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Comparative effects of the toxic dinoflagellate *Karenia brevis* on clearance rates in juveniles of four bivalve molluscs from Florida, USA

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Abstract

The effects of *Karenia brevis* (Gymnodiniales, Gymnodiniaceae) on the feeding activity of juveniles of four species of bivalve mollusc were examined in the laboratory to assess the potential impacts on these important shellfish populations from Florida. Clearance rates were determined under short-term (one hour) static and long-term (two days) flow-through conditions using both whole and lysed cultures of *K. brevis*. Under short-term conditions, the bay scallop, *Argopecten irradians*, was the most sensitive species, exhibiting a 79% reduction in clearance rate at 1000 cells ml^{-1} of whole *K. brevis* culture compared to the control (no *K. brevis*). The eastern oyster, *Crassostrea virginica*, was the least responsive, showing a 38% reduction in clearance rate between the same treatments. The green mussel, *Perna viridis*, and the northern quahog, *Mercenaria mercenaria*, displayed intermediate responses. Similar results were also observed during long-term exposures to a continuous supply of *K. brevis*. Bay scallops showed a significant decline in clearance rate at 100 cells ml^{-1} after 24 h exposure; clearance rate of oysters was not affected by *K. brevis* at this concentration. No mortality was observed for any species during these brief exposures. The prospect for recovery of bay scallop populations in Florida estuaries where they were once abundant may be hampered by recurring blooms of *K. brevis*. Reduced clearance rates in *M. mercenaria* at high *K. brevis* densities could translate into poor growth of cultured Florida hard clams. On the other hand, *P. viridis*, which also showed reduced clearance rates at high *K. brevis* concentrations, might be negatively impacted by *K. brevis* blooms, thereby affecting their ability to spread into estuaries hampered by recurring toxic algal blooms.

Keywords: Bivalves; Clearance rate; Toxic dinoflagellate; *Karenia brevis*; Florida

1. Introduction

The impact of diets which include toxic dinoflagellates on feeding in bivalve molluscs has received increased attention in the past 20 years (Shumway and Cucci, 1987; Gainey and Shumway, 1988; Bricelj and Shumway, 1998; Landsberg,

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2002). The recurring conclusion is that bivalve responses are species-specific (Shumway and Cucci, 1987; Gainey and Shumway, 1988; Shumway, 1990; Lesser and Shumway, 1993; Smolowitz and Shumway, 1997), and depend upon a variety of factors, including the algal species encountered (Shumway and Cucci, 1987; Gainey and Shumway, 1988; Shumway, 1990; Lesser and Shumway, 1993), algal toxicity (Bricelj et al., 1991, 1996; Bardouil et al., 1993; Lassus et al., 1996; Li and Wang, 2001), algal concentration (Li et al., 2002), cell size and selectivity (Shumway et al., 1985, 1990; Lesser and Shumway, 1993; Matsuyama et al., 1997), history of exposure (Shumway and Cucci, 1987; Chebib et al., 1993; Bricelj et al., 1996), season (Lesser and Shumway, 1993) and differences in digestive function (Wikfors and Smolowitz, 1993).

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 a suite of 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). The principal toxins in *K. brevis* are PbTx-1 (the most potent), PbTx-2 (the most abundant), and brevenal, an antagonist that suppresses the effects of brevetoxins (Landsberg, 2002; Bourdelais et al., 2004).

Blooms of *K. brevis* pose a serious threat to shellfish resources and the growing bivalve aquaculture industry in Florida. These blooms may be especially harmful to bay scallops (*Argopecten irradians*) (Summerson and Peterson, 1990), and could jeopardize efforts to restore Florida's dwindling bay scallop populations (Geiger and Arnold, 2003; Leverone et al., 2005) and the potential for a successful aquaculture program (Blake et al., 2000). The burgeoning hard clam (= quahog) aquaculture industry in Florida (Adams and Sturmer, 2004) has many lease sites in Pine Island Sound (Lee County), an estuary with a history of repeated red tide outbreaks (Tester and Steidinger, 1997). The non-indigenous green mussel, *Perna viridis*, became established in Tampa Bay in 1999 (Ingrao et al., 2001), and has since spread south along the Florida gulf coast (Benson et al., 2001), the same geographic area where blooms of *K. brevis* are most frequent (Tester and Steidinger, 1997). Lastly, restoration and creation of oyster habitats (*Crassostrea virginica*) is receiving increased attention within this same region (Savarese et al., 2004). The effects of *K. brevis* on oyster populations in Florida have not yet been examined.

The purpose of this study was to determine the effects of the toxic dinoflagellate, *K. brevis*, on the clearance rate of juveniles of four species of common bivalve molluscs from Florida: the bay scallop (*A. irradians*), northern quahog (= hard clam, *Mercenaria mercenaria*), eastern oyster (*C. virginica*) and green mussel (*P. viridis*). Separate static (1 h) and flow-through (two day) experiments were conducted for each species to investigate short-term and long-term exposure effects, respectively. We also examined the effects of whole culture (intact cells) and lysed culture (disrupted cells) of *K. brevis* on clearance rate to distinguish between the effects of the dinoflagellate and its toxins.

2. Materials and methods

2.1. Maintenance of algal cultures

Batch cultures of *K. brevis* (= *Gymnodinium breve*) (Wilson clone) were grown in NH15 media without aeration. Cultures of *Isochrysis galbana* (Tahitian clone) were grown in f/2 media plus Trimsa minus silica with aeration. Seawater was collected locally, filtered through cartridge filters to remove particles $>0.2\,\mu m$, passed through an activated charcoal filter, sterilized with ultraviolet light and autoclaved. Cultures were maintained at a temperature of 24–26 °C, a salinity of 33–35 ppt and an irradiance of 60–80 μmol photons m^{-2} s PAR. Cultures of *K. brevis* were maintained on a 12:12 hour light: dark cycle, while *I. galbana* cultures were exposed to constant illumination. All experiments used cultures in stationary growth phase, generally achieved two weeks after inoculation.

2.2. Algal cell and toxin concentration and preparation of lysed cultures

Algal cell concentration was determined by a Coulter® Multisizer IIE fitted with a 100 μm orifice. Brevetoxin concentration and composition were determined by high performance liquid chromotography (HPLC) interfaced with a mass spectrometer detector (Pierce et al., 2005).

Each experiment was carried out using both whole and lysed culture preparations of *K. brevis*. Lysed preparations were produced by exposing the algal culture to ultrasonic disruption at 750 W for

4 min using a Sonics® Vibracell with 5 mm microtip probe. A small subsample (<1 ml) was observed microscopically to verify that the cells had been disintegrated. The amount (ml) of *K. brevis* added to each treatment was determined by the cell concentration of whole culture. The same volume of lysed culture was added to respective treatments. Triplicate 500 ml samples of whole and lysed culture were analyzed for brevetoxins.

2.3. Collection and maintenance of bivalves

Four species of juvenile bivalve molluscs were used in these experiments: the bay scallop (*A. irradians*), eastern oyster (*C. virginica*), northern quahog (= hard clams) (*M. mercenaria*) and green mussel (*P. viridis*). Eastern oysters and green mussels were collected from the Gandy Bridge support pilings (Old Tampa Bay, FL) which have been free of *K. brevis* blooms for decades (Tester and Steidinger, 1997). Bay scallops were obtained from the University of South Florida (St. Petersburg, FL) shellfish hatchery and northern quahogs from the Bay Shellfish Company hatchery (Palmetto, FL). We believe all bivalves had no previous exposure to *K. brevis*. All epibionts were cleaned off after shellfish were transferred to the laboratory. Animals were maintained in aerated aquaria at 25 °C and fed *I. galbana* (Tahitian strain) continuously (20,000 cells ml^{-1}) prior to experimentation. All bivalves were starved for 24 h prior to the initiation of each experiment.

2.4. Feeding rates of bivalves

Separate static (short-term) and flow-through (long-term) experiments were conducted for each species to calculate feeding responses under different scenarios of exposure to *K. brevis*.

Static experiments were initiated by placing individuals in separate beakers containing 500 ml filtered seawater and allowing each bivalve to acclimate for 1 h. Each beaker was lightly aerated. Treatments consisted of three concentrations and two culture preparations of *K. brevis* and a control (no *K. brevis* added). Cell densities were 10, 100 and 1000 cells ml^{-1} and culture preparations included lysed and whole culture. The alga, *I. galbana*, was added to each beaker at an optimal concentration of 2×10^3 cells ml^{-1} (Lu and Blake, 1996) to encourage feeding. Reductions in both *I. galbana* and *K. brevis* cell concentrations were measured. Clearance rate was calculated from the decline in *I. galbana* after 1 h.

At the end of each experiment, dry weight (mg) was determined by removing the soft tissues from the shells and drying at 70 °C for 24 h, and weight-specific clearance and filtration rates were calculated (Coughlan, 1969). A two-way analysis of variance (ANOVA) with equal replication was used to test for significant differences in weight-specific clearance rate among cell concentrations and culture treatments. Multiple comparison analyses were performed using Tukey's ω procedure (Zar, 1996). A three-way ANOVA on pooled data was performed to determine significant differences in clearance rate among the four bivalve species.

Continuous-flow experiments were conducted using the test system of Singer et al. (1990). The system pumps test solutions through 18 separate enclosed exposure vessels (290 ml volume). Treatment solutions were prepared in 20-L glass carboys, allowed to mix by gentle stirring and introduced into each chamber at a flow rate of 2 ml min^{-1}. Experimental animals sat on a porous (70 μm) shelf in the middle of the chamber which allowed for the passage of unfiltered algal cells but not feces or pseudofeces.

Experiments consisted of two treatments ($n = 6$), a control ($n = 5$) and a blank. Treatment concentrations were 100 and 1000 cells ml^{-1} *K. brevis*. Each experiment lasted two days and clearance rates were calculated twice daily, once at 9 A.M and again at 5 P.M. Separate experiments using lysed and whole *K. brevis* culture had to be conducted for each species due to the limited number of exposure vessels in the system ($n = 18$).

Tissue dry weight (mg) was determined at the termination of the experiment. Weight-specific clearance rate was calculated according to Widdows and Salkeld (1993) for a flow-through system. Since different *K. brevis* cultures were used in subsequent flow-through experiments, separate repeated measures ANOVA's were employed to test for differences among species, concentration, and duration of exposure for experiments using whole and lysed cultures of *K. brevis*, respectively.

3. Results

Cell concentrations of *K. brevis* cultures ranged from $1.8–2.2 \times 10^4$ cells ml^{-1} for static experiments and from $2.0–2.5 \times 10^4$ cells ml^{-1} for flow through experiments (Table 1). Static experiments (run

Table 1
Experimental conditions, bivalve species, sample matrix, cell and brevetoxin concentrations of laboratory cultures of *K. brevis* (Wilson Clone) used for juvenile feeding experiments

Experiment	*K. brevis* culture		Brevetoxin amount ($\mu g\,L^{-1}$)				Total
	Matrix	(cells ml^{-1})	PbTx-1	PbTx-2	PbTx-3	Brevenal	
Static							
Bay scallops	Whole	22,000	n.d.	32.9	1.0	33.9	67.8
(*Argopecten irradians*)	Lysed		n.d.	12.2	4.1	24.9	41.2
Green mussel	Whole	21,650	1.9	17.6	3.6	—	23.1
(*Perna viridis*)	Lysed		1.9	20.0	6.5	—	28.4
Northern quahog	Whole	22,000	0.7	17.4	22.2	—	40.3
(*Mercenaria mercenaria*)	Lysed		5.9	36.2	18.4	—	60.5
Eastern oyster	Whole	21,300	5.9	36.6	21.1	—	63.5
(*Crassostrea virginica*)	Lysed		7.6	52.7	20.1	—	80.3
Flow-through							
Bay scallops	Whole	19,600	n.d.	30.4	4.7	31.9	67.0
(*Argopecten irradians*)	Lysed	21,800	n.d.	32.9	1.0	33.9	67.8
Green mussel	Whole	21,400	n.d.	10.7	9.2	9.7	29.7
(*Perna viridis*)	Lysed	23,800	n.d.	34.4	5.5	13.9	53.8
Northern quahog	Whole	21,500	n.d.	32.9	1.0	33.9	67.8
(*Mercenaria mercenaria*)	Lysed	23,100	n.d.	43.2	12.1	19.8	75.1
Eastern oyster	Whole	24,600	n.d.	24.8	5.3	31.8	61.9
(*Crassostrea virginica*)	Lysed	23,300	n.d.	36.2	5.9	18.4	60.5

n.d.= not detected.

simultaneously) used the same culture for each species, while flow through experiments (run consecutively) required separate cultures. Total brevetoxin concentrations ranged from 41.2–67.8 $\mu g\,L^{-1}$ for static experiments and 49.7–75.1 $\mu g\,L^{-1}$ for flow through experiments. PbTx-2 and PbTx-3 were the most abundant brevetoxins in *K. brevis* cultures used in all experiments. PbTx-1, which was detected only in static experiments, was present in concentrations $<8\,\mu g\,L^{-1}$. Brevenal, a putative inhibitor of brevetoxin action, was not identified prior to flow through experiments; however, it is possible, even likely, that it was present, yet undetected, in *K. brevis* cultures used in static experiments. Total brevetoxin was typically higher after a culture was lysed.

Table 2 summarizes the decline in *I. galbana* for each bivalve species exposed to different concentrations and preparations of *K. brevis* under static conditions. Table 3 summarizes filtration and clearance rates for each species. The results on clearance rates are also presented graphically in Fig. 1. No pseudofeces production was observed in any treatment. Results for each species are discussed separately.

Bay scallop (*A. irradians*): Mean dry weights for juvenile bay scallops ranged from 16.9–19.5 mg dry wt. Clearance rate was highest in the control (11.19 $ml\,h^{-1}\,mg\,dry\,wt^{-1}$) and lowest in the Whole-1000 treatment (2.33 $ml\,h^{-1}\,mg\,dry\,wt^{-1}$) (Fig. 1A). This equals a 79% reduction in clearance rate between the two treatments. There was a significant difference in clearance rate among treatments (ANOVA; $p<0.001$). A two-factor ANOVA showed a concentration effect ($p<0.001$), a treatment effect ($p<0.001$), and an interaction effect ($p<0.001$). Bay scallops filtered 3% of *K. brevis* over 1 h at 1000 cells ml^{-1} (Table 2).

Green mussel (*P. viridis*): Mean dry weights for juvenile green mussels ranged from 40.3–46.5 mg dry wt. Mean clearance rate was highest in the control (16.39 $ml\,h^{-1}\,mg\,dry\,wt^{-1}$) and lowest in the Whole-1000 treatment (4.37 $ml\,h^{-1}\,mg\,dry\,wt^{-1}$) (Fig. 1B), a 73% reduction in clearance rate between the two treatments. There was a significant difference in clearance rate among treatments (ANOVA; $p<0.001$). A two-factor ANOVA showed a concentration effect ($p<0.001$), a treatment effect ($p<0.001$), and an interaction effect ($p<0.001$). Green mussels filtered 32% of *K. brevis* over 1 h at 1000 cells ml^{-1} (Table 2).

Northern quahog (*M. mercenaria*): Mean dry weights for juvenile northern quahogs ranged from 13.8–16.3 mg dry wt. Clearance rate was highest in

Table 2
Decline in *Isochrysis galbana* cell counts (cells ml^{-1}) for juvenile bivalve molluscs exposed to different concentrations and preparations of *Karenia brevis*

Treatment	Mean dry wt (mg) (S.D.)	Cell concentration ($t = 0$ h) (cells ml^{-1})		Cell concentration ($t = 1$ h) (cells ml^{-1})		Reduction in cell concentration mg dry wt^{-1} h^{-1} (cells ml^{-1})
		T. iso	*K. brevis*	*T. iso*	*K. brevis*	
Perna viridis						
Control	43.5 (3.87)	20,736	—	5005	—	362
Whole-10	44.9 (4.38)	21,547	27	5589	14	355
Lysed-10	43.3 (3.49)	20,928	—	5388	—	359
Whole-100	43.4 (6.18)	21,524	117	10,023	111	265
Lysed-100	44.4 (1.90)	21,285	—	6704	—	328
Whole-1000	48.5 (7.21)	22,298	1432	14,891	770	153
Lysed-1000	40.3 (3.16)	20,944	—	7092	—	344
Crassostrea virginica						
Control	43.94 (5.88)	19,210	—	2647	—	176
Whole-10	40.55 (8.99)	18,986	9	3304	8	173
Lysed-10	57.13 (15.84)	18,800	—	3555	—	142
Whole-100	47.08 (15.43)	19,251	108	5207	63	145
Lysed-100	50.85 (6.54)	19,864	—	5289	—	145
Whole-1000	48.27 (8.14)	20,447	1035	12,957	760	74
Lysed-1000	55.68 (14.75)	20,493	—	8453	—	122
Mercenaria mercenaria						
Control	14.9 (1.43)	22,988	—	15,727	—	487
Whole-10	16.3 (2.41)	23,600	13	16,601	13	429
Lysed-10	16.0 (1.81)	24,098	—	17,610	—	406
Whole-100	15.3 (1.01)	23,207	99	17,467	55	375
Lysed-100	13.8 (0.98)	24,154	—	17,094	—	512
Whole-1000	16.1 (1.43)	22,820	979	19,897	544	182
Lysed-1000	15.2 (0.79)	23,787	—	17,370	—	422
Argopecten irradians						
Control	19.5 (0.92)	25,758	—	16,671	—	466
Whole-10	18.8 (1.34)	25,574	32	17,355	22	437
Lysed-10	16.9 (1.95)	25,334	—	17,751	—	449
Whole-100	18.2(1.75)	25,343	142	22,181	115	174
Lysed-100	18.4 (1.50)	25,728	—	21,520	—	229
Whole-1000	17.7 (1.28)	25,261	1568	23,260	1466	113
Lysed-1000	18.4 (1.02)	25,651	—	21,414	—	230

Starting seawater volume in each replicate was 500 ml.

the control (12.91 ml h^{-1} mg dry wt^{-1}) and lowest in Whole-1000 (4.28 ml h^{-1} mg dry wt^{-1}), or a 73% reduction in clearance rate (Fig. 1C). There was a significant difference in clearance rate among treatments (ANOVA; $p<0.001$). A two-factor ANOVA showed a concentration effect ($p<0.001$), a treatment effect ($p<0.001$), and an interaction effect ($p<0.001$). Northern quahogs filtered 9% of *K. brevis* over 1 h at 1000 cells ml^{-1} (Table 2).

Eastern oyster (*C. virginica*): Mean dry weights for juvenile oysters ranged from 40.6–50.6 mg dry wt. Clearance rate was highest in the control (13.57 ml h^{-1} mg dry wt^{-1}) and lowest in the Whole-1000 treatment (8.42 ml h^{-1} mg dry wt^{-1}) (Fig. 1D). This equals a 38% reduction in clearance rate between the two treatments. There was a significant difference in clearance rate among treatments (ANOVA; $p<0.001$). A two-factor ANOVA showed a concentration effect ($p<0.001$) but no treatment effect ($p = 0.73$). Oysters filtered 54% of *K. brevis* over 1 h at 1000 cells ml^{-1} (Table 2).

Differences in mean clearance rate among the four bivalve species are summarized in Fig. 2A for whole cultures and Fig. 2B for lysed cultures. Significant differences were found among species, *K. brevis* concentration and culture ($p<0.001$).

Table 3
Filtration rate and clearance rates of juvenile bivalve molluscs exposed to whole and lysed culture of *Karenia brevis*

Treatment	Dry tissue (mg) Mean (S.D.)	Filtration rate (cells h^{-1})	Clearance rate (ml h^{-1})	Weight-specific clearance rate (ml h^{-1} mg dry wt^{-1})
Perna viridis				
Control	43.5 (3.87)	15,731	714	16.39
Whole-10	44.9 (4.38)	15,958	679	15.13
Lysed-10	43.3 (3.49)	15,540	682	15.80
Whole-100	43.4 (6.18)	11,501	385	9.01
Lysed-100	44.4 (1.90)	14,581	580	13.06
Whole-1000	48.5 (7.21)	7407	205	4.37
Lysed-1000	40.3 (3.16)	13,852	545	13.60
Crassostrea virginica				
Control	43.94 (5.88)	16,563	613	13.44
Whole-10	40.55 (8.99)	15,683	530	12.56
Lysed-10	57.13 (15.84)	15,244	497	11.72
Whole-100	47.08 (15.43)	14,045	755	8.79
Lysed-100	50.85 (6.54)	14,574	612	8.77
Whole-1000	48.27 (8.14)	7489	245	2.86
Lysed-1000	55.68 (14.75)	12,040	548	6.19
Mercenaria mercenaria				
Control	14.9 (1.43)	7261	191	12.91
Whole-10	16.3 (2.41)	6999	176	10.99
Lysed-10	16.0 (1.81)	6488	157	9.93
Whole-100	15.3 (1.01)	5739	142	9.31
Lysed-100	13.8 (0.98)	7060	173	12.54
Whole-1000	16.1 (1.43)	2923	69	4.28
Lysed-1000	15.2 (0.79)	6417	157	10.37
Argopecten irradians				
Control	19.5 (0.92)	9087	218	11.19
Whole-10	18.8 (1.34)	8219	194	10.40
Lysed-10	16.9 (1.95)	7583	178	10.54
Whole-100	18.2 (1.75)	3161	67	3.69
Lysed-100	18.4 (1.50)	4207	89	4.89
Whole-1000	17.7 (1.28)	2001	41	2.33
Lysed-1000	18.4 (1.02)	4236	90	4.93

Starting seawater volume in each replicate was 500 ml.

There were also significant interaction differences ($p<0.001$) among all factors (Multifactor ANOVA; univariate test of significance for clearance rate).

Fig. 3 summarizes clearance rates for all species under long-term continuous flow-through exposure to whole (top) and lysed (bottom) cultures of *K. brevis*.

Bay scallop (*A. irradians*): Mean clearance rate of juvenile *A. irradians* was significantly reduced ($p<0.05$) at *K. brevis* concentrations of 100 cells ml^{-1} and higher in both whole (Fig. 3A) and lysed (Fig. 3B) experiments. The bay scallop was the only bivalve species to show a concentration effect of lysed *K. brevis* culture on clearance rate. This effect was delayed until day two, when there was a significant decrease in clearance rate at 100 cells ml^{-1} and higher.

Green mussel (*P. viridis*): Mean clearance rate of *P. viridis* exposed to whole *K. brevis* culture (Fig. 3B) was significantly lower ($p<0.05$) at 1000 cells ml^{-1}. There was no significant difference ($p>0.05$) in clearance rate with lysed *K. brevis* over time, although rates increased slightly during the two-day exposure.

Northern quahog (*M. mercenaria*): Mean clearance rate of *M. mercenaria* exposed to whole culture was significantly lower ($p<0.05$) at 1000 cells ml^{-1} (Fig. 3C). There was no significant difference ($p>0.05$) in clearance rate when *M. mercenaria* was exposed to lysed (Fig. 3C) *K. brevis*.

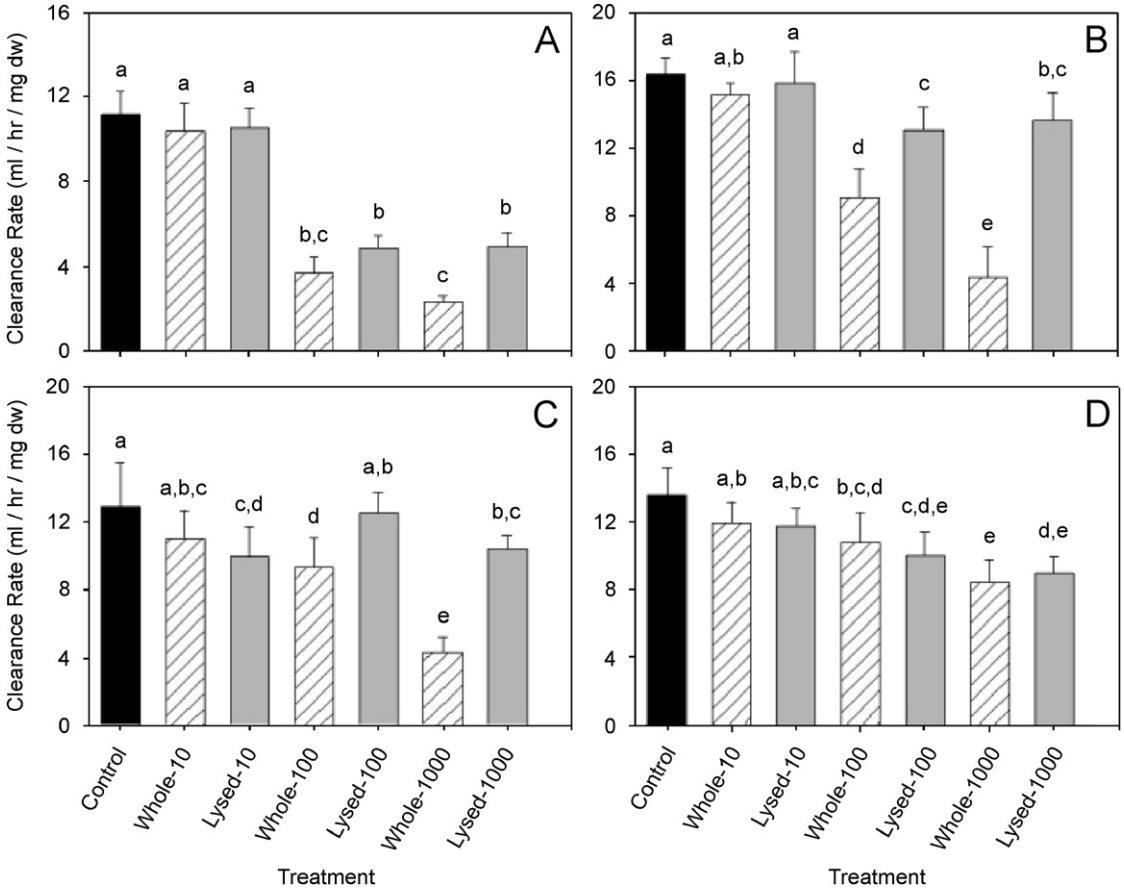

Fig. 1. Mean (±S.D.) clearance rate (ml h^{-1} mg dry wt^{-1}) for juvenile bivalves of (A) *Argopecten irradians*, (B) *Perna viridis*, (C) *Mercenaria mercenaria*, and (D) *Crassostrea virginica*. Treatments consisted of three concentrations and two preparations of *Karenia brevis*. Treatments with the same letter are not significantly different ($p > 0.05$) $n = 10$. Two-way ANOVA; Tukey's Multiple Comparison Test.

Eastern oyster (*C. virginica*). There was no significant difference ($p > 0.05$) in clearance rate of juvenile *C. virginica* exposed to different concentrations of lysed (Fig. 3D) or whole (Fig. 3D) *K. brevis* over time.

Repeated measures ANOVA showed significant differences among species ($p < 0.001$) and concentrations ($p < 0.01$) for bivalves exposed to lysed culture of *K. brevis* under continuous flow-through conditions. There was no effect of exposure time. For experiments with whole culture of *K. brevis*, there were significant differences among species ($p < 0.001$), concentrations ($p < 0.001$) and exposure times ($p < 0.01$).

4. Discussion

The species-specific response of bivalve molluscs to the presence of toxic or noxious algae in their diet (Shumway and Cucci, 1987; Shumway, 1990) is supported in the current laboratory study. Each of the four species responded differently when exposed to *K. brevis* at different concentrations and culture preparations. Furthermore, we found that each species responded similarly under two very different exposure regimes: short-term (1 h) static exposure to a nonreplenished supply of *K. brevis* and long-term (2 day) flow-through exposure to a continuous supply of *K. brevis*.

In the present study, the bay scallop (*A. irradians*) was the most sensitive to the presence of *K. brevis* in terms of clearance rate. This was the only species that showed a significant reduction in clearance rate when fed *K. brevis* at a concentration of 100 cells ml^{-1}, independent of culture preparation. The response was immediate when exposed to intact cells, but took 24 h to be manifested with lysed cells. Poor growth, histopathologies and mortality of

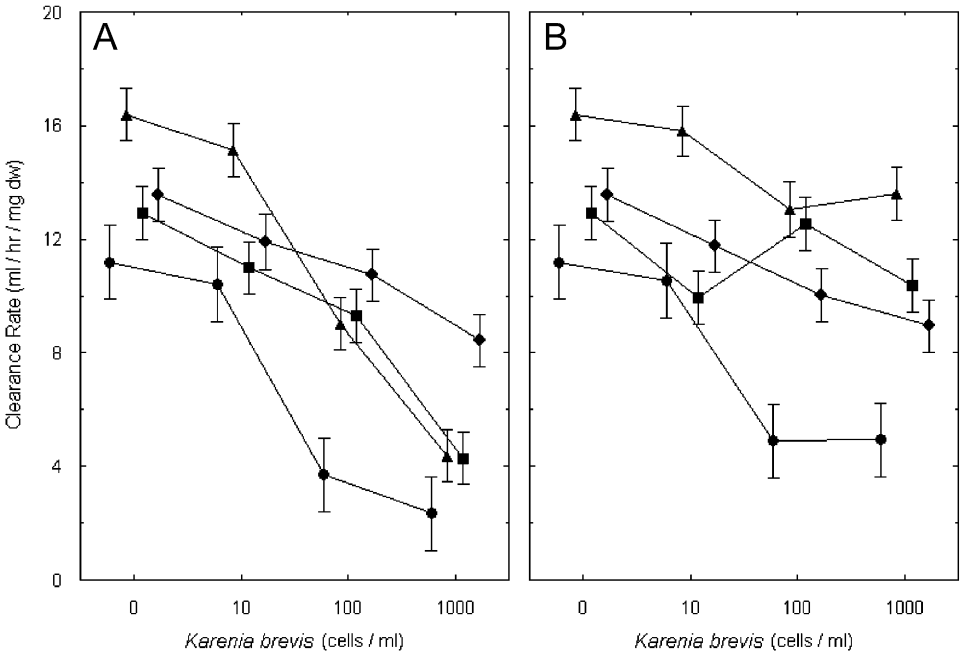

Fig. 2. Mean (±S.D.) clearance rate (ml h^{-1} mg dry wt^{-1}) for juvenile bivalves exposed to (A) whole and (B) lysed *K. brevis* culture under static conditions. (●) = *Argopecten irradians*; (▲) = *Perna viridis*; (■) = *Mercenaria mercenaria*; and (♦) = *Crassostrea virginica*. ($n = 10$).

A. irradians exposed to other toxic dinoflagellates suggest a systemic toxic effect (Wikfors and Smolowitz, 1993; Smolowitz and Shumway, 1997; Lesser and Shumway, 1993). The delayed feeding response to lysed *K. brevis* in our study was not related to any observed behavioral changes (e.g., shell valve closure Shumway and Cucci, 1987), but likely indicates an unknown cytotoxic or neurotoxic effect.

Green mussels (*P. viridis*) and northern quahogs (*M. mercenaria*) were intermediate in their feeding responses when exposed to *K. brevis*. Both species showed significantly reduced clearance rates at 1000 cells ml^{-1} whole culture while neither species was affected by lysed culture. In fact, the clearance rate of *P. viridis* increased gradually during the two-day exposure to lysed culture, regardless of concentration. Clearance rate in juvenile *P. viridis* was also unaffected by another toxic dinoflagellate, *Alexandrium tamarense* (Li et al., 2002); however, the congener, *P. canaliculus*, was able to clear, ingest and absorb laboratory cultures (EPA-JR strain) of *K. brevis* (Ishida et al., 2004). The effects of toxic algae on feeding activity in the northern quahog (*M. mercenaria*) are more species-specific. While *M. mercenaria* can ingest and survive exposure to potentially toxic strains of *Prorocentrum* (Wikfors and Smolowitz, 1993), ingestion of *Alexandrium fundyense* was low and could only be induced by the addition of a nontoxic diatom (Bricelj et al., 1990). Additionally, feeding rates of *M. mercenaria* fed *A. tamarense* and *Gyrodinium aureolum* were low compared to rates when fed *I. galbana,* and exposure to *G. aureolum* resulted in significant mortalities (Lesser and Shumway, 1993).

Eastern oysters (*C. virginica*) were the least responsive bivalve when exposed to *K. brevis* with respect to clearance rate, although there was a significant concentration effect in the static experiment. Of the four species of bivalve tested, oysters removed the highest percentage of *K. brevis* cells from the surrounding media. Sievers (1969) showed that Eastern oysters maintained normal shell valve activity at high densities of *K. brevis* in the laboratory. During red tides in the Gulf of Mexico, oysters became toxic (Cummins et al., 1971), easily accumulating (Dickey et al., 1999) and metabolizing (Poli et al., 2000) brevetoxins. Our results support the view that eastern oysters are relatively unharmed by exposure to bloom concentrations of *K. brevis* (Shumway et al., 1990).

Overall, whole cultures of *K. brevis* (intact cells) had a greater effect than lysed cultures (disrupted cells) on clearance rate in all species except

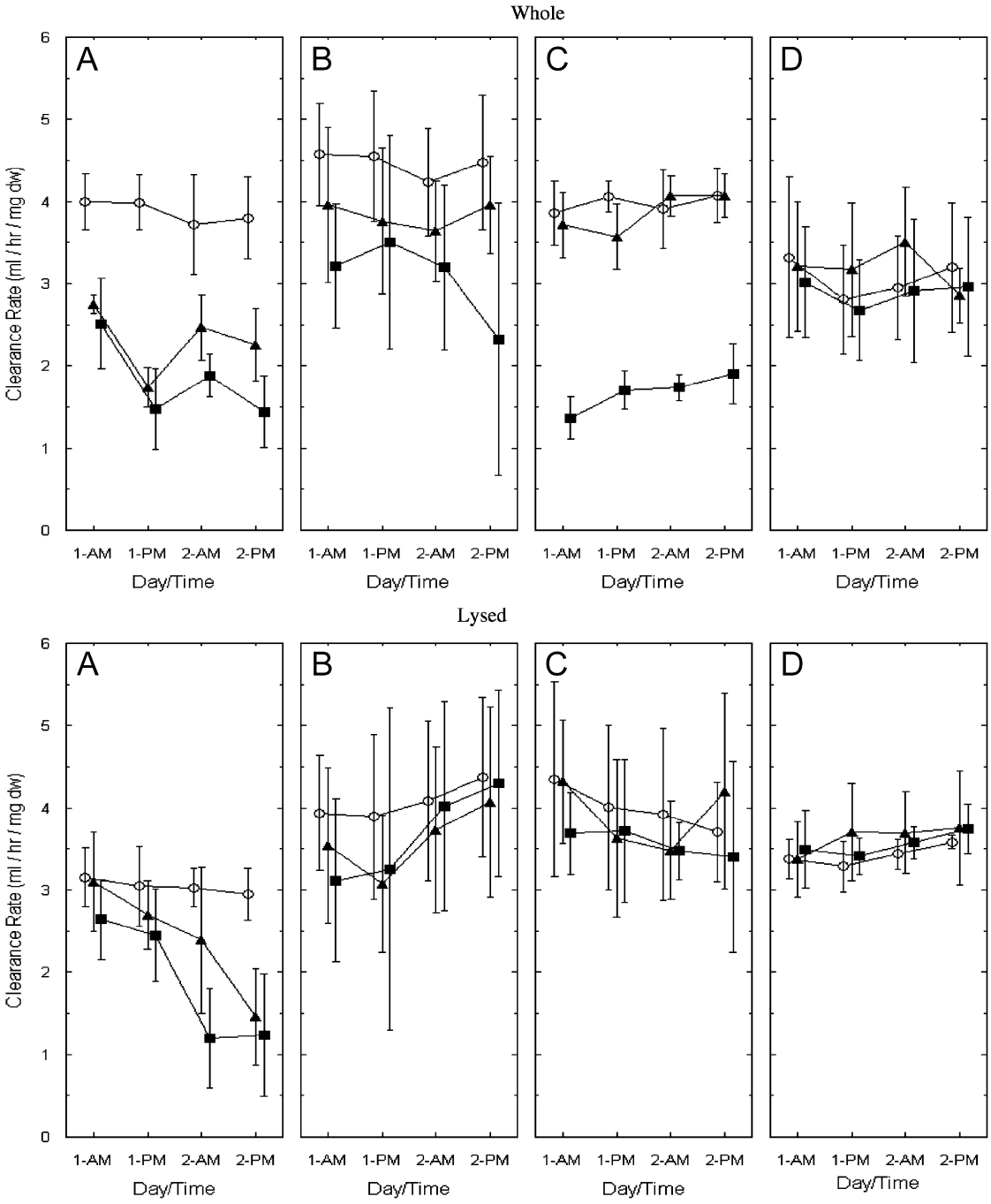

Fig. 3. Mean (±S.D.) clearance rate (ml h^{-1} mg dry wt^{-1}) of juvenile bivalves exposed to whole (top) and lysed (bottom) cultures of *K. brevis* under flow-through conditions. Species include (A) *Argopecten irradians*, (B) *Perna viridis*, (C) *Mercenaria mercenaria*, and (D) *Crassostrea virginica. K. brevis* exposure concentrations: Control (○), 100 (▲) and 1000 (■) cells ml^{-1}. ($n = 6$). Clearance rates were measured twice a day (9 A.M. and 5 P.M.) and calculated from inflow and outflow concentrations of *Isochrysis galbana*.

C. virginica, even though the amount of total brevetoxin was similar between the two preparations, suggesting that encounters with the dinoflagellate interfered with filtering capability. The New Zealand cockle (*Austrovenus stutchbury*) and the greenshell mussel (*P. canaliculus*) were shown to assimilate brevetoxins from *K. brevis* culture as well as from the supernatant from disrupted culture

(Ishida et al., 2004), but the effects of these preparations on feeding was not investigated. Additional studies using recently isolated strains of *K. brevis*, including a nontoxic Wilson clone and two new isolates from Sarasota Bay (Florida, USA), could further elucidate these differences in bivalve feeding behavior.

There was close within-species agreement in clearance rates between static and flow-through systems; however, the effects of *K. brevis* on *A. irradians* was shown to be significantly affected by exposure time, whereby clearance rates at both medium ($100\,cells\,ml^{-1}$) and high ($1000\,cells\,ml^{-1}$) densities declined only after 24 h exposure. For this reason, continuous flow-through systems are generally preferred over static systems when measuring physiological performance. With static systems, conditions are not held constant and therefore clearance rates may be affected if algal concentrations fall below a critical level (Widdows and Salkeld, 1993). Conditions in flow-through systems can be held constant (i.e., algal concentration), thus enabling continuous monitoring of clearance rate over extended time periods which more closely reflect environmental conditions during algal blooms. Additionally, flow-through systems allow for the monitoring of possible behavioral or physiological changes associated with long term exposure to toxic algae (Lassus et al., 1999). Bardouil et al. (1996) suggested that longer exposure times are necessary to assess the effects of toxic algae on algal ingestion and toxin absorption in bivalve shellfish.

Recurring blooms (= red tides) of *K. brevis* are common along the Florida west coast (Tester and Steidinger, 1997; Kirkpatrick et al., 2004). Our results showed that the effects of laboratory cultures of *K. brevis* on clearance rates of juveniles of four important bivalves were species-specific, suggesting that the ecological and fisheries impacts from these algal blooms could be quite different depending upon bivalve species, bloom concentration and duration. The most sensitive species in the present study was the bay scallop, *A. irradians*. A rare bloom of *K. brevis* in North Carolina during 1987–1988 was implicated in the massive mortality and subsequent recruitment failure of local bay scallop populations (Summerson and Peterson, 1990). Recently, bay scallops have been the focus of restoration activities in several southwest Florida estuaries (Geiger and Arnold, 2003; Wilbur et al., 2005; Leverone et al., 2005). In 2001, a restoration project was irrevocably compromised when a dense (10^5–$10^7\,cells\,L^{-1}$) bloom of *K. brevis* infiltrated Sarasota Bay, FL, resulting in complete mortality of captive scallops (Leverone, unpublished). While more precise studies are necessary to resolve the relationship between red tide intensity and duration on bay scallop mortality, prediction and monitoring of algal blooms would be beneficial in identifying potential restorations sites that are less prone to chronic *K. brevis* blooms. Florida's hard clam (*M. mercenaria*) aquaculture industry would also benefit from improved red tide prediction and monitoring. Relocating lease sites to areas less susceptible to red tides would benefit the industry twofold: (1) reduce the deleterious effects of high *K. brevis* concentrations on feeding rates which, in turn, would affect growth rates; and (2) reduce the probability that cultured clams will be prevented from reaching the market due to harvest closures (Shumway, 1990). Similarly, reduced feeding rates in the green mussel (*P. viridis*) at high *K. brevis* concentrations may make it more difficult for populations to become established in estuaries where red tides are more frequent and/or severe. Finally, the relative insensitivity of *C. virginica* feeding rates to *K. brevis* suggests that the structure and function of Eastern oyster habitats in southwest Florida should not suffer serious negative impacts from *K. brevis* blooms.

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