ	3.67	0.24	+
2228:SP-1	2228	0.62(0.05)	35.71(2.20)	1.60(0.19)	NA
	SP-1	-	-	-	NA
Egg production					
2228:Wilson (High)	2228	1.76(0.33)	11.67(2.28)	0.12(0.03)	+
	Wilson	0.11(0.13)	1.27(0.82)	0.01(0.01)	+
2228:SP-1	2228	1.51(0.10)	13.15(1.55)	0.11(0.02)	+
	SP-1	NQ	NQ	-	-

2228 during the CCMP 2228:Wilson (High) food removal experiment and lower but detectable levels of PbTx-1, -2 and -3 during the egg production experiment (Table 2). Brevetoxins were not detected in strain SP-1 during the food removal experiment; however, low, yet non-quantifiable levels of PbTx-1 and PbTx-2 were present during the egg production experiment (Table 2). A peak for the antagonist compound brevetoxin was also present during analysis of *K. brevis* strains CCMP 2228 and Wilson with particularly broad peaks in the Wilson strain and CCMP 2228 during the egg production experiment. The concentration of the compound could not be determined due to the unavailability of a brevetoxin standard.

Food removal experiments

In the CCMP 2228:Wilson food removal experiments (High and Medium), no significant differences were found in the survivorship of *Acartia tonsa* among treatments regardless of toxicity (Fig. 1A,B; ANOVA, $F_5 = 1.84, 2.68, p = 0.179, 0.075$, respectively). Significant differences in *A. tonsa* survivorship were found during the CCMP 2228:SP-1 food removal experiment (Fig. 1C; ANOVA, $F_5 = 6.56, p = 0.0037$). Significantly lower survivorship was found in the 100% 2228 treatment, averaging only $90.2 \pm 2.2\%$.

Grazing rates appeared to be affected by the toxicity of the diet. In the CCMP 2228:Wilson (High and Medium) experiments, *Acartia tonsa* had significantly different clearance rates among the treatments (Fig. 2A,B; ANOVA, $F_4 = 5.63, 4.17, p = 0.012,$

0.031, respectively). High variability was found in the CCMP 2228:Wilson (High) experiment with negative clearance rates for the 75% 2228:25% Wilson and 25% 2228:75% Wilson treatments; however, comparable clearance rates were calculated for the highly toxic 100% 2228 and lower toxicity 100% Wilson treatments (Fig. 2A). In the CCMP 2228:Wilson (Medium) experiment, negative clearance rates of -0.46 ± 0.12 ml ind.⁻¹ h⁻¹ were calculated for copepods exposed to the highly toxic 100% 2228 treatment (Fig. 2B). Pairwise comparisons indicate that this was significantly lower than clearance rates on the 25% 2228:75% Wilson treatment, the treatment with the

highest clearance rates of 0.55 ± 0.41 ml ind.⁻¹ h⁻¹. The effects of toxins on copepod grazing rates were more pronounced during the CCMP 2228:SP-1 food removal experiment. *A. tonsa* clearance rates were significantly different among the treatments (Fig. 2C; ANOVA, $F_4 = 16.98, p = 0.0002$), and positive grazing rates were observed only for diets dominated by the non-toxic strain SP-1. Positive clearance rates of 0.10 ± 0.16 and 0.49 ± 0.01 ml ind.⁻¹ h⁻¹ were measured for the 25% 2228:75% SP-1 and 100% SP-1 treatments, respectively. Pairwise comparisons show that clearance rates on the 100% SP-1 treatment were significantly higher than for all other treatments.

Similar patterns were observed for calculated ingestion rates (prey number and carbon-based) during the food removal experiments. In the CCMP 2228:Wilson (High) experiment, negative ingestion rates were observed in the 75% 2228:25% Wilson and 25% 2228:75% Wilson treatments, while high ingestion rates of 541.7 ± 265.4 cells ind.⁻¹ h⁻¹ or 319.2 ± 156.4 ng C ind.⁻¹ h⁻¹ were measured for the 100% 2228 treatment (Fig. 3A,D; ANOVA, $F_4 = 5.78, 6.8, p = 0.011, 0.007$, respectively). In the CCMP 2228:Wilson (Medium) experiment, negative ingestion rates were measured for the highly toxic 100% 2228 treatment. Differences among the treatments were only marginally significant on a prey number basis (Fig. 3B; ANOVA, $F_4 = 3.36, p = 0.055$), but were more strongly significant on a carbon basis (Fig. 3E; ANOVA, $F_4 = 4.37, p = 0.027$). Ingestion rates in all other treatments were positive and were highest in the 25% 2228:75% Wilson treatment (Fig. 3B,E; 282.2 ± 266.7 prey ind.⁻¹ h⁻¹ or 220.3 ± 208.1 ng C ind.⁻¹ h⁻¹).

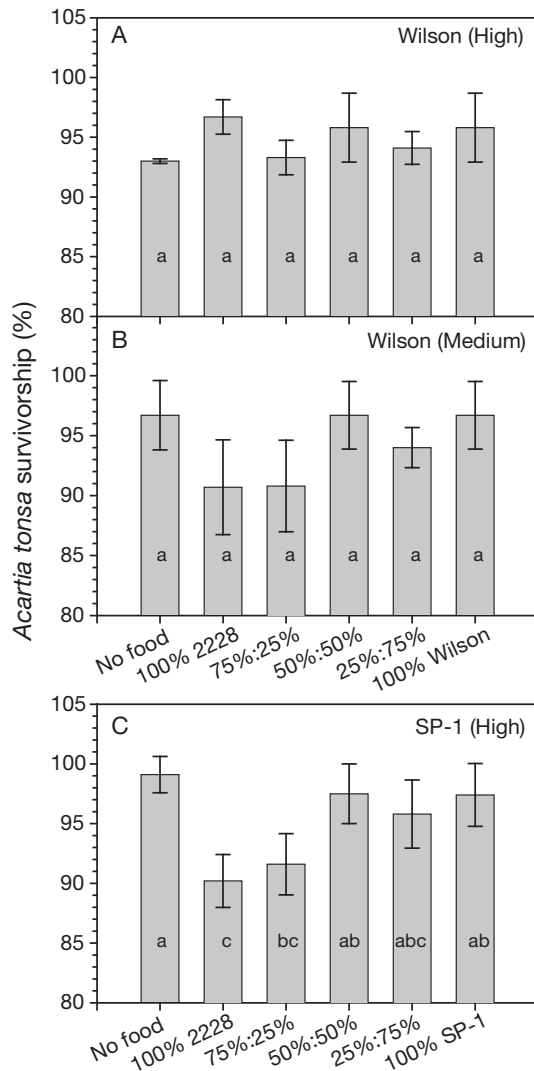


Fig. 1. *Acartia tonsa* and *Karenia brevis*. Mean survivorship (%) of *A. tonsa* following food removal experiments from *K. brevis* (A) 2228:Wilson (High), (B) 2228:Wilson (Medium), and (C) 2228:SP-1 (High) food removal experiments. 75%:25%, 50%:50%, 25%:75% on the x-axis: first value is CCMP 2228; second value is (A,B) Wilson or (C) SP-1. Error bars indicate ± 1 SD. Different letters (a, b, c) on the bars indicate significant differences among treatments according to post-hoc Tukey's honestly significant difference (HSD), $p < 0.05$

Acartia tonsa ingestion rates were also significantly different among treatments within the CCMP 2228:SP-1 experiment. Significantly higher ingestion rates were observed on the non-toxic 100% SP-1 treatment than on the toxic 100% 2228, 75% 2228:25% SP-1, and 50% 2228:50% SP-1 treatments (Fig. 3C,F; ANOVA, $F_4 = 8.52, 7.52, p = 0.0029, 0.0046$, respectively). Positive ingestion rates of 111.6 ± 182.0 prey ind.⁻¹ h⁻¹ (64.8 ± 105.7 ng C ind.⁻¹ h⁻¹) and 381.5 ± 16.7 prey ind.⁻¹ h⁻¹ (209.7 ± 9.2 ng C

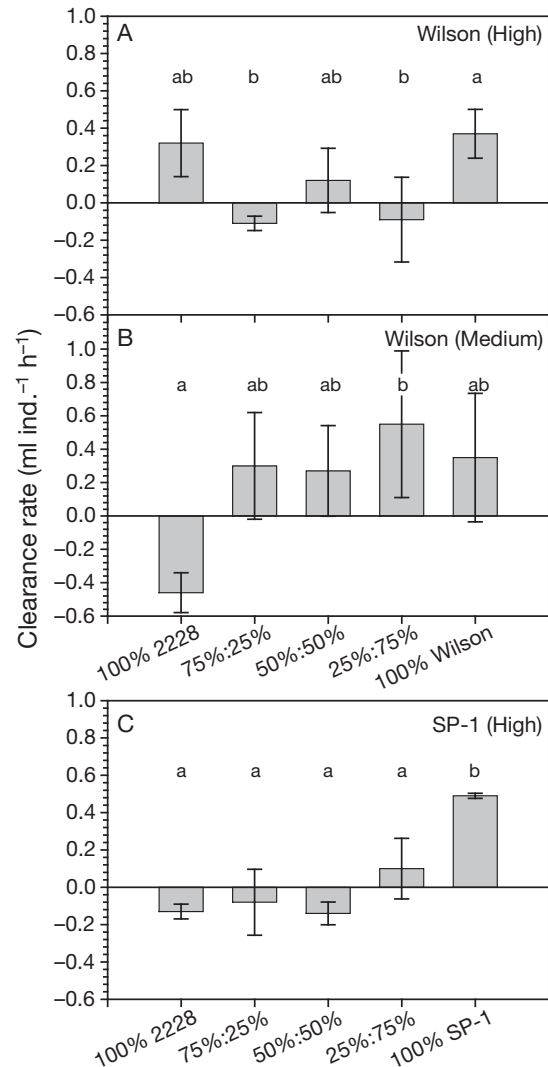


Fig. 2. *Acartia tonsa* and *Karenia brevis*. Mean clearance rates (ml ind.⁻¹ h⁻¹) of *A. tonsa* on monoalgal and mixed algal diets of *K. brevis* during (A) 2228:Wilson (High), (B) 2228:Wilson (Medium), and (C) 2228:SP-1 (High) food removal experiments. See Fig. 1 for further details

ind.⁻¹ h⁻¹) were measured for the 25% 2228:75% SP-1 and 100% SP-1 treatments, respectively.

Egg production experiments

In the control egg production experiment with *Karenia brevis* CCMP 2228:*Rhodomonas salina*, copepods survived equally well in all treatments after 24 h (Fig. 4A; ANOVA, $F_3 = 0.24, p = 0.866$); however, significant differences in *Acartia tonsa* survivorship were found after 48 h of exposure, with the greatest survival ($94.2 \pm 0.7\%$) in the 100% *R. salina*

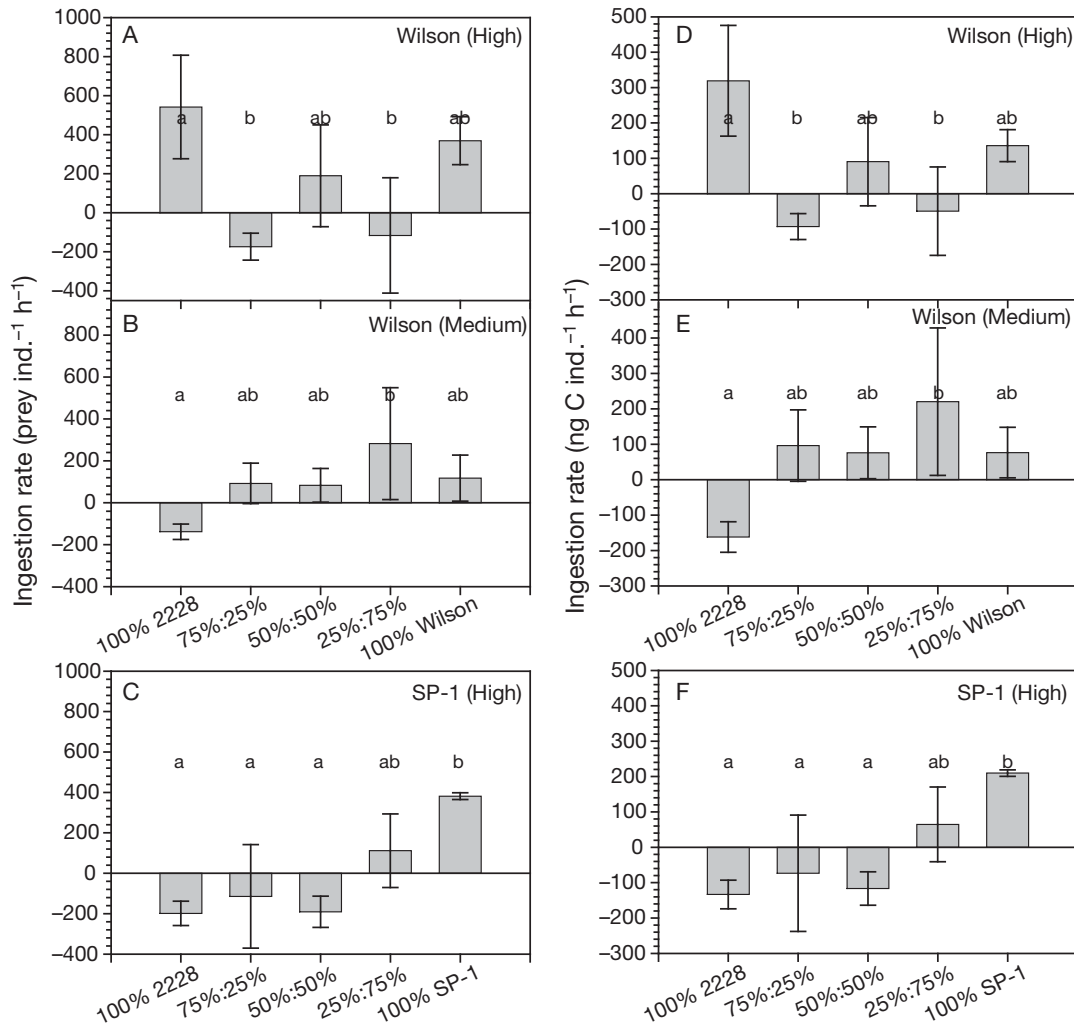


Fig. 3. *Acartia tonsa* and *Karenia brevis*. Mean ingestion rates (prey ind.⁻¹ h⁻¹ and ng C ind.⁻¹ h⁻¹) of *A. tonsa* on monoalgal and mixed algal diets of *K. brevis* during (A,D) 2228:Wilson (High), (B,E) 2228:Wilson (Medium), and (C,F) 2228:SP-1 (High) food removal experiments. 75%:25%, 50%:50%, 25%:75% on the x-axis: first value is CCMP 2228; second value is (A,B,D,E) Wilson or (C,F) SP-1. See Fig. 1 for further details

treatment and the lowest ($66.6 \pm 4.8\%$) in the 100% *K. brevis* CCMP 2228 treatment (Fig. 4B; ANOVA, $F_3 = 6.63$, $p = 0.015$). Although no significant differences in copepod survival were found among treatments in the CCMP 2228:Wilson egg production experiment (Fig. 4C,D; ANOVA, $F_5 = 1.01$, 0.83 , $p = 0.451$, 0.552 , respectively), there was a notable decline in copepod survival in all treatments from a mean of $87.8 \pm 9.1\%$ after 24 h to $73.5 \pm 12.9\%$ after an additional 24 h. After a 24 h exposure to the diets in the CCMP 2228:SP-1 experiment, no significant differences were apparent in copepod survival (Fig. 4E; ANOVA, $F_5 = 2.01$, $p = 0.148$); however, *A. tonsa* survival from 24 to 48 h was significantly different among treatments (Fig. 4F; ANOVA, $F_5 = 10.22$, $p < 0.001$). Low-

est survivorship was found in the 100% 2228 ($65.0 \pm 8.7\%$) and the 75% 2228:25% SP-1 ($72.2 \pm 6.9\%$) treatments. Pairwise comparisons indicate significantly higher survival of over 90% in the 'no food', 25% 2228:75% SP-1, and 100% SP-1 treatments (Fig. 4F).

Copepods in the *Karenia brevis* CCMP 2228: *Rhodomonas salina* control experiment had positive and significantly higher rates of clearance and algal ingestion (both prey and carbon based) of *R. salina* in the 100% *R. salina* treatment (Fig. 5A–C; ANOVA, $p = 0.031$, 0.005 , 0.006 , respectively). Positive grazing rates on *R. salina* were also measured in the 50% 2228:50% *R. salina* treatment, but this was not significantly different than grazing rates on CCMP 2228

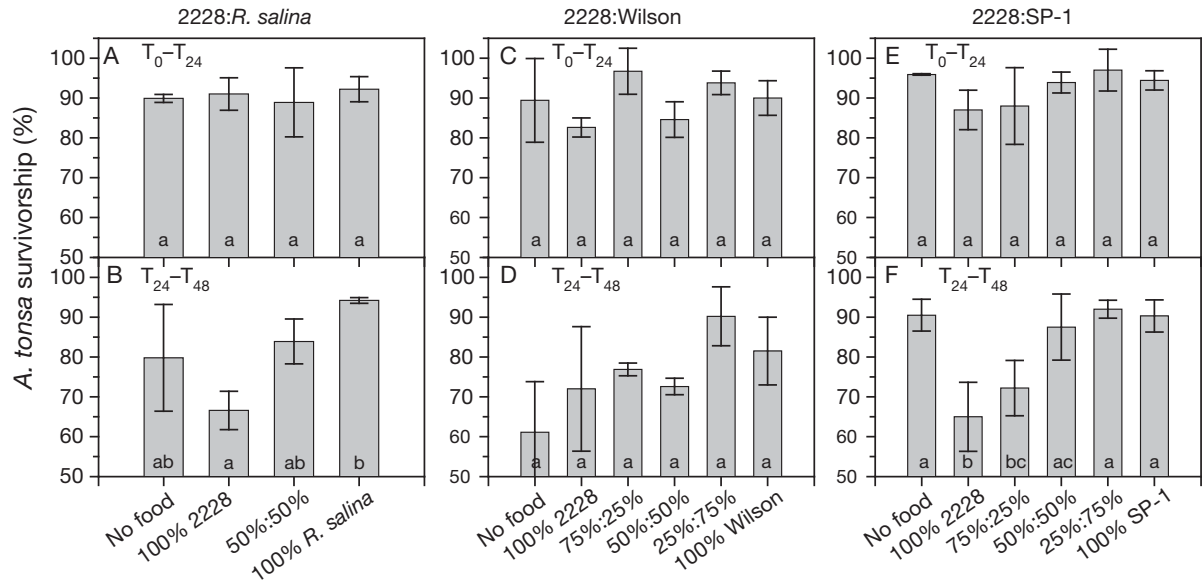


Fig. 4. *Acartia tonsa*, *Karenia brevis* and *Rhodomonas salina*. Mean survivorship (%) of *A. tonsa* exposed to monoalgal and mixed algal diets of *K. brevis* CCMP 2228 and (A,B) *R. salina*, (C,D) *K. brevis* Wilson, and (E,F) *K. brevis* SP-1 during egg production experiments. Exposure time (T) was (A,C,E) 0 to 24 h or (B,D,F) 24 to 48 h. 75%:25%, 50%:50%, 25%:75% on the x-axis: first value is CCMP 2228; second value is (A,B) *R. salina*, (C,D) Wilson, or (E,F) SP-1. See Fig. 1 for further details

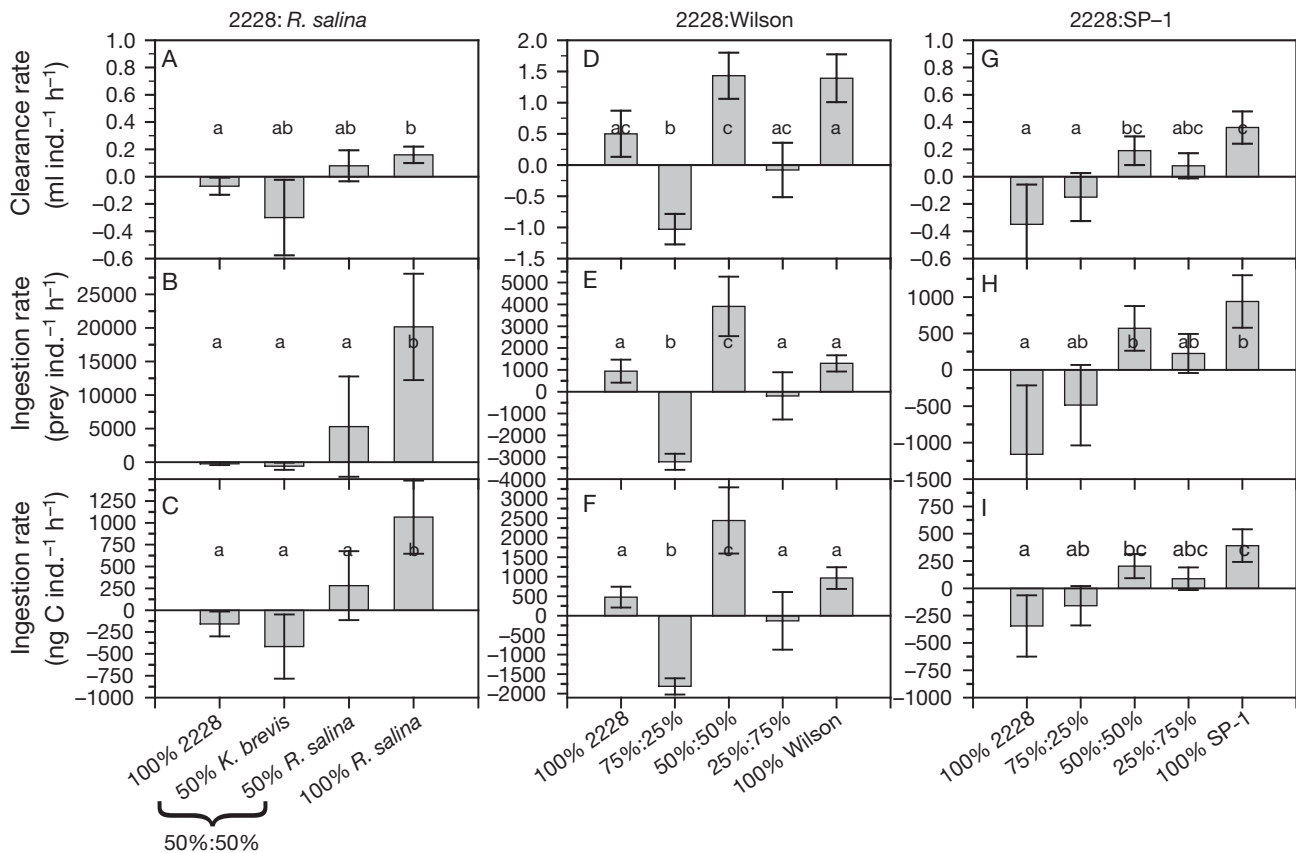


Fig. 5. *Acartia tonsa*, *Karenia brevis* and *Rhodomonas salina*. Grazing rates of *A. tonsa* during *K. brevis* CCMP 2228 and (A,C) *R. salina*, (D–F) *K. brevis* Wilson, and (G–I) *K. brevis* SP-1 egg production experiments reported as (A,D,G) mean clearance rates ($\text{ml ind.}^{-1} \text{h}^{-1}$), (B,E,H) mean ingestion rates ($\text{prey ind.}^{-1} \text{h}^{-1}$), and (C,F,I) mean ingestion rates ($\text{ng C ind.}^{-1} \text{h}^{-1}$). Note differences in the y-axis scaling among the experiments. 75%:25%, 50%:50%, 25%:75% on the x-axis: first value is CCMP 2228; second value is (D–F) Wilson or (G–I) SP-1. See Fig. 1 for further details

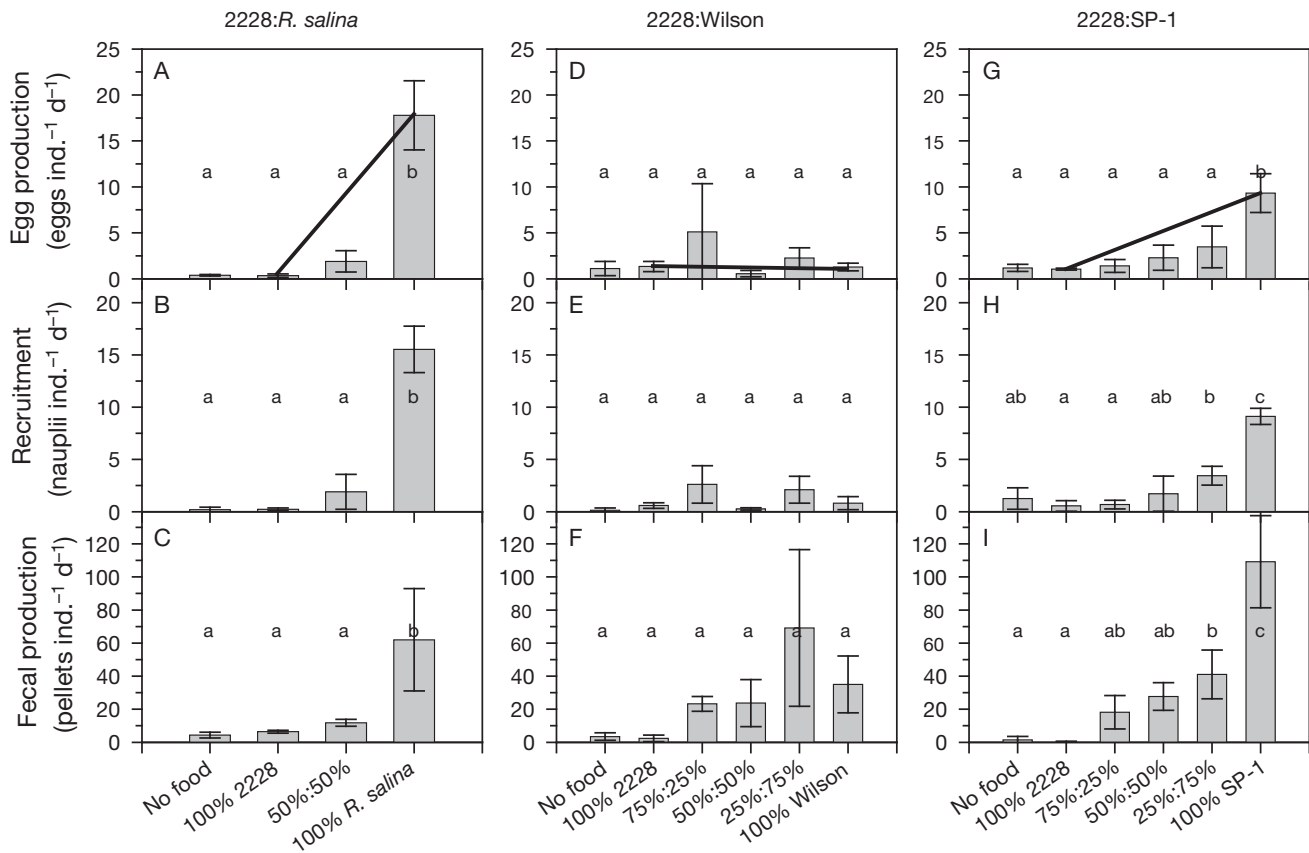


Fig. 6. *Acartia tonsa*, *Karenia brevis* and *Rhodomonas salina*. Offspring and fecal pellet production. (A,D,G) Egg production (eggs ind.⁻¹ d⁻¹), (B,E,H) recruitment (nauplii ind.⁻¹ d⁻¹), and (C,F,I) fecal pellet production (pellets ind.⁻¹ d⁻¹) of *A. tonsa* during *K. brevis* CCMP 2228 and (A–C) *R. salina*, (D–F) *K. brevis* Wilson, and (G–I) *K. brevis* SP-1 egg production experiments. Black line in (A,D,G) represents reference line connecting production at 100% suspect and 100% control diets. 75%:25%, 50%:50%, 25%:75% on the x-axis: first value is CCMP 2228; second value is (A,C) *R. salina*, (D–F) Wilson, or (G–I) SP-1. See Fig. 1 for further details

(Fig. 5A–C). *Acartia tonsa* consumed an average of 20146.9 ± 7918.5 *R. salina* ind.⁻¹ h⁻¹ (1065.4 ± 418.8 ng C ind.⁻¹ h⁻¹).

Similar to the variation measured in the CCMP 2228:Wilson high cell density food removal experiment, a high degree of variability in *Acartia tonsa* grazing rates was observed among treatments in the CCMP 2228:Wilson egg production experiment (Fig. 5D–F). Positive clearance and ingestion rates (prey- and carbon-based) were measured for the 100% 2228, 50% 2228:50% Wilson, and 100% Wilson treatments. Significantly higher ingestion rates of 3908 ± 1360.9 *Karenia brevis* ind.⁻¹ h⁻¹ (2441.7 ± 850.1 ng C ind.⁻¹ h⁻¹) were measured for the 50% 2228:50% Wilson treatment (Fig. 5B,C; ANOVA, $F_4 = 28.02, 24.85, p < 0.001, 0.001$, respectively).

Acartia tonsa clearance and ingestion (prey- and carbon-based) rates were significantly different among treatments within the CCMP 2228:SP-1 egg

production experiment (Fig. 5G–I; ANOVA, $F_4 = 7.80, 7.11, 8.12, p = 0.004, 0.006, 0.004$, respectively). Positive grazing rates were measured only for the 50% 2228:50% SP-1, 25% 2228:75% SP-1, and 100% SP-1 treatments. Highest clearance and ingestion rates were observed in the 100% SP-1 treatment with copepods consuming 939.1 ± 359.9 *Karenia brevis* ind.⁻¹ h⁻¹ (390.9 ± 149.8 ng C ind.⁻¹ h⁻¹).

In the control egg production experiment with *Karenia brevis* CCMP 2228:*Rhodomonas salina*, copepods produced significantly more eggs (17.8 ± 3.8 eggs ind.⁻¹ d⁻¹) when exposed to the 100% *R. salina* diet than all other treatments (Fig. 6A; ANOVA, $F_3 = 55.78, p < 0.001$). Additionally, the 100% *R. salina* diet supported a significantly greater number of nauplii (15.5 ± 2.2 nauplii ind.⁻¹ d⁻¹; Fig. 6B; ANOVA, $F_3 = 84.92, p < 0.001$) and fecal pellet production (62.0 ± 31.0 fecal pellets ind.⁻¹ d⁻¹; Fig. 6C; ANOVA, $F_3 = 9.34, p = 0.005$). In contrast, very

few eggs or nauplii were produced during the CCMP 2228:Wilson experiment (Fig. 6D,E). No significant differences in egg production rates (Fig. 6D; ANOVA, $F_5 = 1.63$, $p = 0.225$) were found among the treatments. Recruitment ranged from 0.1 ± 0.2 to 2.6 ± 1.8 nauplii ind.⁻¹ d⁻¹ and was significantly different among treatments, with greatest numbers found in the 75 % 2228:25 % Wilson and 25 % 2228:75 % Wilson treatments (Fig. 6E; ANOVA, $F_5 = 3.48$, $p = 0.036$). Fecal pellet production varied significantly among treatments (Fig. 6F; $F_5 = 3.93$, ANOVA, $p = 0.024$), with greatest production in the 25 % 2228:75 % Wilson treatment (69.1 ± 47.4 fecal pellets ind.⁻¹ d⁻¹) and lowest rates in the 'no food' and 100 % 2228 treatments (3.5 ± 2.3 and 2.4 ± 2.0 fecal pellets ind.⁻¹ d⁻¹, respectively).

In the CCMP 2228:SP-1 experiment, significant differences were found among treatments for egg production, recruitment, and fecal pellet production (Fig. 6G–I). Copepods exposed to the 100 % SP-1 diet produced significantly more eggs (9.3 ± 2.1 eggs ind.⁻¹ d⁻¹) and more nauplii (9.1 ± 0.8 nauplii ind.⁻¹ d⁻¹) than in all other treatments (Fig. 6G,H; ANOVA, $F_5 = 14.98$, 33.56 , respectively, $p < 0.001$ for each). Additionally, copepods produced significantly more fecal pellets in the 100 % SP-1 treatment (109.2 ± 27.9 fecal pellets ind.⁻¹ d⁻¹) when compared with all other treatments, and more fecal pellets in the 25 % 2228:75 % SP-1 treatment (41.1 ± 14.7 fecal pellets ind.⁻¹ d⁻¹) than in the 'no food' and 100 % 2228 treatments (Fig. 6I; ANOVA, $F_5 = 25.14$, $p < 0.001$). In the CCMP 2228:*Rhodomonas salina* and CCMP 2228:SP-1 experiments, low egg production rates for mixed diet treatments indicate that CCMP 2228 is either a toxic prey or that *Acartia tonsa* actively selects against CCMP 2228 (Fig. 6A,G). Similarities in egg production rates in the CCMP 2228:Wilson experiment indicate that CCMP 2228 and Wilson strains offer equally poor nutrition or toxicity to *Acartia tonsa* (Fig. 6D).

DISCUSSION

Is *Karenia brevis* toxic to copepod grazers or merely nutritionally inadequate?

Both toxicity and nutritionally inadequate food reduce the grazing and egg production rates of *Acartia tonsa* when fed toxic *Karenia brevis*. Three lines of evidence support the role of toxicity in compromising grazing rates of *A. tonsa*. First, copepod survivorship was significantly reduced after exposure to the

highly toxic *K. brevis* CCMP 2228 for more than 24 h. This was particularly apparent during experiments involving a non-toxic alternative food (*Rhodomonas salina* or SP-1). In these experiments, copepods had a higher survival rate when starved than when exposed to 100 % CCMP 2228. Second, grazing rates on all prey were significantly suppressed in treatments dominated by toxic CCMP 2228 (Figs. 2 & 4). Finally, copepods exposed to toxic strains of *K. brevis* (CCMP 2228 and Wilson) exhibited severe reductions in both egg and fecal pellet production. In addition, egg production on the non-toxic *K. brevis* strain SP-1 was only half of that on a nutritionally adequate food source, *R. salina* (Fig. 6).

Toxic effects of *Karenia brevis*

The conclusions above were made, in part, by applying the experimental approach provided by Jónasdóttir et al. (1998), wherein we were able to examine whether *Karenia brevis* is beneficial, nutritionally poor, or toxic to *Acartia tonsa*. Jónasdóttir et al.'s model requires that grazers be offered monoalgal and mixed algal diets of suspect and nutritionally sufficient prey, as done in the current study using the brevetoxin-laden *K. brevis* CCMP 2228 as our suspect food. In egg production experiments using the non-toxic, nutritionally replete, *Rhodomonas salina* as the control food, results showed that *K. brevis* CCMP 2228 was truly toxic to exposed copepods. Not only was egg production depressed relative to what would be expected for a nutritionally insufficient food (Fig. 6A), but low ingestion rates (Fig. 5A–C) and high mortality (Fig. 4B) all indicate toxic effects of *K. brevis* CCMP 2228 exposure.

Analysis of the egg production experiments using the non-toxic *Karenia brevis* SP-1 and low toxicity *K. brevis* Wilson as the control food provides additional insight into the inimical effects of *K. brevis* CCMP 2228. Given adequate concentrations of nutritionally sufficient food, egg production rates of *Acartia tonsa* typically range from 20 to 60 eggs female⁻¹ d⁻¹ (Jónasdóttir 1994, Kleppel et al. 1998, Hazzard & Kleppel 2003). Egg production rates on a diet of SP-1 are only half of those measured on the nutritional food, *Rhodomonas salina*, suggesting that brevetoxins are not the only factor limiting reproductive output. If *K. brevis* SP-1 was a nutritious food source, we would expect egg production rates similar to those on *R. salina*. When SP-1 dominated the prey field (25 % 2228:75 % SP-1), prey switching most likely occurred, as indicated by the

increased survivorship (Fig. 5F), positive ingestion rates (Fig. 5G–I), and higher egg and fecal pellet production (Fig. 6G–I); however, if CCMP 2228 is >50% of the prey field, toxic effects result in mortality and decreased ingestion. Copepods had higher clearance rates on SP-1 than on *R. salina*; however, due to different cell concentrations and carbon content in the prey, this amounted to lower carbon-based ingestion rates on SP-1 vs. *R. salina*. Interestingly, copepods fed 100% *R. salina* ingested 2.7 times the carbon of copepods fed 100% SP-1. This may potentially contribute to the reduced egg production rates of copepods in the SP-1 diet. Results of the egg production experiment involving the 2 toxic *K. brevis* strains, CCMP 2228 and Wilson, clearly indicated that the 2 diets were equivalent food items and neither was nutritionally sufficient to support egg production. Despite variable, yet sometimes high grazing rates, egg production and fecal pellet production on diets of toxic *K. brevis* remained low and similar to those of starved copepods (Fig. 6D–F).

Nutritional quality of *Karenia brevis*

While the C:N ratios from these experiments indicate that all strains of *Karenia brevis* were nitrogen-replete, the source of nutritional inadequacy likely stems from the inability of *K. brevis* to produce sterols essential for copepod growth and reproduction. Cholesterol is the dominant sterol found in copepod tissues and make up 89 to 99% of the total sterol composition in wild and lab-reared populations of *Acartia tonsa* (Goad 1981, Ederington et al. 1995). Reductions in copepod egg production result from a lack of dietary sterols, thus supporting the current assumption that copepods are incapable of de novo synthesis of membrane sterols and must rely on dietary sources to sustain development (Goad 1981, Ederington et al. 1995, Crockett & Hassett 2005). Giner et al. 2003 and Mooney et al. 2007 determined that *K. brevis* produces large quantities of rare, unsaturated sterols which are likely to be non-nutritious for zooplankton grazers due to their unusual structure. Further, *K. brevis* lacks the 27-methyl group necessary for their sterols to be converted into cholesterol, the dominant sterol in calanoid copepods. This offers further evidence that even non-toxic strains of *K. brevis* would be insufficient to nutritionally support lasting copepod production during a bloom event (Giner et al. 2003).

Grazing on *Karenia brevis*

Results of other researchers have indicated varying responses of copepods to *Karenia brevis* exposure. Turner & Tester (1989) found that during a rare bloom event off the North Carolina coast, the co-occurring copepod species *Acartia tonsa*, *Oncaea venusta*, and *Labidocera aestiva* all consumed *K. brevis* at rates increasing with cell concentration, while *Centropages typicus* and *Paracalanus quasimodo* ceased grazing; however, in prey selection experiments, *A. tonsa*, *L. aestiva*, and *C. typicus* all selected against *K. brevis* in favor of consuming *Skeletonema costatum*. Prey selection results do favor the conclusion that *K. brevis* is either toxic or not a sufficient food for grazers, a fact supported by our results and many other studies (Collumb & Buskey 2004, Speekmann et al. 2006, Breier & Buskey 2007). Low to negligible grazing rates on monoalgal diets of *K. brevis* have been documented repeatedly for *A. tonsa* populations from the GOM (Collumb & Buskey 2004, Speekmann et al. 2006, Breier & Buskey 2007). These low grazing rates were coupled with reduced egg production rates similar to starved copepods, further supporting the conclusion that *K. brevis* is not a nutritionally viable food source, or is toxic. Cohen et al. (2007) examined the effects of *K. brevis* and brevetoxins on populations of *A. tonsa*, *C. typicus*, and *Temora turbinata* from inshore North Carolina waters. Variable grazing rates were measured with low mortality upon exposure to either *K. brevis* cells or brevetoxins, yet significant sublethal behavioral effects were observed for *C. typicus* and *T. turbinata*, attributable to a combined effect of starvation and *K. brevis* toxicity.

Several reasons may explain the disparity among the results of previous grazing experiments. First, the biogeographic source of the experimental copepod population may factor into the observed responses. Experiments using copepod populations from the western GOM repeatedly show no significant mortality, yet show low egg production and grazing rates on monoalgal diets of *Karenia brevis*, with high grazing on mixtures of *K. brevis* and a nutritious algal species (Collumb & Buskey 2004, Speekmann et al. 2006, Breier & Buskey 2007). These populations experience more frequent exposure to *K. brevis* blooms than counterparts from the US SAB. Although *K. brevis* does extend into US SAB waters, only one documented bloom has been reported offshore North Carolina where copepods were collected for the current experiment and those done by Turner & Tester (1998) and Cohen et al. (2007). In the GOM, long-term

exposure to *K. brevis* may have selected for a population of *Acartia tonsa* more resistant to brevetoxins. Genetic subpopulations of *A. tonsa* have been identified in the GOM and along the US Atlantic coast (Caudill & Bucklin 2004, Chen & Hare 2008). Resistance to the toxic dinoflagellate *Alexandrium fundyense* has been documented for historically exposed populations of the congeneric copepod *Acartia hudsonica* from Maine and has been induced in naïve populations from the waters off New Jersey (Colin & Dam 2004, Avery & Dam 2007). Given this information, it is highly likely that *A. tonsa* populations in the GOM may be better evolved to tolerate toxic *K. brevis* than those in the US SAB. However, the present study indicates that *K. brevis* has evolved 2 effective strategies to deter grazers: toxin production and a nutritional state that does not support reproductive efforts of co-occurring copepod grazers. These 2 tactics may work synergistically to deter multiple grazers and promote the formation and maintenance of monospecific bloom events.

Variability in toxin content

Variability in *Karenia brevis* populations may also affect observed results during predator–prey experiments. Toxin levels of *K. brevis* vary among and within strains depending on culture conditions and growth phase (Baden & Tomas 1988, Roszell et al. 1990, Loret et al. 2002). In the present experiments, we measured the brevetoxin content of cells on the day of experimentation. Our results showed variability in toxin content both within and among strains, most probably due to culture growth conditions. Of the 3 strains used during the experiments, CCMP 2228 was the most toxic; however, PbTx-2 concentrations varied by 6-fold over the experiments. Levels of PbTx-2 varied by almost 3-fold within the Wilson strain. Fluctuations in toxin production have been reported previously for *K. brevis* (Baden & Tomas 1988, Roszell et al. 1990, Loret et al. 2002) and other phytoplankton species (Johansson & Granéli 1999a,b, Adolf et al. 2009). Shifts in both brevetoxin congeners and total toxin content have been documented for *K. brevis* over different growth phases (Baden & Tomas 1988, Roszell et al. 1990). Additionally, Loret et al. (2002) reported significantly different toxin contents for 5 strains of *K. brevis* cultured under identical conditions and significant toxin levels (12.23 ± 0.02 pg PbTx-2 cell⁻¹) were found in strain SP-1, which has proven to be non-toxic during the present study.

Toxic *Karenia brevis* strains (CCMP 2228 and Wilson) also produced detectable levels of the brevetoxin antagonist brevenal. Although, the brevenal concentrations were not quantified, it is interesting to note the apparent absence of brevenal in the non-toxic strain SP-1. The implication here is that perhaps brevenal serves as a self-protective compound for *K. brevis*, and concentrations may vary along with the potency of brevetoxins being produced. It will be important in future work to quantify brevenal concentrations to determine if this is a mediating factor counteracting the lethal and sublethal effects of brevetoxins on grazers.

It is imperative that grazing and production experiments involving toxic phytoplankton also include concurrent toxin analysis since toxins vary with culture condition, growth phase, nutrient availability, and between strains (Baden & Tomas 1988, Roszell et al. 1990, Loret et al. 2002, present study). Also, toxin potency varies with presence/absence of antagonists such as brevenal and gambieric acid, which could have direct consequences on the behavior and mortality of grazers. The results described here indicate that care should be taken before assumptions regarding an entire species are made based on results of experiments using a single strain under a single set of culture conditions.

Karenia brevis disrupts top-down grazer control

The results of the present study indicate that *Karenia brevis* has evolved mechanisms to reduce grazing pressure and alter food web dynamics. Further, *K. brevis* fits the 3 criteria necessary to be classified as an ecosystem disruptive algal bloom (EDAB) species (Sunda et al. 2006). First, it is widely known that *K. brevis* forms massive, nearly monospecific blooms on an almost annual basis in the GOM West Florida Shelf. Second, results presented here and by other researchers indicate that zooplankton exhibit decreased grazing and reproductive rates due to both the toxic and nutritional state of *K. brevis*. The third condition required by the EDAB model is the formation of feedbacks, as evidenced by the reduction in grazing, increased copepod mortality, decreased egg production, and potential changes in copepod behavior when exposed to toxic *K. brevis* diets. These factors suggest that zooplankton-mediated nutrient recycling would be interrupted, thereby reducing nutrient concentrations and favoring the proliferation of a low nutrient-adapted species such as *K. brevis*. As witnessed by field observa-

tions, these blooms often persist for weeks or months at a time. *K. brevis* may act as an EDAB species via both reduced grazing and nutrient recycling (Mechanism 1), or increased toxin production under nutrient limitation (Mechanism 2) (Sunda et al. 2006), which has been shown to occur in other toxic dinoflagellates including the haptophytes *Chrysochromulina polylepis* and *Prymnesium parvum* (Johansson & Granéli 1999a,b) and the 'brown tide' alga *Aureococcus anophagefferens* (Bricelj et al. 2001).

These adaptive mechanisms could favor the development of an EDAB during each stage of the bloom event (Steidinger & Vargo 1988, Steidinger et al. 1998). During the initiation stage of a bloom, *Karenia brevis* is introduced to an area, yet exists at background concentrations. Given the results presented here and by previous researchers, copepods and other herbivores should continue to graze on the available phytoplankton community, selecting for nutritional, non-toxic food sources over *K. brevis* (Turner & Tester 1998, Collumb & Buskey 2004, Speekmann et al. 2006). Since copepods would be selectively eliminating algal competitors of *K. brevis*, this should facilitate the transition to the growth stage of the bloom allowing k-selected, low-nutrient-adapted *K. brevis* populations to grow to a state of dominance. As the bloom evolves, nutrient limitation increases with decreasing rates of grazing and grazer-linked nutrient recycling, and *K. brevis* comes to dominate, which further reduces grazing rates. This inherent positive feedback mechanism should promote further bloom proliferation and maintenance—Stage 3 of the bloom. As seen in the present study, exposure to toxic *K. brevis* strains leads to increased copepod mortality, reduced ingestion rates, and reduced egg production. These combined effects should further contribute to declining grazer populations, the release of *K. brevis* populations from top-down control, and a subsequent reduction in nutrient recycling thus favoring k-selected species such as *K. brevis*. These positive feedback mechanisms will continue to maintain the *K. brevis* bloom until some physical mechanisms such as a change in wind or currents act to disperse the bloom, leading to the final bloom stage of termination. However, if grazing is completely eliminated and recycling completely stops, the *K. brevis* will deplete local nutrients and cells may senesce from apoptosis or necrosis.

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