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Sublethal effects of the toxic dinoflagellate *Karenia brevis* on marine copepod behavior

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Apart from grazing interactions, little is known regarding the sublethal effects of Karenia brevis *cells on copepod behavior. We conducted grazing and mortality experiments with* K. brevis *cells and brevetoxins (PbTx-2), establishing routes of toxicity for the copepods* Acartia tonsa, Temora turbinata *and* Centropages typicus. *Subsequent behavioral experiments determined whether copepod swimming and photobehavior, both behaviors involved in predator avoidance, were impaired at sublethal* K. brevis *and PbTx-2 levels. Copepods variably grazed toxic* K. brevis *and non-toxic* Prorocentrum minimum *at bloom concentrations. Although copepods accumulated brevetoxins, significant mortality was only observed in* T. turbinata *at the highest test concentration (1×10^7* K. brevis *cells L^{-1}). Acartia tonsa exhibited minimal sublethal behavioral effects. However, there were significant effects on the swimming and photobehavior of* T. turbinata *and* C. typicus *at the lowest sublethal concentrations tested (0.15 μg PbTx-2 L^{-1}, 1×10^5* K. brevis *cells L^{-1}). Although physiological incapacitation may have altered copepod behavior, starvation likely played a major role as well. These data suggest that sublethal effects of* K. brevis *and brevetoxin on copepod behavior occur and predicting the role of zooplankton grazers in trophic transfer of algal toxins requires knowledge of species-specific sublethal effects.*

INTRODUCTION

Blooms of the athecate dinoflagellate *Karenia brevis* (= *Gymnodinium breve*) adversely affect human and ecological health through their production of lipid-soluble brevetoxins. These toxins (PbTx 1–9) bind and activate voltage-gated sodium channels at nanomolar to picomolar concentrations resulting in severe neurological symptoms in affected organisms (Baden, 1989). Human exposure to brevetoxins occurs through contaminated shellfish (neurotoxic shellfish poisoning) and by breathing aerosols in the proximity of a *K. brevis* bloom (Martin and Chatterjee, 1969; Kirkpatrick *et al.*, 2004). The best documented ecological health effects include mass mortalities of invertebrates, finfish, birds and marine mammals, either through direct exposure or incorporation of brevetoxins in marine food webs (Gunter *et al.*, 1948; Tester *et al.*, 2000; Landsberg, 2002; Flewelling *et al.*, 2005). These mortalities are most evident during the intense blooms ($>0.5–10 \times 10^6$ cells L^{-1}), which develop along the continental shelf off the west coast of Florida (USA) in late summer or early autumn and persist from less than a month to more than a year (Steidinger, 1975; Geesey and Tester, 1993; Tester and Steidinger, 1997; Steidinger *et al.*, 1998).

The effects of *K. brevis* on lower trophic levels are poorly studied. In theory, *K. brevis* could cause direct mortality or adversely affect the behavior of any organism with a nervous system. This implies that the ecological impacts of *K. brevis* blooms begin at the plankton community level and extend to upper trophic levels (Tester *et al.*,

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2000). A key intermediary group of organisms that may be impacted are the copepods. These small crustaceans often dominate marine zooplankton communities and serve as keystone intermediates in the trophic transfer of carbon (Sommer *et al.*, 2002). This study specifically examined whether environmentally relevant concentrations of *K. brevis* and brevetoxins caused direct mortality of copepods or adversely altered fundamental behaviors that play a role in their ability to avoid predators.

Previous studies on the interactions between copepods and toxic dinoflagellates have largely examined mortality, feeding and reproduction in order to assess whether or not copepods ingest toxic cells and quantify lethal ingestion levels and to determine what, if any, effects ingested toxins have on fecundity (reviewed by Turner and Tester, 1997). Some of these studies have incorporated toxin measurement to evaluate assimilation and depuration of algal toxins (e.g. Turner *et al.*, 1998; Teegarden *et al.*, 2001; Guisande *et al.*, 2002); however, none of these studies has documented explicit sublethal effects of toxic phytoplankton on copepod behavior other than altered grazing effects.

The present study used two copepod species sympatric with *K. brevis* (*Acartia tonsa* and *Temora turbinata*) and one allopatric copepod species (*Centropages typicus*) for feeding, mortality and behavioral experiments in order to characterize effects of *K. brevis* on zooplankton grazers. Grazing studies with *A. tonsa*, *T. turbinata* and *C. typicus* established whether these species ingest *K. brevis* cells at bloom concentrations. Mortality studies evaluated dissolved brevetoxin in the fluid medium and intracellular brevetoxin of *K. brevis* cells as potential routes of toxin exposure and established lethal concentrations/doses of dissolved brevetoxin and *K. brevis* cells. Copepods were then exposed to sublethal levels of dissolved brevetoxins and *K. brevis* cells and tested for alterations in swimming behavior and in behavioral responses to light (photobehavior) associated with predator avoidance. Altered swimming and photobehavior could potentially leave copepods more vulnerable to predation, which is important because copepod grazers have the potential to alter the bloom dynamics of harmful algal species through their feeding pressure (Collumb and Buskey, 2004; Campbell *et al.*, 2005). If altered behavior correlates with high toxin body burdens, then copepod-mediated transfer of algal toxins to higher trophic levels (e.g. White, 1981; Doucette *et al.*, 2006) may be influenced by the extent and type of behavioral effect. Alternatively, the copepod species that have evolved a tolerance for brevetoxin (e.g. Colin and Dam, 2005) may accumulate the greatest body burdens and represent the principal vector for transport of toxins to higher trophic levels. Accordingly, understanding these sublethal effects and relative resistance to brevetoxins by various copepod species will be fundamental to predicting the ecological impact of *K. brevis* blooms at higher trophic levels.

METHOD

Grazing experiments

Karenia brevis (Wilson clone; diameter 20.42 ± 0.42 μm, $n = 2243$) and a local, non-toxic isolate of *Prorocentrum minimum* (diameter 10.46 ± 0.34 μm, $n = 469$) were grown in filtered Gulf Stream seawater diluted with distilled water to a salinity of 30, enriched with f/2 nutrients (Guillard and Ryther, 1962) at 22°C. *Prorocentrum minimum* co-occurs with *K. brevis* (Millie *et al.*, 1997) and served as a non-toxic dinoflagellate control. Negative bioassays with bay scallops (G. Wikfors, personal communication, National Marine Fisheries Service, Milford, CT, USA) and negative toxicity assays for hepatotoxic and diarrhetic shellfish toxins (Shimizu, 1987) completed by Luckas (Friedrich-Schiller-Universität Jena) confirmed our local isolate of *P. minimum* as non-toxic. Cultures were kept on a 12 L:12 D period with maximum irradiance of 100–120 μmol photons $m^{-2} s^{-1}$ (cool-white fluorescent lighting) and harvested during log-phase growth for use in the grazing experiments. Carbon and nitrogen contents of both prey species were measured using a Costech Analytical Technologies Inc. Model 4010 200A C:H:N analyzer. Carbon content was used to convert cell densities to biomass (discussed subsequently). The $C{:}N$ ratio (mol:mol) for *K. brevis* (9.41 ± 0.24, SD) and *P. minimum* (7.55 ± 0.05, SD) was above the Redfield ratio (6.625), suggesting that both species were nitrogen replete under the culture conditions.

Three copepod species (*A. tonsa*, 5.33 μg dry wt.; *T. turbinata*, 8.77 μg dry wt. and *C. typicus*, 11.15 μg dry wt.) were collected using a 333 μm mesh plankton net suspended from the NOAA pier on Pivers Island, NC, (USA) ~2.5 km inshore of Beaufort Inlet (34°41.8′N, 76°40.2′W). Sampling was limited to 15–20 min tows on daytime ebb tides. Zooplankton samples were diluted with ambient water and acclimated overnight to 22°C and a salinity of 30. Actively swimming adult female copepods were isolated for experiments with a micropipette under a dissecting microscope. Only adult females were used to reduce any variations due to sex and growth stage (age), and adult females are generally larger, having greater energy requirements due to egg production (Mauchline, 1998). Copepods were placed

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in either a starved or fed acclimation condition for 12 h prior to grazing experiments at 22°C under the ambient L:D cycle; 'starved' copepods were maintained in filtered seawater (FSW), whereas 'fed' copepods were maintained in FSW supplemented with $1-10 \times 10^6$ *Rhodomonas* sp. cells L^{-1}. FSW was prepared by GF/F (Whatman) filtration of seawater collected ~25 km offshore of Beaufort Inlet (NC, USA).

The experimental protocol for the grazing studies followed Tester and Turner (Tester and Turner, 1990) and Tester *et al.* (Tester *et al.*, 2000). Twenty *A. tonsa* or *T. turbinata*, or 15 *C. typicus*, were placed in 400 mL bottles on an ambient L:D cycle. Grazing experiments (12–18 h) were conduced in triplicate using three concentrations of *K. brevis* cells (~1×10^6, 5×10^6 and 1×10^7 cells L^{-1}). These cell concentrations for the grazing experiments were chosen to parallel the concentrations used in the mortality experiments (below) to help determine potential routes of toxin exposure. Triplicate positive control treatments were run using the same copepod densities at three similar concentrations of non-toxic *P. minimum*. Triplicate phytoplankton controls with no copepods were incubated under the same conditions to correct for phytoplankton growth. *Karenia brevis* (860.5 ± 45.9 pg C $cell^{-1}$) and *P. minimum* (133.2 ± 4.3 pg C $cell^{-1}$) cells were converted to carbon, and ingestion rates were computed as ng C consumed $copepod^{-1}\ h^{-1}$ according to Frost (Frost, 1972). Differences in ingestion rates among treatments were tested using two-factor analysis of variance (ANOVA) tests with Holm–Sidak *post hoc* testing (SigmaStat 3.10, Systat Software, Inc.).

Mortality experiments

Copepods were collected using a stationary 333 μm mesh 0.75 m diameter plankton net set prior to maximum current near the sampling site described for grazing experiments. All net samples were brought to the laboratory and diluted with ambient seawater (salinity 36; range at sampling site = 30–36). For experiments with *A. tonsa* and *T. turbinata* conducted during the spring and autumn when water temperatures were ~21°C, copepods were sorted immediately following collection and used in experiments. *Centropages typicus* experiments were conducted during the winter when water temperatures were ~10°C. Accordingly, copepods were slowly acclimated to experimental temperatures (21°C) in the presence of ambient phytoplankton as food over 2 days, then sorted for experiments on the third day. Prior to experimentation, copepods of a given test species were sorted from net samples and either: (i) fed with 1×10^6 *Rhodomonas* sp. cells L^{-1} in FSW or (ii) starved in FSW. Fed and starved copepods (300–700) were maintained in an incubator (Sherer model CEL 4–4; 21°C; 6.2×10^{19} photons $m^{-2}\ s^{-1}$ cool-white fluorescent lighting, ambient L:D) in 2 L beakers with slow aeration.

After 24 h of either feeding or starvation, adult female copepods that appeared healthy were sorted into groups of 10 individuals in 1 mL FSW. A 70 mL culture flask was filled with 50 mL of a treatment solution, a group of copepods was gently added and then the flask was filled with an additional 20 mL of the appropriate treatment solution. Five replicate flasks were prepared for each of the following treatment solutions: (i) FSW, (ii) 0.015 μg PbTx-2 L^{-1}, (iii) 0.15 μg PbTx-2 L^{-1}, (iv) 1.5 μg PbTx-2 L^{-1}, (v) 15 μg PbTx-2 L^{-1}, (vi) 1×10^7 *P. minimum* cells L^{-1}, (vii) 1×10^5 *K. brevis* cells L^{-1}, (viii) 1×10^6 *K. brevis* cells L^{-1}, (ix) 5×10^6 *K. brevis* cells L^{-1} (*A. tonsa* and *T. turbinata* only) and (x) 1×10^7 *K. brevis* cells L^{-1}. All treatment solutions were prepared by diluting dinoflagellate cultures (discussed earlier) with FSW or dissolving purified PbTx-2 (Calbiochem) in FSW. Treatments encompassed a range of *K. brevis* cell and dissolved brevetoxin concentrations occurring in natural bloom conditions (Tester *et al.*, in press). Flasks were arbitrarily placed in rows in an incubator (21°C and ambient L:D, irradiance as described earlier) for 24 h, after which the number of copepods alive (showing any sign of movement) or dead (showing no sign of movement) was counted. Copepod mortality after 24 h was calculated either relative to the *P. minimum* control treatment (1×10^7 *P. minimum* cells L^{-1}) for experiments with *K. brevis* cells or relative to the FSW control for experiments with purified PbTx-2. To calculate relative mortality for a given treatment flask, the percentage mortality during the experiment was divided by the mean percentage mortality of the appropriate control (either *P. minimum* or FSW, $n = 5$). Relative mortality calculated in this way yields a value of 1 for a flask with the same mortality as the control, a value of 2 for a flask with double the mortality of the control, etc. Significant mortality was determined for each copepod species using Kruskal–Wallis ANOVAs on Ranks for brevetoxin and *K. brevis* cell treatments, with Dunnett's *post hoc* testing versus the appropriate control treatment (FSW or *P. minimum* cells) (SigmaStat 3.10, Systat Software, Inc.).

Copepods, water and cells (*K. brevis* treatments only) from the highest concentration of PbTx-2 (15 μg L^{-1}) and the highest and lowest concentrations of *K. brevis* cells (1×10^5 and 1×10^7 cells L^{-1}) were assayed for brevetoxins (PbTx-2, PbTx-3 and PbTx-5). The number of brevetoxin samples that could be processed was limited by the high cost of sample analysis; these

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treatments represent the maximum PbTx-2 concentration and the range of *K. brevis* cell concentrations in the morality experiments. Contents from three of the five mortality experiment replicates were vacuum-filtered under low pressure through GF/F filters. Copepods were gently picked from the filters, washed in FSW to remove adsorbed toxin, placed onto clean filters and frozen at −80°C until analysis. Filters with cells (*K. brevis* treatments only) and the cell-free filtrate were likewise frozen at −80°C until analysis. Brevetoxins were analyzed using micellar electrokinetic capillary chromatography, as described by Shea (Shea, 1997). Differences in toxin content between fed and starved copepods of the three species were tested using two-factor ANOVAs with Holm–Sidak *post hoc* testing (SigmaStat 3.10, Systat Software, Inc.).

Behavioral experiments

Copepods were collected as described for mortality experiments, except that all sampling was done at night in order to collect copepods in the ascent phase of nocturnal diel vertical migration (e.g. Cohen and Forward, 2005). Adult female copepods of each species were fed *Rhodomonas* sp. for 24 h as described earlier and then sorted into groups of 25 individuals and added to 70 mL flasks containing the following sublethal treatment solutions: (i) cell-free and brevetoxin-free FSW, (ii) 0.15 μg PbTx-2 L^{-1}, (iii) 1.5 μg PbTx-2 L^{-1}, (iv) 15 μg PbTx-2 L^{-1}, (v) 1×10^7 *P. minimum* cells L^{-1}, (vi) 1×10^5 *K. brevis* cells L^{-1}, (vii) 5×10^5 *K. brevis* cells L^{-1} (*T. turbinata* only), (viii) 1×10^6 *K. brevis* cells L^{-1}, (ix) 5×10^6 *K. brevis* cells L^{-1} (*T. turbinata* only) and (x) 1×10^7 *K. brevis* cells L^{-1} (*A. tonsa* and *C. typicus* only).

Copepods remained in treatment solutions for 24 h in the incubator under the ambient L:D cycle, after which their swimming speeds and photobehaviors were tested in an apparatus simulating the angular light distribution underwater (Cohen and Forward, 2005). The test sequence involved gently transferring the contents of a flask (copepods and treatment solution) into an acrylic cuvette (4 × 4 × 5 cm) placed at the horizontal center of a water bath (50 × 50 × 25 cm) with inside walls painted flat black. The bath was filled with deionized water at a level just below the upper edge of the cuvette. Light entered the bath from the top and passed through a diffuser plate, creating a uniform light field such that the walls of the bath were outside the critical angle (zenith ± 48.6°), as viewed from the bottom of the cuvette. The light source was a 300 W incandescent bulb, filtered to the blue–green spectral region with a Corning no. 4-96 filter (Kopp Glass), which are wavelengths encompassed by the spectral sensitivities of marine copepods (Cohen and Forward, 2002). Copepods were initially kept in darkness for 5 min, and then were stimulated for 8 s at increasing irradiances from 2×10^{13} to 2×10^{16} photons $m^{-2}\,s^{-1}$, with 3 min darkness between each light stimulus. Preliminary experiments indicated that this stimulus duration was adequate to evoke a photobehavioral response in the test species, and 3 min in darkness between stimuli was sufficient for copepods to resume normal swimming behavior and sensitivity to light.

Copepod behavior was recorded with a closed-circuit video system. The camera's depth of field was sufficiently large to reduce the width of the cuvette to two-dimensions. Copepods were illuminated with far-red light (maximum transmission = 774 nm), which does not alter or induce copepod photoresponses (Cohen and Forward, 2002). Video was digitized, and aspects of movement in the XY-plane were determined for each copepod in the field of view using a PC-based motion analysis system (CellTrak Software, Motion Analysis, Inc.). The percentage of copepods swimming in the upper 90% of the test chamber (i.e. not inactive on the bottom) was determined in darkness prior to irradiance stimuli. Swimming speeds and directions of copepods near the center of the chamber (i.e. away from walls and top/bottom) were measured in darkness (control values) over a 6 s period prior to the first light stimulus (2×10^{13} photons $m^{-2}\,s^{-1}$) and averaged for all copepods in the field of view. The swimming direction of each copepod in the field of view was also measured again 1 s after the onset of each stimulus for an equivalent duration. The percentage of copepods swimming upwards toward the stimulus light ± 60° (positive phototaxis) was calculated.

Swimming activity and speed in darkness were compared among brevetoxin and *K. brevis* cell treatments for each species using one-factor ANOVAs, with Holm–Sidak *post hoc* testing for significant differences among all treatments (SigmaStat 3.10, Systat Software, Inc.). Phototactic response data for each treatment were analyzed using one-factor repeated measures ANOVAs (RM ANOVAs). Holm–Sidak *post hoc* testing was again used to determine significant differences among positive phototaxis at each irradiance level when compared with upward swimming in darkness (SigmaStat 3.10, Systat Software, Inc.).

RESULTS

Grazing

Copepod feeding state prior to the grazing experiments (fed for 12 h in FSW with 1 – 10×10^6 *Rhodomonas*

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sp. cells L^{-1}, or starved for 12 h in FSW) did not uniformly affect ingestion rates (ng C copepod h^{-1}) on *P. minimum* and *K. brevis* among copepod species (Table I). For *A. tonsa*, no effect of copepod feeding state on ingestion rate was observed with either dinoflagellate prey type at the carbon concentrations offered [two-factor ANOVAs, $P = 0.630$ (*P. minimum*), and $P = 0.904$ (*K. brevis*) for feeding state *x* prey concentration]. In contrast, starved *T. turbinata* had higher carbon ingestion rates than fed copepods at the highest carbon concentration offered for both *P. minimum* and *K. brevis* [two-factor ANOVAs, $P = 0.018$ (*P. minimum*), $P = 0.036$ (*K. brevis*) for feeding state *x* prey concentration, Holm–Sidak *post hoc* tests). *Centropages typicus* ingestion rates were not statistically different between fed and starved copepods over the carbon concentrations offered when feeding on the non-toxic *P. minimum* (two-factor ANOVA, $P = 0.87$ for feeding state *x* prey concentration), but when feeding on the toxic *K. brevis*, ingestion rates of fed *C. typicus* were higher at the highest carbon concentration offered (two-factor ANOVA, $P = 0.002$ for feeding state *x* prey concentration, Holm–Sidak *post hoc* tests). Only starved *C. typicus* grazed upon the non-toxic control dinoflagellate, *P. minimum*, with ingestion rates increasing with increasing carbon concentrations offered (Table I). Both *A. tonsa* and *T. turbinata* grazing on *P. minimum* reached saturating food levels at intermediate carbon concentrations offered ($7–8 \times 10^5$ ng C L^{-1}).

Fed *A. tonsa* ingested *K. brevis* only at the lowest concentrations offered ($\sim 8 \times 10^5$ cells L^{-1} or 6.9×10^5 ng C L^{-1}) (Table I). Starved *A. tonsa* ingested *K. brevis* at a rate of 78–105 ng C copepod^{-1} h^{-1} at cell concentrations between $\sim 1 \times 10^6$ and 5×10^6 cell L^{-1}. Fed *T. turbinata* ingested *K. brevis* offered at 7.0×10^5–4.3×10^6 ng C L^{-1} (8.0×10^5 – 5.0×10^6 cells L^{-1}). Starved *T. turbinata* ingested *K. brevis* erratically. It should be noted that for both *A. tonsa* and

Table I: Copepod grazing on toxic (K. brevis) and non-toxic (P. minimum) dinoflagellates

Species	Prey species	Fed/starved	Initial cells offered cells L^{-1}	Initial carbon offered ng C L^{-1}	Ingestion rate ng C copepod^{-1} h^{-1}
A. tonsa	*P. minimum*	Fed	1.0E + 07	1.3E + 06	0.868 (0.290)
			5.4E + 06	7.2E + 05	0.716 (0.154)
			1.1E + 06	1.4E + 05	0.210 (0.036)
A. tonsa	*P. minimum*	Starved	9.8E + 06	1.3E + 06	0.708 (0.056)
			5.0E + 06	6.9E + 05	0.693 (0.103)
			1.1E + 06	1.4E + 05	0.213 (0.165)
A. tonsa	*K. brevis*	Fed	1.1E + 07	9.4E + 06	0
			5.2E + 06	4.4E + 06	0
			8.0E + 05	6.9E + 05	157.7 (67.1)
A. tonsa	*K. brevis*	Starved	8.9E + 06	7.7E + 06	0
			4.5E + 06	3.9E + 06	104.5 (327.5)
			9.5E + 05	8.2E + 05	78.4 (12.7)
C. typicus	*P. minimum*	Fed	9.2E + 06	1.2E + 06	0.201 (0.155)
			4.6E + 06	6.1E + 05	0.850 (0.079)
			6.2E + 05	8.2E + 04	0.162 (0.026)
C. typicus	*P. minimum*	Starved	8.9E + 06	1.2E + 06	2.800 (0.565)
			3.9E + 06	5.2E + 05	1.219 (0.100)
			8.8E + 05	1.2E + 05	0.265 (0.026)
C. typicus	*K. brevis*	Fed	8.9E + 06	7.7E + 06	2,372.3 (387.3)
			3.6E + 06	3.1E + 06	663.2 (247.8)
			7.4E + 05	6.4E + 05	162.2 (42.9)
C. typicus	*K. brevis*	Starved	9.2E + 06	7.9E + 06	829.7 (320.4)
			4.6E + 06	3.6E + 06	663.2 (247.8)
			9.0E + 05	7.7E + 05	214.4 (18.7)
T. turbinata	*P. minimum*	Fed	9.9E + 06	1.3E + 06	0.409 (0.084)
			5.6E + 06	7.5E + 05	1.222 (0.067)
			1.3E + 06	1.7E + 05	0.318 (0.007)
T. turbinata	*P. minimum*	Starved	1.0E + 07	1.3E + 06	1.596 (0.731)
			5.8E + 06	7.7E + 05	1.506 (0.084)
			1.2E + 06	1.6E + 05	0.193 (0.012)
T. turbinata	*K. brevis*	Fed	1.0E + 07	8.7E + 06	0
			5.0E + 06	4.3E + 06	290.3 (610.9)
			7.9E + 05	6.8E + 05	223.9 (40.4)
T. turbinata	*K. brevis*	Starved	1.1E + 07	9.1E + 06	974.0 (438.4)
			5.4E + 06	4.4E + 06	0
			9.6E + 05	8.3E + 05	80.9 (35.0)

Means (± SD for rate calculations) for three replicates are shown. An ingestion rate of 0 indicates the calculated mean was negative, resulting from the Frost equations when cell concentrations are higher in controls than in grazing containers at the end of grazing experiments.

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T. turbinata, the standard deviations of the ingestion rates are far higher in the *K. brevis* feeding experiments than in experiments when *P. minimum* was used as the prey (Table I). It is likely that *A. tonsa* and *T. turbinata* cease feeding at high concentrations of *K. brevis* or feed only intermittently. *Centropages typicus* grazed *K. brevis* at all concentrations offered (7.4×10^5–9.2×10^6 cells L^{-1}, 6.4×10^5–7.9×10^6 ng C L^{-1}); grazing rates increased with increasing *K. brevis* concentrations and only began to satiate in starved copepods (Table 1).

Mortality

Mortality of copepods in each treatment was calculated as relative mortality by dividing the percentage mortality in each flask by the mean percentage mortality of the appropriate control [either 1×10^7 *P. minimum* cells L^{-1} or 0 μg PbTx-2 L^{-1} (FSW)]. In this way, relative mortality represents the multiplicative effect of a given treatment on copepod mortality. Exposure for 24 h to dissolved brevetoxin (PbTx-2) did not result in significant mortality for any of the species tested, regardless of the feeding condition (Kruskal–Wallis ANOVA on Ranks, $P > 0.05$ for each species and feeding condition) (Fig. 1a and b). Mortality did increase with increasing PbTx-2 concentration in fed *C. typicus* (Fig. 1a); however, this increase was not statistically significant (Kruskal–Wallis ANOVA on Ranks, $P = 0.365$).

Species-specific differences in copepod mortality were observed after 24 h exposure to *K. brevis* cells (Fig. 1c and d). Both fed and starved *T. turbinata* showed a significant increase in mortality as *K. brevis* cell concentration increased (Kruskal–Wallis ANOVA on Ranks, fed: $P = 0.005$, starved: $P = 0.018$), with mortality becoming significantly higher at 1×10^7 *K. brevis* cells L^{-1} than in the 1×10^7 *P. minimum* cells L^{-1} control treatments (Dunnett's tests, $P < 0.05$). The effect was greater in fed *T. turbinata* (Fig. 1c) than in those starved for 24 h prior to *K. brevis* treatments (Fig. 1d). Both fed and starved *A. tonsa* had higher mortality at higher *K. brevis* cell concentrations, but these were not significantly higher than controls (Kruskal–Wallis ANOVA on Ranks, fed: $P = 0.360$, starved: $P = 0.173$) and were considerably lower than those observed in

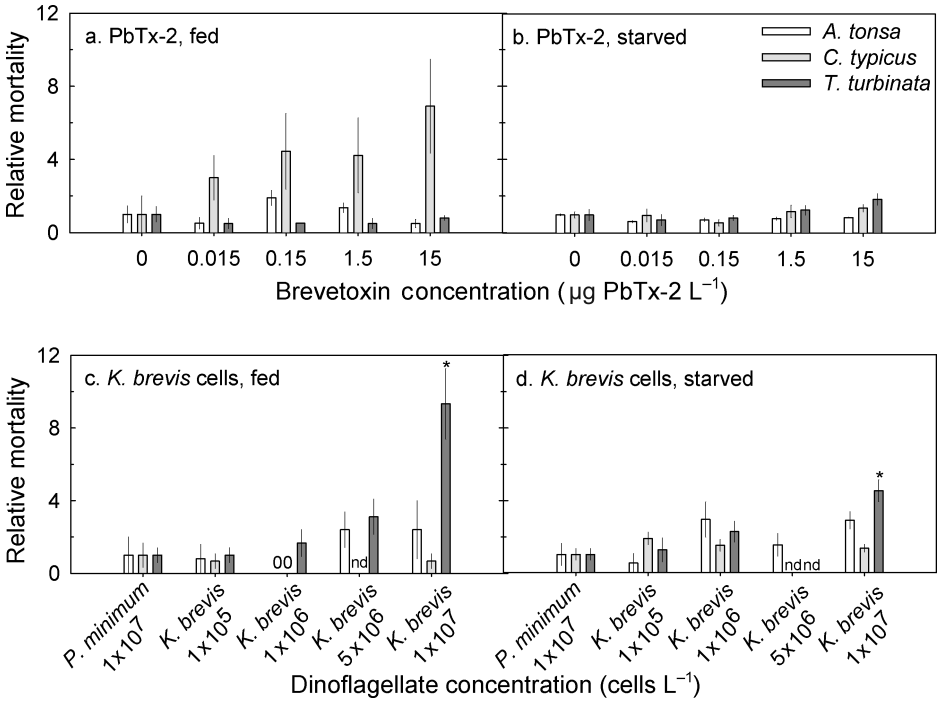

Fig. 1. Copepod mortality after 24 h exposures to PbTx-2 or *K. brevis* cells. Mortality was calculated relative to controls for (a) copepods fed *Rhodomonas* sp. for 24 h prior to PbTx-2 exposure (control = 0 μg PbTx-2 L^{-1} = FSW), (b) copepods starved in FSW for 24 h prior to PbTx-2 exposure (control = 0 μg PbTx-2 L^{-1} = FSW), (c) copepods fed *Rhodomonas* sp. for 24 h prior to *K. brevis* cell exposure (control = 1×10^7 *P. minimum* cells L^{-1}) and (d) copepods starved in FSW for 24 h prior to *K. brevis* cell exposure (control = 1×10^7 *P. minimum* cells L^{-1}). The relative mortality calculation is described in the text; a value of 1 for a given treatment represents the same mortality as the control. Data for *A. tonsa* are plotted as open bars, *C. typicus* as light grey bars and *T. turbinata* as dark grey bars. All bars are means (± SEM) for five replicates. Asterisks denote a significant difference between the treatment and either the FSW or *P. minimum* control ($P < 0.05$, Kruskal–Wallis ANOVA, Dunnett's test versus control). Mortalities of zero are indicated as 0, whereas 'nd' indicates no mortality data were obtained for that species/concentration combination.

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T. turbinata (Fig. 1c and d). *Centropages typicus* appeared unaffected by 24 h exposure to *K. brevis* cells as mortality was low in all treatments (Fig. 1c and d) (Kruskal–Wallis ANOVA on Ranks, fed: $P = 0.437$, starved: $P = 0.347$).

Brevetoxin content of copepod bodies (PbTx-2, PbTx-3 and PbTx-5) was assayed after mortality experiments (Fig. 2). For copepods exposed to 15 μg PbTx-2 L^{-1}, only PbTx-2 was detected. Starved copepods as a group had higher PbTx-2 body burdens than those that were fed prior to PbTx-2 exposure (two-factor ANOVA, $P = 0.018$, Holm–Sidak *post hoc* test), but body burdens for starved copepods within each species were not significantly greater than those of fed copepods (two-factor ANOVA, $P = 0.969$; Fig. 2). *Acartia tonsa* had significantly higher PbTx-2 body burdens than either *C. typicus* or *T. turbinata* (two-factor ANOVA, $P < 0.001$, Holm–Sidak *post hoc* test). For mortality experiments with *K. brevis* cells, brevetoxins (PbTx-2, PbTx-3 and PbTx-5) were not detected in bodies of copepods exposed to 1×10^5 *K. brevis* cells L^{-1}; however, copepods exposed to 1×10^7 *K. brevis* cells L^{-1} contained all three compounds. Among the brevetoxins, PbTx-2 was detected in the highest quantities (Table II). Fed copepods in the 1×10^7 *K. brevis* cells L^{-1} treatment had PbTx-2 body burdens similar to those exposed to 15 μg PbTx-2 L^{-1} (Fig. 2), whereas

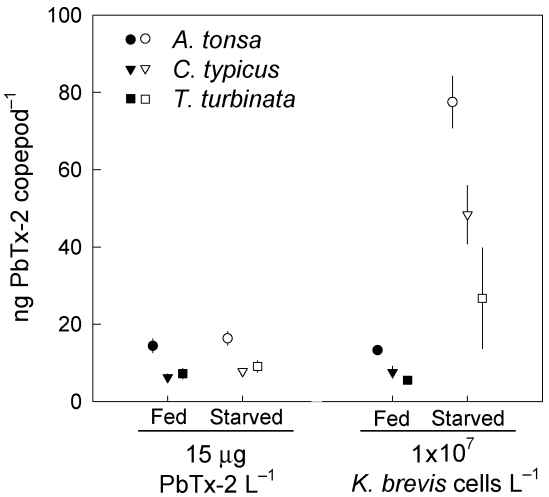

Fig. 2. Copepod brevetoxin body burdens after 24 h exposures to PbTx-2 or *K. brevis* cells in mortality experiments. Brevetoxin content of copepods (ng PbTx-2 copepod^{-1}) from mortality experiments is plotted for animals fed (Fed; solid symbols), or starved (Starved; open symbols), as described in Fig. 1. Copepods were briefly rinsed in FSW prior to freezing for toxin analysis, but were not allowed to clear their guts. Brevetoxins were not present at detectable levels in copepods exposed to the lowest *K. brevis* concentration (1×10^5 *K. brevis* cells L^{-1}), but were present in the two other treatments that were assayed (15 μg PbTx-2 L^{-1} and 1×10^7 *K. brevis* cells L^{-1}). Data are means (± SD) for three replicate mortality experiment flasks.

Table II: Brevetoxin body burdens of fed and starved copepods exposed for 24 h to 1×10^7 K. brevis cells L^{-1}

Copepod species	Brevetoxin	Fed	Starved
A. tonsa	PbTx-2	13.28 (0.75)	77.49 (6.69)
	PbTx-3	3.95 (1.18)	16.32 (0.84)
	PbTx-5	1.73 (0.70)	8.18 (3.15)
C. typicus	PbTx-2	7.57 (1.56)	48.33 (7.51)
	PbTx-3	2.33 (0.76)	9.77 (0.85)
	PbTx-5	0.93 (0.31)	3.77 (0.25)
T. turbinata	PbTx-2	5.50 (0.85)	26.70 (13.05)
	PbTx-3	0.80 (0.80)	4.30 (2.55)
	PbTx-5	BLD	1.43 (1.29)

Data are means (± SD) in units of ng PbTx copepod^{-1} for three replicate groups of copepods. BLD, below level of detection.

PbTx-2 content of starved copepods in the 1×10^7 *K. brevis* cells L^{-1} treatment was higher (two-factor ANOVA, $P < 0.001$, Holm–Sidak *post hoc* test) and more variable (Fig. 2). Starved *A. tonsa* had significantly higher PbTx-2 body burdens than the other species, whereas fed *A. tonsa* did not (two-factor ANOVA, starved: $P < 0.001$, fed: $P > 0.05$, Holm–Sidak *post hoc* test).

Behavior

A general assessment of copepod swimming activity is provided by the percentage of copepods actively swimming in the test chamber in darkness (i.e. not remaining inactive on the bottom of the chamber). For *A. tonsa*, approximately half of the copepods in the chamber were present in the upper 90% of the water column, with no difference among treatment conditions (one-factor ANOVA, $P = 0.952$) (Fig. 3). *Centropages typicus* swimming activity was dependent on treatment condition (Fig. 3); low and intermediate PbTx-2 concentrations (0.15 and 1.5 μg L^{-1}) and low and high *K. brevis* cell concentrations (1×10^5 and 1×10^7 cells L^{-1}) suppressed swimming relative to the FSW control, whereas swimming activity was elevated in both the non-toxic dinoflagellate *P. minimum* (1×10^7 cells L^{-1}) and the intermediate *K. brevis* cell concentrations (1×10^6 cells L^{-1}) (one-factor ANOVA, $P < 0.001$, Holm–Sidak pairwise *post hoc* tests). For *T. turbinata*, all treatments lacking dinoflagellate cells (0–15 μg PbTx-2 L^{-1}) had approximately half of the copepods swimming, whereas more copepods were swimming in all treatments with cells, with the exception of the highest *K. brevis* cell concentration (5×10^6 cells L^{-1}), in which only ~25% of the copepods were actively swimming (Fig. 3) (one-factor ANOVA, $P < 0.001$, Holm–Sidak pairwise *post hoc* tests).

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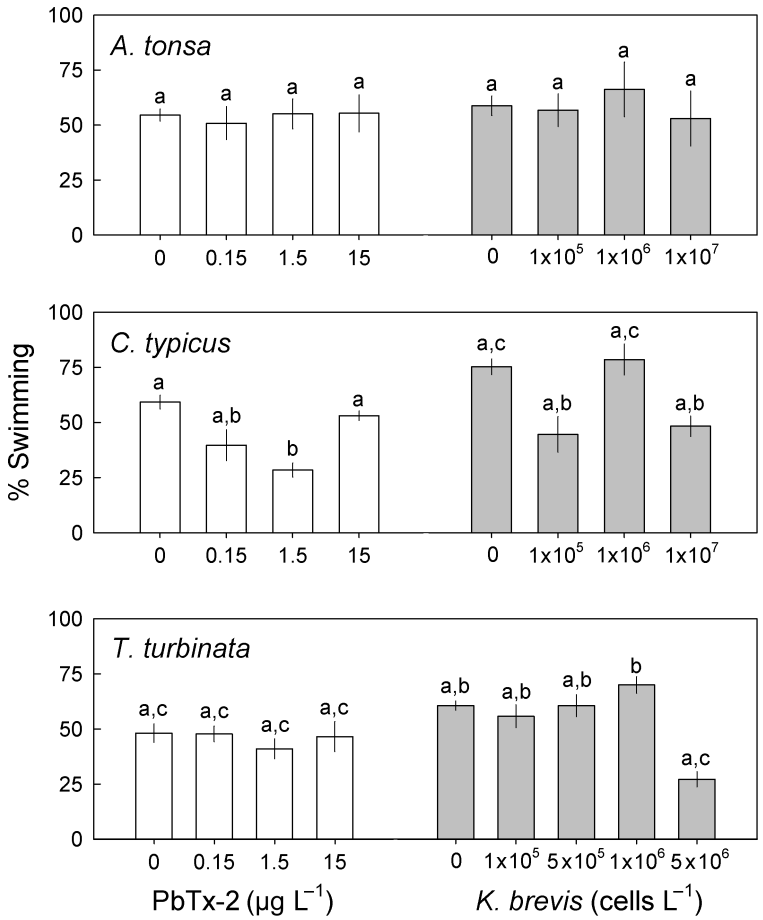

Fig. 3. Copepod swimming activity after 24 h sublethal exposures to PbTx-2 and *K. brevis* cells. The mean (± SEM, $n = 5$) percentage of copepods swimming in the upper 90% of the test chamber in darkness prior to first irradiance stimulus is plotted as a function of PbTx-2 concentration (µg L^{-1}; open bars) and *K. brevis* cell concentration (cells L^{-1}; shaded bars). Toxin-free control treatments are 0 µg PbTx-2 L^{-1} (= FSW) and 0 *K. brevis* cells L^{-1} (= 1×10^7 *P. minimum* cells L^{-1}). Letters (a, b and c) denote significantly different treatment groups within each panel (one-factor ANOVA, Holm–Sidak pairwise *post hoc* tests among all treatments); treatments with the same letter are not significantly different.

Comparing copepod swimming speeds in darkness among treatments provides a behavioral measure of motor coordination, which is essential for effective escape behavior. For both *A. tonsa* and *T. turbinata*, there was no significant difference in swimming speed among copepods in all treatments (one-factor ANOVA, $P = 0.516$, 0.666), although there is some indication of increased variability in *A. tonsa* swimming speed at the intermediate PbTx-2 concentration (1.5 µg L^{-1}) and with increasing *K. brevis* cell concentration (Fig. 4). In contrast, swimming speed of *C. typicus* did vary among treatments (Fig. 4; one-factor ANOVA, $P < 0.001$, Holm–Sidak pairwise *post hoc* tests), with the fastest speeds occurring in the *P. minimum* control (1×10^7 cells L^{-1}) and elevated speeds in both the highest PbTx-2 concentration (15 µg L^{-1}) and the intermediate *K. brevis* cell concentration (1×10^6 cells L^{-1}).

Analyzing the behavioral responses of copepods to light (photobehavior) provides a measure of sensory/motor coordination, particularly as it pertains to light-mediated predator–avoidance behaviors such as diel vertical migration (Cohen and Forward, 2005). The ability to swim toward a light source in a simulated natural underwater angular light distribution (positive phototaxis) was altered in *T. turbinata* and *C. typicus*, but not in *A. tonsa*, after 24 h exposure to sublethal concentrations of either dissolved PbTx-2 or *K. brevis* cells (Figs 5 and 6). *Acartia tonsa* did not display a positive phototactic response, as there was no significant change in the percentage of positive phototaxis with increasing irradiance in the FSW control or in any of the PbTx-2 treatments (0.15, 1.5 and 15.0 µg L^{-1}) (Fig. 5a; Table III). Similarly, the percentage of copepods exhibiting positive phototaxis was the same for all stimuli in

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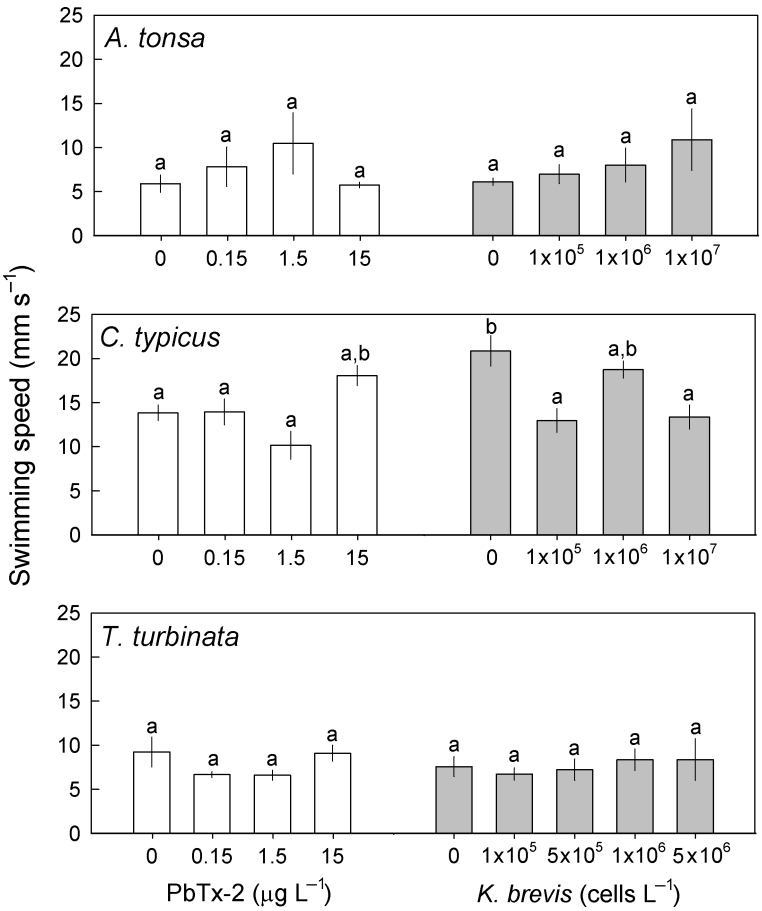

Fig. 4. Copepod swimming speed after 24 h sublethal exposures to PbTx-2 and *K. brevis* cells. The mean (± SEM, $n = 5$) speed (mm s^{-1}) of copepods swimming in darkness in the center of the test chamber is plotted as a function of PbTx-2 and *K. brevis* cell concentrations, as described in Fig. 3.

the *P. minimum* control (1×10^7 cells L^{-1}) and in all *K. brevis* cell treatments (1×10^5, 1×10^6 or 1×10^7 cells L^{-1}) (Fig. 6a; Table III).

For *C. typicus* in the FSW control, positive phototaxis increased to a maximum at 10^{14} photons m^{-2} s^{-1}, then decreased at higher irradiance stimuli (Fig. 5b; Table III). Exposure to low and intermediate PbTx-2 concentrations (0.15 and 1.5 μg L^{-1}) resulted in an increase in positive phototaxis in darkness, and generally more erratic swimming, whereas exposure to the highest PbTx-2 concentration (15.0 μg L^{-1}) resulted in a phototactic response at all irradiances similar to that in FSW (Fig. 5b; Table III). For *C. typicus* fed *P. minimum* (1×10^7 cells L^{-1}), positive phototaxis at 10^{14} photons m^{-2} s^{-1} was not significantly greater than in darkness, but the positive phototactic response increased with exposure to all tested concentrations of *K. brevis* cells (Fig. 6b; Table III).

Temora turbinata in the FSW control had increasing positive phototaxis with increasing irradiance and a maximum response at 10^{15} photons m^{-2} s^{-1} (Fig. 5c; Table III). This pattern was also present in *T. turbinata* exposed to low and intermediate PbTx-2 concentrations (0.15 and 1.5 μg L^{-1}), but exposure to the highest PbTx-2 concentration (15.0 μg L^{-1}) resulted in a loss of photosensitivity and reduced positive phototaxis at 10^{15} photons m^{-2} s^{-1} (Fig. 5c; Table III). For *T. turbinata* fed the non-toxic *P. minimum* control (1×10^7 cells L^{-1}), positive phototactic responses to all irradiance stimuli (10^{13} – 10^{16} photons m^{-2} s^{-1}) were significantly higher than the response in darkness (Fig. 6c; Table III). Exposure of *T. turbinata* to sublethal concentrations of *K. brevis* cells resulted in an increase in upward swimming in darkness and highly variable responses at the highest *K. brevis* cell concentration (5×10^6 cells L^{-1}) (Fig. 6c; Table III).

DISCUSSION

The present experiments suggest that although copepods will graze to some extent upon *K. brevis* at bloom

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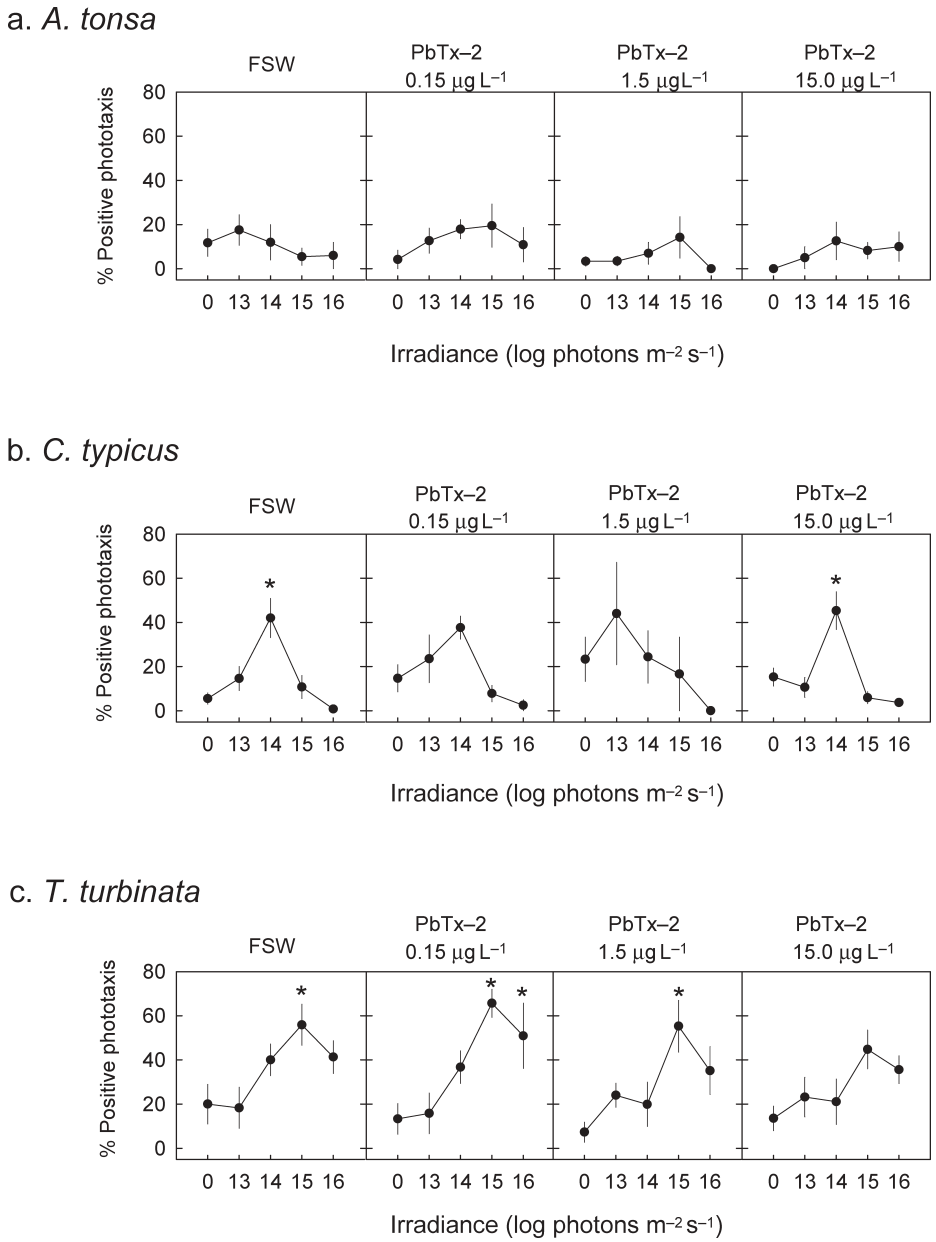

Fig. 5. Copepod photobehavior after 24 h sublethal exposure to dissolved PbTx-2. The mean (± SEM, $n = 5$) percentage of copepods undergoing positive phototaxis is plotted as a function of irradiance stimulus (log photons m^{-2} s^{-1}). Treatment condition is indicated above each panel, where FSW is brevetoxin-free FSW, and PbTx-2 concentration is in µg L^{-1}. Asterisks denote a significant difference within a treatment (one-factor RM ANOVA, Holm–Sidak *post hoc* test versus control) between the phototactic response at a given irradiance and the response in darkness (plotted at 0 log photons m^{-2} s^{-1}).

concentrations and that mortality is generally low with exposure to either *K. brevis* cells or brevetoxin, some copepod species exhibit behavioral effects upon sublethal exposure resulting from starvation as well as physiological incapacitation. The route of toxicity includes both ingestion of *K. brevis* cells and exposure to dissolved brevetoxins. These behavioral results fit with a variety of other studies examining the short-term interactions (grazing rates) and long-term consequences (survival and egg production rates) of copepods exposed to a variety of toxic phytoplankton species (reviewed in Turner and Tester, 1997; Marinho da Costa and Fernández, 2002).

Of the copepod species tested, *A. tonsa* appears the least vulnerable when exposed to *K. brevis* or brevetoxins. This species is extremely common in coastal and

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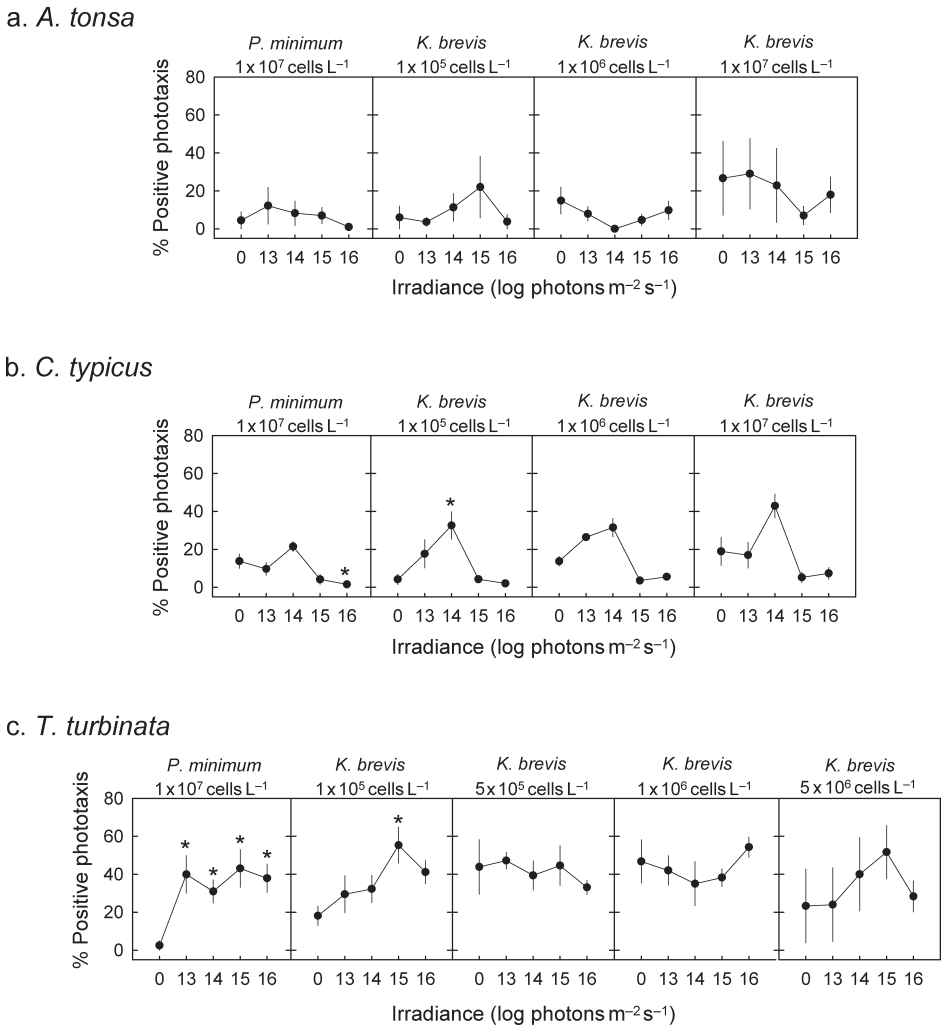

Fig. 6. Copepod photobehavior after 24 h sublethal exposure to *K. brevis* cells. The mean (± SEM, $n = 5$) percentage of copepods undergoing positive phototaxis is plotted as a function of irradiance stimulus as in Fig. 5. Treatment condition is indicated above each panel, where non-toxic (*P. minimum*) and toxic (*K. brevis*) dinoflagellate concentrations are in cells L^{-1}.

estuarine habitats throughout the year, particularly during summer and autumn, along the southeastern US and in the Gulf of Mexico. With females ranging in length from 1.3 to 1.5 mm (Johnson and Allen, 2005), *A. tonsa* was the smallest species tested. *Acartia tonsa* was found to be among the dominant copepods associated with *K. brevis* blooms in the Gulf of Mexico (Lester *et al.*, in press). Ingestion of *K. brevis* cells at bloom concentrations by *A. tonsa* is generally low (Table I). This result is similar to that of Turner and Tester (Turner and Tester, 1989), who reported that *A. tonsa* and two other copepod species (*Onceaea venusta* and *Labidocera aestiva*) grazed on *K. brevis* in natural water samples collected during a bloom off North Carolina, but selected against *K. brevis* when offered in combination with the diatom *Skeletonema costatum* at natural cell concentrations. Although recent studies differ in the extent that *A. tonsa* graze *K. brevis* cells, it is generally agreed that *K. brevis* is a low quality food for this copepod, with effects on egg production and hatching similar to starvation (e.g. Collumb and Buskey, 2004; Prince *et al.*, 2006) and to the effects of grazing on unialgal and nitrogen-deplete diatom and dinoflagellate diets (e.g. Jones and Flynn, 2005). Our study extends observations of *K. brevis* interactions with *A. tonsa* to behavior (Figs 3–6) and provides further evidence of only minor *K. brevis* toxicity for this copepod species.

Interestingly, *A. tonsa* consistently had the highest PbTx-2 body burdens (PbTx-2 copepod^{-1}) of the copepod species tested (Fig. 2). *Acartia tonsa*, therefore, accumulates brevetoxins, particularly starved copepods in the presence of *K. brevis* cells, without killing them,

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Table III: Statistical analysis of copepod photobehavior experiments

			Holm–Sidak Test (versus dark control)			
			log photons m^{-2} s^{-1}			
Copepod	Treatment	RM ANOVA *P*-value	13	14	15	16
A. tonsa	FSW	0.644	—	—	—	—
	0.15 μg PbTx-2 L^{-1}	0.562	—	—	—	—
	1.5 μg PbTx-2 L^{-1}	0.338	—	—	—	—
	15 μg PbTx-2 L^{-1}	0.537	—	—	—	—
	1×10^7 *P. minimum* cells L^{-1}	0.723	—	—	—	—
	1×10^5 *K. brevis* cells L^{-1}	0.213	—	—	—	—
	1×10^6 *K. brevis* cells L^{-1}	0.259	—	—	—	—
	1×10^7 *K. brevis* cells L^{-1}	0.877	—	—	—	—
C. typicus	FSW	<0.001	ns	*	ns	ns
	0.15 μg PbTx-2 L^{-1}	0.018	ns	ns	ns	ns
	1.5 μg PbTx-2 L^{-1}	0.313	ns	ns	ns	ns
	15 μg PbTx-2 L^{-1}	<0.001	ns	*	ns	ns
	1×10^7 *P. minimum* cells L^{-1}	0.002	ns	ns	ns	*
	1×10^5 *K. brevis* cells L^{-1}	0.001	ns	*	ns	ns
	1×10^6 *K. brevis* cells L^{-1}	<0.001	*	*	*	*
	1×10^7 *K. brevis* cells L^{-1}	0.003	ns	*	ns	ns
T. turbinata	FSW	0.027	ns	ns	*	ns
	0.15 μg PbTx-2 L^{-1}	0.003	ns	ns	*	*
	1.5 μg PbTx-2 L^{-1}	0.024	ns	ns	*	ns
	15 μg PbTx-2 L^{-1}	0.069	—	—	—	—
	1×10^7 *P. minimum* cells L^{-1}	0.002	*	*	*	*
	1×10^5 *K. brevis* cells L^{-1}	0.033	ns	ns	*	ns
	5×10^5 *K. brevis* cells L^{-1}	0.774	—	—	—	—
	1×10^6 *K. brevis* cells L^{-1}	0.233	—	—	—	—
	5×10^6 *K. brevis* cells L^{-1}	0.778	—	—	—	—

The *P*-value for each one-factor RM ANOVA is provided, and where significant ($P < 0.05$), results are given for Holm–Sidak *post hoc* testing versus a control (control = positive phototaxis in darkness, 0 log photons m^{-2} s^{-1}). Holm–Sidak test results are given as significant difference (*), no significant difference (ns) or no test because of insignificant RM ANOVA (—).

and with only negligible sublethal effects on swimming and photobehavior. Similarly, no obvious physiological incapacitation was evident for *A. tonsa* grazing on a natural population of *K. brevis* during a bloom off North Carolina (Turner and Tester, 1989). These findings suggest that *A. tonsa* would make an excellent model organism for studies into mechanisms of brevetoxin resistance. Mutations in sodium channel proteins confer saxitoxin resistance to the clam *Mya arenaria* (Bricelj *et al.*, 2005); perhaps, a similar mechanism confers brevetoxin resistance to *A. tonsa*. Indeed, preliminary evidence exists for sodium channel mutations in a congeneric copepod, *A. hudsonica*, which may be related to saxitoxin resistance (H. G. Dam, S. Lin and H. Zhang, University of Connecticut, personal communication). Although high PbTx body burdens could make *A. tonsa* good vectors for trophic transfer of toxins (e.g. Tester *et al.*, 2000; Doucette *et al.*, 2005), their defensive behaviors (i.e. swimming speed/escape response and diel vertical migration) would remain functional at the exposure levels of most blooms, suggesting that their vulnerability to predation will not increase during the bloom periods. Nonetheless, they could be potent vectors for toxin to higher trophic levels.

Like *A. tonsa*, *T. turbinata* is common on the west Florida continental shelf and is associated with *K. brevis* blooms (Grice, 1960; Sutton *et al.*, 2001; Lester *et al.*, in press). *Temora turbinata*, also like *A. tonsa*, has been shown previously to graze on *K. brevis* and accumulate brevetoxins (Tester *et al.*, 2000). Similar to our results for *A. tonsa*, we found that *K. brevis* was ingested by *T. turbinata* at relatively low levels (Table I). In mortality experiments (Fig. 1), *K. brevis* was only toxic to *T. turbinata* with 24 h exposure to 1×10^7 *K. brevis* cells L^{-1}, suggesting that exposure to dissolved toxins or to whole cells at low-to-moderate bloom densities ($1 \times 10^3 - 1 \times 10^6$ *K. brevis* cells L^{-1}; Florida Fish and Wildlife Conservation Commission) is not sufficient to directly kill the copepods. However, sublethal effects on photobehavior were found at the lowest PbTx-2 and *K. brevis* cell concentrations tested (0.15 μg PbTx-2 L^{-1} and 1×10^5 *K. brevis* cells L^{-1}; Figs 5 and 6). This represents a striking difference between *A. tonsa* and *T. turbinata*; although *A. tonsa* was minimally affected behaviorally by *K. brevis* or brevetoxins, *T. turbinata* behavior appeared vulnerable

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to each at concentrations commonly found during blooms.

The observed photobehavioral alterations could result from either starvation or physiological incapacitation (Cronin and Forward, 1980; Ives, 1987; Colin and Dam, 2002). Starvation likely affected photobehavior at low *K. brevis* and brevetoxin concentrations (1×10^5 cells L^{-1}, 0.15–1.5 μg PbTx-2 L^{-1}), given similarities in the phototactic responses between the FSW treatment and low *K. brevis*/brevetoxin treatments (Figs 5 and 6). However, physiological incapacitation likely occurred at higher concentrations, as the positive phototactic response became increasingly erratic (5×10^5–5×10^6 cells L^{-1}) or dampened (15.0 μg L^{-1} PbTx-2), suggesting loss of sensory/motor control (Figs 5 and 6). Photobehavior underlies copepod predator-avoidance behaviors such as diel vertical migration (Cohen and Forward, 2005); therefore, difficulties in the ability of *T. turbinata* to orient in the underwater light field may increase its vulnerability to predators. Interestingly, *T. turbinata* swimming activity and speed were not significantly affected by exposure to brevetoxin or whole cells, with the exception of depressed swimming activity, but not routine swimming speed, at the highest *K. brevis* cell concentration (5×10^6 cells L^{-1}; Figs 3 and 4). *Temora turbinata* is capable of tracking vertically migrating nontoxic dinoflagellates in laboratory columns over several days (Bird and Kitting, 1982), and there is some suggestion that *T. turbinata* in offshore waters of the west Florida Shelf actively avoid dense *K. brevis* patches (Lester *et al.*, in press). Perhaps, this behavior provides some refuge from *K. brevis* toxicity. The present study did not investigate how long sublethal effects continue in copepods if the animals are placed in less toxic or toxin-free water, and this information is needed to evaluate the function of migration as a refuge from *K. brevis* toxicity. However, depuration of brevetoxins by zooplankton appears fairly rapid; 4 h depuration studies with zooplankton samples (333 μm mesh) collected during a *K. brevis* bloom suggested considerable (~75%) loss of PbTx-2 over this time period (P. Tester, unpublished results).

Centropages typicus differs from *A. tonsa* and *T. turbinata* in that it is a larger copepod (1.3–1.8 mm) (Johnson and Allen, 2005) and is not regularly found in the Gulf of Mexico; therefore, it does not typically co-occur with *K. brevis* (Turner and Tester, 1989). Previous grazing studies with *C. typicus* collected at the same location as in the present study found that this species avoided *K. brevis* cells and exhibited no apparent ill effects from *K. brevis* exposure (Turner and Tester, 1989). In contrast, *C. typicus* in the present study readily grazed *K. brevis* cells and accumulated brevetoxins (Table I; Fig. 2). Distinct sublethal effects on *C. typicus* swimming behavior and photobehavior were also observed (Figs 3–6). Some of these sublethal effects are consistent with starvation. For example, the heightened photosensitivity and suppressed swimming activity and speed at 1×10^5 and 1×10^7 *K. brevis* cells L^{-1}, are similar to the FSW treatment. However, other effects suggest some physiological incapacitation, such as suppressed swimming activity at 0.15 and 1.5 μg L^{-1}, and erratic photosensitivity at 1.5 μg L^{-1}. Given the biogeographical range of *C. typicus*, ecological conclusions drawn from these experiments are not particularly valuable. However, its congener, *C. velificatus*, is common in the Gulf of Mexico and co-occurs with *K. brevis* blooms at high cell concentrations (~5×10^6 cells L^{-1}) (K. M. Lester *et al.*, University of South Florida, unpublished results). If *C. typicus* and *C. velificatus* are similar in their response to *K. brevis* cells and brevetoxins, then *C. velificatus* would be expected to ingest *K. brevis* cells and accumulate brevetoxins, with resulting behavioral effects that could influence their predator-avoidance capability.

All copepod species tested in the present study accumulated brevetoxins from *K. brevis* cell and dissolved PbTx-2 exposure, with some species (e.g. *A. tonsa*) accumulating more toxin with little apparent sublethal behavioral effects and others (e.g. *T. turbinata*) accumulating less toxin with severe sensory/motor effects. This variability among species is interesting, and future studies into the molecular mechanisms of variable brevetoxin resistance in copepods will hopefully provide a physiological explanation. Examining the physiology and behavior of zooplankton grazers and their trophic interactions *in situ* is warranted, as these data suggest that copepods may act as vectors for toxin to higher trophic levels either by high accumulation of toxin with little change in vulnerability to predation or by lower toxin accumulation but weakened defenses against predators.

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