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Effects of the dinoflagellate *Karenia brevis* on larval development in three species of bivalve mollusc from Florida

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Abstract

The effects of *Karenia brevis* (Wilson clone) on larval survival and development of the northern quahog, *Mercenaria mercenaria*, eastern oyster, *Crassostrea virginica* and bay scallop, *Argopecten irradians*, were studied in the laboratory. Larvae were exposed to cultures of whole and lysed cells, with mean total brevetoxin concentrations of 53.8 and 68.9 $\mu g\,L^{-1}$, respectively. Survival of early (3-day-old) larvae was generally over 85% for all shellfish species at *K. brevis* densities of 100 cells ml^{-1} or less, and not significantly different between whole and lysed culture. At 1000 cells ml^{-1}, survival was significantly less in lysed culture than whole culture for both *M. mercenaria* and *C. virginica*. Survival of late (7-day-old) larvae in all three species was not significantly affected by *K. brevis* densities of 1000 cells ml^{-1} or less. At 5000 cells ml^{-1}, however, survival was reduced to 37%, 26% and 19% for *A. irradians*, *M. mercenaria* and *C. virginica*, respectively. Development of *C. virginica* and *M. mercenaria* larvae was protracted at *K. brevis* densities of 1000 cells ml^{-1}. These results suggest that blooms of *K. brevis*, and particularly their associated brevetoxins, may have detrimental consequences for Florida's shellfisheries by disrupting critical larval processes. Special attention should be paid to blooms of *K. brevis* where these shellfish occur naturally or where aquaculture and restoration activities are either ongoing or planned.

Keywords: *Karenia brevis*; Brevetoxins; Bivalves; Larvae; Florida

1. Introduction

Recurring and persistent harmful algal blooms have raised increasing concerns about the long-term effects on local fisheries, including critical species of bivalve shellfish (Shumway and Cucci, 1987; Shumway, 1990; Landsberg, 1996). The toxic dinoflagellate, *Karenia brevis* (= *Gymnodinium breve*) Hansen and Moestrup, causes periodic and extensive red tides along the south-central Gulf coast of Florida (Steidinger et al., 1995) and produces potent neurotoxins (= brevetoxins). These brevetoxins are lethal to fish and cause neurotoxic shellfish poisoning (NSP) in humans from the consumption of contaminated shellfish (Baden, 1988; Steidinger et al., 1998). At the same time, *K. brevis* blooms pose a potential threat to Florida's shellfish resources and growing bivalve aquaculture industry (Blake et al., 2000; Adams and Sturmer, 2004). Red tides occur more frequently during the summer and fall along

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the Florida west coast (Steidinger, 1975) at a time when native shellfish species are spawning (Barber and Blake, 1983; Hesselman et al., 1989). Shellfish populations could thus be exposed to *K. brevis* blooms at a critical stage in their life history, and the relative success or failure of recruitment could depend on whether there are detrimental effects of exposure to *K. brevis* on the developmental stages of affected shellfish species. We therefore examined the effects of the NSP-producing alga, *K. brevis*, on survival and development of larvae from three species of bivalve molluscs: the northern quahog (= hard clams) (*Mercenaria mercenaria*), the bay scallop (*Argopecten irradians*) and the Eastern oyster (*Crassostrea virginica*). We also sought to distinguish whether differences in survival were due to the dinoflagellate itself or its constituent toxins.

2. Materials and methods

2.1. Shellfish larvae

Shellfish larvae were kindly supplied from the following hatcheries: *M. mercenaria* (Bay Shellfish Company, Palmetto, FL), *C. virginica* (Louisiana Sea Grant Oyster Hatchery, Grand Isle, LA), *A. irradians* (University of South Florida Shellfish Hatchery, St. Petersburg, FL). Broodstock from each hatchery used to produce larvae for this study came from populations having no prior history of exposure to *K. brevis*.

2.2. Algal cultures

Batch cultures of *K. brevis* (= *Gymnodinium breve*) (Wilson clone) were grown in NH15 media without aeration. Seawater was collected locally, filtered to remove particles $>0.2\,\mu m$, passed through activated charcoal, UV sterilized and autoclaved. (Incoming seawater is routinely monitored twice daily for the presence of *K. brevis*; the seawater system is switched to recirculation mode whenever cells are present.) Cultures were maintained at 24–26 °C and 33–35 ppt on a 12:12 h light: dark cycle. Algae were harvested at stationary phase and cell concentration determined by a Coulter® Multisizer IIE fitted with a 100 μm orifice. Cultures of *Isochrysis galbana* (Tahitian clone) were grown in *f*/2 media plus Trimsa minus silica with aeration under constant illumination.

2.3. Culture preparations

Whole and lysed culture preparations of *K. brevis* (obtained from the same batch culture) were used as experimental treatments. Whole culture treatments required no special preparation. Lysed culture treatments were produced by exposing *K. brevis* culture to ultrasonic disruption at 750 W for four minutes using a Sonics® Vibracell with 5 mm microtip probe. A subsample was observed microscopically to verify that cells had been disintegrated. A 500 ml sample of both preparations was processed for analysis of brevetoxins.

2.4. Brevetoxin analysis

Brevetoxins were extracted by passing the culture sample (whole or lysed) through a C-18 solid-phase extraction disk under vacuum according to the procedure of (Pierce et al., 2005). Brevetoxin analyses were performed by LC–MS (Pierce et al., 2005).

2.5. Three-day exposure experiment

Separate experiments were conducted for each species of shellfish. Three-day-old larvae were exposed to *K. brevis* for three days. Each experiment had three concentrations (10, 100 and 1000 cells ml^{-1}) and two treatments (whole and lysed culture) of *K. brevis* plus a control (no *K. brevis*). Concentrations of *K. brevis* were achieved by adding a specific amount of batch culture (known concentration) to reach the desired final concentration (v/v). The same volume (ml) of lysed culture was added to the lysed treatments as whole culture was added to the whole treatments. In addition, larvae were fed 10^4 cells ml^{-1} of the chrysophyte, *I. galbana*, which was replenished daily.

Initially, ca. 500 larvae were transferred to a 100-ml graduated cylinder containing 50-ml filtered seawater. Filtered seawater was added to bring the volume to the desired level before innoculation with *K. brevis* and *I. galbana*. The contents were transferred to a 100-ml petri dish and inoculated with the appropriate amount of freshly prepared *K. brevis* and *I. galbana*, covered, and left undisturbed for 24 h. Final density was 5 larvae ml^{-1}. Each day, the contents of each dish were poured through a 35-μm sieve and gently rinsed with filtered seawater. Larvae were again transferred to a 100-ml graduated cylinder, the volume brought to the appropriate volume prior to reinoculation, and

returned to their respective dish. Dishes were reinoculated with freshly prepared *K. brevis* and *I. galbana*, covered, and left undisturbed for 24 h. The experiment was terminated after three days and the larvae preserved in 2% buffered formalin. The number of live and dead larvae (determined by marked morphological disintegration) and developmental stage were determined.

2.6. Seven-day exposure experiment

Separate experiments were conducted for each species. Seven-day-old larvae were exposed to *K. brevis* for seven days. Each experiment consisted of four concentrations (10, 100, 1000, and 5000 cells ml^{-1}) of whole *K. brevis* culture and a control. Approximately 500 larvae were transferred to a 250-ml graduated cylinder containing 200-ml filtered seawater. Filtered seawater was added to bring the volume to the desired level before innoculation with *K. brevis* and *I. galbana*. The contents were transferred to a 250-ml finger bowl and inoculated with the appropriate amount of freshly prepared *K. brevis* and *I. galbana*, covered, and left undisturbed for 24 h. Final density was 2–3 larvae ml^{-1}. Each day, the contents of each bowl were poured through a 53-μm sieve and gently rinsed with filtered seawater. Larvae were transferred to a 250-ml graduated cylinder containing 200 ml of filtered seawater and brought to the appropriate volume prior to reinoculation. Bowls were reinoculated with *K. brevis* and *I. galbana*, covered, and left undisturbed for 24 h. The experiment was terminated after seven days and the larvae preserved in a 2% buffered formalin.

The number of live and dead larvae (determined by marked morphological disintegration) was determined for each developmental stage. Developmental stage depended upon the bivalve species and included straight-hinged veliger, umbonal veliger, pediveliger, and spat (Sastry, 1965 for *A. irradians*; Carriker, 2001 for *M. mercenaria*; Waller, 1981 for *C. virginica*).

2.7. Statistics

The square root of the proportion of live and dead larvae was arcsine transformed to satisfy the assumption of normality when dealing with percentages (Zar, 1996). Differences in mortality among cell concentration and culture treatments in the 3-day-exposure experiments were determined by two-way ANOVA with repeated measures. Significant differences among treatments were analyzed using Tukey's multiple comparison test. A single factor ANOVA was performed to determine significant differences in mortality among cell concentrations in the 7-day-exposure experiments.

3. Results

3.1. Toxin profile of dinoflagellate cultures

Toxin profiles of *K. brevis* cultures used in experiments are summarized in Table 1. Three brevetoxin compounds were present in each culture: Pbtx-2, Pbtx-3, and brevenal, a recently idenfitied brevetoxin antagonist. Cell density, brevetoxin composition and total toxin concentration were similar among cultures used for experiments for each shellfish species. Total toxin concentration was higher after cultures had been lysed.

3.2. The effects of Karenia brevis on 3-day-old larvae

A. irradians (Bay scallop). Survival of *A. irradians* larvae exposed to *K. brevis* was >90% in

Table 1
Cell density, sample matrix and brevetoxin composition of *Karenia brevis* (Wilson Clone) culture used in experiments for each species

Species	*K. brevis* culture		Brevetoxin amount (ug L^{-1})			
	(Cells/ml)	Matrix	Pbtx-2	Pbtx-3	Total	Brevenal
Bay scallop (*Argopecten irradians*)	12,000	Whole	20.03	5.07	55.3	30.23
		Lysed	26.31	8.11	68.9	34.47
Northern quahog (*Mercenaria mercenaria)*	12,800	Whole	23.36	2.46	55.0	29.17
		Lysed	32.33	2.42	70.1	35.32
Eastern oyster (*Crassostrea virginica*)	10,000	Whole	22.19	4.10	51.2	24.93
		Lysed	32.94	0.97	67.8	33.90

all treatments and >80% of surviving larvae reached the umboveliger stage (Table 2). Survival was significantly lower ($p<0.001$) at the highest *K. brevis* concentration in both whole and lysed treatments (Table 5). There was no treatment effect on survival of *A. irradians* larvae.

M. mercenaria (Northern quahog = hard clam). Survival of *M. mercenaria* larvae was >88% in all treatments and >86% of surviving larvae reached the umboveliger stage (Table 3). Percent survival was significantly lower ($p<0.001$) in the Lysed-1000 cells ml^{-1} treatment (Table 5). There was a significant ($p<0.05$) concentration and culture effect on larval survival.

C. virginica (Eastern oyster). Survival of *C. virginica* larvae ranged from 75% to 94% (Table 4), with <1% reaching the umboveliger stage in all treatments, including the control. Survival decreased with increasing *K. brevis* concentration; survival was lower in lysed treatments than whole treatments at the same *K. brevis* concentration. There was a significant ($p<0.05$) concentration and culture effect on larval survival (Table 5).

3.3. The effects of Karenia brevis on 7-day-old larvae

A. irradians (Bay scallop). Exposure of *A. irradians* larvae to *K. brevis* concentrations up to 1000 cells ml^{-1} for 7 days did not greatly impact survival (Fig. 1). When the quantity was increased to 5000 cells ml^{-1}, survival declined to 37%. All larvae had reached the pediveliger stage at the termination of the experiment.

M. mercenaria (Northern quahog). Survival of *M. mercenaria* larvae gradually decreased with increasing *K. brevis* concentration up to 1000 cells ml^{-1} (Fig. 2). Larval survival at 5000 cells ml^{-1}

Table 2
Mean (±SD) number of live and dead larvae, larval stage and percent survival for *Argopecten irradians* after 3 days exposure to *Karenia brevis*

	Straight-Hinged Veliger		Umboveliger		Larvae	Survival (%)	
Treatment	Live	Dead	Live	Dead	Total	Total	Umboveliger
Control	41.0 (24.7)	3.6 (3.0)	395.8 (125.6)	4.0 (3.1)	444.4 (128.3)	98.3 (0.8)	89.1 (5.9)
Whole-10	45.8 (19.4)	7.2 (2.9)	410.8 (140.9)	3.2 (2.0)	467.0 (125.0)	97.8 (1.4)	88.0 (7.8)
Lysed-10	47.6 (7.1)	3.6 (3.6)	350.0 (70.8)	3.6 (0.9)	404.8 (74.9)	98.2 (0.6)	86.5 (2.5)
Whole-100	61.0 (33.8)	4.6 (2.3)	475.6 (92.5)	1.2 (1.1)	542.4 (115.5)	98.9 (0.2)	87.7 (4.3)
Lysed-100	57.6 (5.5)	10.2 (2.8)	384.2 (95.9)	1.8 (2.7)	453.8 (101.5)	97.4 (0.7)	84.7 (2.4)
Whole-1000	39.4 (16.4)	22.8 (10.4)	430.0 (76.4)	3.8 (2.8)	496.0 (77.9)	94.6 (2.3)	86.7 (4.5)
Lysed-1000	63.2 (33.6)	21.8 (11.6)	402.2 (123.0)	5.4 (2.3)	492.6 (111.9)	94.5 (2.5)	81.6 (10.2)

Treatments consisted of whole and lysed cultures of *K. brevis* at three concentrations: 10, 100 and 1000 cells ml^{-1}. Each treatment consisted of five replicates.

Table 3
Mean (±SD) number of live and dead larvae, larval stage and percent survival for *Mercenaria mercenaria* after three days exposure to *Karenia brevis*

	Straight-Hinged Veliger		Umboveliger		Larvae	Survival (%)	
Treatment	Live	Dead	Live	Dead	Total	Total	Umboveliger
Control	8.4 (2.0)	4.0 (2.9)	448.4 (41.6)	46.2 (17.0)	507.0 (53.8)	90.1 (2.1)	88.4 (0.7)
Whole-10	7.0 (4.2)	3.2 (2.3)	474.4 (90.0)	36.4 (6.9)	521.0 (91.5)	92.4 (1.3)	91.1 (0.5)
Lysed-10	4.8 (3.7)	0.4 (0.6)	428.4 (138.9)	48.8 (10.7)	482.4 (150.9)	89.8 (2.0)	88.8 (0.5)
Whole-100	5.2 (2.7)	1.0 (1.0)	454.0 (155.7)	41.0 (14.9)	501.2 (171.6)	91.6 (0.8)	90.6 (0.5)
Lysed-100	5.4 (2.7)	3.4 (1.3)	463.8 (158.9)	47.2 (19.0)	519.8 (177.9)	90.3 (1.0)	89.2 (0.8)
Whole-1000	10.6 (4.0)	4.6 (2.9)	512.4 (129.5)	49.4 (9.1)	577.0 (142.6)	90.6 (0.8)	88.8 (0.6)
Lysed-1000	7.4 (2.0)	5.0 (4.0)	539.6 (166.1)	68.6 (21.8)	620.6 (191.3)	88.1 (0.9)	86.9 (0.2)

Treatments consisted of whole and lysed cultures of *K. brevis* at three concentrations: 10, 100 and 1000 cells ml^{-1}. Each treatment consisted of five replicates.

Table 4
Mean (±SD) number of live and dead larvae, larval stage and percent survival for *Crassostrea virginica* after 3 days exposure to *Karenia brevis*

	Straight-Hinged Veliger		Umboveliger		Larvae	Survival (%)	
Treatment	Live	Dead	Live	Dead	Total	Total	Pediveliger
Control	241.6 (56.7)	20.4 (6.9)	1.6 (3.0)	10.4 (9.2)	274.0 (59.2)	88.8 (3.1)	0.6 (1.1)
Whole-10	304.8 (74.1)	17.0 (7.9)	1.2 (0.8)	1.2 (1.1)	324.2 (81.1)	94.4 (1.8)	0.4 (0.3)
Lysed-10	259.4 (21.5)	15.8 (8.3)	0.4 (0.5)	0.2 (0.4)	275.8 (27.1)	94.2 (2.9)	0.1 (0.2)
Whole-100	299.0 (19.4)	42.0 (5.5)	0.0	0.4 (0.5)	341.4 (19.5)	87.6 (1.8)	0.0
Lysed-100	226.4 (27.0)	48.4 (10.7)	0.0	1.6 (1.1)	276.4 (34.6)	81.9 (2.3)	0.0
Whole-1000	252.6 (38.1)	46.4 (14.0)	0.6 (1.3)	1.4 (0.9)	301.0 (43.5)	84.1 (4.0)	0.2 (0.4)
Lysed-1000	199.8 (16.8)	65.2 (9.5)	0.0	0.8 (0.8)	265.8 (12.4)	75.2 (4.1)	0.0

Treatments consisted of whole and lysed cultures of *K. brevis* at three concentrations: 10, 100 and 1000 cells ml^{-1}. Each treatment consisted of five replicates.

declined to 26%. A higher percent of larvae from the low dose treatments developed into pediveligers than larvae from higher dose treatments (Fig. 3).

C. virginica (Eastern oyster). Survival of *C. virginica* larvae was 88% in the control, 75% in *K. brevis* concentrations up to 1000 cells ml^{-1}, and only 19% at 5000 cells ml^{-1} (Fig. 4). Larval development was similar among exposure doses up to 1000 cells ml^{-1} (Fig. 5). Approximately 46% were umboveligers after 7 days; 42% developed into pediveligers and 11% had settled as spat. At 5000 cells ml^{-1}, 67% of surviving larvae were still umboveligers, 32% were pediveligers, and only 1% had settled as spat.

4. Discussion

Studies on the interaction between toxic dinoflagellates and bivalves have focused primarily on juvenile and adult life stages (Shumway, 1990; Bricelj and Shumway, 1998; Landsberg, 1996). Recently, attention has begun to focus on the effects of harmful algal on bivalve larvae (Wikfors and Smolowitz, 1995; Matsuyama et al., 2001; Yan et al., 2001; Yan et al., 2003; Jeong et al., 2004). Bivalve larvae, with their planktonic existence and small size, can be expected to respond in unique ways (compared to their post-larval counterparts) when exposed to harmful algal blooms.

In all three species of bivalve (*A. irradians*, *M. mercenaria* and *C. virginica*), survival of 3-day-old larvae in the presence of *K. brevis* was concentration-dependent. At densities of 100 cells ml^{-1} or less, survival was generally over 85% and not affected by treatment preparation; i.e., whole or lysed culture. At 1000 cells ml^{-1}, survival was significantly less in lysed treatments for both *M. mercenaria* and *C. virginica*. Seven-day-old larvae showed a similar survival response after exposure to *K. brevis* for seven days. *A. irradians* and *C. virginica* survival was not significantly reduced at *K. brevis* concentrations up to 1000 cells ml^{-1}, while survival of *M. mercenaria* larvae was significantly lower at 1000 cells ml^{-1}. Survival in all three species was significantly reduced at 5000 cells ml^{-1}. Matsuyama et al. (2001) reported lethal effects of *Alexandrium tamarense*, *A. taylori*, *Gymnodinium mikimotoi* and *Heterocapsa circularisquama* on larvae of the Pacific oyster, *C. gigas*, at cell densities of 100–1000 cells ml^{-1}.

The process by which *K. brevis* affects larval survival is not clear, but several possible mechanisms may be involved. Direct cell-to-cell contact with microalgae, either through exposure to toxins present on the cell surface or through mechanical damage to sensitive organs, particularly gills, may negatively affect bivalve larvae (Gallager et al., 1989; Landsberg, 1996). Mortality of *C. virginica* larvae in the presence of the dinoflagellate *Cochlodinium heterolobatum* was thought to be a result of increased physical contact between larvae and algal cells (Ho and Zubkoff, 1979). Contact with toxic algal cells may also release an unknown inhibitory factor which could negatively affect survival (Yan et al., 2001). Ultrasonic disruption (= lysing) produces cellular fragments as well as releasing intracellular toxins to the environment, thus making them available for encounters with bivalve larvae.

Consumption (or ingestion) of toxic algal cells by bivalve larvae is dependent on a variety of factors,

Table 5
Effect of *Karenia brevis* concentration and culture preparation on percent survival in 3-day-old shellfish larvae

(A) Two-way ANOVA ($\alpha = 0.05$)

Source of variation	SS	df	MS	F	P-value	F crit
Argopecten irradians						
Concentration	0.0630	2	0.0315	23.20	$2.43E^{-6}$	3.40
Culture preparation	0.0015	1	0.0015	1.14	0.2971	4.26
Interaction	0.0089	2	0.0045	3.28	0.0549	3.40
Within	0.0326	24	0.0014			
Total	0.1061	29				
Mercenaria mercenaria						
Concentration	17.0532	2	8.5266	4.75	0.0183	3.4028
Culture preparation	34.8101	1	34.8101	19.39	0.0002	4.26
Interaction	3.6143	2	1.8072	1.01	0.3804	3.40
Within	43.0858	24	1.7952			
Total	98.5635	29				
Crassostrea virginica						
Concentration	0.2837	2	0.1418	69.35	$1.06E^{-10}$	3.40
Culture preparation	0.0308	1	0.0308	15.07	0.0007	4.26
Interaction	0.0164	2	0.0082	4.02	0.0312	3.40
Within	0.0491	24	0.0020			
Total	0.3800	29				

(B) Tukey's multiple comparison tests

Argopecten irradians

k =	7	q(alpha) =	4.541			
v =	28	Sy =	0.016079			
a =	0.05	w =	0.073013			
L-1000	W-1000	L-100	W-10	Control	L-10	W-100

Mercenaria mercenaria

k =	7	q(alpha) =	4.464			
v =	28	Sy =	0.682228			
a =	0.05	w =	3.045466			
L-1000	L-10	Control	L-100	W-1000	W-100	W-10

Crassostrea virginica

k =	7	q(alpha) =	4.541			
v =	28	Sy =	0.0205			
a =	0.05	w =	0.0929			
L-1000	L-100	W-1000	W-100	Control	L-10	W-10

Underlined treatments are not significantly different ($p > 0.05$).

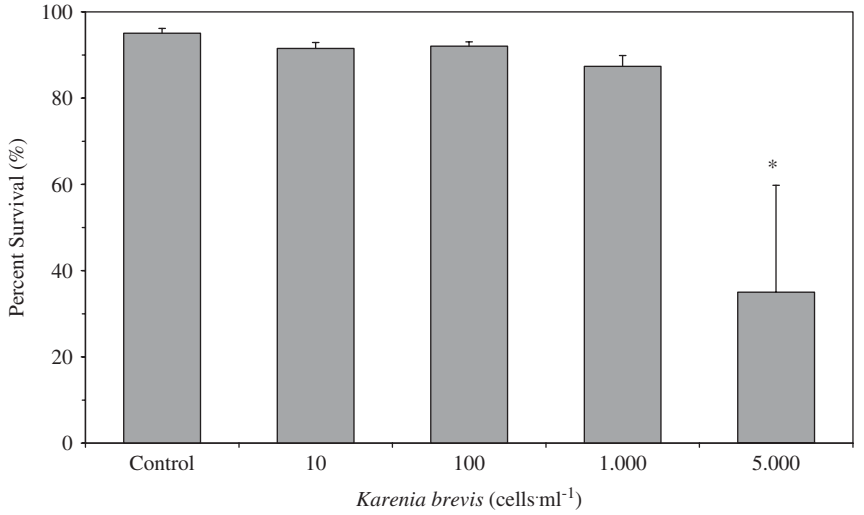

Fig. 1. Percent survival (Mean ± SD) of *Argopecten irradians* larvae after exposure to *Karenia brevis* for 7 days. Treatment with an asterisk was significantly different ($p < 0.05$). Larvae were seven days old at start of experiment.

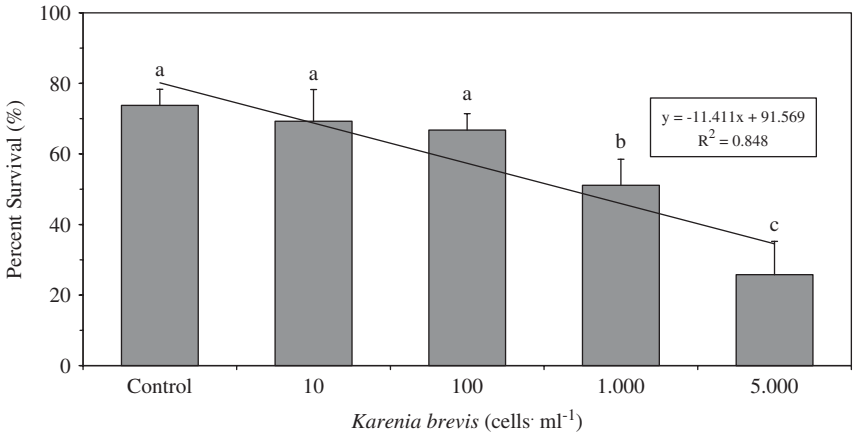

Fig. 2. Percent survival (mean ± SD) of *Mercenaria mercenaria* larvae after exposure to *Karenia brevis* for 7 days. Treatment with the same letter were not significantly different ($p < 0.05$). Survival was significantly affected by *K. brevis* cell concentration ($p < 0.01$; $R^2 = 0.85$). Larvae were seven days old at start of experiment.

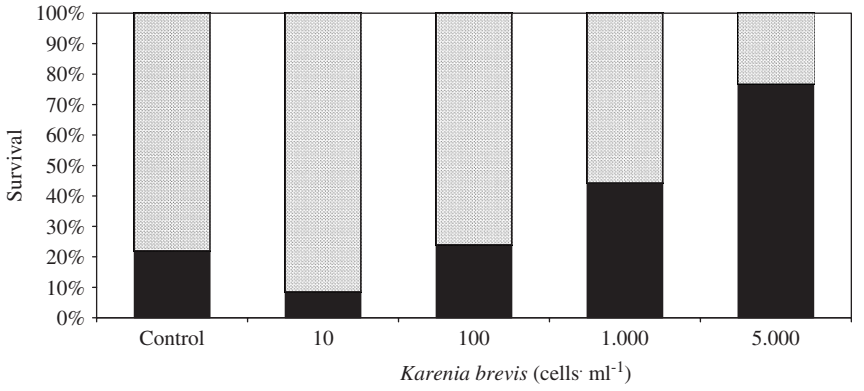

Fig. 3. Percent of surviving *Mercenaria mercenaria* larvae as umboveligers (■) and pediveligers (⬚) after exposure to *Karenia brevis* for 7 days. Larvae were 7-day-old umboveligers at beginning of experiment.

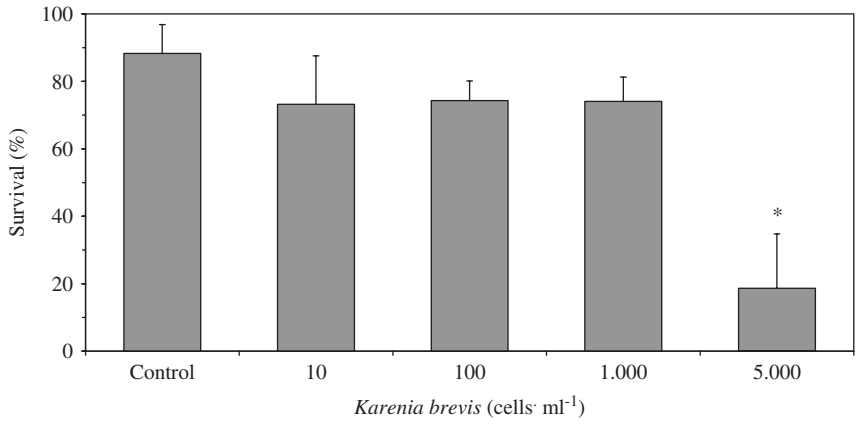

Fig. 4. Percent survival (mean ± SD) of *Crassostrea virginica* larvae after exposure to *Karenia brevis* for 7 days. Treatment with an asterisk was significantly different ($p < 0.05$). Larvae were 7 days old at start of experiment.

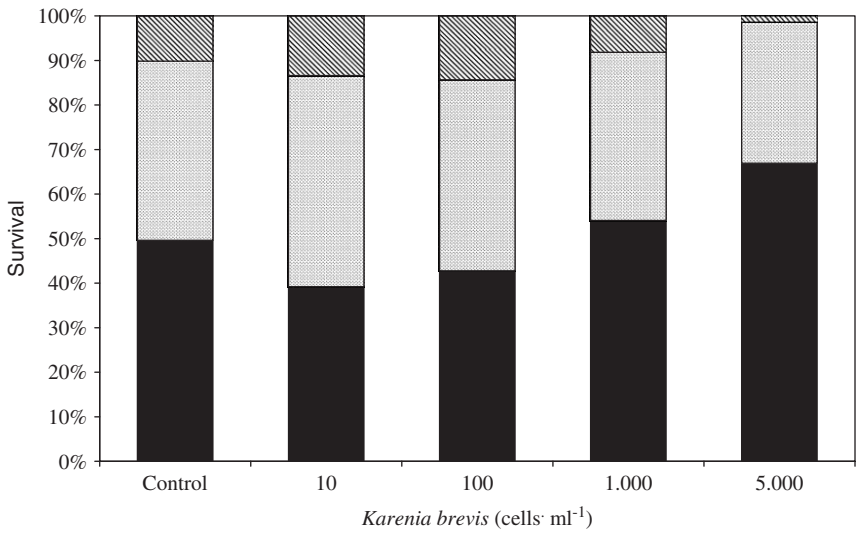

Fig. 5. Percent of surviving *Crassostrea virginica* larvae as umboveligers (■), pediveligers (⬚) and spat (▨) after exposure to *Karenia brevis* for seven days. Larvae were 7-day-old umboveligers at beginning of experiment.

including algal species, cell size and concentration, and larval species and age. Consumption of *K. brevis* cells may also explain the observed inhibitory effects on larval survival. Larvae of the mussel, *Mytilus galloprovincialis*, readily ingested cells of several species of toxic dinoflagellates with mean equivalent spherical diameters of 12–38 μm (Jeong et al., 2004). Eastern oyster (*C. virginica*) larvae ingested *P. minimum* cells, although algal filtration was depressed in the presence of this toxic algae (Wikfors and Smolowitz, 1995), and ingestion of this toxic alga resulted in cytological changes in digestive tissues, including the deleterious development of cuboidal and squamous epithelial cells in the stomach and intestine, reductions in the size of absorptive cells, and the presence of dense inclusions in the cytoplasm, indicating possible phagolytic reactions to dinoflagellate debris (Wikfors and Smolowitz, 1995). Early D-shape larvae of two scallop species (*A. irradians concentricus* and *Chlamys farreri*) were unable to feed on *Alexandrium tamarense* cells due to its relatively large size (Yan et al., 2001; Yan et al., 2003). During the current study, larvae were fed an optimal ration (Lu and

Blake, 1996) of the chrysophyte, *I. galbana*, a common alga used in bivalve culture, in addition to *K. brevis*. Although larval feeding rates were not measured nor *K. brevis* consumption investigated, ingestion of *K. brevis* cells was most likely negligible due to the relatively large cell size (ESD = 14–26 μm) and low density compared to *I. galbana*. This critical distinction regarding the capability of bivalve larvae to filter and consume *K. brevis* cells necessitates further study. In either case, the presence of *K. brevis*, especially at higher concentrations, could interfere with bivalve larvae by altering activity patterns (Yan et al., 2003) and/or feeding rates (Jeong et al., 2004), resulting in increased mortality and retarded metamorphosis (Matsuyama et al., 2001).

Exposure of seven-day-old larvae to *K. brevis* had an effect on survival, development and metamorphosis. Even though overall survival was identical in *C. virginica* larvae exposed to 100 and 1000 cells ml^{-1}, a higher proportion from 100 cells ml^{-1} had (a) reached the pediveliger stage and (b) completed larval development (i.e., settled as spat) than larvae from 1000 cells ml^{-1}. Almost 90% of larvae subjected to 5000 cells ml^{-1} did not live beyond the umboveliger stage. Larval development of *M. mercenaria* was also affected by the presence of *K. brevis* cells. In this case, progress to the pediveliger stage was inversely related to *K. brevis* concentration. Similarly, larvae of the Pacific oyster, *C. gigas*, which did not show significant mortality when exposed to *Cochlodinium polykrikoides*, did suffer retarded metamorphosis to the D-shaped larvae (Matsuyama et al., 2001). Development of *C. virginica* larvae was also delayed when exposed to a laboratory clone of the dinoflagelatte, *P. minimum* (Wikfors and Smolowitz, 1995). While the mechanism for increased mortality of bivalve larvae remains unanswered, it is easy to see how the added stress associated with *K. brevis* and/or its toxins could be reflected in suboptimum development.

Sixty percent of brevetoxins in laboratory cultures of *K. brevis* are extracellular in nature (Pierce et al., 2001). Ultrasonic disruption, which releases the remaining intracellular toxins, resulted in a 20–24% increase in total brevetoxin in the current study. Three brevetoxin compounds were present in each culture: Pbtx-2, Pbtx-3, and brevenal, a recently idenfitied brevetoxin antagonist (Bourdelais et al., 2004). The proportion of each brevetoxin remained unchanged after the cultures were lysed. Except for the absence of Pbtx-1, the relative brevetoxin composition of laboratory cultures closely resembled that from water samples collected during a red tide outbreak along the Gulf Coast of Sarasota, FL in 2003 (Pierce et al., 2005).

Larvae of all three bivalve species in this study responded similarly, but with different sensitivities, to cells of *K. brevis* and its suite of toxins. Mortality was not necessarily dependent on ingestion of algal cells; rather it appears that the toxins were at least partially responsible for increased mortality and delayed larval development. The presence of *K. brevis* cells at high densities may interfere with larval feeding processes, resulting in suboptimal clearance, inhibited growth and development, and mortality.

Brevetoxins may persist in coastal waters for more than a month after the dissipation of a *K. brevis* bloom (Flewelling et al., 2005). Our results clearly indicate that when these toxins persist, shellfish larvae are at greater risk of mortality and may continue to be adversely affected even after the disappearance of *K. brevis* cells. While *K. brevis* blooms may not directly cause mortality in adult shellfish, they do have the ability to disrupt a critical phase in the life cycle and consequently have important ramifications for recruitment and population stability. The failure of bay scallops to successfully recruit in North Carolina, USA, was attributed to a bloom of *Ptychodiscus brevis* (= *K. brevis*), which interfered with either adult spawning, larval survival and settlement, or survival of newly settled spat (Summerson and Peterson, 1990). Since we demonstrated negative impacts of *K. brevis* on larvae of northern quahogs (= hard clams) and eastern oysters, we might expect blooms of *K. brevis* to negatively impact recruitment in these species as well. Thus, there is a clear need for continued research on the relationship between *K. brevis* and bivalve larvae, ranging from the mechanisms of toxicity to the effects on recruitment and population stability.

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