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Comparative effects of the toxic dinoflagellate, Karenia brevis, on bivalve molluscs from Florida

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Comparative Effects of the Toxic Dinoflagellate, *Karenia brevis*, on Bivalve Molluscs from Florida

by

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Dedication

This dissertation is wholeheartedly dedicated to my gracious wife, Barbara. Throughout my graduate studies, she has steadfastly supported me through all my trials and tribulations. Whenever an experimental procedure went awry, especially in the beginning, she was always there to put things in perspective, reminding me that through apparent failures, progress and learning took root. And at times when I was not making even the slightest progress or was not paying proper attention to my work, she would gently push me to keep going. Even at times when I had doubts, she never wavered. She had complete trust and confidence in me at times when I questioned myself. Furthermore, she always maintained a convivial home and family life which provided a welcome respite from my studies. I will be forever grateful to her for her support and hope she may share in the joy of my accomplishment.

May this work be an inspiration to any student contemplating a return to graduate school later in life. May you have a partner who will encourage and support you as my wife did me.

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Abstract

The effects of the toxic dinoflagellate, *Karenia brevis* (Wilson clone), on larval survival and development of the northern quahog (= hard clam, *Mercenaria mercenaria*), eastern oyster (*Crassostrea virginica*) and bay scallop (*Argopecten irradians*) were studied in the laboratory. The effects of *K. brevis* on feeding activities of juveniles from these species plus the green mussel (*Perna viridis*) were also examined. Finally, adult bay scallops were exposed to *K. brevis* for two weeks to investigate possible cytotoxic effects.

Survival of 3-day-old larvae was generally > 85% for all shellfish species at *Karenia brevis* densities of 100 cells · ml^{-1} or less, and not significantly different between whole and lysed culture. At 1,000 cells · ml^{-1}, survival was significantly less in lysed culture than whole culture for both *M. mercenaria* and *C. virginica*. Survival of 7-day-old larvae in all species was not significantly affected at densities up to 1,000 cells · ml^{-1}. At 5,000 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 1,000 cells · ml^{-1}.

Clearance rates of juveniles were determined under static and flow-through

conditions using whole and lysed cultures of *K. brevis*. The bay scallop was most sensitive, exhibiting a 79% reduction in clearance rate at 1,000 cells · ml^{-1} of whole culture. The eastern oyster was least responsive, showing a 38% reduction in clearance rate between the same treatments. The green mussel and the northern quahog displayed intermediate responses. Similar results were observed during longer (2 day) exposures to a continuous supply of *K. brevis*. Bay scallops showed a significant decline in clearance rate at 100 cells · ml^{-1} after 24 hr 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.

Adult bay scallops exposed to *K. brevis* for two weeks showed degenerative and inflammatory changes in the digestive gland, including reduced thickness of the epithelium, increased size of digestive tubule lumens and hemocytic infiltration. The prospect for recovery of bay scallop populations in Florida may be hampered by recurring blooms of *K. brevis*.

Chapter One

Introduction

The dinoflagellate, *Karenia brevis* (formally *Gymnodinium breve*, Davis) (Daugbjerg *et al*., 2001) is responsible for one of the oldest reported harmful algal blooms in North America (Ingersoll, 1882), yet shellfish poisonings in the Gulf of Mexico from this algal species were considered rare and infrequent as late as forty years ago. At that time, it was not known whether shellfish could actually feed upon *K. brevis* or accumulate the toxins in their tissues. In 1967, laboratory experiments in Texas (Ray and Aldrich, 1967) and field studies from Sarasota, FL (Cummins *et al*., 1971) both demonstrated that eastern oysters (*Crassostrea virginica*) could consume *K. brevis* and become toxic. Partly as a result of these findings, the state of Florida began monitoring shellfish for toxicity from outbreaks of harmful algae in the 1970's.

Today, advances are being made in our knowledge and comprehension of the human health impacts from exposure to Florida red tides, which occur either through the consumption of contaminated shellfish or by inhalation of toxin-laden aerosols (Pierce *et al*., 1990; Pierce *et al*., 2005). We now know that *K. brevis* produces at least twelve and possibly fourteen potent neurotoxins (=brevetoxins) that are lethal to fish and cause neurotoxic shellfish poisoning (NSP) in humans from the consumption of contaminated shellfish (Baden, 1988; Steidinger *et al*., 1998; Bourdelais *et al*., 2004). Through these human health related studies, we are learning more about how shellfish accumulate, metabolize and eliminate brevetoxins. Of particular interest is the discovery that eastern

oysters may remain toxic for several months after dissipation of a bloom (Dickey *et al*., 1999; Wang, 2004). The literature on the human health effects of Florida red tides has been recently reviewed by Kirkpatrick *et al*. (2004).

Not surprisingly, progress in understanding how blooms of *K. brevis* affect the health of individual bivalve species lag considerably behind the human health ramifications of these same blooms; today, however, these red tides have led to greater concerns about the long-term effects these blooms are having on local fisheries, including critical species of bivalve molluscan shellfish (Landsberg, 1996). For instance, we currently do not know whether *K. brevis* affects critical early life stages, growth and development of juveniles, or reproductive development and fecundity of adults for any species of bivalve mollusc from Florida. Information on possible cytotoxic effects of *K. brevis* on bivalves is also critically lacking. Does exposure to *K. brevis* affect feeding and behavior in bivalves, or render them more susceptible to predation? What are the effects on population dynamics, particularly larval dispersal and recruitment? We still do not even know if *K. brevis* causes mortality in any species of bivalve, particularly the bay scallop, *Argopecten irradians*. Any deleterious effect of exposure to blooms of *K. brevis* would potentially threaten Florida's valuable shellfish resources and negatively impact the state's growing bivalve aquaculture industry (Blake *et al*., 2000; Adams and Sturmer, 2004).

The objectives of this research were to investigate the effects of the toxic dinoflagellate, *Karenia brevis*, on four important species of bivalve mollusc from Florida. The bivalve species selected for study were the bay scallop (*Argopecten irradians*), northern quahog (= hard clam, *Mercenaria mercenaria*), eastern oyster (*Crassostrea*

virginica) and green mussel (*Perna viridis*). This research was divided into the following separate investigations of the specific effects of *K brevis* on bivalves: 1) survival and development in larvae, 2) feeding rates in juveniles, and 3) histopathology in adult bay scallops from long-term, sublethal exposure. All studies were conducted under controlled laboratory conditions using a specific culture (Wilson clone) of *K. brevis*. In each instance, experiments were designed to distinguish between effects caused by the toxic dinoflagellate and the effects of its associated toxins (=brevetoxins).

Chapter Two

Literature Review

The initial motivation for research on toxigenic algae was the potential human health impacts associated with these blooms (Hemmert, 1975; Bicknell and Walsh, 1975; Price *et al*., 1991; Todd, 1993; Fremy *et al*., 1999; Fernandez, 2000; Garthwaite, 2000). Human health problems generally result from the consumption of bivalve molluscan shellfish rendered toxic by filtering and ingesting harmful microalgae (Shumway, 1995; Bricelj and Shumway, 1998) or by the inhalation of aerosolized brevetoxins incorporated in marine aerosol by bubble-mediated (Pierce *et al*., 1990; Pierce *et al*, 2005). Not very long ago, bivalves were thought to accumulate toxins in their tissues without any apparent negative consequences (Prakash *et al*., 1971; Quayle, 1969). That impression, however, has since been abandoned as researchers have taken a more thorough look at the chronic, sublethal effects of harmful algae on bivalves and how these interactions affect shellfish populations, mariculture activities and coastal ecosystems (Shumway *et al*., 1985; Shumway and Cucci, 1987; Shumway and Cembella, 1993; Bricelj and Shumway, 1998; see reviews by Shumway, 1990 and Landsberg, 2002).

Bivalves accumulate microalgal toxins in their tissues through filter-feeding. The way in which they respond to the presence of toxic algae depends upon the species of bivalve as well as the algal species encountered (Shumway and Cucci, 1987; Smolowitz

and Shumway, 1997; Gainey and Shumway, 1988; Bricelj *et al*., 1991; Lesser and Shumway, 1993). In turn, behavioral and physiological differences among bivalves in feeding response also depend upon a number of factors. These factors include, but are not limited to, history of exposure (Shumway and Cucci, 1987; Bricelj *et al*., 2000), season (Lesser and Shumway, 1993), algal toxicity (Bricelj *et al*., 1996) toxin content (Bricelj *et al*., 1991; Li and Wang, 2001), algal cell concentration (Bricelj *et al*., 2004), cell selectivity (Shumway *et al*., 1985; Shumway *et al*., 1990), cell size (Lesser and Shumway, 1993), and differences in digestive function (Wikfors and Smolowitz, 1993).

Bivalve Mortality Associated with Harmful Algal Species

Global accounts of the lethal, sublethal and chronic effects of harmful algal blooms on shellfish and other molluscs may be found in Shumway and Cucci (1987) and also in two comprehensive reviews (Shumway, 1990; Landsberg, 2002). Reports of massive shellfish mortality linked to harmful dinoflagellate blooms must be interpreted with caution since there can be other unfavorable events or conditions associated with algal blooms. Most often, prolonged periods of low dissolved oxygen (=hypoxia) or even the absence of oxygen (=anoxia) in bottom waters will accompany or follow an algal bloom as cells lyse or fish decompose. These conditions generate a high biochemical oxygen demand, which may also be a causative factor in shellfish mortalities associated with toxic algal blooms.

In a report on red water organisms (= dinoflagellates) from the Pacific Northwest, Nightengale (1936) listed some of the earliest records of harmful algal blooms and

shellfish that were "destroyed" (see Table). In 1929, Nightengale (1936) personally

Table of historical harmful algal events and affected bivalves reported in Nightengale (1936)

Year	**Locality**	**Harmful Alga**	**Shellfish Affected**
1891	Pt. Jackson, Australia	*Glenodinium rubrums*	Oysters and mussels
1893	Gokasho Bay, Japan	*Gymnodinium* (?)	Pearl oysters
1902	Santa Barbara to San Diego, CA	*Gonyaulax* species	Fish and bottom fauna
1907	San Pedro to San Diego ,CA	*Gonyaulax polyedra*	Pearl oysters, fish and shellfish
1910	Gokasho Bay, Japan	*Gymnodinium* (?)	Pearl oysters

observed losses of oysters in Oakland Bay, Washington during a bloom of *Gymnodinium splendens*. Although the cause of mortality (toxins or oxygen depletion) in all of these instances was not established, decomposition of organic debris (and concomitant depletion of oxygen?) was suspected as the primary cause. The only reported bivalve mortalities associated with *Alexandrium catenella* were white mussels (*Donax serra*) and black mussels (*Chloromytilus meridionalis*) off the southern coast of South Africa (Horstman, 1981). Koray (1992) reported unidentified shellfish mortalities due to *Alexandrium minutum* in Izmir Bay, Turkey. Wardle *et al*. (1975) observed dead surfclams (*Spisula solidissima*) and eastern oysters (*Crassostrea virginica*) among an assemblage of invertebrate and fish fatalities associated with a bloom of *Gonyaulax monilata* off Galveston, Texas from 1971-72. In this instance, affected species were either sessile, sedentary or weakly motile, suggesting the more motile species were able to avoid the bloom area before accumulating lethal amounts of toxin. Unfortunately, dissolved oxygen was not monitored during these mass mortality events. Species of *Alexandrium*, reportedly toxic to a host of pectinid (= scallop) species (see Table 1 in

Shumway and Cembella, 1993), have not been linked to scallop mortalities in nature.

Mass mortalities of bivalves are occasionally associated with blooms of nontoxic algal species. Mortality in these instances is more often than not due to the subsequent decline in dissolved oxygen that accompanies these blooms. A bloom of the dinoflagellate, *Ceratium tripos*, in New York Bight during the summer of 1976 was followed by mass mortalities of surfclams (*Spisula solidissima*), ocean quahog (*Arctica islandica*), sea scallops (*Placopecten magellanicus*), American lobster (*Homarus americanus*) and fish (Mahoney and Steimle, 1979). Mortalities from this event were attributed to extensive oxygen depletion resulting from degradation of the algal bloom, and not a toxic response to the algal bloom. Several species of *Gonyaulax* have been implicated in shellfish mortalities worldwide even when other environmental factors, particularly low dissolved oxygen, were at least partially at play during these events. In South Africa, both *Gonyaulax grindleyi* (=*Protoceratium reticulatum*) and *G. polygramma* blooms resulted in massive quantities of dead invertebrates and fish, including a variety of mussels and abalone (Grindley and Nel, 1968; Grindley and Taylor, 1964). Separate *G. polygramma* blooms were associated with mussel mortalities in Venezuela (Ferraz-Reyes *et al*., 1979; La Barbera-Sanchez *et al*., 1993), Japan (Koizumi *et al*., 1996; Schwimmer and Schwimmer, 1968) and Hong Kong (Lam and Yip, 1990). Forbes (1990) reported shellfish mortality in connection with a bloom of *G. spinifera* in 1990. In nearly all of these events, mortality was associated with low dissolved oxygen levels; thus, the cause of death could not be directly attributed to the dinoflagellate. Furthermore, during a PSP event in Venezuela during 1988, HPLC analysis of *G. polygramma* samples did not reveal any toxins, supporting the idea that

two other species, namely *A. tamarensis* and *G. catenatum*, which were present during that PSP event, were the toxigenic organisms (La Barbera-Sanchez *et al*., 1993).

Species of *Gymnodinium*, on the other hand, have been implicated in shellfish mortalities, especially the queen scallop (*Pecten maximus*) in European waters. High mortalities of larvae, post-larvae and juveniles (Lassus and Berthome, 1988; Erard-LeDenn *et al*., 1990) and inhibited growth and reproduction in adults (Erard-LeDenn *et al*., 1990) have been documented from France and Ireland in association with *Gymnodinium* cf. *aureolum* blooms. Abbott and Ballantine (1957) detailed queen scallop mortality in the presence of *G. veneficum* in the laboratory. The softshell clam, *Mya arenaria*, suffered 30-40% mortality as a result of a *Gyrodinium aureolum* (=*Gymnodinium aureolum*) bloom in Maquoit Bay, Brunswick, Maine in 1988 (Heinig and Campbell, 1992). The blue mussel, *Mytilus edulis*, was also affected. Again, mortality was attributed to both low dissolved oxygen and toxin production by *G. aureolum*. Mortality of (unidentified) shellfish was observed in association with a bloom of *Gymnodinium* sp. in southern Brazil during April, 1978. Shellfish collected during this episode were found to contain saxitoxins (Machado, 1979).

In 1987, North Carolina experienced its first recorded red tide outbreak, caused by the dinoflagellate, *Gymnodinium breve* (=*Karenia brevis*) (Tester *et al*., 1991). Strong circumstantial evidence suggested that the red tide caused mortality of both adult and newly recruited bay scallops (Summerson and Peterson, 1990), leading to recruitment failure in subsequent years in the state's most productive scallop beds (Peterson and Summerson, 1992). No other information on the effects of *K. brevis* on bay scallops has been published.

Mass mortality of marine animals due to *Karenia brevis* red tides was first reported from the west coast of Florida in 1946-47 by Gunter *et al*. (1947). The total number of dead fish from the entire area was estimated to be over 50,000,000. Accounts of dead invertebrates were limited to the statements: "Oysters, clams, crabs, shrimp, barnacles, and coquinas were also killed. The clam industry at Marco … [did] not appear to be involved". During a red tide outbreak near Sarasota, Florida in 1978, Tiffany and Heyl (1978) reported numerous surf clams (*Spisula solidissima similis*) and coquina (*Donax variabilis*) washed up on local beaches. Peak *Gymnodinium breve* (= *Karenia brevis*) cell counts during the outbreak reached 5×10^6 cells $\cdot L^{-1}$. Sufficient dissolved oxygen was present in offshore bottom waters during the red tide suggesting that mortality was due to the toxic dinoflagellate. Simon and Dauer (1972) observed the near complete destruction of the benthic infaunal community in Old Tampa Bay during a severe (up to 17.7×10^6 cells $\cdot L^{-1}$) *K. brevis* bloom in 1971. Oxygen depletion accompanying the massive fish kill could not be ruled out as a causative factor. Interestingly, the bivalve *Mulinia lateralis* was one of the least impacted species in the assemblage, but this may be due to the ability of this species to withstand short periods of anoxia (Shumway et al., 1983). The authors concluded that laboratory studies were needed to confirm the field observations. Under laboratory conditions, Sievers (1969) showed that *Crassostrea virginica* exhibited no mortality when exposed to *K. brevis* concentrations up to 9.9×10^6 cells $\cdot L^{-1}$ for 48 hours. Additional laboratory studies are necessary to delineate the direct effects of *K. brevis* on mortality in Florida shellfish populations.

Behavioral Responses of Bivalves to Harmful Algal Species

Measures of behavioral responses of organisms can provide insight into how they cope with potentially harmful conditions; in this case, exposure to harmful or toxic algae. Behavior patterns can provide a useful index of *in vivo* sensitivity to these algae and their toxins (Bricelj *et al*., 1996). Many species of bivalve mollusc exhibit a variety of responses to the presence of toxic dinoflagellates, including changes in shell valve activity, siphon retraction, byssal thread production, burrowing activity, oxygen consumption, and heart rates (Shumway and Cucci, 1987).

Shell valve activity in the Pacific oyster (*Crassostrea gigas*) was inhibited when oysters were fed the toxic dinoflagellate *Alexandrium minutum* for 8 to 15 days (Lassus *et al*., 1999), but there was an immediate and significant increase in shell valve activity when the *A. minutum* diet was followed by a nontoxic food source. This same oyster species, however, displayed vigorous clapping of shell valves when exposed to *A. catenella* (Dupay and Sparks, 1968), although two other oyster species (*Crassostrea virginica* and *Ostrea edulis*) remained open and continued to filter in the presence of *Alexandrium tamarensis* (Shumway and Cucci, 1987), each species exhibited unique behaviors in the presence of this toxic dinoflagellate. In *O. edulis*, there was an initial, partial adduction of the shell valves followed by periodic 'snaps' which continued until clean seawater was introduced. Eastern oysters (*C. virginica*) responded by initially closing their shell valves, followed by a gradual reopening. This pattern repeated itself, although complete closure never occurred. Eastern oysters rarely opened and did not filter when exposed to *Gonyaulax monilata* (=*Alexandrium monilatum*), but opened

frequently in the presence of *Karenia brevis* (Ray and Aldrich, 1967).

It was first suggested by Twarog and Yamaguchi (1975), and given further credence by Shumway and Cucci (1987), that a number of molluscan species from locales that periodically experience toxic algal blooms have evolved mechanisms that allow them to exploit the toxic algae as a food source. Mussels (*Mytilus edulis*) from Maine, which have a history of exposure to toxic algal blooms, showed no evidence of shell-valve closure when exposed to *Protogonyaulax tamarensis* (= *Alexandrium tamarense*) in the laboratory, while the majority of mussels from Rhode Island and Spain, which are not subjected to toxic algal blooms in their native locales, showed initial erratic shell-valve activity followed by complete shell closure (Shumway and Cucci, 1987). In addition, exhalent siphons from many of the mussels from both Rhode Island and Spain were closed, while the mantle edges in otherwise 'open' animals were retracted. Mussels from Rhode Island and Spain also produced copious amounts of a white, mucus-like material in the presence of *A. tamerense*. Many eventually died.

Populations of bivalves from the west coast of Florida (northern quahogs and eastern oysters, in particular) have become established in locations which are undoubtedly exposed to a higher frequency of recurring blooms of *K. brevis* than other populations which rarely, if ever, come in contact with these blooms. An understanding of a species' exposure history to harmful algal episodes must be taken into account when attempting to explain the interactions between a toxic alga and a particular bivalve species.

The sea scallop (*Placopecten magellanicus*) showed dramatic activity patterns when exposed to *Alexandrium tamarense* (Shumway and Cucci, 1987). Most individuals

exhibited an immediate closure of the shell valves followed by either violent swimming activity, partial, sustained shell-valve closure, or a combination of the two. Swimming / clapping activity lasted less than one hour, possibly a result of fatigue. Sea scallops showed a similar response when subjected to starfish extracts, in addition to an accelerated heart rate, increased stroke volume and an enhanced cardiac output (Thompson *et al*., 1980). Sea scallops also produced copious amounts of a white mucus-like substance when exposed to bloom conditions of *A. tamerense* (Shumway and Cucci, 1987). Activity patterns of bay scallops (*Argopecten irradians*) in the presence of toxic dinoflagellates have not been investigated.

The northern quahog (= hard clam) *Mercenaria mercenaria*, showed an initial retraction of the siphons followed by complete closure of the shell valves in the presence of *Alexandrium tamarense* (Shumway and Cucci, 1987). The animals did not re-open until they were returned to clean sea water.

Feeding Responses of Bivalves Exposed to Harmful Algal Species

Harmful algae have significant impacts on the feeding activity of bivalve molluscs (Shumway and Cucci, 1987; Bricelj *et al*., 1996; Lassus *et al*., 1996; Lassus *et al*., 1999; Li and Wang, 2001). The exact response depends on the species of bivalve as well as the algal species encountered (Shumway and Cucci, 1987; Gainey and Shumway, 1988; Bricelj *et al*., 1996; Lesser and Shumway, 1993; Bardouil *et al*., 1993; Bardouil *et al*., 1996; Smolowitz and Shumway, 1997; Lassus *et al*., 1999). Changes in feeding activity can provide an indication of the sensitivity of bivalves to toxins and thus

potential for toxin uptake of various species (Bricelj *et al*., 1996). Feeding rate measurements may also be useful in looking at the response within a species to variation in dinoflagellate cell toxicity (Bricelj and Shumway, 1998).

The simplest and most common measure of feeding activity in bivalve molluscs is the clearance rate, which is a measure of the volume of water "cleared" of particles per unit time. Filtration rate is usually defined as the number of particles removed from suspension per unit time. The clearance rate is a measure of an individual's ability to remove particles from suspension. When no pseudofeces are produced (no captured particles are rejected), the clearance rate equals the ingestion rate.

Oysters. Early studies on oysters led to erroneous conclusions that they were able to avoid the accumulation of toxic algae and were thus considered to be less prone to becoming toxic than other bivalves (see Shumway *et al.*, 1990 for review). Today, however, there is much more convincing evidence demonstrating that feeding rates in oysters are, indeed, affected by toxic dinoflagellates. These feeding responses, particularly clearance rates, may be quite variable and sometimes contrary (Bardouil *et al*., 1993; Shumway and Cucci, 1987). The European oyster, *Ostrea edulis*, exhibited significantly higher clearance rates when fed a mixed nontoxic algal diet plus *Alexandrium tamarense*, when compared to an identical diet without the toxic dinoflagellate (Shumway and Cucci, 1987). The opposite was true for *Crassostrea virginica* when subjected to identical experimental conditions; the diet containing *A. tamarense* resulted in lower clearance rates (Shumway and Cucci, 1987).

Bardouil *et al*. (1993) offered unialgal cultures of toxic and nontoxic microalgae to *Crassostrea gigas* under conditions similar in design to Shumway and Cucci (1987).

The same batch of adult *C. gigas* was exposed successively to non-toxic and toxic *Alexandrium tamarense*, and a different group was exposed to nontoxic *Scrippsiella trochoidea* and toxic *A. minutum*. Mean algal concentrations were based on field values observed during red tide phenomena. Mean clearance rates showed significant differences between toxic and nontoxic *A. tamarense* as well as between *S. trochoidea* and *A. minutum*. Clearance rates of juvenile *C. virginica* fed separately on unialgal cultures of two toxic dinoflagellates (*A. tamarense* and *Gyrodinium* (= *Gymnodinium*) *aureolum*) were significantly different from one another and significantly lower than the clearance rates on the nontoxic alga *Isochrysis* sp. (Lesser and Shumway, 1993). In the same experiment, post-hoc multiple comparison tests showed clearance rates of *Ostrea edulis* which were fed the same two toxic dinoflagellates grouped together, and were also significantly lower than clearance rates of *Isochrysis* sp.

Bardouil *et al*. (1996) continued their studies on the feeding habits of *Crassostrea gigas* by investigating the effects of two strains (toxic and nontoxic) of the dinoflagellate *Alexandrium tamarense* in combination with the diatom *Thalassiosira weissflogii*. When compared to a unialgal diet of *T. weissflogii*, a diatom/toxic dinoflagellate ratio as low in biomass as 90/10 reduced clearance rates in *C. gigas* by twenty percent. Clearance rates were slightly, but significantly, lower for a 50/50 diatom/toxic dinoflagellate mixture, while a unialgal toxic dinoflagellate diet resulted in complete inhibition of filtration, ingestion and absorption (Bardouil *et al*., 1996).

Following the work of Bardouil *et al*. (1996), Lassus *et al*. (1999) evaluated the feeding behavior of *Crassostrea gigas* on toxic algal diets over a longer time period (up to 30 days) to ascertain whether physiological behavior returned to normal during

detoxification. Oysters fed *Alexandrium minutum* for 8 to 15 days exhibited significant reductions in clearance rate, filtration rate and biodeposition rate as compared to a nontoxic dinoflagellate, *Scrippsiella trochoidea*. When the *A. minutum* diet was followed by a diet of either flagellates or diatoms, clearance and filtration rates did not return to their pre-exposure levels (Lassus *et al*., 1999).

The probability of Eastern oysters becoming exposed to *Karenia brevis* (=*Gymnodinium breve*) is less than other shellfish because oyster beds are typically located in the more oligohaline, upper reaches of an estuary. In the Gulf of Mexico, optimum growth and reproduction occurs in oyster reefs with a salinity of 12 to 30 ppt, but oyster abundance is greatest at salinities between 10 and 20 ppt (Butler, 1954). The salinity preference of *K. brevis* is > 24 ppt (Tester and Fowler, 1990), which effectively provides a salinity barrier against *K. brevis* for many oysters. Ray and Aldrich (1965) first noted that the optimum salinity for oysters is lower than that for *K. brevis*; however, this does not mean that oysters are never exposed to *K. brevis* nor are they always free of brevetoxins (Shumway *et al*., 1990). Cummins and Hill (1969) found that oysters accumulated 89 – 95 % of *K. brevis* in 2-4 hours with an original concentration of 2.4 x 10^6 cells $\cdot$ L^{-1}. Eastern oysters not only survived, but exhibited normal behavior during a 48 hour exposure to *K. brevis* at cell concentration of 9.9 x 10^6 cells $\cdot$ L^{-1} (Sievers, 1969). Brevetoxin metabolites have now been isolated and identified in *C. virginica* from shellfish harvesting areas after a bloom of *K. brevis* in the Gulf of Mexico in 1996 (Dickey *et al*., 1999) and in Sarasota Bay in 2001 (Pierce *et al*., 2004). No data have been published on the effects of *K. brevis* on clearance rates in eastern oysters.

Mussels. Following a bloom of *Gyrodinium (=Gymnodinium) aureolum* off

Plymouth, England in 1978, Widdows *et al*. (1979) examined the effects of this dinoflagellate on the physiological and cytological responses of the mussel *Mytilus edulis* in the laboratory. They showed that the dinoflagellate either produced or contained a substance which was cytotoxic to *M*. *edulis*. It had an acute effect on the clearance rate and caused marked cellular damage to the gut; however, the mussels were capable of rapid recovery when *G. aureolum* cell concentrations declined.

Shumway and Cucci (1987) examined the effects of the toxic dinoflagellate *Protogonyaulax tamerensis* (=*Alexandrium tamarense*) on feeding in several species of commercially important bivalve mollusc. Each species was fed a mixture of nontoxic algae and clearance rates were calculated. The same individuals were purged in filtered seawater overnight and fed an identical algal mixture with the addition of *A. tamerense* (clone GT 429) the following day. The mussel, *Mytilus edulis*, showed relatively little change in clearance rates when exposed to *A. tamerense*, but this response seemed partly affected by the locality of each mussel population. Mussel populations from Maine showed no selection for or against the toxic dinoflagellates; *A. tamerense* was readily filtered and appeared in both the pseudofeces and feces. Mussels from other populations showed varying behavioral responses to the presence of *A. tamerense* (summarized in the previous section), suggesting that feeding rates in these populations were negatively affected by this particular dinoflagellate culture.

Clearance rates of juvenile *Mytilus edulis* fed unialgal cultures of either *Alexandrium tamarense* or *Gyrodinium aureolum* were significantly lower than clearance rates on a unialgal diet of *Isochrysis* sp. (Lesser and Shumway, 1993). Results were similar in experiments conducted during the winter (5^{o} C) and spring (10^{o} C).

In Japan, the clearance rate of the mussel *Mytilus galloprovincialis* was significantly reduced when exposed to *Heterocapsa circularisquama*, even at a dinoflagellate cell density of only 50 cells · ml^{-1} (Matsuyama *et al*., 1997). The reduction in clearance rate did not seem to be caused by size, density, or shape of *H. circularisquama* cells since no inhibitory effect was observed when mussels were exposed to other morphologically similar dinoflagellates. Repression of clearance rate was not observed when *M. galloprovincialis* were exposed to a filtrate of *H. circularisquama* culture or cultures where the cell walls had been removed by centrifugation, indicating that the source of toxicity of *H. circularisquama* is localized on the cell surface (Matsuyama *et al*., 1997).

Li and Wang (2001) employed a radiotracer technique to determine the selective feeding behavior of the mussel *Perna viridis* and the clam *Ruditapes philippinarum* on an algal mixture containing both toxic and nontoxic *Alexandrium tamarense*. Both bivalves had similar clearance and ingestion rates between the two *A. tamarense* cultures. No selective ingestion of either algal preparation was observed, indicating that the two bivalves were unable to distinguish the particles based on their toxicity. These findings support earlier reports on *Mytilus californianus*, which was observed to ingest both toxic and nontoxic dinoflagellates in natural sea water (Buley, 1936; Fox and Coe, 1943).

Scallops. Shumway and Cembella (1993) provide a comprehensive review of the impact of toxic algae on scallop culture and fisheries. Less is known about the effects of toxic algae on scallop feeding physiology, particularly clearance and filtration rates, compared to other bivalve molluscs.

Li and Wang (2000) suggested that high PSP toxin levels in the scallop *Chlamys*

nobilis were partly due to its relatively high clearance rate of *Alexandrium tamarense*. Feeding behavior in the king scallop (*Pecten maximus*), after switching from a diet of *A. tamarense* to a nontoxic diet, changed drastically depending on the algal species used to detoxify the scallops (Bougrier *et al*., 2000). A diet based on *Tetraselmis suecica* appeared to stimulate clearance and filtration rates, whereas one based on *Isochrysis galbana* had the opposite effect.

The sea scallop, *Placopecten magellanicus*, continued to feed normally when exposed to toxic *Alexandrium tamarense* (Shumway and Cucci, 1987). However, most exhibited striking behavioral responses including violent swimming activity. While this increased activity may provide temporary escape from predators, it does not provide protection from prolonged toxic algal blooms. The associated increase in heart rate, stroke volume and other physiological stresses may actually prove to be detrimental to *P. magellanicus* Thompson *et al*., 1980). Feeding rates for *P. magellanicus* on *A. tamarense* and *Gyrodinium* (=*Gymnodinium*) *aureolum* were significantly different from one another, but still significantly lower than feeding rates on *Isochrysis* sp. (Lesser and Shumway, 1993).

Juvenile and adult bay scallops (*Argopecten irradians*) did not exhibit feeding inhibition during a two week exposure to the toxic epibenthic dinoflagellate *Prorocentrum lima*, a known producer of diarrhetic shellfish poisoning (DSP) (Bauder *et al*., 2001). Clearance rates were similar for bay scallops exposed to equivalent biovolume cell concentrations of *P. lima* and the non-toxic diatom *Thalassiosira weissflogii*. In contrast, clearance rates of *A. irradians* fed two species of toxic dinoflagellate (*Alexandrium tamarense* and *Gyrodinium aureolum*) were significantly higher than bay scallops fed *Isochrysis* sp. (Lesser and Shumway, 1993). No information is available on the effects of *Karenia brevis* on feeding behavior in bay scallops (Shumway, personal communication).

Clams. Northern quahogs (*Mercenaria mercenaria*) retracted their siphons and completely closed their shell valves when exposed to *Alexandrium tamarense* (Shumway *et al*., 1985). The valves remained closed and clams did not feed until the addition of clean seawater. In a separate study, however, *M. mercenaria* was shown to clear *A. tamarense* at very low clearance rates during spring and even less so during winter (Lesser and Shumway, 1993). When exposed to a diet composed solely of *A. fundyense, M. mercenaria* closed their shells and did not resume pumping until a low density of *Thallasiosira weissflogii* was added (Bricelj *et al*. 1990). Quahogs ingested *T. weissflogii* and *A. fundyense* in the same proportion as offered in the algal suspension, thus exhibiting no ingestion selectivity. Quahogs ingested up to 3.4×10^5 toxic cells $\cdot g^{-1} \cdot day^{-1}$ (Bricelj *et al*. 1990). From these studies it appears that a supply of nontoxic algae is necessary to entice northern quahogs to extend their siphons and commence feeding in the presence of certain species of toxic dinoflagellate.

The softshell clam, *Mya arenaria*, filtered 7.6×10^3 cells h^{-1} g dry wt^{-1} in the

presence of *Gonyaulax* (=*Alexandrium*) *tamarense*, a 47% reduction compared to the same individuals fed the same diet without the toxic dinoflagellate (Shumway and Cucci, 1987). *Mya arenaria* were not able to clear nontoxic algal cells in the presence of *A. tamarense*.

During a 1973-74 *Karenia brevis* bloom in Sarasota, FL, shellfish suspected of being contaminated were processed and analyzed for the presence of toxins (Hemmert, 1975). The surfclam (*Spisula solidissima raveneli*) had a toxicity of 75 MU and the southern hard clam (*Mercenaria campechaenis*) had a toxicity of 96 – 118 MU, indicating their ability to filter and ingest *K. brevis* and to accumulate brevetoxins.

Cytotoxic Effects of Harmful Algal Species to Bivalves

While the role of harmful algal blooms is well documented in human shellfish poisonings and aquatic organism mass mortality events, there is far less information concerning chronic, lethal or sublethal effects on shellfish caused by bioaccumulated or biomagnified algal toxins. This paucity of information also concerns whether such harmful algal blooms render shellfish susceptible to disease (Landsberg, 1996).

One of the earlier studies demonstrating the cytotoxic impacts of harmful algae on bivalves was conducted by Widdows *et al*. (1979) who observed cellular damage to the gut of adult *Mytilus edulis* after a short (< 24 h) exposure to bloom conditions of *Gyrodinium (=Gymnodinium) aureolum*.

More comprehensive studies have centered on the pathological effects of bivalve exposure to the toxic dinoflagellate, *Prorocentrum minimum* (Bardouil *et al*., 1993;

Luckenbach *et al*., 1993; Wikfors and Smolowitz, 1993; Wikfors and Smolowitz, 1995). Wikfors and Smolowitz (1993) showed that *P. minimum*, when offered as a food source in combination with *Isochrysis* sp., was poorly ingested by juvenile bay scallops (*A. irradians*) and resulted in 100% mortality after a four-week exposure. After one week, there were appreciable differences in tissues between scallops feeding on *Isochrysis* sp. and those fed a mixed *Isochrysis/P. minimun* diet. Affected scallops had poorly developed digestive diverticula, severe attenuation of epithelial cells associated with absorptive-cell necrosis and sloughing of cells into central lumens. Residual cells were more numerous. Large melanized hemocyte clots were present in the open vascular system of the mantle, digestive diverticula, heart, gill, and kidney tissues. Collectively, these findings suggested that *P. minimum* produced an enterotoxin that gradually affected absorptive cells (Wikfors and Smolowitz, 1993). Juvenile hard clams (*Mercenaria mercenaria*), on the other hand, ingested both *P. minimum* and the congener *P. micans* under identical conditions and survived well under all feeding conditions. No histological examinations of hard clam tissues were made (Wikfors and Smolowitz, 1993).

Luckenbach *et al*. (1993) observed that juvenile eastern oysters (*C. virginica*) exposed to 100% *P. minimum* bloom density (8.9×10^3 to 2.5×10^5 cells $\cdot$ ml^{-1})died within 14 days, and 43% exposed to 33% bloom density died within 22 days, but oysters exposed to 5% bloom density had good shell growth and no mortality. Wikfors and Smolowitz (1995) further examined the histopathology of embryos, larvae, spat and juveniles of the eastern oyster (*C. virginica*) fed different diet combinations of *P. minimum* and *Isochrysis* sp. Feeding larvae showed poor growth and poor development

of the digestive system on a *P. minimum* diet only. Growth of oyster larvae fed a 1:1 *P. minimum/Isochrysis* diet was intermediate, while a 1:2 *P. minimum/Isochrysis* diet resulted in distinctive changes in the anatomy of the digestive system (Wikfors and Smolowitz, 1995). Oyster spat fed a 1:2 *P. minimum/Isochrysis* diet showed an abnormal accumulation of lipid in the stomach epithelium. Absorptive cells in the digestive glands of both larvae and spat contained accumulation bodies, often with a laminated, fibrous appearance. Accumulation bodies were periodic acid-Schiff (PAS) positive and may have corresponded to autolysosomal bodies within *P. minimum* cells. The linkage of accumulation bodies within absorptive cells of oyster digestive diverticula and dinoflagellate autolysosomal bodies suggests a mechanism by which some dinoflagellates interfere with feeding in phytoplankton grazers (Wikfors and Smolowitz, 1995).

Flat oysters (*Ostrea rivularis*), which had died following a *Prorocentrum* bloom in southern China (Yomgjia *et al*. 1995), exhibited a pathology consistent with a systemic toxicosis resulting from the absorption of toxins by the digestive gland (Landsberg, 1996). The most intense lesion was formed by hemocytes that accumulated in and around the hemolymph channels, infiltrated the walls of the blood sinus, and formed intravascular thrombi. Interestingly, this pathology was similar to that found in *C. virginica* by Wikfors and Smolowitz (1993). These studies suggest that *Prorocentrum* spp. may induce pathological effects in the hematopoietic system of oysters (Landsberg, 1996). Finally, spat of the Pacific oyster (*C. gigas*) showed abnormal histopathologies in the gut and gill when exposed for 21 days to a diet of cultured *Prorocentrum rhathymum* at 1.2×10^4 cells $\cdot L^{-1}$ (Pearce *et al*., 2005).

Other toxic algae have caused histopathologies in bivalves. Elevated lysosomal

destabilization rates, which indicate damage to the hepatopancreas, have been reported in eastern oysters (*C. virginica*) exposed to algal blooms dominated by the raphidophyte *Heterosigma akashiwo* (Keppler *et al*., 2005) as well as the dinophyte, *Kryptoperidinium foliaceum* (Lewitus *et al*., 2003).

Smolowitz and Shumway (1997) examined gut tissues from juveniles of eight species of bivalve that had been exposed to *Gyrodinium aureolum* and found the impact to be species-specific. The eastern oyster (*Crassostrea virginica*) and the bay scallop (*Argopecten irradians*) were most severely affected. Several *C. virginica* showed mantle and gill lesions. Bay scallops exhibited decreased height of absorptive cells and increased lumen diameter after exposure, suggesting that *G. aureolum* was of poor food quality. Evidence of toxic effects was not identified in the digestive gland. Several bay scallops also showed variable amounts of inflammation in the kidney associated with protozoal infestations and variable amounts of predominately rod-shaped bacteria within the urinary space. Another pectinid, the king scallop (*Pecten maximus*) developed obvious STX neoformation in kidneys after exposure to PSP toxins (Bougrier *et al*., 2000). Pectinids are apparently unaffected by disseminated neoplasia (Landsberg, 1996), and only one case of germinoma has been reported in bay scallops (Peters *et al*., 1996).

Brevetoxins produced by *Karenia brevis* and which are responsible for neurotoxic shellfish poisoning (NSP) in the Gulf of Mexico, have not yet been shown to play a role in the development of neoplasia in bivalves (Landsberg, 1996). Although brevetoxins are well known for their role in fish kills (Steidinger *et al*., 1973; see Landsberg, 2002 for review), their effect in developing histopathologies in bivalve molluscs is unknown.

Chapter Three

Larval Studies

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*, 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 usually occur in the late summer and fall along the Florida west coast (Kirkpatrick *et al*., 2004) 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. Therefore, the effects of the NSP-producing alga, *K. brevis*, on survival and development of larvae were examined for three species of bivalve molluscs: the northern quahog (= hard clams) (*Mercenaria mercenaria*), the bay scallop (*Argopecten irradians*) and the Eastern oyster (*Crassostrea virginica*). Experiments were designed to specifically investigate whether differences in survival were due to the dinoflagellate itself or its constituent toxins.

Materials and Methods

Collection and Maintenance of Bivalves

All adult bivalves used for broodstock were collected from the Florida Gulf Coast. Bay scallops were collected from the Anclote estuary while northern quahogs were collected from Tampa Bay. Oyster larvae were provided by John Supan, manager of the Grand Isle (Louisiana) Bivalve Hatchery. Adults were transferred to one of the following facilities for maturation and spawning: University of South Florida, St. Petersburg (bay scallops); Bay Shellfish Co., Palmetto, FL (northern quahogs). Larvae were transferred to Mote Marine Laboratory, Sarasota, FL immediately after spawning for subsequent larval studies. Larvae were maintained in 2L flasks at a density of five larvae $\cdot$ ml^{-1} under gentle aeration and fed *Isochrysis galbana* daily at a density of 10,000 cells $\cdot$ ml^{-1} prior to experimentation.

Maintenance of Algal Cultures

Batch cultures of *Karenia 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 μm, passed through an activated charcoal filter, sterilized with ultraviolet light and autoclaved. Cultures were maintained at a temperature of 24 to 26^{o} C and a salinity of 33 to 35 ppt (parts per thousand). Lighting was provided by a combination of Cool-white and Gro-lite bulbs. 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.

Preparation of Lysed Culture

Larval experiments were conducted using both whole and lysed culture preparations of *Karenia brevis*. Lysed preparations were produced by exposing a sample of *K. brevis* culture to ultrasonic disruption at 750 W for four minutes 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. A 500 ml sample of lysed culture was then subjected to brevetoxin analysis by the same procedure as whole culture samples.

Determination of Cell and Brevetoxin Concentrations

Cell concentration for each algal culture was determined prior to each experiment employing a Coulter® Multisizer IIE fitted with a 100 μm orifice. The number of particles in a 500 μl sample of culture was ascertained for *Isochrysis galbana* (size range: 3.4 – 8.1 μm) and *Karenia brevis* (size range: 14.2 – 30.0 μm). Three replicate counts were made and the mean value determined. Cell concentrations were verified by microscopic enumeration using a counting chamber (Hausser Scientific Company).

Brevetoxin concentration of each culture of *K. brevis* was determined by high performance liquid chromotography (HPLC). Brevetoxins were extracted by passing a known volume (≅ 500 ml) of culture through a C-18 extraction disc placed inside a Teflon® filtering apparatus under vacuum (20 psi). The C-18 disc was eluted with methanol to recover the brevetoxins and placed in a flask. The flask was then placed on a Labonco® rotary evaporator and reduced to dryness. Methanol was added to produce a final volume of 3 ml and the sample injected into a Shimadzu® LC-600 HPLC with a Shimadzu® SPD-M6A photodiode array UV-VIS detector. Total brevetoxin concentrations were quantified using a C-18 column and an 85:15 methanol: water (1 ml/min) isocratic elution at 215 nm according to the procedure of Pierce *et al*. (2005).

Three-Day Static Exposure

The first series of experiments consisted of exposing larvae to *Karenia brevis* for

three days. Bivalve species used for larval studies were bay scallops (*Argopecten irradians*), northern quahogs (*Mercenaria mercenaria*) and eastern oysters (*Crassostrea virginica*). Each experiment employed three concentrations of both whole and lysed culture of *K. brevis* and a control. *K. brevis* concentrations were 10, 100 and 1,000 cells · ml^{-1}. Each treatment consisted of five replicates.

Larvae were three days old at the start of the experiment. For each replicate, approximately 500 larvae were transferred to a petri dish containing 100 ml filtered seawater. Each dish was supplied with 1 x 10^4 cells · ml^{-1} of *Isochrysis galbana*. *K. brevis* (whole or lysed culture) was added to each treatment to obtain the desired exposure concentration. After inoculation with *K. brevis*, a small subsample (0.5 ml) was withdrawn and algal cells counted with a hemocytometer under a compound microscope to verify that the desired concentration was obtained. Each dish was covered and left undisturbed. Dishes were neither stirred nor aerated.

Each day, the contents of each dish were poured through a 35 μm sieve and gently rinsed with filtered seawater. Larvae were transferred to a 100 ml graduated cylinder containing 50 ml of filtered seawater and brought to the appropriate volume prior to reinoculation. Dishes were reinoculated with *K. brevis* and *I. galbana*, covered, and left undisturbed for 24 hours.

The experiment was terminated after seventy-two hours and the larvae preserved in 2% buffered formalin. Straight-hinged larvae which have been dead for as little as one hour have few internal details visible through the transparent shell and are easily separated from larvae preserved while living. Larvae were considered dead if either empty shells or shells with decomposing tissues were found. Larvae were considered

live if tissues were discernable and differentiated under magnification. Developmental stage for each live and dead larva was determined. A two-way analysis of variance with repeated measures was performed for each experiment to determine significant differences in mortality among cell concentration and culture treatment.

Seven-Day Static Exposure

Seven-day-old larvae were used in this series of experiments to investigate the effects of *K. brevis* on mortality, development and metamorphosis of each bivalve species. Each experiment consisted of four concentrations of whole *K. brevis* culture and a control. Each treatment consisted of five replicates. The four *K. brevis* concentrations were: 10, 100, 1,000, and 5,000 cells $\cdot$ ml^{-1}. In each case, approximately 500 larvae were transferred to a 250 ml glass finger bowl (density = 5 larvae $\cdot$ ml^{-1}). Each bowl was supplied with 2 x 10^4 cells $\cdot$ ml^{-1} of *Isochrysis galbana* as a food source. Whole culture of *K. brevis* was added to each bowl to obtain the desired exposure concentration. A small subsample (0.5 ml) was withdrawn after inoculation and cells counted with a hemocytometer under a compound microscope to confirm that the desired concentration was achieved. All bowls were covered and left undisturbed. Bowls were neither stirred nor aerated.

Each day, the contents of each bowl were poured through a 50 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 hours. The experiment was terminated after seven days and the larvae preserved in 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*).

Statistical Analyses

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 three-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 seven-day-exposure experiments.

Results

Toxin Profile of *Karenia brevis* Cultures

Toxin profiles of *K. brevis* cultures used in experiments are summarized in Table 1. Two brevetoxins and one antagonist were present in each culture: PbTx-2, PbTx-3, and brevenal, a recently identified brevetoxin antagonist (Bourdelais *et al*., 2004). 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.

Three-Day Static Exposure

Survival of bay scallop larvae exposed to *K. brevis* was > 90% in 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.

Survival of *M. mercenaria* larvae was > 88% in all treatments (Table 3). Most larvae survived to the umboveliger stage. Percent survival was significantly lower ($p < 0.001$) in the Lysed-1,000 cells $\cdot$ ml^{-1} treatment (Table 5). There was a significant ($p < 0.05$) concentration and culture effect on larval survival.

Larval survival for *C. virginica* ranged from 94 to 75%, with < 1% reaching the umboveliger stage (Table 4). 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).

Seven-Day Static Exposure

Exposure of *A. irradians* larvae to *K. brevis* concentrations up to 1,000 cells · ml^{-1} for seven days did not greatly impact survival (Fig. 1). When the quantity was increased to 5,000 cells · ml^{-1}, survival declined to 37%. All larvae had reached the pediveliger stage at the termination of the experiment.

Survival of *M. mercenaria* larvae gradually decreased with increasing *K. brevis* concentration up to 1,000 cells · ml^{-1} (Fig. 2). Larval survival at 5,000 cells · ml^{-1} declined to 26%. A higher percent of larvae from the low dose treatments developed into pediveligers than larvae from higher dose treatments (Fig. 3)

Total survival of *C. virginica* larvae was 88% in the control, 75% in *K. brevis* concentrations up to 1,000 cells · ml^{-1}, and only 19% at 5,000 cells · ml^{-1} (Fig 4). Larval development was similar among exposure doses up to 1,000 cells · ml^{-1} (Fig. 5). Approximately 46% were umboveligers after seven days; 42% developed into pediveligers and 11% had settled as spat. At 5,000 cells · ml^{-1}, 67% of surviving larvae were still umboveligers, 32% were pediveligers, and only 1% had settled as spat.

Table 6 summarizes the results from one-way ANOVA and Tukey's Multiple

Comparison Test for each species. All three species showed a significant difference in mortality at *K. brevis* concentrations of 5,000 cells · ml^{-1}. Only *M. mercenaria* showed a significant difference in mortality at *K. brevis* concentrations of 1,000 cells · ml^{-1}.

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). Not until more recently has attention begun to focus on the effects of harmful algae 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 three-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 1,000 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 1,000 cells/ml, while survival of *M. mercenaria* larvae was significantly lower at 1,000 cells · ml^{-1}. Survival in all three species was significantly reduced at 5,000 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 100-1,000 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; see Landsberg, 2002 for review). 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, 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 (Jeong *et al*., 2004), 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 (*Argopecten 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*. However, 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 1,000 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 1,000 cells · ml^{-1}. Almost ninety percent of larvae subjected to 5,000 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 dinoflagellate, *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. Two brevetoxins and one antagonist were present in each culture: PbTx-2, PbTx-3, and brevenal, a recently identified 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.

Blooms of *K. brevis* may persist in coastal waters for many months (Steidinger *et*

al., 1995). Our results clearly indicate that when these blooms and their 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.

Table 1

Cell density, sample matrix and brevetoxin composition of *Karenia brevis* (Wilson clone) cultures used in larval experiments for each species. (Brevenal is considered a brevetoxin antagonist).

Species	*K. brevis* Culture (Cells/ml)	Matrix	Brevetoxin Amount (ug · L^{-1}) PbTx-2	PbTx-3	TOTAL	Brevenal
Bay scallop (*Argopecten irradians*)	12,000	Whole	20.03	5.07	25.10	30.23
		Lysed	26.31	8.11	34.42	34.47
Northern quahog (*Mercenaria mercenaria)*	12,800	Whole	23.36	2.46	25.82	29.17
		Lysed	32.33	2.42	35.32	35.32
Eastern oyster (*Crassostrea virginica*)	10,000	Whole	22.19	4.10	26.29	24.93
		Lysed	32.94	0.97	33.91	33.90

Table 2

Mean (± SD) number of live and dead larvae, larval stage and percent survival for *Argopecten irradians* after exposure for three days to *Karenia brevis*. Treatments consisted of whole and lysed cultures of *K. brevis* at three concentrations: 10, 100 and 1,000 cells · ml^{-1}. (n = 5).

	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-1,000	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-1,000	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)

Table 3

Mean (± SD) number of live and dead larvae, larval stage and percent survival for *Mercenaria mercenaria* after exposure for three days to *Karenia brevis*. Treatments consisted of whole and lysed cultures of *K. brevis* at three concentrations: 10, 100 and 1,000 cells · ml^{-1}. (n = 5).

	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-1,000	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-1,000	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)

Table 4

Mean (± SD) number of live and dead larvae, larval stage and percent survival for *Crassostrea virginica* after exposure for three days to *Karenia brevis*. Treatments consisted of whole and lysed cultures of *K. brevis* at three concentrations: 10, 100 and 1,000 cells · ml^{-1}. (n = 5).

	Straight-Hinged Veliger		**Umboveliger**		**Larvae**	**Survival (%)**	
Treatment	Live	Dead	Live	Dead	Total	Total	Umboveliger
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-1,000	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-1,000	199.8 (16.8)	65.2 (9.5)	0.0	0.8 (0.8)	265.8 (12.4)	75.2 (4.1)	0.0

Table 5

Effect of *Karenia brevis* concentration and culture preparation on percent survival in three-day-old bivalve larvae. A) Two-way ANOVA ($\alpha = 0.05$). B) Tukey's (ω) multiple comparison test. Underlined treatments are not significantly different ($p > 0.05$).

Argopecten irradians

Source of Variation	SS	df	MS	F	P-value	F crit
Concentration	0.063	2	0.0315	23.20	0.0000	3.40
Treatment	0.002	1	0.0015	1.14	0.2971	4.26
Interaction	0.009	2	0.0045	3.28	0.0549	3.40
Within	0.033	24	0.0014			
Total	0.106	29				

k =	7	q(alpha)=	4.541			
v =	28	Sy =	0.0161			
α =	0.05	ω =	0.0731			
L-1,000	W-1,000	L-100	W-10	Control	L-10	W-100

Mercenaria mercenaria

Source of Variation	SS	df	MS	F	P-value	F crit
Concentration	17.053	2	8.5266	4.75	0.0183	3.40
Treatment	34.810	1	34.8101	19.39	0.0002	4.26
Interaction	3.614	2	1.8072	1.01	0.3804	3.40
Within	43.086	24	1.7952			
Total	98.563	29				

k =	7	q(alpha)=	4.464			
v =	28	Sy =	0.6822			
α	0.05	ω =	3.0455			
L-1,000	L-10	Control	L-100	W-1,000	W-100	W-10

Crassostrea virginica

Source of Variation	SS	df	MS	F	P-value	F crit
Concentration	0.284	2	0.1418	69.35	0.0000	3.40
Treatment	0.031	1	0.0308	15.07	0.0007	4.26
Interaction	0.016	2	0.0082	4.02	0.0312	3.40
Within	0.049	24	0.0020			
Total	0.380	29				

k =	7	q(alpha)=	4.541			
v =	28	Sy =	0.0205			
a =	0.05	ω =	0.0929			
L-1,000	L-100	W-1,000	W-100	Control	L-10	W-10

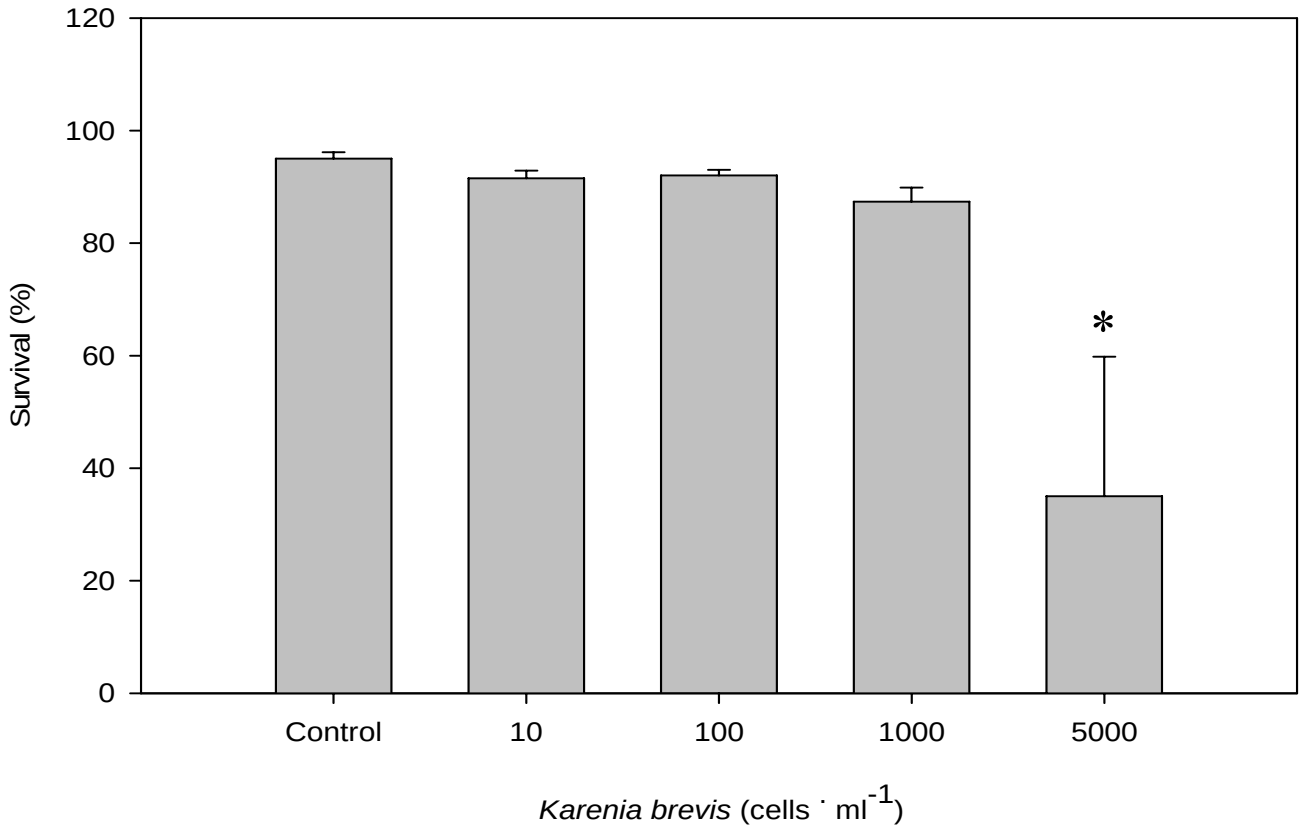

Figure 1. Percent survival (mean ± SD) of *Argopecten irradians* larvae after exposure to *Karenia brevis* for seven days. Treatment with an asterisk was significantly different ($p < 0.05$). Larvae were seven days old at start of experiment.

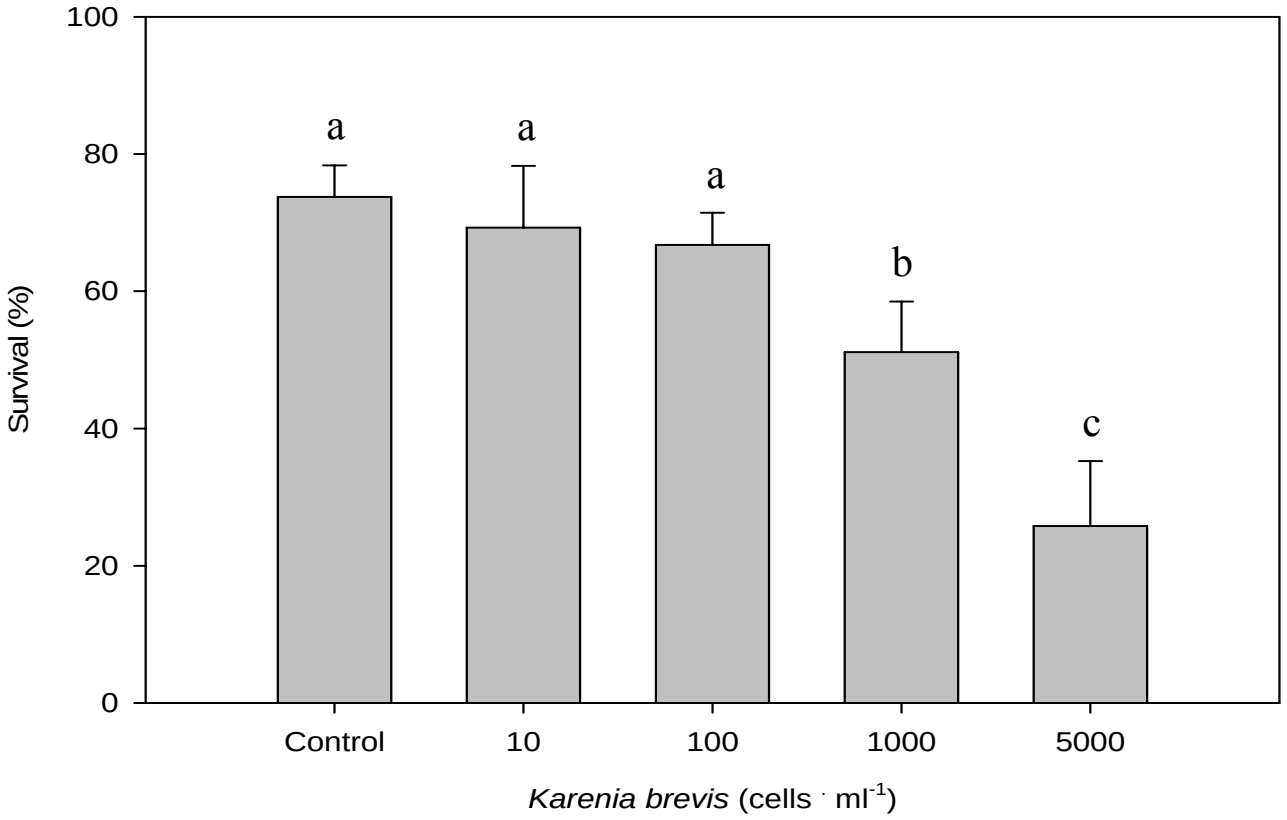

Figure 2. Percent survival (mean ± SD) of *Mercenaria mercenaria* larvae after exposure to *Karenia brevis* for seven days. Treatments with the same letter were not significantly different ($p < 0.05$). Larvae were seven days old at start of experiment.

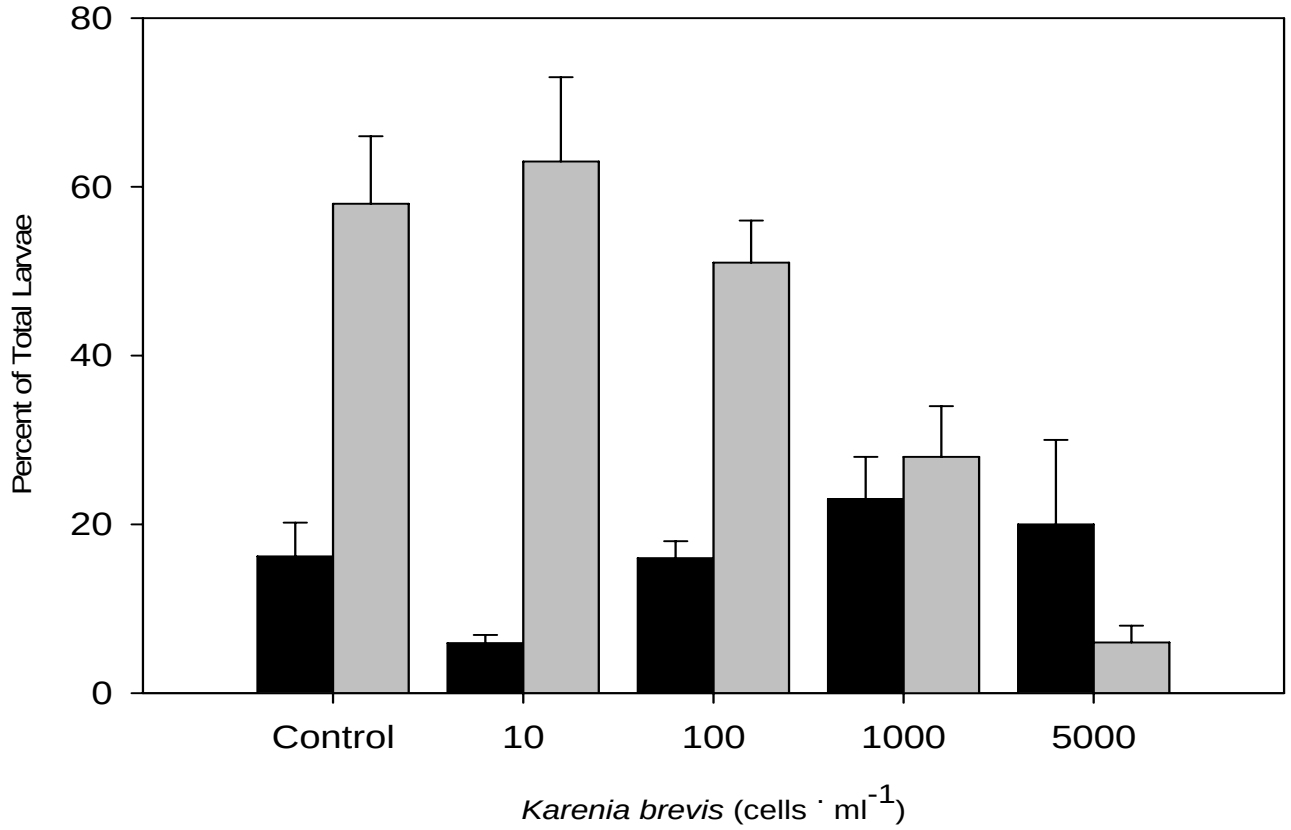

Figure 3. Percent of total *Mercenaria mercenaria* larvae that survived to the umboveliger (■) and pediveliger (□) stages after exposure to *Karenia brevis* for seven days. Larvae were seven-day-old umboveligers at beginning of experiment.

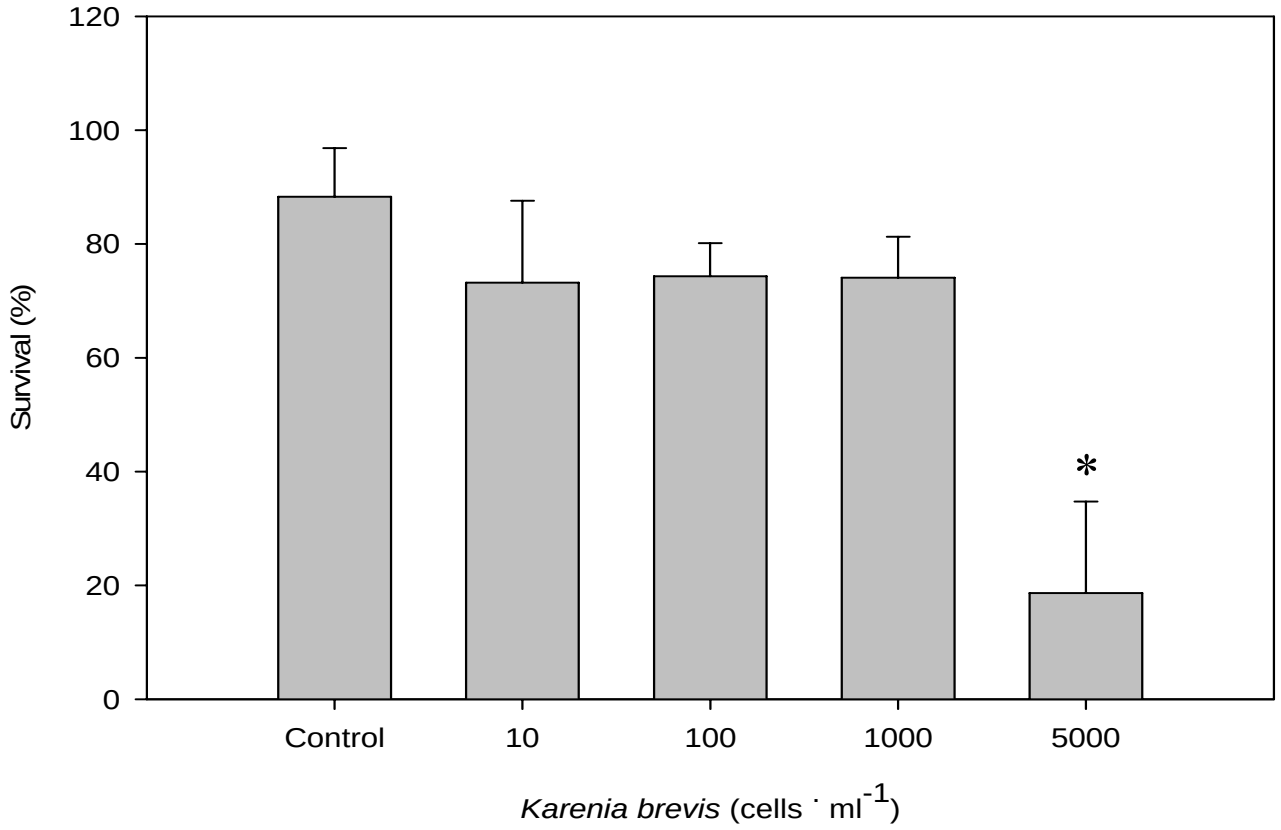

Figure 4. Percent survival (mean ± SD) of *Crassostrea virginica* larvae after exposure to *Karenia brevis* for seven days. Treatment with an asterisk was significantly different ($p < 0.05$). Larvae were seven days old at start of experiment.

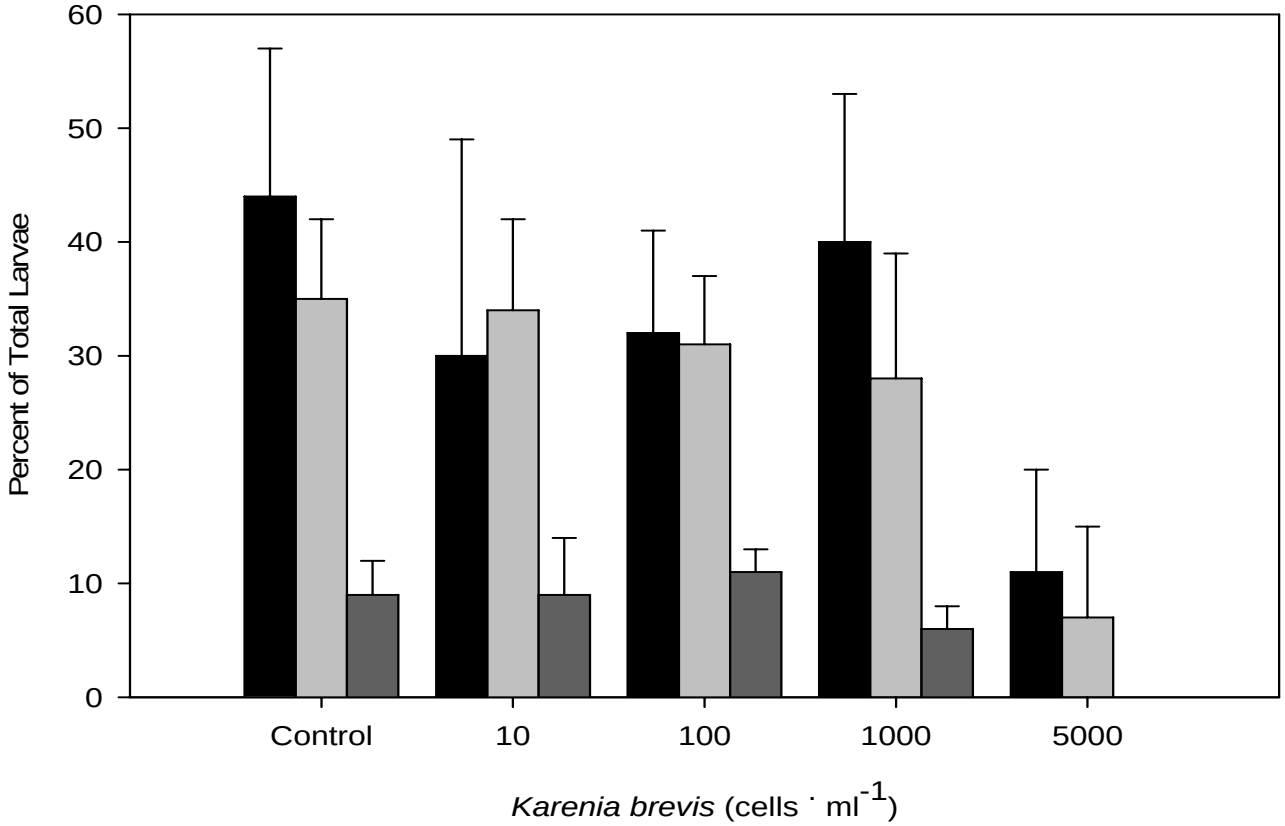

Figure 5. Percent of total *Crassostrea virginica* larvae that survived to the umboveliger ( ), p liveliger (and spat ( ) stages after exposure to *Karenia brevis* for seven days. Larvae were seven-day-old umboveligers at start of experiment.

Table 6

Effect of *Karenia brevis* concentration and culture preparation on percent survival in seven-day-old shellfish larvae. A) Two-way ANOVA ($\alpha = 0.05$). B) Tukey's (ω) multiple comparison test. Underlined treatments are not significantly different ($p > 0.05$).

A) Two-Way ANOVA

Argopecten irradians

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.8261	4	0.4565	28.42	5.35E-08	2.87
Within Groups	0.3213	20	0.0161			
Total	2.1475	24				

Mercenaria mercenaria

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.8554	4	0.2139	31.92	2.00E-08	2.87
Within Groups	0.1340	20	0.0067			
Total	0.9894	24				

Crassostrea virginica

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.6176	4	0.4044	17.54	7.2665	2.96
Within Groups	0.3920	17	0.0231			
Total	2.0096	21				

B) Tukey's Multiple Comparison Test

Argopecten irradians

k =	5	*q(alpha)* =	4.232
v =	20	Sy =	0.0567
a =	0.05	w =	0.2399

5000 1000 10 100 Control

Mercenaria mercenaria

k =	5	*q(alpha)* =	4.232
v =	20	Sy =	0.0366
a =	0.05	w =	0.1549

5000 1000 100 10 Control

Crassostrea virginica

k =	5	*q(alpha)* =	4.303
v =	17	Sy =	0.0679
a =	0.05	w =	0.2922

5000 1000 100 10 Control

Chapter Four

Juvenile Studies

Introduction

The effects of diets that include toxic dinoflagellates on feeding in bivalve molluscs have received increased attention in the past twenty years (Shumway and Cucci, 1987; Gainey and Shumway, 1988; Bricelj *et al*., 1996; Lassus *et al*., 1996; Lassus *et al*., 1999; Li and Wang, 2001; Lesser and Shumway, 1993; Bricelj and Shumway, 1998). 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; Bardouil *et al*., 1993; Bricelj *et al*., 1996; Lassus *et al*., 1996; Li and Wang, 2001), algal concentration (Li *et al*., 2002), cell size and selectivity (Shumway *et al*., 1985; Shumway *et al*., 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).

Blooms of *K. brevis* 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 nonindigenous 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.

This study was undertaken to determine the effects of the toxic dinoflagellate, *Karenia brevis*, on the clearance rate of juveniles of four species of common bivalve molluscs from Florida: the bay scallop (*Argopecten irradians*), northern quahog (= hard clam , *Mercenaria mercenaria*), eastern oyster (*Crassostrea virginica*) and green mussel (*Perna viridis*). Both short-term (one hour) and long-term (two day) effects were investigated. 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.

Materials and Methods

Collection and Maintenance of Juveniles

Juveniles of four species of bivalve were used in these experiments: the bay scallop (*Argopecten irradians*), eastern oyster (*Crassostrea virginica*), northern quahog (*Mercenaria mercenaria*) and green mussel (*Perna viridis*). Bay scallops and northern quahogs were obtained directly from the hatchery. Eastern oysters and green mussels were collected from upper Tampa Bay, which had not experienced blooms of *K. brevis* for decades. All bivalves were maintained in aerated aquaria at 25° C and fed *Isochrysis galbana* daily at a density of 2×10^4 cells $\cdot$ ml^{-1} prior to experimentation.

Maintenance of Algal Cultures

Batch cultures of *Karenia 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 μm, passed through an activated charcoal filter, sterilized with ultraviolet light and autoclaved. Cultures were maintained at a temperature of 24 to 26° C and a salinity of 33 to 35 ppt (parts per thousand). Lighting was provided by a combination of Cool-white and Gro-lite bulbs. 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.

Preparation of Lysed Culture

Larval and juvenile bivalve experiments were conducted using both whole and lysed culture preparations of *Karenia brevis*. Lysed preparations were produced by exposing a sample of *K. brevis* culture to ultrasonic disruption at 750 W for four minutes 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. A 500 ml sample of lysed culture was then subjected to brevetoxin analysis by the same procedure as whole culture samples.

Determination of Cell and Brevetoxin Concentrations

Cell concentration for each algal culture was determined prior to each experiment employing a Coulter® Multisizer IIE fitted with a 100 μm orifice. The number of particles in a 500 μl sample of culture was ascertained for *Isochrysis galbana* (size range: 3.4 – 8.1 μm) and *Karenia brevis* (size range: 14.2 – 30.0 μm). Three replicate counts were made and the mean value was determined. Cell concentrations were verified by microscopic enumeration using a counting chamber (Hausser Scientific Company).

Brevetoxin concentration of each culture of *K. brevis* was determined by high performance liquid chromotography (HPLC). Brevetoxins were extracted by passing a known volume (≅ 500 ml) of culture through a C-18 extraction disc placed inside a

Teflon® filtering apparatus under vacuum (20 psi). The C-18 disc was eluted with methanol to recover the brevetoxins and placed in a flask. The flask was then placed on a Labonco® rotary evaporator and reduced to dryness. Methanol was added to produce a final volume of 3 ml and the sample injected into a Shimadzu® LC-600 HPLC with a Shimadzu® SPD-M6A photodiode array UV-VIS detector. Total brevetoxin concentrations were quantified using a C-18 column and an 85:15 methanol: water (1 ml min^{-1}) isocratic elution at 215 nm according to the procedure of Pierce *et al*., 2005).

Clearance Rate Studies

All feeding experiments were carried out at a temperature of 25° C and a salinity of 30 o/oo. Two separate sets of feeding experiments were conducted for each bivalve species. The first set consisted of short-term experiments under static conditions. Individuals were exposed to an initial set of conditions and left undisturbed for one hour. Feeding rates were calculated at the end of the hour. The second set consisted of long-term experiments under flow-through conditions. Individuals were subjected to experimental conditions that were continuously replenished over a 48 hour period. Feeding rates were calculated twice each day. Complete details for each set of experiments are provided below.

Static Exposure Experiments

Separate static feeding experiments were carried out for each bivalve species under identical conditions. Individuals were placed in separate beakers containing 500 ml filtered seawater and allowed to acclimate for one hour. Each beaker was lightly aerated.

Experimental treatments included three concentrations and two culture preparations of *K. brevis* and a control (no *K. brevis* added). Each treatment consisted of five replicates. Cell densities of *K. brevis* were 10, 100, and 1,000 cells $\cdot$ ml^{-1} and culture preparations included lysed and whole cultures. *Isochrysis galbana*, a common food alga, was added to each beaker at an initial concentration of 2-4 x 10^4 cells $\cdot$ ml^{-1}. Beakers were left undisturbed and the reduction in cell concentration of both *I. galbana* and *K. brevis* was determined after one hour.

At the end of each experiment, tissue dry weight (mg) was determined by drying soft tissues in preweighed aluminum pans at 103^o C for 24 hours. Weight specific clearance rates were calculated as: CR_{dw} (ml/hr) = (volume in ml) $*$ (ln C_1 – ln C_2) / t, where C_1 and C_2 are the cell concentrations at the beginning and end of each time increment. Weight-specific filtration rate (if no pseudofeces production was observed) was calculated as: FR_{dw} (cells/l/hr) = CR_{dw} $*$ (C_1 + C_2)/2, where C_1 and C_2 are the cell concentrations at the beginning and end of each time increment.

A two-way analysis of variance with equal replication was performed to determine significant differences in weight-specific filtration and ingestion rates among cell concentrations and culture treatments. Multiple comparison analyses were

performed using Tukey's ω procedure (designed to prevent Type I errors) and Dunnett's τ (designed to compare all means with a control).

The amounts of brevetoxin to which individual bivalves were exposed were determined by multiplying the total toxin concentration of the culture (Table 10) by the amount (volume) of culture added to each treatment. In this way, the relationship between clearance rate and toxin exposure could be determined for whole and lysed cultures of *K. brevis* for each species. Significant differences between regression lines (whole vs. lysed cultures) were tested by a simple F test (for both residual variance and slope).

Flow-Through Exposure Experiments

Continuous-flow feeding experiments were conducted using a test system developed by Singer *et al*. (1990). This system operated by pumping the algal solution for each treatment through an enclosed 290-ml exposure vessel. There were eighteen separate vessels available for any given experiment.

Each test vessel consisted of two halves which are sealed using a silicon O-ring and a full-circumference clamp. The top half had two ports. One port was used as the inflow line and the second port was available for feeding or treatment injection if required. When not in use, the second port was sealed with a Teflon-lined cap. The bottom half had a single port, which served as the outflow port. The treatment solution flowed through the inlet into the top half of the vessel, through a fritted glass disk (40-60 μm pore size) which was permanently fastened within the lower half of the vessel and finally through the outflow port.

Treatment solutions of *K. brevis* were prepared in 20L polypropylene carboys and allowed to mix by gentle aeration. Treatment solutions were introduced into each chamber using an ISMATIC® MCP Pumpsystem Model 78002 multihead pump at a flow rate approximately two to three times the clearance rate of the control bivalves (i.e., the cell concentration in the outflow fell between 50 and 80% of the inflow concentration). All tubing used throughout the pump system was made of platinum-cured silicon. The outflow (waste) solution was treated with bleach before disposal.

Each experiment consisted of two treatments (six replicate chambers), a control (five replicate chambers) and a blank (one chamber). Treatment concentrations were 100 and 1,000 cells $\cdot$ ml^{-1} of *K. brevis*. *Isochrysis galbana*, a common food alga, was added to each beaker at an initial concentration of 2-4 x 10^4 cells $\cdot$ ml^{-1}. Since there were a limited number of chambers available for a given experiment, consecutive experiments were run for each bivalve species, the first using whole cultures of *K. brevis* and the second using lysed cultures. Each experiment ran for two days and feeding rates were calculated twice a day (9 AM and 5 PM). At the end of each experiment, tissue dry weight (mg) was determined.

Weight-specific clearance rates were calculated as: CR_{dw} (ml $\cdot$ hr^{-1}) = flow rate (ml $\cdot$ hr^{-1}) $*$ (C_i - C_o) / C_i, where C_i is the inflow concentration of *I. galbana* and C_o is the outflow concentration from each experimental chamber. Weight-specific filtration rates were calculated as: FR_{dw} (cells $\cdot$ hr^{-1}) = $CR_{dw} * (C_i + C_o)/2$, where C_i and C_o are the inflow and outflow *I. galbana* cell concentrations during each feeding rate determination.

A single factor analysis of variance was performed to determine significant differences in weight-specific clearance and filtration rates among cell concentrations.

Results

Cell concentration of *K. brevis* cultures ranged from 2.1 – 2.2 x 10^4 cells · ml^{-1} for static experiments and from 2.0 – 2.5 x 10^4 cells · ml^{-1} for flow through experiments (Table 7). Static experiments (run simultaneously for each species) used the same culture while flow-through experiments (run consecutively for each species) required separate cultures. Total brevetoxin concentration ranged from 23.1 – 80.3 μg · L^{-1} for static experiments and 29.7 – 75.1 μg · L^{-1} for flow-through experiments. PbTx -2 and PbTx-3 were the most abundant brevetoxins in cultures of *K. brevis* for all experiments. PbTx-1, which was detected only cultures used in the static experiments, was present in concentrations < 8 μg · L^{-1}. Brevenal, a putative inhibitor of brevetoxin action, was not identified prior to the flow-through experiments; however, it is possible, even likely, that it was present, yet undetected, in cultures of *K. brevis* used in the static experiments. Total brevetoxin was typically higher after a culture was lysed.

Static Exposure Experiments

Table 8 summarizes the decline in *I. galbana* for each bivalve species exposed to different concentrations and preparations of *K. brevis* under static conditions. Table 9 summarizes filtration and clearance rates for each species. No pseudofeces production was observed for any species under any treatment condition. Results for each species are discussed separately.

Bay scallops (*Argopecten irradians*)

Mean dry weight for juvenile bay scallops ranged from 16.9 – 19.5 mg dry wt. Clearance rate was highest in the control (11.19 ml · hr^{-1} · mg dry wt^{-1}) and lowest in the Whole-1,000 treatment (2.33 ml · hr^{-1} · mg dry wt^{-1}) (Fig. 6). 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 one hour at 1,000 cells · ml^{-1} (calculated from Table 8).

Green mussels (*Perna viridis*)

Mean dry weight for juvenile green mussels ranged from 40.3 – 46.5 mg dry wt. Mean clearance rate was highest in the control (16.39 ml · hr^{-1} · mg dry wt^{-1}) and lowest in the Whole-1,000 treatment (4.37 ml · hr^{-1} · mg dry wt^{-1}) (Fig. 7), 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 one hour at 1,000 cells · ml^{-1} (calculated from Table 8).

Northern quahogs (*Mercenaria mercenaria*)

Mean dry weight for juvenile northern quahogs ranged from 13.8 – 16.3 mg dry wt. Clearance rate was highest in the control (12.91 ml · hr^{-1} · mg dry wt^{-1}) and lowest in Whole-1,000 (4.28 ml · hr^{-1} · mg dry wt^{-1}), or a 73% reduction in clearance rate (Fig. 8). 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 one hour at 1,000 cells · ml^{-1} (calculated from Table 8).

Eastern oysters (*Crassostrea virginica*)

Mean dry weight for juvenile oysters ranged from 40.6 – 50.6 mg dry wt. Clearance rate was highest in the control (13.57 ml · hr^{-1} · mg dry wt^{-1}) and lowest in the Whole-1,000 treatment (8.42 ml · hr^{-1} · mg dry wt^{-1}) (Fig. 9). 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 one hour at 1,000 cells · ml^{-1} (calculated from Table 8).

Differences in mean clearance rate among the four bivalve species is summarized in Fig. 10A for whole cultures and Fig. 10B for lysed cultures. Significant differences were found among species, *K. brevis* concentration and culture ($p < 0.001$). There were also significant interaction differences ($p < 0.001$) among all factors (Multifactor

ANOVA; univariate test of significance for clearance rate).

Relationships between clearance rate and brevetoxin concentration for each bivalve species are summarized in Figures 11 through 14. Bay scallops showed a significant decrease in clearance rate with increasing brevetoxin concentration for both whole and lysed cultures of *K. brevis* (Fig. 11). There was no significant difference ($p > 0.05$) between the two culture treatments. Green mussels (Fig. 12) and northern quahogs (Fig. 12) showed a decline in clearance rate with increasing brevetoxin concentration only for whole cultures. There was a significant difference ($p < 0.05$) between the two cultures for both species. Finally, eastern oysters showed a slight decline in clearance rate for both whole and lysed cultures of *K. brevis* (Fig. 14). There was no significant difference ($p > 0.05$) between the two culture treatments.

Flow-Through Exposure Experiments

Figure 15 summarizes clearance rates for all species under continuous flow-through exposure to whole (top) and lysed (bottom) cultures of *K. brevis.*

Mean clearance rate of juvenile *A. irradians* was significantly reduced ($p < 0.05$) at *K. brevis* concentrations of 100 cells $\cdot$ ml^{-1} and higher in both whole (Fig 15A) and lysed (Fig 15B) 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 $\cdot$ ml^{-1} and higher.

Mean clearance rate of *P. viridis* exposed to whole *K.* brevis culture (Fig 15B)

was significantly lower ($p < 0.05$) at 1,000 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.

Mean clearance rate of *M. mercenaria* exposed to whole culture was significantly lower ($p < 0.05$) at 1,000 cells · ml^{-1} (Fig 15C). There were no significant differences ($p > 0.05$) in clearance rate when *M. mercenaria* was exposed to lysed (Fig 15C) *K. brevis*.

There was no significant difference ($p > 0.05$) in clearance rate of juvenile *C. virginica* exposed to different concentrations of lysed (Fig 15D) or whole (Fig 15D) *K. brevis* over time.

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, each species responded similarly under two very different exposure regimes: short-term (1 hr) exposure to a non-replenished supply of *K. brevis* and long-term (2 day) 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 hr to be manifested with lysed cells. Poor growth, histopathologies and mortality of *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 (*Perna 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 1,000 cells $\cdot$ 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 bivalves 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. Oysters were more toxic than clams taken at the same time from the same location during a red tide outbreak in North Carolina (Tester and Fowler, 1990). 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 *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. viridis*) 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 non-toxic 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 (1,000 cells · ml^{-1}) densities declined only after 24 hr 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 1987-88 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 $\cdot$ 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: 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). Locating aquaculture sites in lower salinity waters might reduce the frequency and duration of exposure to red tides, which typically initiate in more saline offshore waters. If a red tide does penetrate the estuary, the lower salinity further into the bay could serve as a potentially effective salinity barrier to a bloom of K. brevis. Similarly, reduced feeding rates in the green mussel (*P. viridis*) at high *K. brevis* concentrations should theoretically make it more difficult for mussel populations to remain established in estuaries where red tides are more frequent and/or severe. Emperical observations, however, suggest a different outcome. An intense red tide during 2005-06 resulted in high mortality of green mussels attached to pilings and other structures in lower Tampa Bay (personal observation). Intense recolonization by juvenile

green mussels, however, was observed in late 2006, several months after the bloom had dissipated. The prolific and dynamic recruitment rates of green mussels and their ability to rapidly recolonize a previously inhabited space after a red tide has disappeared suggests populations of this exotic species have no difficulty overcoming the temporary effects of exposure to K, brevis. 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.

Table 7

Experimental conditions, bivalve species, sample matrix, cell and brevetoxin concentration of laboratory cultures of *K. brevis* (Wilson Clone) used for juvenile feeding experiments. n.d. = not detected.

EXPERIMENT		*K. brevis* Culture	Brevetoxin Amount (ug · L^{-1})				
Static	Matrix	(cells · ml^{-1})	PbTx-1	PbTx-2	PbTx-3	Brevenal	TOTAL
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

Table 8

Decline in *Isochrysis galbana* cell counts (cells · ml^{-1}) for juvenile bivalve molluscs exposed to different concentrations and preparations of *Karenia brevis* under static conditions. Starting seawater volume in each replicate was 500 ml.

Treatment	Mean Dry Wt (mg) (SD)	Cell concentration (t=0 hr) (cells · ml^{-1}) *T. iso*	Cell concentration (t=0 hr) (cells · ml^{-1}) *K. brevis*	Cell concentration (t=1 hr) (cells · ml^{-1}) *T. iso*	Cell concentration (t=1 hr) (cells · ml^{-1}) *K. brevis*	Reduction in cell concentration · mg dry wt^{-1} · hr^{-1} (cells · ml^{-1})
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-1,000	17.7 (1.28)	25,261	1,068	23,260	1,036	113
Lysed-1,000	18.4 (1.02)	25,651	-----	21,414	-----	230
Perna viridis						
Control	43.5 (3.87)	20,736	-----	5,005	-----	362
Whole-10	44.9 (4.38)	21,547	27	5,589	14	355
Lysed-10	43.3 (3.49)	20,928	-----	5,388	-----	359
Whole-100	43.4 (6.18)	21,524	117	10,023	111	265
Lysed-100	44.4 (1.90)	21,285	-----	6,704	-----	328
Whole-1,000	46.5 (7.21)	22,298	1,132	14,891	770	153
Lysed-1,000	40.3 (3.16)	20,944	-----	7,092	-----	344
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-1,000	16.1 (1.43)	22,820	979	19,897	889	182
Lysed-1,000	15.2 (0.79)	23,787	-----	17,370	-----	422
Crassostrea virginica						
Control	43.9 (5.88)	19,210	-----	2,637	-----	378
Whole-10	40.6 (8.99)	18,986	9	3,193	8	389
Lysed-10	47.1 (5.84)	18,800	-----	3,355	-----	328
Whole-100	47.1 (5.43)	19,251	108	3,987	33	324
Lysed-100	50.6 (6.54)	20,364	-----	4,439	-----	315
Whole-1,000	48.3 (8.14)	20,447	1,035	5,557	560	308
Lysed-1,000	45.7 (4.75)	20,493	-----	5,053	-----	338

Table 9

Filtration and clearance rates of juvenile bivalve molluscs exposed to whole and lysed culture of *Karenia brevis* under static conditions. Starting seawater volume in each replicate was 500 ml.

Treatment	Dry tissue (mg) Mean (SD)	Filtration Rate (cells · hr^{-1})	Clearance Rate (ml · hr^{-1})	Weight-Specific Clearance Rate (ml · hr^{-1} · mg dry wt^{-1})
		Argopecten irradians		
Control	19.5 (0.92)	9,087	218	11.19
Whole-10	18.8 (1.34)	8,219	194	10.40
Lysed-10	16.9 (1.95)	7,583	178	10.54
Whole-100	18.2 (1.75)	3,161	67	3.69
Lysed-100	18.4 (1.50)	4,207	89	4.89
Whole-1,000	17.7 (1.28)	2,001	41	2.33
Lysed-1,000	18.4 (1.02)	4,236	90	4.93
		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-1,000	46.5 (7.21)	7,407	205	4.37
Lysed-1,000	40.3 (3.16)	13,852	545	13.60
		Mercenaria mercenaria		
Control	14.9 (1.43)	7,261	191	12.91
Whole-10	16.3 (2.41)	6,999	176	10.99
Lysed-10	16.0 (1.81)	6,488	157	9.93
Whole-100	15.3 (1.01)	5,739	142	9.31
Lysed-100	13.8 (0.98)	7,060	173	12.54
Whole-1,000	16.1 (1.43)	2,923	69	4.28
Lysed-1,000	15.2 (0.79)	6,417	157	10.37
		Crassostrea virginica		
Control	43.9 (5.88)	16,573	613	13.57
Whole-10	40.6 (8.99)	15,794	530	11.91
Lysed-10	47.1 (5.84)	15,444	497	11.76
Whole-100	47.1 (5.43)	15,265	755	10.74
Lysed-100	50.6 (6.54)	15,924	612	10.02
Whole-1,000	48.3 (8.14)	14,889	245	8.42
Lysed-1,000	45.7 (4.75)	15,440	548	8.95

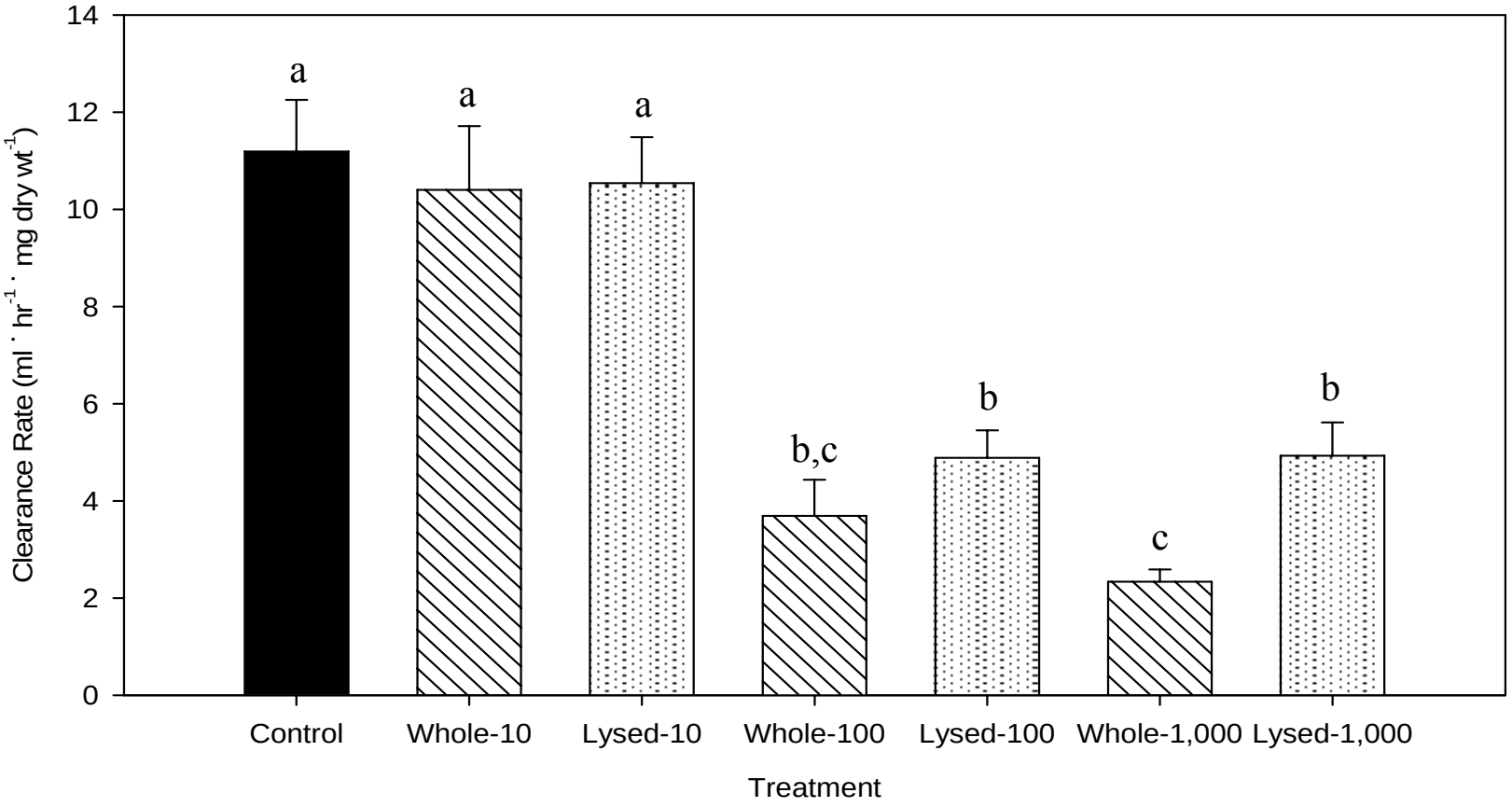

Figure 6. Mean (± S.D.) clearance rate (ml · hr^{-1} · mg dry wt^{-1}) for juvenile bay scallops (*Argopecten irradians*) exposed to three concentrations and two preparations of *Karenia brevis*. Treatments with the same letter are not significantly different ($p > 0.05$). n = 10. Two-way ANOVA; Tukey's Multiple Comparison Test.

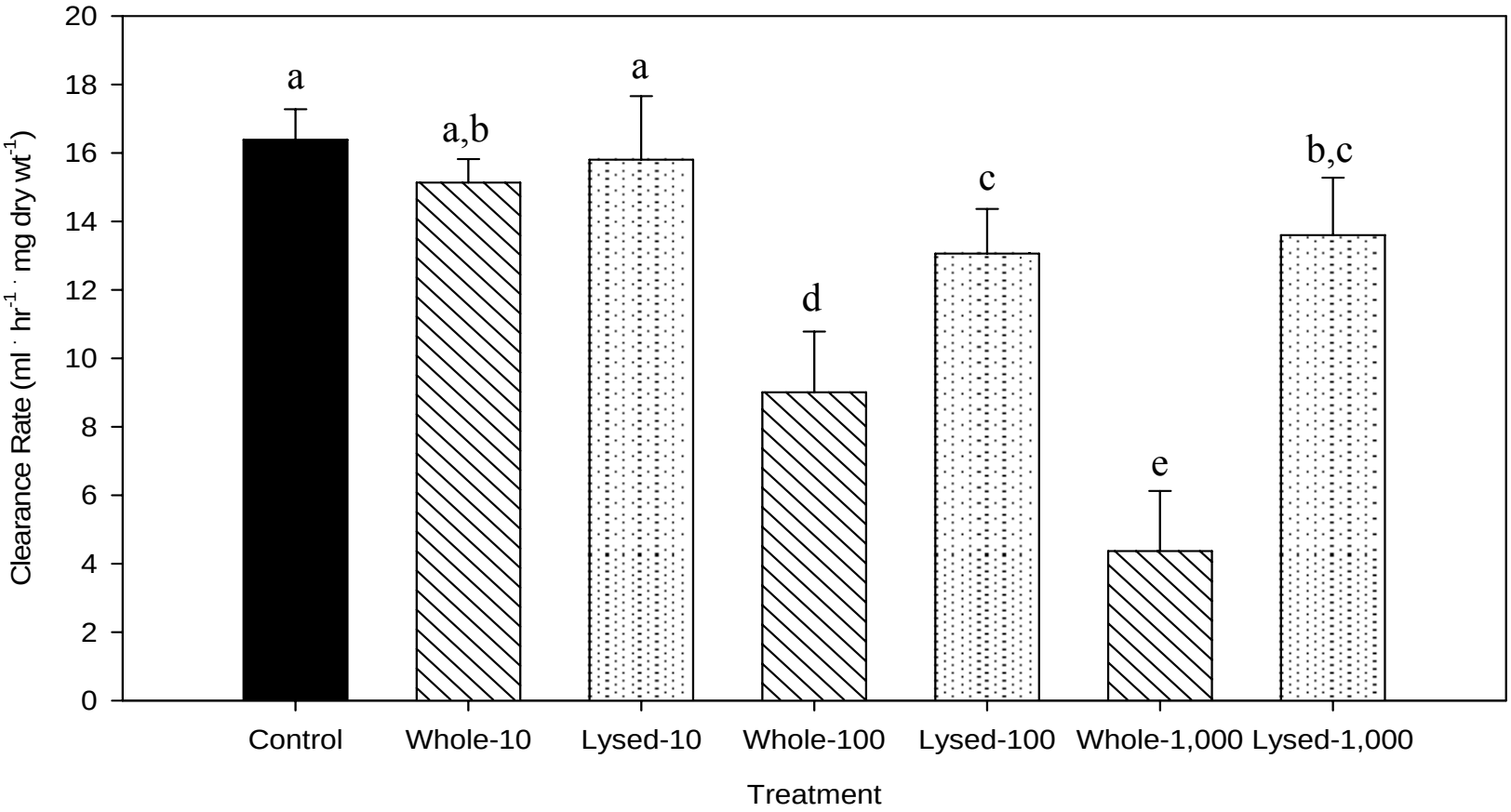

Figure 7. Mean (± S.D.) clearance rate (ml · hr^{-1} · mg dry wt^{-1}) for juvenile green mussels (*Perna viridis*) exposed to three concentrations and two preparations of *Karenia brevis*. Treatments with the same letter are not significantly different ($p > 0.05$). n = 10. Two-way ANOVA; Tukey's Multiple Comparison Test.

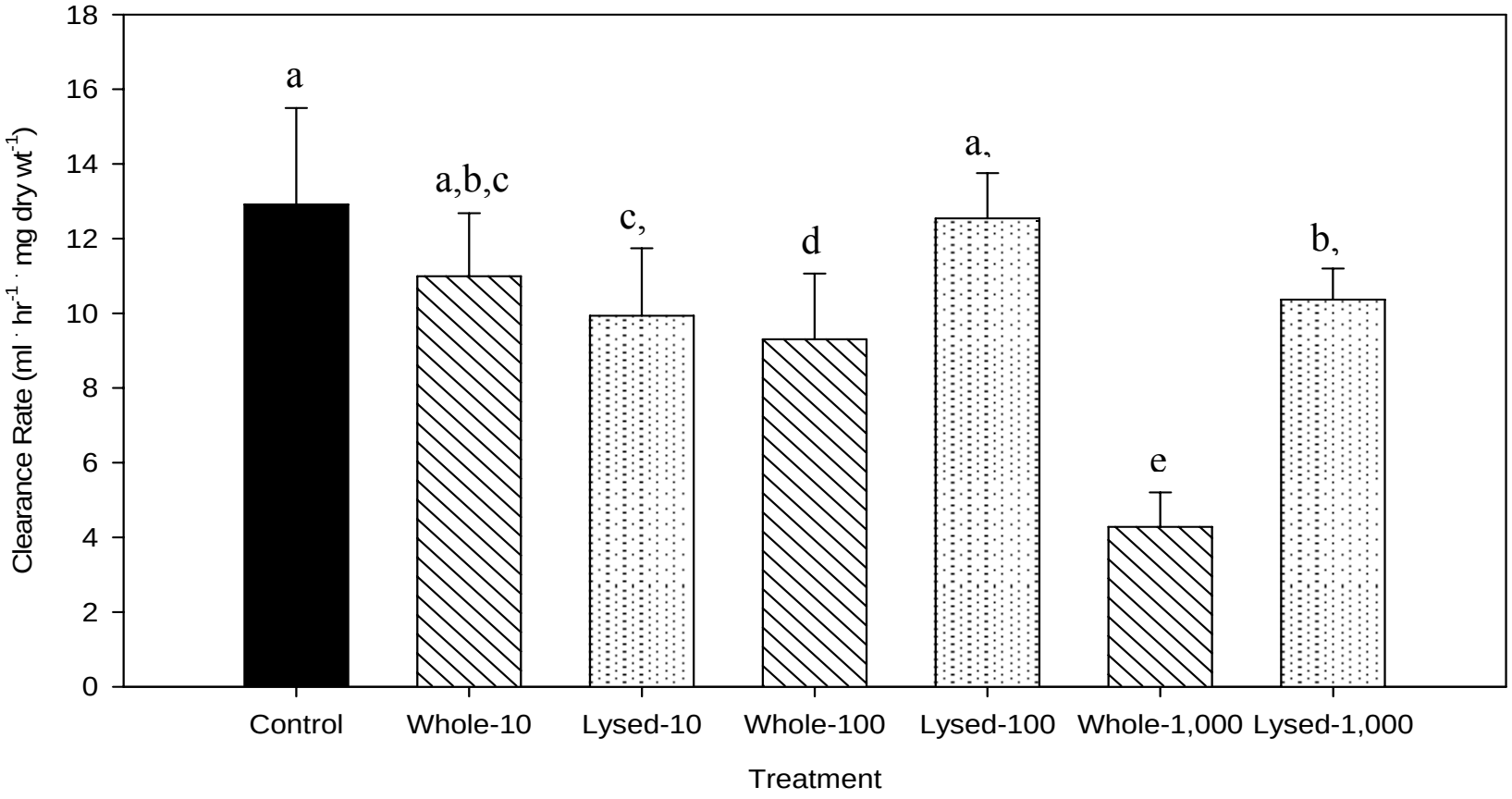

Figure 8. Mean (± S.D.) clearance rate (ml · hr^{-1} · mg dry wt^{-1}) for juvenile northern quahogs (*Mercenaria mercenaria*) exposed to three concentrations and two preparations of *Karenia brevis*. Treatments with the same letter are not significantly different ($p > 0.05$). n = 10. Two-way ANOVA; Tukey's Multiple Comparison Test.

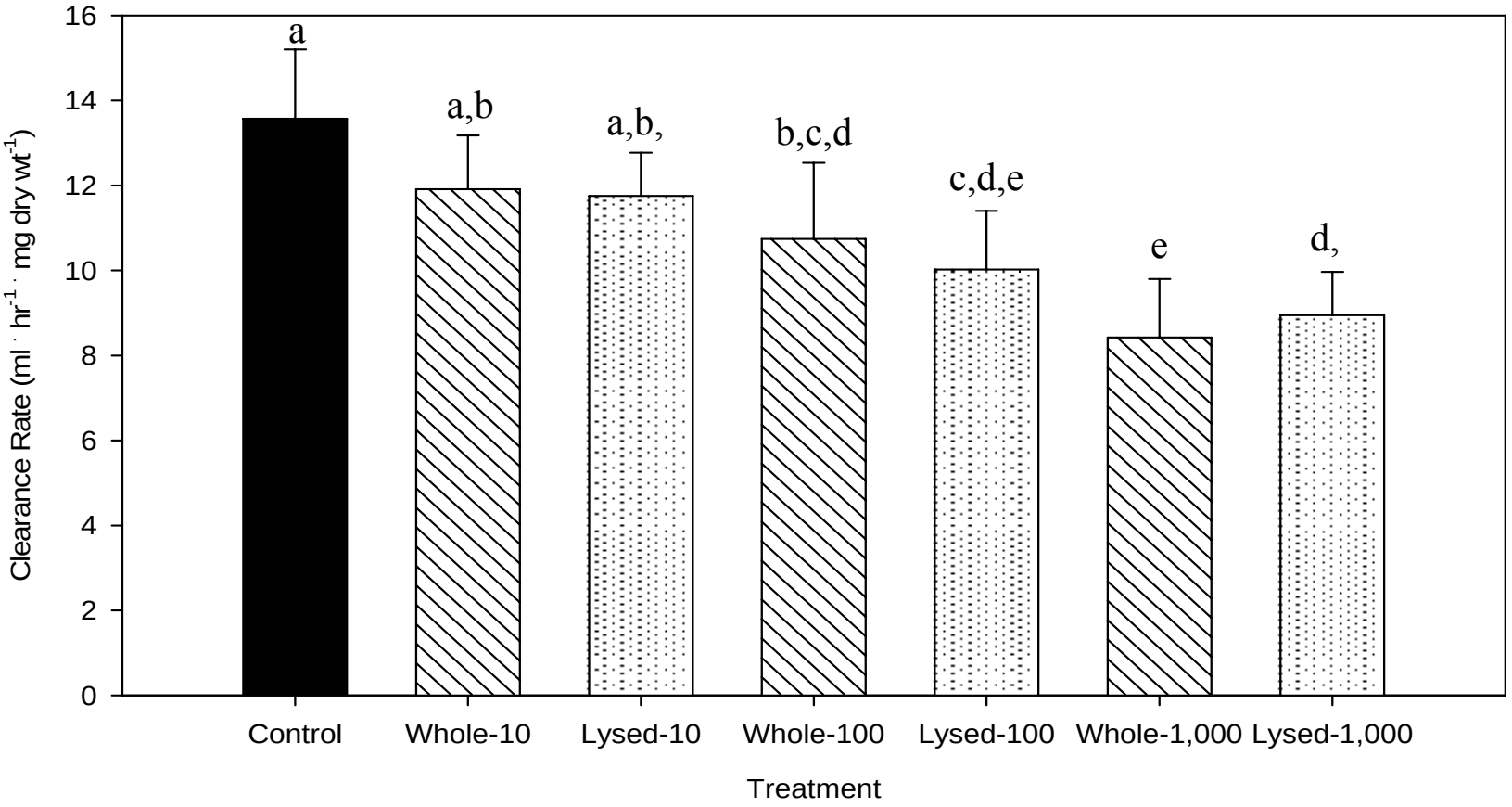

Figure 9. Mean (± S.D.) clearance rate (ml · hr^{-1} · mg dry wt^{-1}) for juvenile eastern oysters (*Crassostrea virginica*) exposed to three concentrations and two preparations of *Karenia brevis*. Treatments with the same letter are not significantly different ($p > 0.05$). n = 10. Two-way ANOVA; Tukey's Multiple Comparison Test.

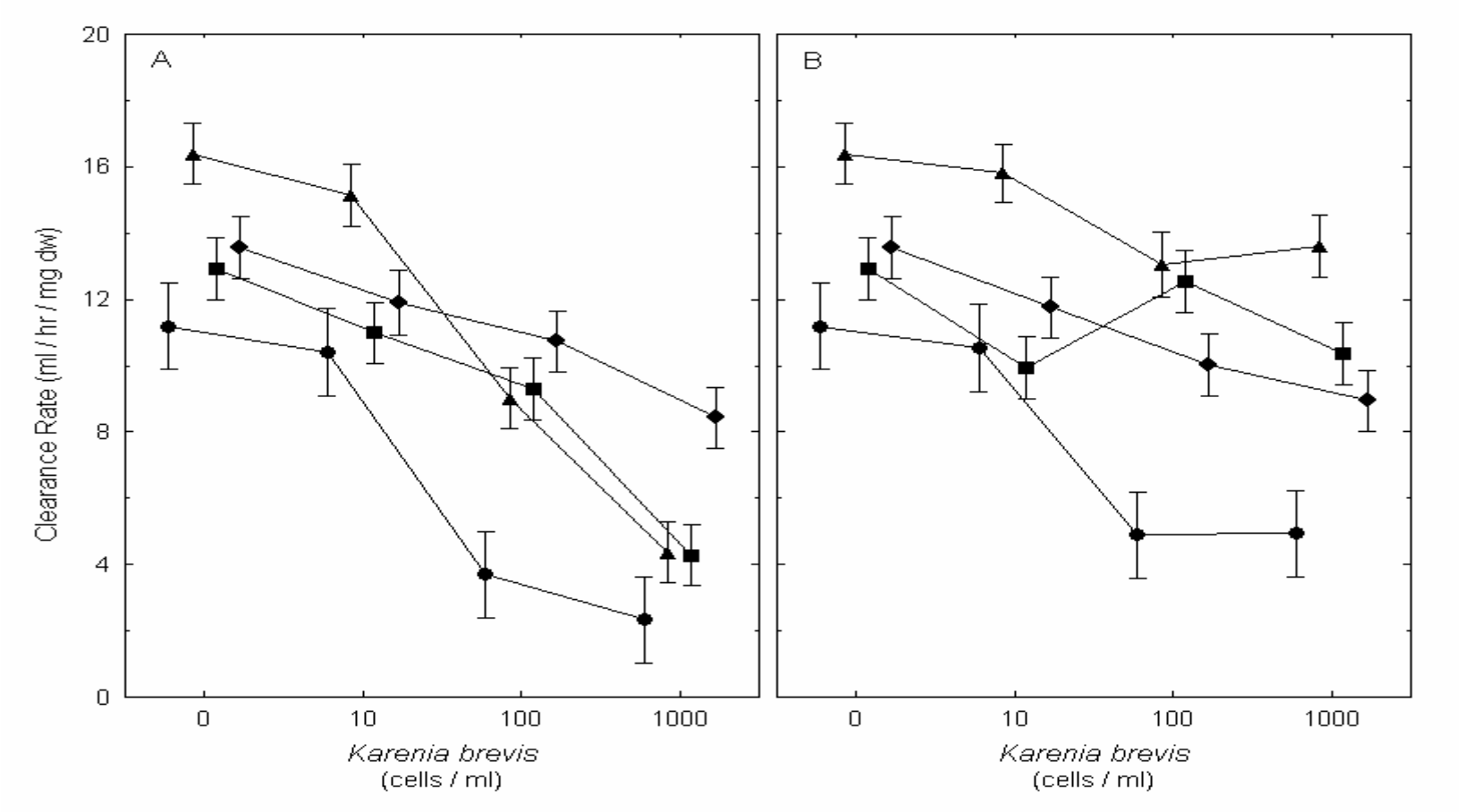

Figure 10. Mean (± S.D.) clearance rate (ml · hr^{-1} · mg dry wt^{-1}) for juvenile bivalves exposed to (A) whole and (B) lysed cultures of *Karenia brevis* under static conditions. ● = *Argopecten irradians*; ▲ = *Perna viridis*; ■ = *Mercenaria mercenaria*; and ◆ = *Crassostrea virginica*. (n = 10).

Table 10

Species, treatment, clearance rate and amount of toxin (µg) each species was exposed to during static feeding experiments. Amount of toxin exposure is based on culture cell concentration and amount of toxic (w/v) in each culture.

Species	Treatment (cells · ml^{-1})	Clearance Rate (ml · min^{-1} · mg dry wt^{-1}) Whole	Lysed	Culture Toxins (µg · L^{-1}) 67.8 Whole	41.2 Lysed
Bay scallop (*Argopecten irradians*)	10	10.4	10.54	0.015	0.009
	100	3.69	4.89	0.154	0.094
	1,000	2.33	4.93	1.541	0.936

	Treatment (cells · ml^{-1})	Clearance Rate (ml · min^{-1} · mg dry wt^{-1}) Whole	Lysed	Culture Toxins (µg · L^{-1}) 23.1 Whole	28.4 Lysed
Green mussel (*Perna viridis*)	10	15.13	15.8	0.005	0.007
	100	9.01	13.06	0.053	0.066
	1,000	4.37	13.6	0.533	0.656

	Treatment (cells · ml^{-1})	Clearance Rate (ml · min^{-1} · mg dry wt^{-1}) Whole	Lysed	Culture Toxins (µg · L^{-1}) 40.3 Whole	60.5 Lysed
Northern quahog (*Mercenaria mercenaria*)	10	10.99	9.93	0.009	0.014
	100	9.31	12.54	0.092	0.138
	1,000	4.28	10.37	0.916	1.375

	Treatment (cells · ml^{-1})	Clearance Rate (ml · min^{-1} · mg dry wt^{-1}) Whole	Lysed	Culture Toxins (µg · L^{-1}) 63.5 Whole	80.5 Lysed
Eastern oyster (*Crassostrea virginica*)	10	11.91	11.76	0.015	0.019
	100	10.74	10.02	0.149	0.189
	1,000	8.42	8.95	1.491	1.890

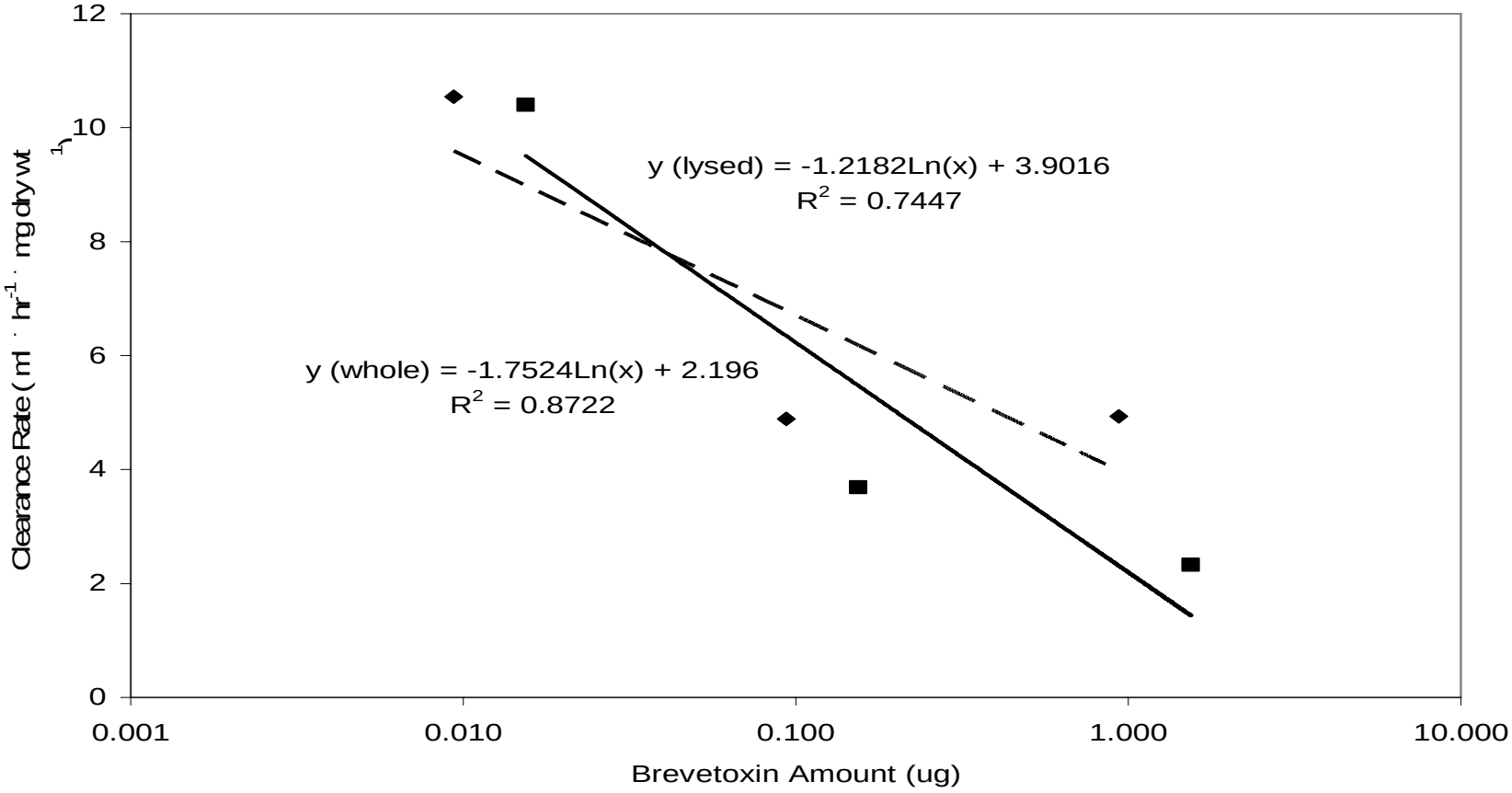

Figure 11. Regression of clearance rates for juvenile *Argopecten irradians* against the amount of brevetoxin exposure under static conditions. Solid line represents regression for whole culture of *Karenia brevis* (■); dashed line represents regression for lysed culture of *K. brevis* (♦). Regression equation shown for each line. (n = 5).

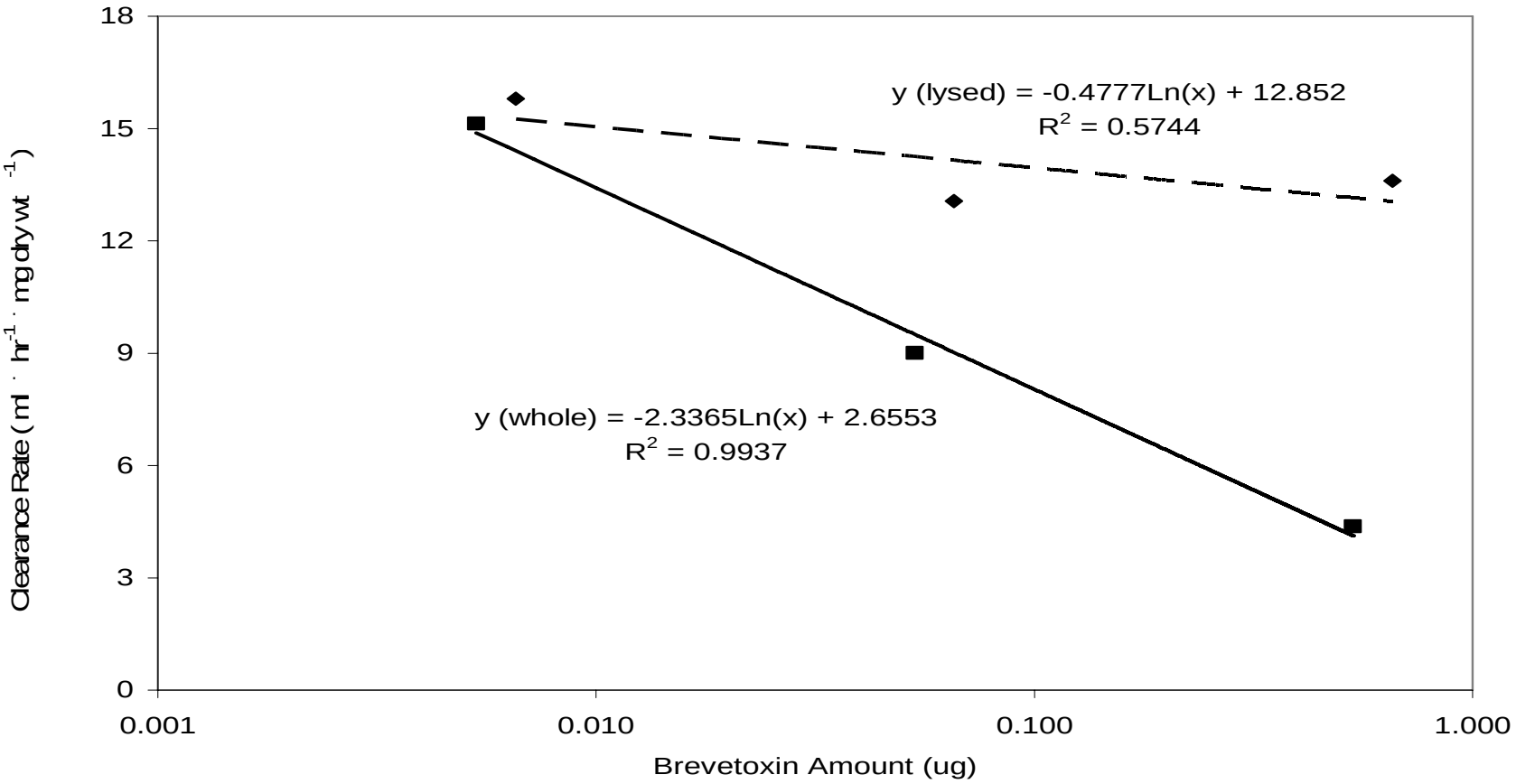

Figure 12. Regression of clearance rates for juvenile *Perna viridis* against the amount of brevetoxin exposure under static conditions. Solid line represents regression for whole culture of *Karenia brevis* (■); dashed line represents regression for lysed culture of *K. brevis* (♦). Regression equation shown for each line (n = 5).

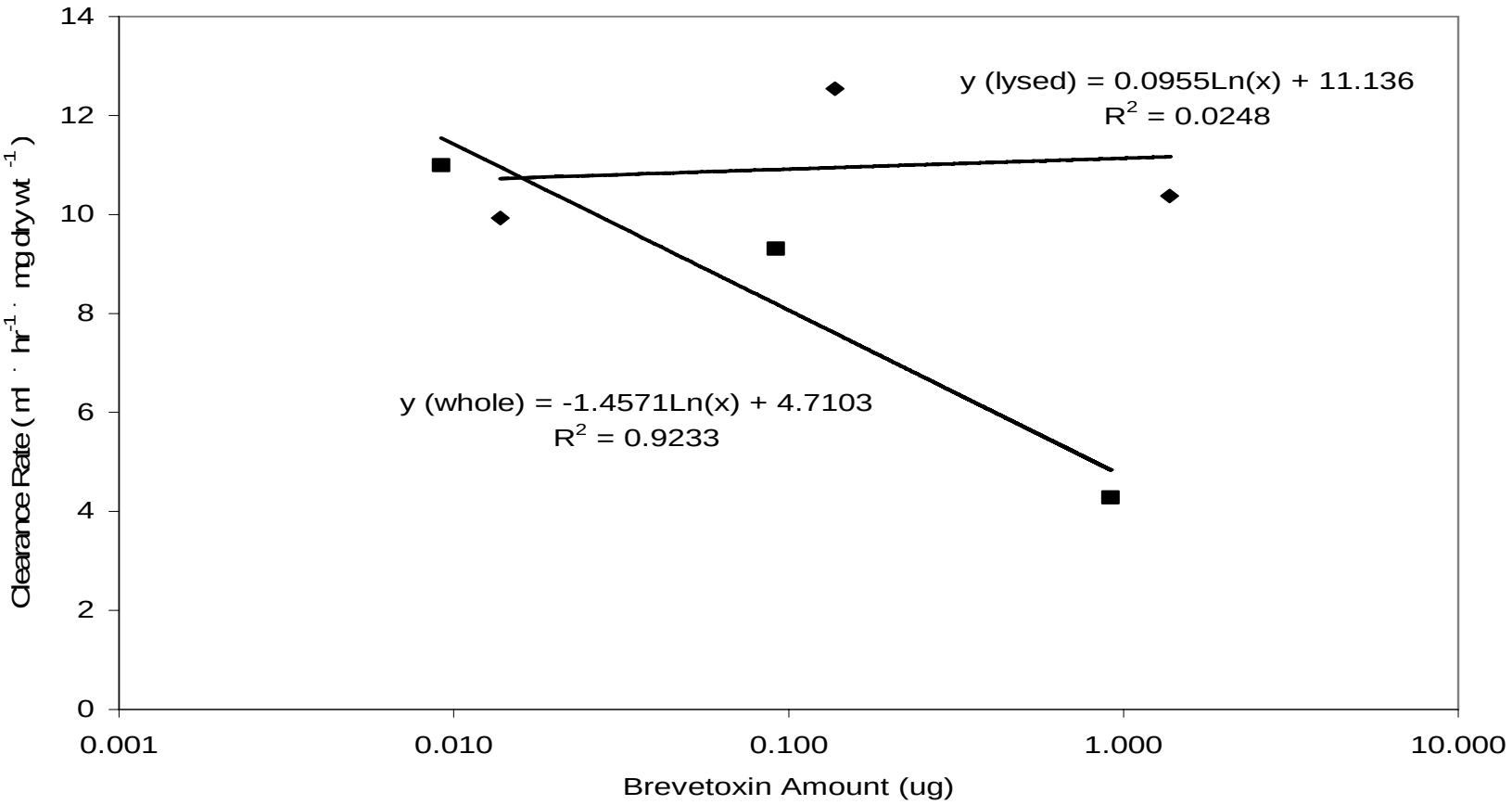

Figure 13. Regression of clearance rates for juvenile *Mercenaria mercenaria* against the amount of brevetoxin exposure under static conditions. Solid line represents regression for whole culture of *Karenia brevis* (■); dashed line represents regression for lysed culture of *K. brevis* (♦). Regression equation shown for each line (n = 5).

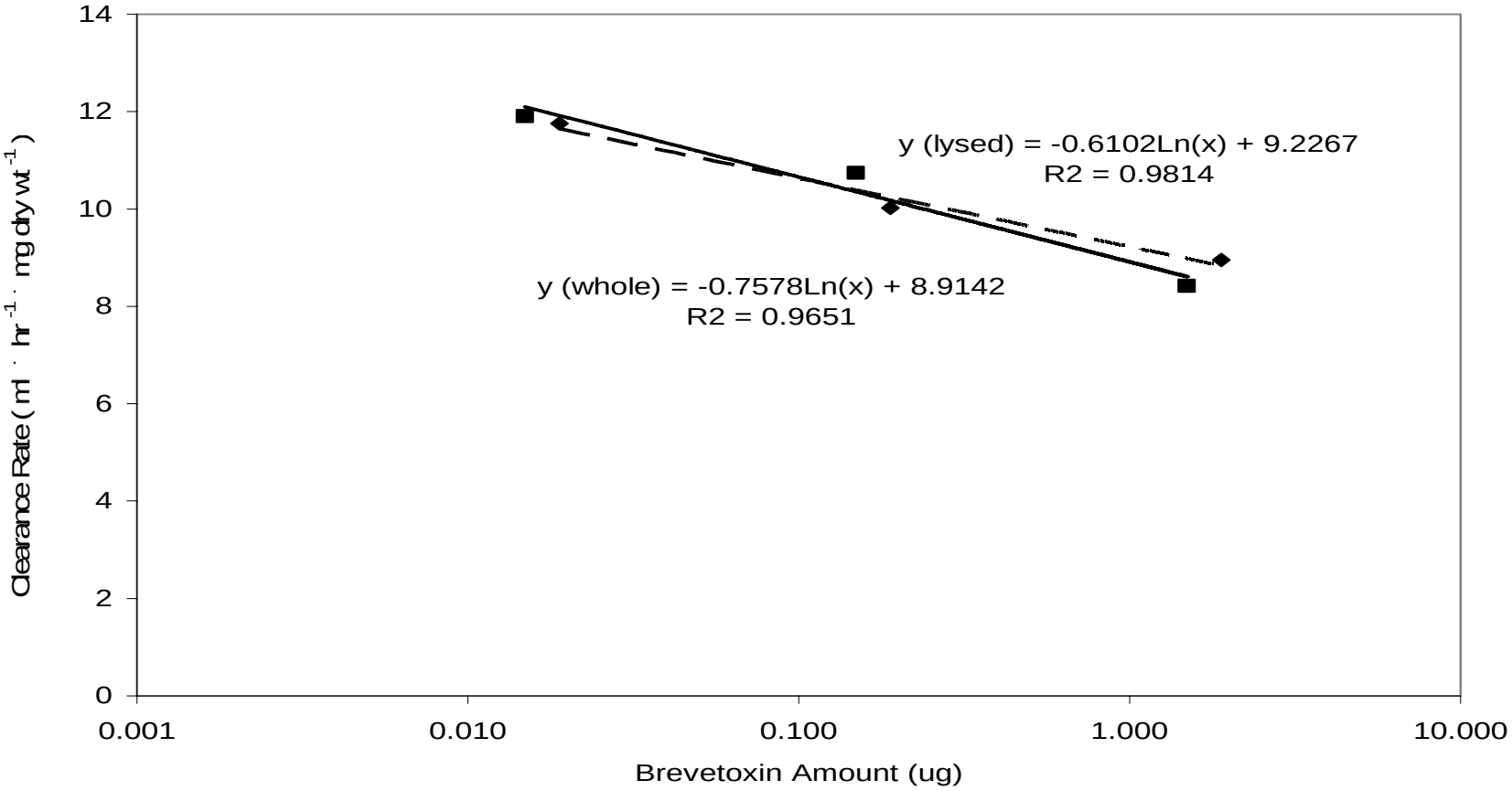

Figure 14. Regression of clearance rates for juvenile *Crassostrea virginica* against the amount of brevetoxin exposure under static conditions. Solid line represents regression for whole culture of *Karenia brevis* (■); dashed line represents regression for lysed culture of *K. brevis* (♦). Regression equation shown for each line (n = 5).

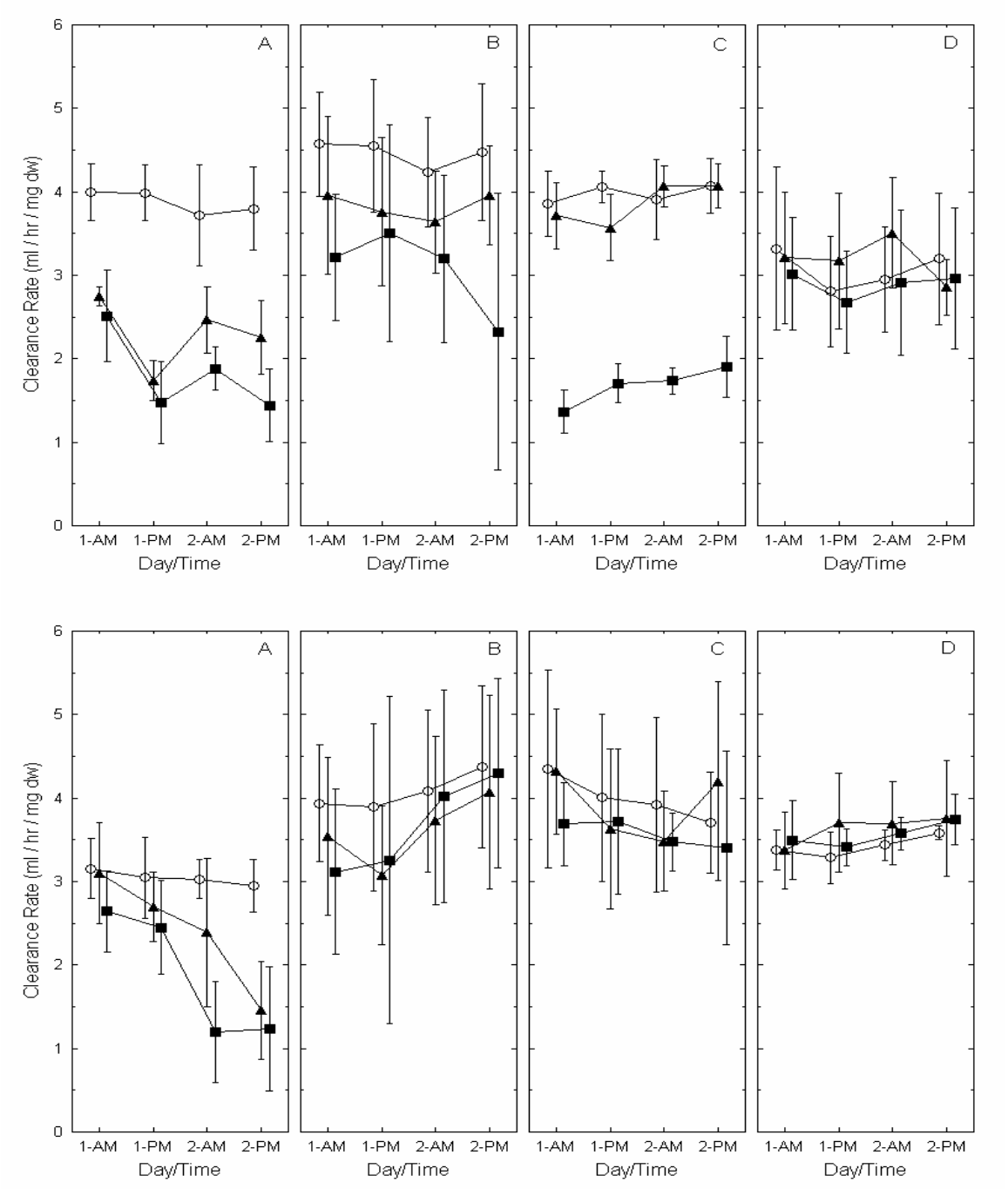

Figure 15. Mean (± S.D.) clearance rate (ml · hr^{-1} · mg dry wt^{-1}) of juvenile bivalves exposed to whole (top) and lysed (bottom) cultures of *Karenia brevis* under flow-through conditions. Species include (A) *Argopecten irradians*, (B) *Perna viridis*, (C) *Mercenaria mercenaria*, and (D) *Crassostrea virginica*. Concentrations of *K. brevis* in each treatment are: Control (○), 100 (▲) and 1,000 (■) 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 a supplemental food algae, *Isochrysis galbana*.

Chapter Five

Histopathology Studies

Introduction

Studies on bivalve exposure to harmful microalgae have mostly focused on short-term acute and lethal effects. The consequences of more long-term, chronic or sublethal contact with toxic algae and/or bioaccumulated toxins have received less attention (Shumway and Cucci, 1987; Shumway, 1990; Landsberg, 1996). Chronic exposure to biotoxins typically leads to impaired feeding, avoidance behaviors, physiological dysfunction, weakened immune function and reduced growth and reproduction (Shumway, 1990; Wikfors and Smolowitz, 1993), which in turn may lead to an increased susceptibility to disease, abnormal development, histopathologies and the induction of neoplasia (Landsberg, 1996). Types of histopathologies that have been observed include mantle and gill lesions (Nielsen and Strømgren, 1991; Smolowitz and Shumway, 1997), cellular changes and increased lumen diameter within the digestive diverticula (Wikfors and Smolowitz, 1993), reproductive abnormalities, protozoan and/or bacterial infections (Smolowitz and Shumway, 1997) and disseminated neoplasia and germinomas (see Landsberg, 1996 for review; Barber, 2004).

The impact of *Gyrodinium aureolum* on the histology of gut tissue from eight species of juvenile bivalve was species-specific (Smolowitz and Shumway, 1997). The

eastern oyster (*Crassostrea virginica*) and the bay scallop (*Argopecten irradians*) were the most severely affected species. Several *C. virginica* showed mantle and gill lesions. Bay scallops exhibited a decrease in the height of absorptive cells and an increase in lumen diameter after exposure, suggesting *G. aureolum* is of poor food quality. Evidence of toxic effects was not identified in the digestive gland. Several bay scallops also showed variable amounts of inflammation in the kidney associated with protozoal infestations and variable amounts of predominately rod-shaped bacteria within the urinary space.

Bay scallops exposed to a *Prorocentrum* isolate also showed tissue abnormalities (Wikfors and Smolowitz, 1993). Bay scallops fed mixed diet of *P. minimum* and *Isochrysis galbana* showed distinctive lesions. Control scallops showed normal, well-developed digestive diverticula while experimental scallops exhibited an assortment of abnormalities in this organ, including contracted absorptive cells, abnormal vacuolation, necrosis of absorptive cells and their exfoliation into the lumen. All other organs (gills, muscle, kidney, foot and heart) in the experimental group appeared moderately to well developed (Wikfors and Smolowitz, 1993). Another pectinid, the king scallop (*Pecten maximus*) developed obvious saxitoxin neoformation in kidneys after exposure to paralytic shellfish poisoning toxins (Bougrier *et al*., 2000).

The mussel *Mytilus edulis* was shown to be cytotoxic in the presence of the dinoflagellate, *Gyrodinium aureolum*, which had an acute effect on the clearance rate and caused marked cellular damage to the gut (Widdows *et al*., 1979). Likewise, exposure of juvenile hard clams (*Mercenaria mercenaria*) and blue mussels (*Mytilus edulis*) to a toxic isolate of the picoplankter *Aureococcus anophagefferens* (which causes brown tides in

coastal bays of the mid-Atlantic USA) caused reduction in digestive epithelium height and overall appearance of absorptive cells (Bricelj *et al*. 2004). These observations are similar to those in bivalves that have undergone starvation.

The effects of long-term exposure of bivalves to *Karenia brevis* have not yet been studied. Consequently, we do not know if the brevetoxins produced by *K. brevis*, which are responsible for neurotoxic shellfish poisoning (NSP) in the Gulf of Mexico, have a role in the initiation of any specific pathologies in bivalve tissues (Landsberg, 1996). Although brevetoxins have been well known for their role in fish kills (Steidinger *et al*., 1973), their role in developing histopathologies in bivalve molluscs is unknown. Of the four bivalves studied in the present work, the bay scallop (*Argopecten irradians*) has been the most sensitive to *K. brevis* exposure. Therefore, this chapter focuses on the histological effects of long-term exposure to *K. brevis* in adult bay scallops. Furthermore, the effects of whole and lysed cultures of *K. brevis*, in unialgal and mixed suspensions, are examined in the following tissues: digestive diverticulum, mantle and gill.

Materials and Methods

Adult bay scallops (*Argopecten irradians*) were collected from the Anclote Anchorage (28^{o} 17'N; 82^{o} 45'W) and Hommosassa Springs (28^{o} 43'N; 82^{o} 43'W) on June 30, 2006 and transferred to Mote Marine Laboratory. Scallops were gently scrubbed to remove any attached fauna or debris and equally divided into five separate 25-liter aquaria. Aquaria were mildly aerated and maintained at 32 ppt salinity and 27^{o} C

in a temperature-controlled exposure room. Scallops were suspended above the aquaria bottom by a mesh partition to allow for the settlement of feces. Scallops were allowed to acclimate without food for two days prior to the start of the experiment.

Each aquarium held twenty-five scallops at the start of the experimental exposure. For those treatments receiving *Isochrysis galbana* and *Karenia brevis*, algal concentrations were set at 1×10^5 cells $\cdot$ ml^{-1} and 5×10^2 cells $\cdot$ ml^{-1}, respectively. Experimental conditions are summarized in Table 11. A water exchange (ca. ninety percent) was made each day and algal concentrations adjusted to maintain the desired concentrations. A sample (n = 4) of individuals was removed from each aquaria on days 2, 7 and 14 and fixed for histology. Scallops were observed daily and any individual showing signs of stress or abnormal behavior (i.e., shell gaping or mantle retraction) was immediately removed and fixed.

Shell height was measured before dissection. Scallops were dissected and the mantle, gill and digestive gland were fixed in Davidson's fixative (Howard and Smith, 1983). Tissues remained in fixative for 48 hours before being transferred to 70% ethanol where they remained until embedding. Each tissue was processed in paraffin (Tissue Prep™), five μm sections prepared and stained with hematoxylin and eosin (Howard and Smith, 1983).

Sections were observed under magnification to determine if any abnormalities had developed after a two week exposure to *K. brevis*. Results are descriptive and qualitative in nature. Photomicrographs accompany descriptive pathologies.

Results

Table 12 summarizes the sampling schedule, withdrawals and mortality of *A. irradians* during the two-week exposure to *K. brevis*. Mean shell height was approximately 50 mm in all treatments. All scallops in every treatment survived the first week of exposure. On day nine, several scallops began to show signs of stress, indicated by slight gaping of the shell valves and partial retraction of the mantle edges. These included one scallop from Tank 3 (*K. brevis* only) and two from Tank 5 (lysed *K. brevis* and *T. iso*). At the end of day twelve, only scallops from Tank 1 (Control) and Tank 4 (whole *K. brevis* and *T. iso*) were still alive. Scallops from both of these treatments were still alive on day fourteen when the experiment was terminated.

Various parasitic infections were observed in gill and digestive tissue from scallops in all treatments. The most common infections were ciliates (*Nematopsis* sp?) and Rickettsias-like bacterial infections (Fig. 16). An unidentified parasite within the digestive diverticula is pictured in Fig. 17.

On the other hand, several histomorphologies, particularly in the digestive diverticula, were observed that appear to be associated with several of the exposure scenarios to *K. brevis*. The most noticeable and pervasive of these pathologies was the presence of hemocyte aggregations and infiltrations in the digestive diverticulum. This particular pathology, which is indicative of an inflammatory response, was found to some degree in all treatments, but was particularly associated with scallops that were either starved or exposed to lysed *K. brevis* and *Isochrysis* (Tank 5; Fig. 18). This pathology first appeared on day 2 in starved scallops; in the other treatment it appeared on day 7

and was present through the remainder of the experiment. The relative intensity of this inflammatory response was scored on a scale of 0 – 3+ and summarized in Table 13.

The appearance of the epithelial layer in the digestive diverticula showed a range of atrophic degradation, including variously reduced thickness of the epithelial layer and reduced sizes of the digestive tubules. These changes were pervasive throughout all treatments except the control and were noticeable from as early as day two in starved scallops. The degree of modification of the epithelial layer is shown in Fig. 19.

Gill and mantle tissue from *A. irradians* exposed to *K. brevis* did not show any obvious or noticeable histopathologies. The epithelia of the ordinary filaments in the gill appeared normal, as did the supporting structures, septa and cilliary tracts (Fig 20A). The variously-shaped interconnecting vessels of the dorsal expansion of the gill also appeared normal and healthy (Fig 20B). Finally, the free edge of the mantle, which is divided into three folds and two grooves, showed no deformities or abnormalities (Fig 21).

Discussion

Bivalve parasites, notably the Rickettsiales and Protista, are commonly found in the epithelial cells of the gills and digestive diverticula of many species, including scallops (Chang *et al*., 1980). Most infections appear benign, despite relatively dense colonization. Light to moderate rickettsial-like infections of the gill have been previously found in wild, captive and cultured adult bay and sea scallops by Leibovitz et al. (1984). The coccideans, *Nematopsis ostrearum* and *N. duorari*, have been found in

bay scallops, but no pathogenicity has been described for these infections (Kruse, 1966; Sprague, 1970). In the present study, bacterial infections were not intense, nor were they predominant in any particular treatment or related to time of exposure to *K. brevis*. The presence of bacterial and protist parasites did not appear to be positively associated with any of the observed cytological histopathologies in this study.

This study showed distinctive and pervasive hemocytic infiltrations in the digestive diverticula of *A. irradians*, particularly in individuals that had either been starved or exposed to lysed culture of *K. brevis*. Hemocytes are known to recruit from circulation to sites of inflammation and tissue damage (Cheng, 1967). The fact that a higher incidence of inflammation occurred from exposure to lysed cultures suggests a cytotoxic response rather than a reaction to the actual dinoflagellate. Damage to adsorptive cells in the digestive diverticula and systemic pathologies characteristic of toxin effects has previously been observed in juvenile bay scallops by Wikfors and Smolowitz (1993) after exposure to a diet which included *Prorocentrum minimum*. These scallops suffered rapid mortality. Similar changes in the digestive cells of the mussel *Mytilus edulis* were noted during a bloom of *Gyrodinium aureolum* and were attributed to a toxic response rather than a result of starvation (Widdows et al., 1979). Additional studies are necessary to elucidate the mechanism by which toxicity from harmful algae leads to such rapid mortality in the bay scallop (as opposed to other bivalves).

Decreased height of absorptive cells and increased lumen diameter suggest that *K. brevis* is, at best, a poor quality food (Smolowitz and Shumway, 1997). Starved bivalves display similar changes in epithelia of the absorptive cells (Wikfors and Smolowitz,

1995). Smolowitz and Shumway (1997), however, did not observe sloughing of digestive epithelial cells in juvenile *A. irradians* exposed to *Gyrodinium aureolum*, leading them to conclude that there was probably no toxic effect. In this study, there were signs of epithelial sloughing which lends support to an unknown toxic mechanism (in addition to poor nutritional processes) in the digestive diverticula of *A. irradians* exposed to *K. brevis*. The use of a nontoxic dinoflagellate in addition to *K. brevis* in future feeding studies might help elucidate the histological differences between nutritional and toxic responses in bay scallop digestive tissues.

Table 11. Experimental design for two-week exposure of *Argopecten irradians* to *Karenia brevis*. (*Isochrysis galbana* is a common nutritional chrysophyte algae).

Treatment	**Tank 1**	**Tank 2**	**Tank 3**	**Tank 4**	**Tank 5**
Isochrysis galbana (1×10^5 cells $\cdot$ ml^{-1})	Yes	No	No	Yes	Yes
Karenia brevis (5×10^2 cells $\cdot$ ml^{-1})	No	No	Yes	Yes	Yes
Whole or lysed *Karenia brevis*	None	None	Whole	Whole	Lysed

Table 12. Schedule for the removal of *Argopecten irradians* from each experimental tank during the two-week exposure to *Karenia brevis*. Values represent shell height (mm) of individuals removed for fixation (shaded) or dead (unshaded) on that day.

Day	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5
	Control (*T. iso* only)	**Starved (no algae)**	**Whole *K.b.* only**	**Whole *K.b.* + *T. iso***	**Lysed *K.b.* + *T. iso***
0					
2	54.9	60.8	59.8	56.5	52.5
	52.4	58.9	56.0	57.1	55.6
	53.1	56.1	55.6	53.2	52.0
	46.4	50.1	46.8	50.1	46.5
3					
4					
5					
6					
7	31.3	40.9	44.1	41.4	40.2
	42.1	44.6	53.2	43.9	42.6
	49.7	48.7	48.8	46.1	48.6
	49.3	54.2	47.7	54.1	52.2
8	59.0			43.0	46.2
9			44.9		53.9
			57.3		45.2
10		52.9			52.2
11					
12		53.7	52.7		
		45.2	43.5		
		55.6			
13					
14	53.4			56.4	
	52.3			55.1	
	49.3			48.3	
Mean	**49.7**	**51.5**	**51.4**	**51.5**	**50.8**
S.D.	**6.49**	**5.80**	**4.96**	**5.65**	**5.49**

Table 13. Scoring of hemocyte infiltration intensity in the digestive diverticula of *A. irradians* exposed to various scenarios of *K. brevis*.

	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5
Day	Control	Starved	Whole *K. brevis* only	Whole *K. brevis* & T. *Iso*	Lysed *K. brevis* & T. *Iso*
2	0 0 0 1+	1 - 2+ 2 - 3+ 2+ 1+	0 0 0 - 1+ 1+	0 0 0 - 1+ 2+	0 0 - 1+ 0 0
7	0 1+ 1+ 0	1 - 2+ 1+ 2+ 2+	0 0 - 1+ 0 - 1+ 0	0 - 1+ 1+ 0 - 1 + 3 +	2+ 2+ 3+ 2+
9			0		2+ 3+
10					0
12			0 - 1+ 0		
14	0 - 1+ 0 1+ 0			0 0 - 1+ 0	

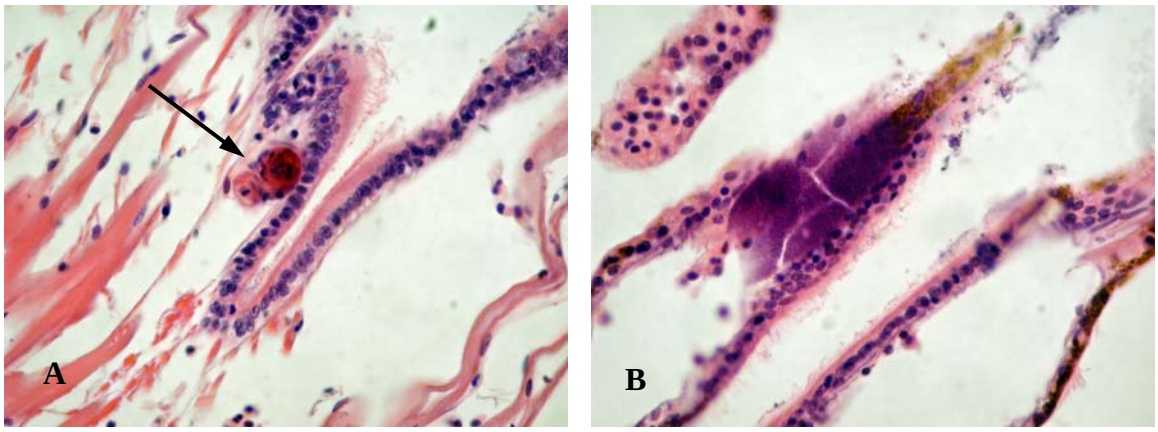

Figure 16. (A) The protist, *Nematopsis* sp? (arrow) and (B) a Rickettsial-like bacterial microcolony in the gill epithelia of *A. irradians*. Magnification equals 400x.

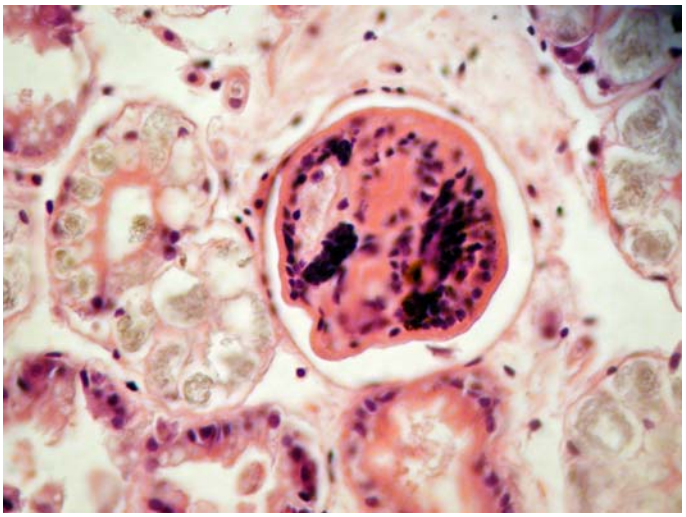

Fig. 17. An unidentified parasite encysted within the digestive diverticula. Magnification equals 400x.

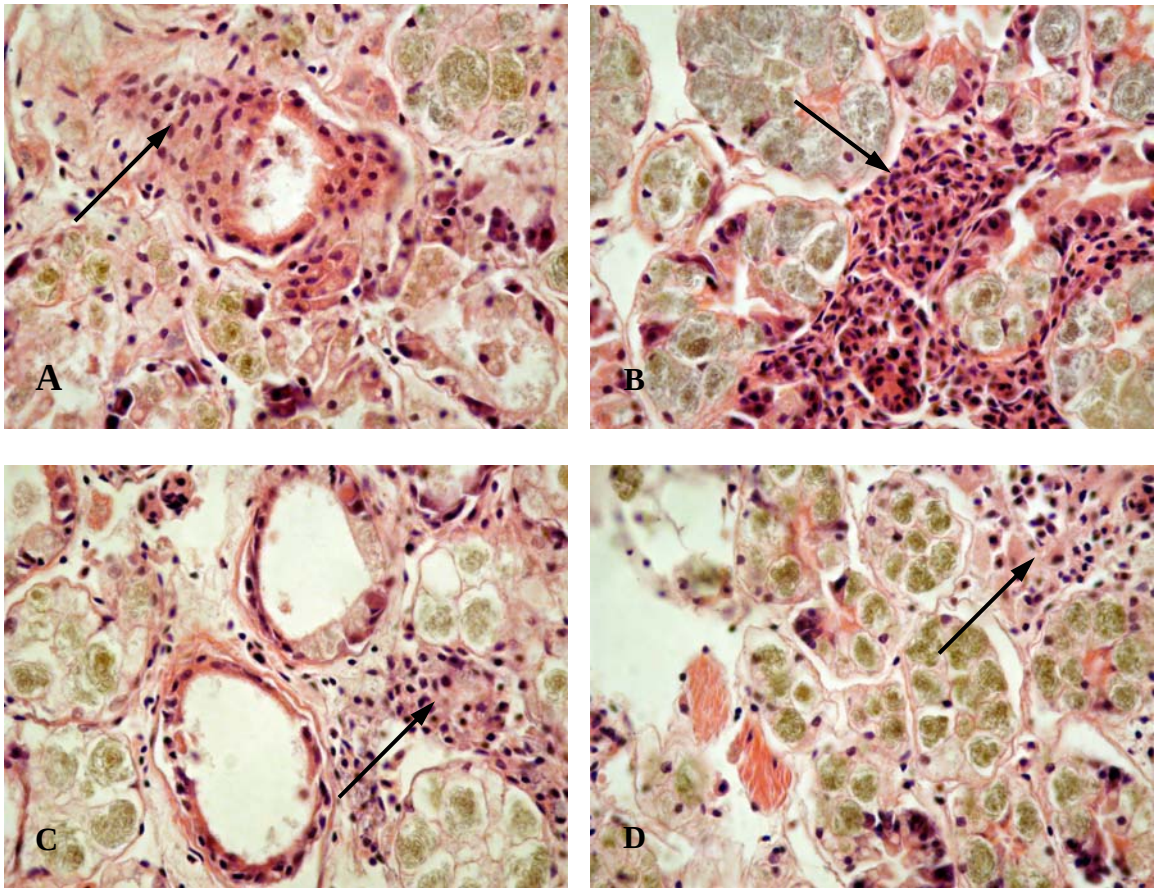

Figure 18. Digestive diverticula from *A. irradians* exposed to lysed culture of *K. brevis* and T. *Isochrysis*. (A and B = day 7; C and D = day 9). Hemocyte aggregations (arrows) displaying inflammatory response. Magnification equals 400x.

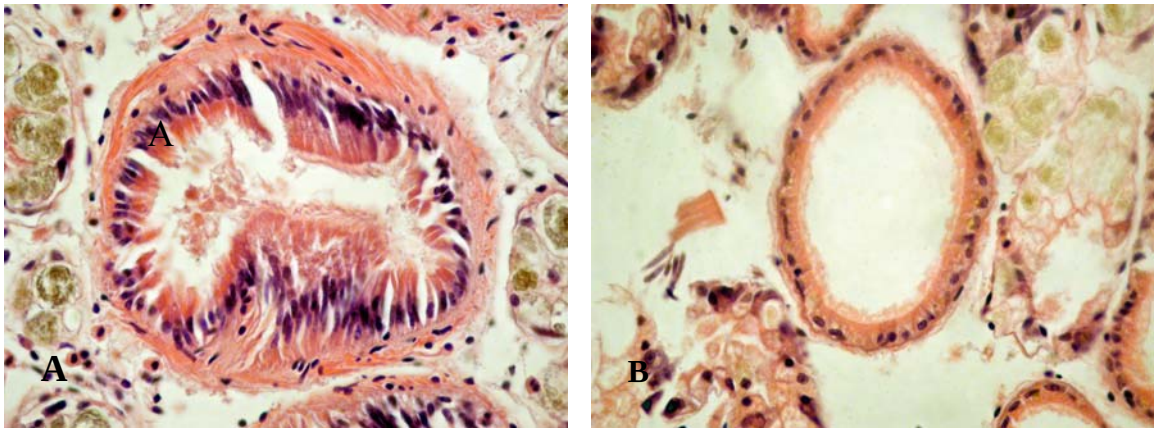

Figure 19. Epithelial layer of the digestive diverticula in *A. irradians* from different exposure scenarios to *K. brevis*. A) control scallop at 14 days; B) scallop exposed to whole culture of *K. brevis* at day 12. Magnification equals 400x.

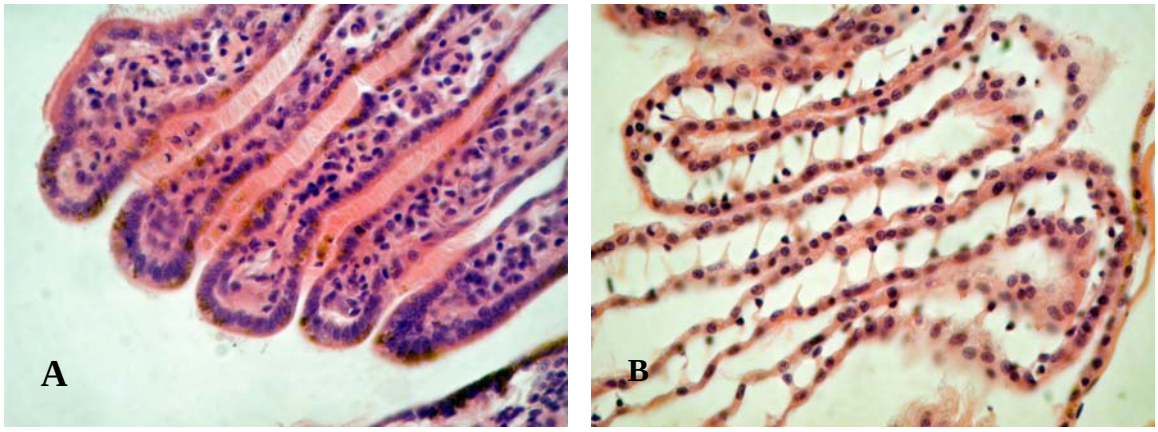

Figure 20. Gill tissue from *A. irradians* showing A) the distal portions of the ordinary filaments (note the lateral cilia) and B) interconnecting vessels of the dorsal expansion.

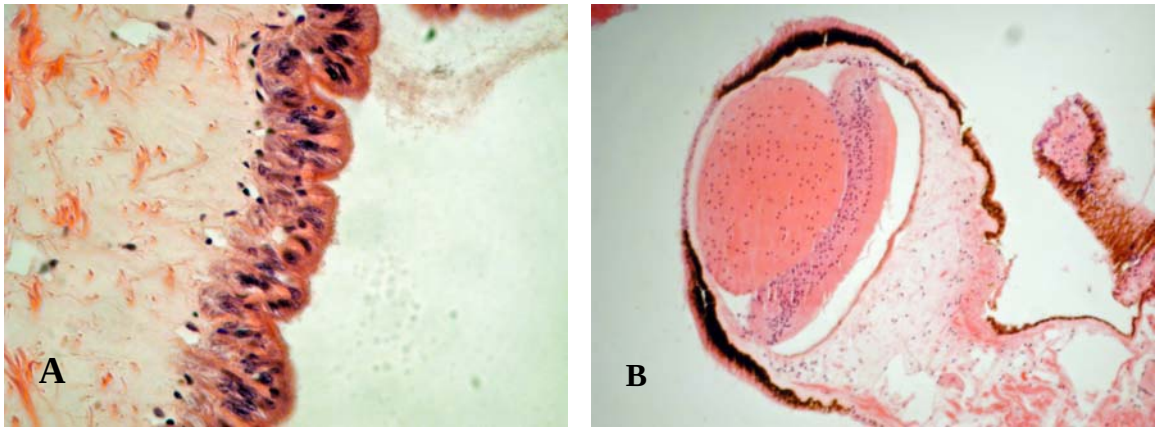

Figure 21. Mantle tissue from *A. irradians* showing A) the epithelia of the mantle margin and B) section through an eye on the middle fold of the mantle margin.

Chapter Six

General Discussion

Florida red tides caused by the dinoflagellate *Karenia brevis* are some of the oldest reported harmful algal blooms, with fish kills being reported since the middle of the nineteenth century (Ingersoll, 1882). These massive fish mortalities are the most consistent observation from reports of red tides, and much has since been learned regarding the toxic mechanisms involved in fish mortality (see Landsberg, 2002 for review). Filter feeding bivalve molluscs also become contaminated with these toxins during blooms of *K. brevis*, and cause a disease called neurotoxic shellfish poisoning in humans who consume contaminated shellfish. The focus of previous research on *K. brevis* and bivalves has been on the human health consequences of eating shellfish contaminated with these toxins, called brevetoxins, or their analogs (Hemmert, 1975). Studies of *K. brevis* and its effects on bivalves themselves are much more limited and can be divided into three categories: 1) observations of bivalve mortalities from natural blooms (Simon and Dauer, 1972; Tiffany and Heyl, 1978); 2) toxicity of shellfish exposed to laboratory cultures (Ray and Aldrich, 1967; Sievers, 1969; Hemmert, 1975) or blooms in the field (Cummins *et al*., 1971; Tester and Fowler, 1990; Wang *et al*., 2004); and 3) the dynamics of toxin uptake, metabolism and elimination after controlled exposure to *K. brevis* cultures and/or pure toxins (Fletcher *et al*., 1998; Plakas *et al*., 2002; Ishida *et al*., 2004). These studies and their findings are summarized in Table 14.

Field observations of mortality from natural blooms demonstrate the ecological consequence of these perturbations on resident fauna, but rarely do they unequivocally relate mortality to the intensity and duration of exposure to *K. brevis*. Invariably, there are other concommitant and complicating conditions that contribute to mortality, most notably severe and prolonged depressions in dissolved oxygen, which may or may not be monitored. Monitoring of shellfish toxicity during red tides has provided valuable information on the toxicity of exposed bivalves, but no consideration has ever been given to the "health" of the shellfish. Recent laboratory studies focusing on the identity of toxins and their derivatives have contributed greatly to our knowledge of how bivalves "process" brevetoxins; however, these studies did not investigate the behavioral or physiological responses, such as changes in feeding rate, that bivalves undergo when confronted with these toxic dinoflagellates.

Not until the early work of Shumway and colleagues was attention given to the impacts of harmful and toxic algae on specific shellfish or to the potential long-term impacts to bivalve fisheries and culture (Shumway and Cucci, 1987; Shumway, 1990; Shumway et al., 1990). These studies revealed no universal effects on bivalves from exposure to toxic algae; rather, the response depends on the interaction between specific alga and bivalve species. It was also demonstrated that bivalve populations which are periodically exposed to toxic algal blooms may have evolved mechanisms permitting them to exploit the toxic organisms as food with no ill effects.

Recurring blooms of *K. brevis* have become common along the Florida west coast (Tester and Steidinger, 1997). These blooms occur almost annually, usually in the late summer and autumn (see Kirkpatrick *et al*., 2004 for review). At the same time,

populations of important bivalve species in Florida are under increasing threat from these persistent algal blooms. For example, projects aimed at restoring bay scallops within several southwest Florida estuaries have increased in recent years (Geiger and Arnold, 2003; Wilbur *et al*., 2005; Leverone *et al*, 2005). The green mussel, *Perna viridis*, which is commercially exploited in its native New Zealand, became established in Tampa Bay in 1999 (Ingrao, 2001), and has since spread throughout the state and has been found as far as South Carolina along the Atlantic coast. The state of Florida has developed a burgeoning clam (*M. mercenaria*) aquaculture industry in the past decade and is responsible for managing leases in coastal areas that coincide with the most frequent episodes of red tide. Specific knowledge regarding the ecological consequences of prolonged or repeated exposure to *K. brevis* in Florida bivalve populations would help tremendously in developing responsible management plans for each of these valuable shellfish species. This dissertation research was undertaken to add to the knowledge of how *K. brevis* affects the different life stages of important bivalve molluscs from Florida.

Bivalve Larvae

Most studies on the interaction between toxic dinoflagellates and bivalves have focused on juvenile and adult life stages (see reviews by Shumway, 1990; Bricelj and Shumway, 1998; Landsberg, 2002). Recently, attention has been given to 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). The veliger is an important and delicate stage in the early development of bivalve shellfish and is generally considered

more sensitive than its post-larval counterparts to perturbations and stressors, including exposure to harmful algal blooms (Yan et al., 2003; Wang et al, 2006).

The research presented in this thesis demonstrated that survival of veliger larvae for all three bivalve species (*Argopecten irradians*, *Mercenaria mercenaria* and *Crassostrea virginica*) was dependent upon the cell concentration of *Karenia brevis*. Overall survival was quite high (85%) at *K. brevis* cell concentrations less than bloom strength (100 cells $\cdot$ ml^{-1} and less), but decreased to roughly 25% at high bloom concentrations (5,000 cells $\cdot$ ml^{-1}). Larval development was also protracted in surviving larvae of *C. virginica* and *M. mercenaria* at *K. brevis* densities of 1,000 cells $\cdot$ ml^{-1}. Larval survival was generally higher when exposed to whole cultures of *K. brevis* compared to lysed cultures.

Matsuyama *et al*. (2001) reported that certain species of harmful algae were lethal to larvae of the Pacific oyster, *C. gigas*, at cell densities (100-1,000 cells $\cdot$ ml^{-1}) similar to those in the present study. While certain dinoflagellates (*Alexandrium tamarense*, *A. taylori*, *Gymnodinium mikimotoi* and *Heterocapsa circularisquama*) were shown to be lethal, four other species (*Chattonella antiqua*, *Gymnodinium catenatum*, *Heterosigma akashiwo* and *Scrippsiella trochoidea*) did not affect the survival rate or development of oyster larvae at the same concentrations. Interestingly, mortality did not necessarily relate to the toxicity of the dinoflagellate. *Alexandrium taylori* had an extreme lethal effect on *C. gigas* larvae, but HPLC analysis of *A. taylori* cultures revealed no PSP toxins. The PSP producer *Gymnodinium catenatum*, however, caused no harmful effects on oyster larvae even at abnormally high (above bloom) concentrations. Exposure of *C. gigas* embryos to unfiltered seawater containing *Gyrodinium aureolum* for two days

resulted in poor (ten percent) survival to the veliger stage (Helm *et al*., 1974), suggesting evidence of toxicity.

The process by which *K. brevis* affects larval survival is not clear, but several possible mechanisms have been suggested and may be involved. The earliest studies on interactions between bivalve larvae and harmful algae suggested that a direct cell-to-cell contact with microalgae was responsible for larval mortality, either through exposure to toxins present on cell surfaces or through mechanical damage to sensitive organs, particularly gills. Gallager *et al*. (1989) suggested that *Argopecten irradians* larvae must ingest or be in contact with whole cells of *Aureococcus anophagefferens* before elevated larval mortality is observed. This same microalgae, however, had no effect on survival for larvae of the northern quahog, *M. mercenaria*, even at bloom conditions (Padilla *et al*., 2006). Mortality of *C. virginica* larvae in the presence of the dinoflagellate *Cochlodinium heterolobatum* was thought to be a result of increased direct contact between larvae and algal cells (Ho and Zubkoff, 1979). Cell-free filtrates of the two *Alexandrium* species (*A. tamarense* and *A. taylori*) had less effect on mortality of *C. gigas* larvae than their whole-cell counterparts (Matsuyama *et al*., 2001), implying the cause of toxicity was localized on the cell surface. Yan *et al*. (2001) suggested that direct contact with toxic algal cells may also release an unknown inhibitory factor which could negatively affect survival. Ultrasonic disruption (=lysing), which produces cellular fragments as well as releasing intracellular toxins, should make the toxins more available for encounters with bivalve larvae. Collectively, these studies strongly suggest that physical cell-to-cell contact between bivalve larvae and toxic dinoflagellates is, at least partially, responsible for observed increases in larval mortality.

In this study, lysed cultures produced higher mortality in *M. mercenaria* and *C. virginica* than whole cultures at the same cell concentrations. Cell fragments in the lysed treatments were not removed from the experimental medium (either by centrifugation or filtration) after sonication. Thus, these fragments (and any released intracellular toxins) were available to interact with the exposed larvae, thereby increasing the frequency of physical contact between "algae" and larvae. This scenario might explain, at least partially, the observed increase in larval mortality associated with lysed treatments of *K. brevis*.

Consumption (or ingestion) of harmful algal cells by bivalve larvae is dependent upon a variety of factors, including algal species, cell size and concentration, and larval species and age. Consumption of *K. brevis* cells could also explain the observed inhibitory effects on larval survival in this study. Larvae of the mussel, *Mytilus galloprovincialis*, readily ingested cells of several species of red-tide dinoflagellates with mean equivalent spherical diameters of 12-38 μm (Jeong *et al*., 2004). However, mussel larvae did not feed on <u>any</u> dinoflagellate until at least nine days after fertilization. Eastern oyster (*C. virginica*) larvae ingested *P. minimum* cells, although filtration was depressed in the presence of this toxic algae (Wikfors and Smolowitz, 1995), and ingestion of these cells 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. All of these symptoms indicate possible phagolytic reactions to dinoflagellate debris (Wikfors and Smolowitz, 1995). Early D-shape larvae of two scallop species (*Argopecten irradians concentricus* and *Chlamys farreri*) were unable to

feed on *Alexandrium tamarense* cells due to the relatively large algal cell size (Yan *et al*., 2001; Yan *et al*., 2003). During this study, larvae were fed an optimal ration (Lu and Blake, 1996) of the chrysophyte, *I. galbana* in addition to *K. brevis*, to ensure that the larvae were well-fed throughout the experiment and that any observed mortality was not due to starvation. Although larval feeding rates were not measured nor *K. brevis* consumption investigated, ingestion of *K. brevis* cells was most likely negligible due to the large cell size (ESD = 14-26 μm) and low density compared to *I. galbana*; however, the presence of *K. brevis*, especially at higher concentrations, could have altered 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). The numerous cellular fragments in the lysed treatments could have been of an appropriate size for filtration and ingestion, further complicating feeding patterns and possibly initiating phagolytic reactions similar to those reported by Wikfors and Smolowitz (1995).

In addition to affecting larval survival, *K. brevis* also negatively impacted larval development and metamorphosis. For example, even though overall survival was identical in *C. virginica* larvae exposed to 100 and 1,000 cells · ml^{-1}, a higher proportion from 100 cells · ml^{-1} had reached the pediveliger stage and completed larval development (i.e., settled as spat) than larvae from 1,000 cells · ml^{-1}. Almost ninety percent of larvae subjected to 5,000 cells · ml^{-1} did not live beyond the umboveliger stage. Development of *M. mercenaria* larvae 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 dinoflagellate, *P. minimum* (Wikfors and Smolowitz, 1995). Heavy metals have also been shown to delay metamorphosis in bivalve larvae. Settlement of oyster (*Crassostrea gigas*) and bay scallop (*Argopecten irradians*) larvae was delayed in the presence of zinc (Boyden et al., 1975; Watling, 1983) and development in northern quahog larvae (*Mercenaria mercenaria*) was delayed by exposure to nickel (Calabrese and Nelson, 1974). 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. Since delayed metamorphosis has been observed in bivalve larvae exposed to heavy metals as well, these results may reflect a more general toxic response rather than one that is attributable to brevetoxins.

Matsuyama *et al*. (2001) organized the effects of harmful algae on (oyster) larvae into three categories:

Type 1 = lethal to larvae at visible bloom density (red tide)

Type 2 = non-lethal effects but induce a delay in metamorphosis

Type 3 = no effect.

Based on these categories, *K. brevis* exhibits a combination of Type 1 and Type 2 responses in the three species of bivalve larvae in these studies.

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 identified 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 in 2003 during a red tide outbreak along the Gulf coast of Sarasota, FL (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. Our results clearly indicate that when *K. brevis* and its 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 collapse of bay scallop populations in North Carolina, USA, in 1989 was attributed to a bloom of *Ptychodiscus brevis* (= *K. brevis*), which was blamed for higher than natural mortality in adults the previous year (Summerson and Peterson, 1990). Depletion of the adult spawner stock led to poor recruitment and failure of local population's ability to recover quickly to previous levels of abundance (Peterson and Summerson, 1992). These observations on population dynamics following a red tide did not even consider the additional, negative effects of *K. brevis* on larval growth and

survival that were ascertained in the present study. Our demonstrated effects of *K. brevis* on the larvae of northern quahogs (= hard clams) and eastern oysters point to the potential for this toxic dinoflagellate 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.

Juvenile Bivalves

There are surprisingly few studies which have focused on the interaction between *K. brevis* and bivalve molluscs. Our current knowledge is limited to general field observations on the toxicity and/or mortality of shellfish during red tides (Gunter *et al*., 1947; Cummins *et al*., 1971; Simon & Dauer, 1972; Hemmert, 1975) and a few laboratory studies on behavioral responses to *K. brevis* cultures (Sievers, 1969; Roberts *et al*. 1979). More recently, a series of studies have focused on brevetoxin uptake and metabolism in the eastern oyster (Dickey *et al*., 1999; Poli *et al*., 2000; Plakas *et al*., 2002; Pierce *et al*., 2004; Wang *et al*., 2004).

Several general relationships between individual bivalve species and *K. brevis* have emerged from these collective studies. During a bloom of *K. brevis* (= red tide), the eastern oyster becomes toxic through the accumulation and metabolizing of brevetoxins, northern quahogs also become toxic and bay scallops succumb to mortality.

The current research lends further support to the species-specific response of bivalve molluscs in the presence of toxic or noxious algae (Shumway and Cucci, 1987;

see Table 1 in Shumway, 1990). 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 hr) exposure to a non-replenished supply of *K. brevis* and long-term (2 day) exposure to a continuous supply of *K. brevis*.

The bay scallop (*A. irradians*) was the most sensitive species 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 $\cdot$ ml^{-1}, independent of culture preparation. The response was immediate when exposed to intact cells, but took 24 hr to be manifested with lysed cells. The delayed feeding response to lysed *K. brevis* in our study likely indicates an unknown cytotoxic or neurotoxic effect. Although no data are available for Florida populations of *A. irradians*, Summerson and Peterson (1990) implicated a bloom of *K. brevis* in massive mortalities of bay scallops in North Carolina. This mass mortality led to recruitment failure of bay scallops in subsequent years (Peterson and Summerson, 1992). No other information is available on how bay scallops are effected by *K. brevis*. Our results showed that *K. brevis* had an appreciable effect on survival or development of *A. irradians* larvae at high (bloom) concentrations. In addition, clearance rates in juvenile bay scallops exposed to *K. brevis* were the most sensitive of the bivalve species we tested. These laboratory findings support reports from North Carolina on bay scallop recruitment failure after a red tide outbreak in that larval mortality is high and feeding in juvenile scallops is compromised.

Longer-term exposure of adult *A. irradians* to *K. brevis* revealed deleterious histological changes in the digestive diverticula; most notably an accumulation of

hemocytes, but also cellular changes in the epithelial layer surrounding the lumen. These observations strongly suggest a combination of poor nutrition and toxic effects from exposure to *K. brevis*. Additional studies are necessary to elucidate these differences.

The ability of green mussels to feed upon and metabolize *K. brevis* cultures was confirmed by Ishida *et al*. (2004) using an EPA strain of *K. brevis* and New Zealand populations of the greenshell mussel, *Perna canaliculus*. Several brevetoxin metabolites have been identified and biosynthetic pathways proposed by Morohashi *et al*. (1995), Murata *et al*. (1998) and Ishida *et al*. (2004). However, no other studies involving *K. brevis* or brevetoxin analyses have been conducted on the greenshell mussel congener, *P. viridis*, which has been a resident of Tampa Bay, FL since 1999. Our data, along with personal observations of local populations, indicate that Florida green mussel populations may be susceptible to blooms of *K. brevis*. In the laboratory, the clearance rate in juvenile *P. viridis* was significantly reduced at moderate (100 cells $\cdot$ ml^{-1}) *K. brevis* cell concentrations, but not by lysed *K. brevis* culture. In the field, a high degree of mortality was observed in green mussel populations throughout lower Tampa Bay during a prolonged red tide outbreak in 2005 (personal observation). Additional studies are clearly needed to better understand the interactions between *K. brevis* and *P. viridis*.

During a 1973-74 red tide in Sarasota, FL, shellfish suspected of being contaminated from a bloom of *K. brevis* were processed and analyzed for the presence of toxins (Hemmert, 1975). Local surfclams (*Spisula solidissima raveneli*) and southern quahogs (*Mercenaria campechaenis*) were found to be toxic. Poli *et al*. (2000) found toxic *Mercenaria* sp. during a rare red tide in the northern Gulf of Mexico. Clams were less toxic than oysters taken at the same time from the same location during an unusual

red tide event in North Carolina (Tester and Fowler, 1990). Pierce *et al*. (2004) found two brevetoxin metabolites in *M. mercenaria* in Sarasota Bay during a 2001 red tide, indicating that quahogs, like oysters, have the ability to consume and metabolize *K. brevis* and its toxins. These are the only published data regarding the ability of clams to filter, ingest and accumulate brevetoxins from exposure to *K. brevis*. Our results are in agreement with this limited information on *M. mercenaria*. While clearance rate was depressed at high *K. brevis* concentrations, quahogs did continue to feed. As a result, no mortality was observed in juvenile quahogs in our studies.

Early laboratory studies showed that eastern oysters exhibited normal feeding behavior during exposure to *K. brevis* (Ray and Aldrich, 1967; Sievers, 1969), while oysters exposed to *K. brevis* during a bloom become toxic (Cummins *et al*., 1971). More recently, Plakas *et al*. (2002) and Wang *et al*. (2004) documented brevetoxin uptake and metabolism in *C. virginica* in the laboratory. Oysters also eliminated brevetoxins once the oysters were removed from the algal source. In the present study, oysters removed the highest percentage of *K. brevis* cells from the surrounding media of all bivalve species examined. We showed that clearance rates in eastern oysters were reduced the least by exposure to *K. brevis*, which supports the general conclusion that *C. virginica* are not critically impacted by *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 *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. viridis*) 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 non-toxic 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, whereas clearance rates at both medium (100 cells $\cdot$ ml^{-1}) and high (1,000 cells $\cdot$ ml^{-1}) densities declined only after 24 hr 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.

Conclusion and Significance

Impacts from Different Culture Preparations

This research sought to determine whether there were differences in larval mortality and juvenile feeding for individual bivalve species, as well as bay scallop cytohistology, when exposed to two different culture preparations of *K. brevis* (Wilson clone). The culture preparations included 1) whole, intact cell cultures and 2) cultures where the cells had been disrupted, or lysed, by ultrasound. Each culture was analyzed for brevetoxin composition and concentration. In essentially every case, lysed cultures had higher reported brevetoxin amounts than the corresponding whole culture. Since the two preparations were obtained from the same batch culture, these differences in brevetoxin amounts are best explained by the extraction and recovery procedures. One explanation for these differences is the possibility that there is better extraction efficiency when cultures are lysed prior to extraction. An important observation from these data, however, is verification that a particular culture of *K. brevis* was not only toxic, but contained ratios of the major brevetoxins (PbTx-2 and PbTx-3) corresponding to previously analyzed cultures (Landsberg, 2002; Pierce *et al*., 2005).

These cultures also contained brevenal, a recently discovered polyether compound which, in fish, has been shown to competitively displace brevetoxin from its binding site thereby inhibiting the toxic effects of brevetoxins (Bourdelais *et al*., 2004). The absolute amount of brevenal reported in each sample is calculated in PbTx-3 equivalents; whereby PbTx-3 is the standard used to develop the response factor upon which all fractions were quantified (Pierce, personal communication). Thus, the amount

of brevenal indicated in a given sample is a relative, rather than absolute concentration. An important observation about the relative amount of brevenal in these cultures is that their concentration was approximately 10x higher than brevenal concentrations from water samples collected off Siesta Key, FL during a 2005 red tide (Pierce, unpublished). These reported differences in brevenal composition suggest that this laboratory culture of *K. brevis* may not be as toxic as natural blooms. Results from this study, therefore, may be considered as conservative estimates of the effects of this toxic dinoflagellate on bivalve molluscs.

Lysed cultures of *K. brevis* did not undergo any additional processing, such as centrifugation or filtering, after sonification. As a result, the solution contained byproducts of the lysing process, including cellular debris and fragments in addition to the liberated toxins. It is reasonable to assume that these toxins, since they are lipophyllic, would adsorb onto these particles as they came in contact. Juvenile bivalves exposed to these conditions during feeding experiments would conceivably have to process this toxin-laden particulate matter in addition to the nontoxic algae. While it could be argued that removing this particulate matter prior to experimentation would have eliminated this variable, in reality, bivalves in nature must deal with intact toxic cells, particulate matter, extracellular toxins and whatever else is present during a bloom; consequently, what is learned from exposing bivalves to lysed treatments as they were prepared in this study are representative of natural exposure conditions.

Juvenile bivalve feeding studies showed a significant effect of culture preparation on clearance rate in the bay scallop, green mussel and northern quahog. This difference was most noticeable in the green mussel and northern quahog at higher cell

concentrations. In both cases, intact *K. brevis* produced significantly lower clearance rates while lysed *K. brevis* had only a slight effect on clearance rate . Two possible explanations for these results are: 1) lysed cultures made the toxins less bioavailable, or 2) the observed effects were due to the presence of the dinoflagellate and not the associated toxins. Since bivalves in the field are exposed to a combination of these conditions during a bloom of *K. brevis*, it could be argued that the observed differences in clearance rate between culture preparations in the laboratory would not be as great in the wild.

Possible Mechanisms of Toxic Activity

Brevetoxins are polyether ladder neurotoxins that bind to voltage-sensitive sodium channels in cell membranes. Binding results in persistent activation of neuronal cells, skeletal muscle cells and cardiac cells (Baden, 1988). The manners in which brevetoxins affect mollusc tissues, or specific ways in which molluscs may respond to brevetoxin exposure, have not been thoroughly investigated.

Observations from this research may be a direct result of brevetoxin interactions with specific tissues, particularly nerve cells, or they may be due to a secondary effect which may or may not include behavioral and physiological responses. What follows is an attempt to explain what these interactions might include.

Juvenile bay scallops (*A. irradians*) were the most sensitive species to the presence of *K. brevis* in terms of clearance rate. Bay scallops are non-siphonate filter feeders and remain partially open when at rest. Scallops also live in seagrass meadows of

the open coast and lower estuary where daily fluctuations in water quality are reduced. Northern quahogs and eastern oysters, on the other hand, can close their valves and survive anaerobically for extended periods of time. Oysters also inhabit the intertidal zone and regularly experience long periods out of the water while quahogs bury in sediments throughout the coastal zone. Oysters and quahogs are subjected to relatively greater daily fluctuations in water quality, particularly oxygen and temperature, than bay scallops.

Differences in morphology and ecology among these bivalves may partially explain how each species responds to *Karenia brevis* in the field. Summerson and Peterson (1990) reported on recruitment failure of the bay scallop during an outbreak of *K. brevis* in North Carolina in 1987. The authors did not, however, mention the mechanism behind these observations. Since there are no other published reports on the effects of *K. brevis* on bay scallops in the field, insight into the physiological response might be obtained by looking at the response of scallops during exposure to brown tides, caused by the picoplantonic alga *Aureococcus anophagefferens*, in eastern Long Island, NY over the past several decades. These responses also included recruitment failure, growth inhibition and decimation of local populations. The negative impacts of this alga were attributed to an unknown, dopamine-mimetic, bioactive/toxic metabolite which suppresses the activity of gill lateral cilia (Gainey and Shumway, 1991) and thus negatively impacted clearance rates. These effects were observed even in the presence of a mixed phytoplankton assemblage containing non-toxic algae similar to results from this research (Bricelj et al., 2004). Unlike the results from this study, however, toxic effects from brown tides required direct contact with the algal cell and did not appear to be

associated with dissolved toxic exudates (Ward and Targett, 1989). Gill cilliary inhibition by brown tide was not demonstrated during *in vitro* trials (Gainey and Shumway, 1991) even though natural populations of this species are known to be adversely affected by brown tides.

The most reasonable conclusion to draw from this body of information is that *K. brevis* also suppresses gill cilliary activity in the scallop. This could occur by direct action on the gill neuronal cells similar to the mechanism described above or by a secondary, indirect action. Perhaps more importantly, the effect of reducing gill cilliary activity also would affect oxygen uptake, thereby compounding the effects of *K. brevis* and/or its toxins on the bay scallop. Scallop mortality in the field has been observed within the first two days after the onset of a red tide (personal observation), indicating that the cause of death is more likely due to a lack of oxygen rather than starvation. Scallops, unlike oysters and quahogs, do not have the ability to close their shells for extended periods and undergo facultative anaerobiosis. Therefore, scallops are more vulnerable to blooms of K. brevis than other bivalves, in part, because of their inability to reduce or eliminate their exposure to this toxic dinoflagellate and its toxins.

Implications for Fisheries Management

Recurring blooms (= red tides) of *K. brevis* are common along the Florida west coast (Tester and Steidinger, 1997; Kirkpatrick *et al*., 2004). Results of these studies clearly demonstrate short-term, negative impacts of *K. brevis* on resident bivalve species, suggesting that the ecological and fisheries impacts from these algal blooms could be quite significant, depending upon bloom intensity and duration, and which bivalve species are exposed.

Results from these studies on bivalve larvae and *K. brevis* are the first reported and while we plainly demonstrated a negative impact of *K. brevis* on larval survival and development, there is a clear need for continued research into the mechanisms underlying these interactions. We also showed that feeding rates in juvenile bivalves were also negatively impacted by exposure to *K. brevis*, and that the response was species-specific.

The most sensitive species in the present study was the bay scallop, *A. irradians*. A rare bloom of *K. brevis* in North Carolina during 1987-88 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 $\cdot$ 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: 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). Locating aquaculture sites in lower salinity waters might reduce the frequency and duration of exposure to red tides, which typically initiate in more saline offshore waters. If a red tide does penetrate the estuary, the lower salinity further into the bay could serve as a potentially effective salinity barrier to a bloom of *K. brevis*. Similarly, reduced feeding rates in the green mussel (*P. viridis*) at high *K. brevis* concentrations should theoretically make it more difficult for mussel populations to remain established in estuaries where red tides are more frequent and/or severe. Emperical observations, however, suggest a different outcome. An intense red tide during 2005-06 resulted in high mortality of green mussels attached to pilings and other structures in lower Tampa Bay (personal observation). Intense recolonization by juvenile green mussels, however, was observed in late 2006, several months after the bloom had dissipated. The prolific and dynamic recruitment rates of green mussels and their ability to rapidly recolonize a previously inhabited space after a red tide has disappeared suggests populations of this exotic species have no difficulty overcoming the temporary effects of exposure to *K. brevis*. 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.

Table 14. Reported impacts of *Karenia brevis* on molluscs

Species	Common name	Field/ Laboratory	Effect/ Observation[1]	Location	*K. brevis* (cells L^{-1})	Reference
Bivalves						
Argopecten irradians	bay scallop	Field	Mortality (?); Recruitment failure	North Carolina	8.2×10^5	Summerson & Peterson (1990)
Austrovenus stutchburyi	New Zealand cockle	Laboratory	Toxic; Brevetoxin metabolism	New Zealand	$6\text{-}12 \times 10^6$	Ishida *et al*. (2004)
Brachidontes recurvus	hooked mussel	Laboratory	Unaffected		9.9×10^6	Sievers (1969)
Chione cancellata		Field	Toxic	Sarasota, FL	N.R.	Poli *et al*. (2000)
Crassostrea virginica	eastern oyster	Laboratory	Unaffected (Normal feeding)	Galveston, TX	N.R.	Ray & Aldrich (1967)
		Laboratory	Unaffected (Normal behavior)		9.9×10^6	Sievers (1969)
		Field	Toxic	Venice, FL	8.2×10^5	Cummins *et al*. (1971)
		Field	Toxic	Beaufort Inlet, NC	$\sim 10^5$	Tester & Fowler, 1990)
		Field	Toxic	Mississippi Sound, Gulf of Mexico	5.6×10^5	Dickey *et al*. (1999)
		Laboratory	Toxic; Brevetoxin metabolism		1.3×10^6	Plakas *et al*. (2002)
		Field	Toxic; Brevetoxin metabolism	Sarasota, FL	6.6×10^5	Pierce *et al*. (2004)
		Laboratory	Toxic; Brevetoxin metabolism		1.5×10^7	Wang *et al*. (2004)
Crassostrea gigas	Pacific oyster	Laboratory	Toxic	New Zealand	$1.0 – 2.5 \times 10^7$	Fletcher *et al*. (1998)
Mulinia lateralis	Cross-barred venus	Field	Mortality	Tampa Bay	$< 1.8 \times 10^7$	Simon & Dauer (1972)

Table 14. (Continued).

Donax variabilis	Variable coquina	Field	Toxic	Venice, FL	8.2×10^5	Cummins *et al*. (1971)
Mercenaria campechiensis	Southern quahog	Field Field Field Laboratory	Toxic Toxic Toxic Toxic	Venice, FL Sarasota Bay, FL Englewood, FL Tampa Bay, FL	8.2×10^5 N.R. N.R. N.R.	Cummins *et al*. (1971) Hemmert (1975) Hemmert (1975) Roberts *et al*. (1979)
Mercenaria mercenaria	Northern quahog	Field Field	Toxic Toxic; Brevetoxin metabolism	Beaufort Inlet, NC Sarasota, FL	$\sim 10^5$ 6.6×10^5	Tester & Fowler, 1990) Pierce *et al*. (2004)
Mercenaria sp.	Quahog	Field	Toxic	Sarasota, FL	N.R.	Poli *et al*. (2000)
Macrocallista nimbosa	Sunray venus	Field	Toxic	Venice, FL	8.2×10^5	Cummins *et al*. (1971)
Perna canaliculus	Greenshell mussel	Laboratory	Toxic; Brevetoxin metabolism	New Zealand	$6\text{-}12 \times 10^6$	Ishida *et al*. (2004)
Spisula solidissma raveneli	Atlantic surfclam	Field	Toxic	Siesta Key, FL	N.R.	Hemmert (1975)
	Oysters	Field	Mortality	Naples to Boca Grande, FL	N.R.	Gunter *et al*. (1947)
	Clams	Field	Mortality	Naples to Boca Grande, FL	N.R.	Gunter *et al*. (1947)
Gastropods						
Busycon contrarium	Whelk	Field	Toxic	Sarasota, FL	N.R.	Poli *et al*. (2000)
Busycon sp.	Whelk	Field	Toxic	Sarasota, FL	6.6×10^5	Pierce *et al*. (2004)
Fasciolaria lilium hunteria	Banded tulip	Laboratory	Loss of muscle control	Tampa Bay, FL	N.R.	Roberts *et a*l. (1979)
Melongena corona	Crown conch	Laboratory	Loss of muscle control	Tampa Bay, FL	N.R.	Roberts *et al*. (1979)
Oliva sayana	Lettered olive	Laboratory	Loss of muscle control	Tampa Bay, FL	N.R.	Roberts *et al*. (1979)

[1]Toxic = containing toxins using the mouse bioassay or analytical methods. Brevetoxin metabolism = the ability to metabolize parent toxins found in *Karenia brevis*.

N.R. = not reported.

Literature Cited

Abbott, B. and D. Ballantine. 1957. The toxin from *Gymnodinium veneficum* Ballantine. J. Mar. Biol. Assoc. U.K. 36: 169-189.

Adams, C. and L. Sturmer. 2004. Hard clam culture: A commercial success story in Florida. World Aquaculture 35: 56-60.

Baden, D.G. 1988. Public health problems of red tides. Pp. 259-277. *In*: A.T. Tu, (ed). Handbook of Natural Toxins, Vol. 3. Dekker, New York.

Barber, B.J. 2004. Neoplastic diseases of commercially important marine bivalves. Aquat. Living Resources 17: 449-466.

Barber, B.J. and N.J. Blake. 1983. Growth and reproduction of the bay scallop, *Argopecten irradians* (Lamark) at its southern distributional limit. J. Exp. Mar. Biol. Ecol. 66, 247-256.

Bardouil, M., M. Bohec, M. Cormerais, S. Bougrier and P. Lassus. 1993. Experimental study of the effects of a toxic microalgal diet on feeding of the oyster *Crassostrea gigas* Thunberg. J. Shellfish Res. 12: 417-422.

Bardouil, M., M. Bohec, S. Bougrier, P. Lassus and P. Truquet. 1996. Feeding responses of *Crassostrea gigas* (Thunberg) to inclusion of different proportions of toxic dinoflagellates in their diet. Ocean. Acta 19: 177-182.

Bauder, A.G., A.D. Cembella, V.M. Bricelj and M.A. Quilliam. 2001. Uptake and fate of diarrhetic shellfish poisoning toxins from the dinoflagellate *Prorocentrum lima* in the bay scallop *Argopecten irradians*. Mar. Ecol. Prog. Ser. 213: 39-52.

Benson, A.J., D.C. Marelli, M.D. Frischer, J.M. Danforth and J.D. Williams. 2001. Establishment of the green mussel, *Perna viridis* (Linnaeus 1758) (Mollusca: Mytilidae) on the west coast of Florida. J. Shellfish Res. 20: 21-29.

Bicknell, W.J. and D.C. Walsh. 1975. The first 'red tide' in recorded Massachusetts history: managing an acute and unexpected public health emergency. In: (V.R. Locicero ed.) Proceedings of the First International Conference on Toxic Dinoflagellate. MSTF, Wakefield, MA.

Blake, N.J., C. Adams, R. Degner and D. Sweat. 2000. Aquaculture and Marketing of the Florida Bay Scallop in Crystal River, Fl. Florida Sea Grant Final Report R/LR-A-20. 99p.

Bougrier, S., P. Lassus, B. Beliaeff, M. Bardouil, P. Masselin, P. Truquet, F. Matignon and C. LeBaut. 2000. Feeding behavior of individuals and groups of king scallops (*Pecten maximus*) contaminated experimentally with PSP and detoxified. Ninth International Conference on Harmful Algal Blooms. Tamsania, Australia. P. 89 (Unpublished).

Bourdelais, A.J., S. Campell, H. Jacocks, J. Naar, J.L.C. Wright, J. Carsi and D.G. Baden. 2004. Brevenal is a natural inhibitor of brevetoxin action in sodium channel receptor binding assays. *Cell. Mol. Neurobio*. 24, 553-563.

Boydon, C.R., H. Watling and I. thornton. 1975. Effect of zinc on the settlement of the oyster Crassostrea gigas. Mar. Biol. 31: 227-234.

Bricelj, V.M. and S.E. Shumway. 1998. Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation. Rev. Fish. Sci. 6: 315-383.

Bricelj, V.M., J.H. Lee, A.D. Cembella and D.M. Anderson. 1990. Uptake of *Alexandrium fundyense* by *Mytilus edulis* and *Mercenaria mercenaria* under controlled conditions. Pp. 269-274 *In*: (E. Graneli, B. Sundstrom, L. Edler and D.M. Anderson, eds.) Toxic Marine Phytoplankton. Elsevier, New York.

Bricelj, V.M., J.H. Lee and A.D. Cembella. 1991. Influence of dinoflagellate cell toxicity on uptake and loss of paralytic shellfish toxins in the northern quahog *Mercenaria mercenaria*. Mar. Ecol. Prog. Ser. 74: 33-46.

Bricelj, V.M., A.D. Cembella, D. Laby, S.E. Shumway and T.L. Cucci. 1996. Comparative physiological and behavioral responses to PSP toxins in two bivalve molluscs, the soft clam, *Mya arenaria*, and surfcalm, *Spisula solidissima*. Pp. 405-408 *In*: (T. Yasumoto, Y. Oshima and Y. Fukuyo, eds.) Harmful and Toxic Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO, Paris.

Bricelj, V.M., S. MacQuarrie and B.M. Twarog. 2000. Differential sensitivity and uptake of PSP toxins within and between softshell clam (*Mya arenaria*) populations from Atlantic Canada. Ninth International Conference on Harmful Algal Blooms. Tamsania, Australia. P. 9 (Unpublished).

Bricelj, M.V., S.P. MacQuarrie and R. Smolowitz. 2004. Concentration-dependent effects of toxic and non-toxic isolates of the brown tide alga *Aureococcus anophagefferens* on growth of juvenile bivalves. Mar. Ecol. Prog. Ser. 282: 101-114.

Buley, H.M. 1936. Consumption of diatoms and dinoflagellates by the mussel. Bull. Scripps Inst. Oceanogr. 4: 19-27.

Burkholder, J.M., H.B. Glasgow, N. Deamer-Melia, J. Springer, M.W. Parrow, C. Zhang and P. Cancellieri. 2001. Species of the toxic *Pfiesteria* complex and the importance of functional type in data interpretation. Environ. Health Perspect. 109 (Suppl.): 667-679.

Butler, P.A. 1954. Summary of our knowledge of the oyster in the Gulf of Mexico. U.S. Fish Wildl. Serv. Fish. Bull. 55: 479-489.

Calabrese, A and D.A. Nelson. 1974. Inhibition of embryonic development of the hard clam, *Mercenaria mercenaria*, by heavy metals. Bull. Environ. Conram. Toxicol. 11: 92-97.

Carriker, M.R., 2001. Functional morphology and behavior of shelled veligers and early juveniles. In: J.N. Kraeuter, J.N., Castagna, M. (Eds), Biology of the hard clam, Elsevier, Amsterdam, pp. 283-303.

Chang, S.C., J.C. Harshbarger and S.V. Otto. 1980. Status of cytoplasmic prokaryote infections and neoplasms in bivalve molluscs. Sixth Food and Drug Science Symposium on Aquaculture: Public Health, Regulatory and Management Aspects, February 12-14, 1980.

Chebib, H.A., A.D. Cembella and P. Anderson. 1993. Differential paralytic shellfish toxin accumulation and detoxification kinetics in transplanted populations of *Mytilus edulis* exposed to natural blooms of *Alexandrium excavatum*. Pp. 383-388 *In*: T.J. Smayda and Y. Shimizu, (eds). Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam.

Cheng, T.C. 1967. Marine molluscs as host for symbioses. Adv. Marine Biol. 5: 1-424.

Cummins, J. M. and W.F. Hill, Jr. 1969. Method for the bioasay of *Gymnodinium breve* toxin(s) in shellfish. Gulf Coast Mar. Health Sci. Lab., Spec. Rep. 69: 1-6.

Cummins, J.M. A.C. Jones and A.A. Stevens. 1971. Occurrence of toxic bivalve molluscs during a *Gymnodinium breve* "red tide". Trans. Amer. Fish. Soc. 100: 112-116.

Duagbjerg, N. G. Hansen, J. Larsen and O. Moestrup. 2001. Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU R-DNA sequence data: including the erection of three new genera of unarmored dynoflagellates. Phycologia 39: 302-317.

Dickey, R., E. Jester, R. Granade, D, Mowdy, C. Moncreiff, D. Rebarchik, M. Robl, S. Musser and M. Poli. 1999. Monitoring brevetoxins during a *Gymnodinium breve* red tide: Comparison of sodium channel specific cytotoxicity assay and mouse

bioassay for determination of neurotoxic shellfish toxins in shellfish extracts. Nat. Toxins 7: 157-165.

Dupay, J.L. and A.K. Sparks. 1968. *Gonyaulax washingtonensis*, its relationship to *Mytilus californianus* and *Crassostrea gigas* as a source of paralytic shellfish toxin in Sequin Bay, Washington. Proc. Nat. Shellfish. Assoc. 58: 2 (Unpublished).

Erard-LeDenn, E., M. Morlaix and J.C. Dao. 1990. Effects of *Gyrodinium* cf. *aureolum* on *Pecten maximus* (post larvae, juveniles and adults). Pp. 132-136 *In*: (E. Graneli, B. Sundstrom, L. Edler and D.M. Anderson, eds.) Toxic Marine Phytoplankton. Elsevier, New York.

Fernandez, M.L. 2000. Regulations for marine microalgal toxins: towards harmonization of methods and limits. S. Afr. J. Mar. Sci. 22: 339-346.

Ferraz-Reyes, E., G. Reyes-Vasquez and I.B. Bruzual. 1979. Dinoflagellate blooms in the Gulf of Cariaco, Venezuela. Pp. 155-160 *In*: (D.L. Taylor and H.H. Seliger, eds.) Toxic Dinoflagellate Blooms. Elsevier, New York.

Fletcher, G.C., B.E. Hay and M.F. Scott. 1998. Detoxifying Pacific oysters (*Crassostrea gigas*) of the neurotoxic shellfish poison (NSP) produced by *Gymnodinium breve*. J. Shellfish Res. 17: 1637-1641.

Forbes, J.R. 1990. Massive bloom of *Gonyaulax spinifera* along the west coast of Vancouver Island. Red Tide Newslett 3: 160-167.

Fox, D.L. and W.R. Coe. 1943. Biology of the California sea mussel (*Mytilus californianus*). J. Exp. Biol. 93: 205-249.

Fremy, J.M., L. Puech, S. Krys and S. Dragacci. 1999. Recent advances in analytical procedures for the detection of diarrhetic phycotoxins: A review. J. Appl. Phycol. 11: 377-384.

Gainey, L.F., Jr. and S.E. Shumway. 1988. Physiological effects of *Protogonyaulax tamerensis* on cardiac activity in bivalve molluscs. Comp. Biochem. Physiol. 91C: 159-164.

Gallager, S.M., Stoecker, D.K., Bricelj, V.M., 1989. Effects of the brown tide alga on growth, feeding physiology and locomotory behavior of scallop larvae (*Argopecten irradians*). In: Cosper, E.M., Bricelj, M.V., Carpenter, E.J. (Eds), Novel phytoplankton blooms, Springer Verlag, Berlin, pp. 511-541.

Garthwaite, I. 2000. Keeping shellfish safe to eat: a brief review of shellfish toxins, and methods for their detection. Trends Food Sci. Technol. 11: 235-244.

Geiger, S.P. and W.S. Arnold. 2003. Restoration of bay scallops in highly modified and relatively pristine habitats on the west coast of Florida, USA. J. Shellfish Res. 22: 331-332.

Grindley, J.R. and F.J.R. Taylor. 1964. Red water and marine fauna mortality near Cape Town. Trans. Roy. Soc. S. Afr. 37: 111-130.

Grindley, J.R. and E.A. Nel. 1968. Mussel poisoning and shellfish mortality on the west coast of South Africa. S. Afr. J. Sci. 64: 420-422.

Gunter, G., F.G.W. Smith and R.H. Williams. 1947. Mass mortality of marine animals on the lower west coast of Florida, November 1946-January 1947. Science 105: 256-257.

Helm, MM, BT Hepper, BE Spencer and PR Walne. 1974. Lugworm mortalities and a bloom of *Gyrodinium aureolum* Hulburt in the eastern Irish Sea, autumn 1971. J. Mar. Biol. Ass. U.K. 54: 857-869.

Heinig, C.S. and D.E. Campbell. 1992. The environmental context of a *Gyrodinium aureolum* bloom and shellfish kill in Maquoit Bay, Maine, September 1988. J. Shellfish Res. 11: 111-122.

Hemmert, W.H. 1975. The public health implications of *Gymnodinium breve* red tides: a review of literature and recent events. Pp. 489-497 *In*: (V.R. LoCicero ed.) Proceedings of the First International Conference on Toxic Dinoflagellate Blooms. Mass. Sci. Tech. Fndn. Wakefield, MA.

Hesselman, D.M., Barber, B.J., Blake, N.J., 1989. The reproductive cycle of adult hard clams, *Mercenaria* spp. in the Indian River Lagoon, Florida. J. Shellfish Res. 8, 43-49.

Ho, M.S. and P.L. Zubkoff. 1979. The effects of a *Cochlodinium heterolobatum* on the survival and calcium uptake by larvae of the American oyster, *Crassostrea virginica*. In: Taylor, D.L., Selinger, H.H. (Eds.), Toxic dinoflagellate blooms, Elsevier/ North Holland, New York, pp. 409-412.

Horstman, D.A. 1981. Reported red-water outbreaks and their effects on fauna of the west and south coasts of South Africa, 1959-1980. Fish. Bull. S. Afr. 15: 71-88.

Howard, D.W. and C.S. Smith. 1983. Histological techniques for marine bivalve mollusks. NOAA Tech. Mem. NMFS-F/NEC-25. Woods Hole, MA. 97 p.

Ingrao, D.A., P.M. Mikkelsen and D.W. Hicks. 2001. Another introduced marine mollusk in the Gulf of Mexico: The Indo-Pacific green mussel, *Perna viridis*, in Tampa Bay, Florida. J. Shellfish Res. 20: 13-19.

Ingersoll, E. 1882. On the fish mortality in the Gulf of Mexico. Proc. U.S. Nat. Mus 4: 74-80.

Ishida, H., A. Nozawa, H. Nukaya, L. Rhodes, P. McNabb, P.Holland and K Tsuji. 2004. Confirmation of brevetoxin metabolism in cockle, *Austrovenus stutchburyi*, and greenshell mussel, *Perna canaliculus*, associated with New Zealand neurotoxic shellfish poisoning, by controlled exposure to *Karenia brevis* culture. Toxicon 43: 701-712.

Jeong, H.J., Song, J.Y., Lee, C.H., Kim, S.T., 2004. Feeding by larvae of the mussel *Mytilus galloprovincialis* on red-tide dinoflagellates. *J. Shellfish Res*. 23, 185-195.

Keppler, C.J., J. Hoguet, K. Smith, A.H. Ringwood and A.J. Lewitus. 2005. Sublethal effects of the toxic alga *Heterosigma akashiwo* on the southeastern oyster (*Crassostrea virginica*). Harmful Algae 4: 275-285.

Kirkpatrick, B., L.E. Fleming, D. Squicciarini, L.C. Backer, R. Clark, W. Abraham, J. Benson, Y.S. Cheng, D. Johnson, R. Pierce, J. Zaias, G.D. Bossart and D.G. Baden. 2004. Literature review of Florida red tide: implications for human health effects. Harmful Algae 3: 99-115.

Koizumi, Y. J. Kohno, N. Matsuyama, T. Uchida and T. Honjo. 1996. Environmental features and the mass mortality of fish and shellfish during the *Gonyaulax polygramma* red tide occurred in and around Uwajima Bay, Japan, in 1994. Nippon Suisan Gakkaishi 62: 217-224.

Koray, T. 1992. Noxious blooms in the Bay of Izmir, Aegean Sea. Harmful Algae News 2: 1-2.

Kruse, D.N. 1966. Life cycle studies on *Nematopsis duorari* n. sp. (Gregarina: Poropsoridae), a parasite of the pink shrimp (*Penaeus duorarum*) and pelecypod molluscs. Diss. Abstr. 27B: 2919-B.

La Barbera-Sanchez, A., S. Hall and E. Ferraz-Reyes. 1993. *Alexandrium* sp., *Gymnodinium catenatum* and PSP in Venezuela. Pp. 281-285 *In*: (T.J. Smayda andY. Shimizu, eds.) Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam.

Lam N.N. and S.S.Y. Yip. 1990. A three-month red tide event in Hong Kong. Pp. 385-390 *In*: (E. Graneli, B. Sundstrom, L. Edler and D.M. Anderson, eds.) Toxic Marine Phytoplankton. Academic Press, New York.

Landsberg, J.H. 1996. Neoplasia and biotoxins in bivalves: Is there a connection? J. Shellfish Res. 15: 203-230.

Landsberg, J.H. 2002. The effects of harmful algal blooms on aquatic organisms. Rev. Fish. Sci. 10: 113-390.

Lassus, P. and J.P. Berthome. 1988. Status of 1987 algal blooms in IFREMER. ICES annex III. C.M. 1988/F:33AL5-13

Lassus, P., D.J. Wildish, M. Bardouil, J.L. Martin, M. Bohec and S. Bougier. 1996. Ecophysioligical study of toxic *Alexandrium* spp effects on the oyster *Crassostrea gigas*. Pp. 409-412. *In*: T. Yasumoto, Y. Oshima and Y. Fukuyo (eds.). Harmful and Toxic Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO.

Lassus, P., M. Bardouil, B. Beliaeff, P. Masselin, M. Naviner and P. Truquet. 1999. Effect of a continuous supply of the toxic dinoflagellate *Alexandrium minutum* Halim on the feeding behavior of the Pacific oyster (*Crassostrea gigas* Thunberg). J. Shellfish Res. 18: 211-216.

Leibovitz, L., E.F. Schott and R.C. Karney. 1984. Diseases of wild, captive and cultured scallops. J. World Maricul. Soc. 15: 269-283.

Lesser, M.P. and S.E. Shumway. 1993. Effects of toxic dinoflagellates on clearance rates and survival in juvenile bivalve molluscs. J. Shellfish Res. 12: 377-381.

Leverone, J.R., W.S. Arnold, S.P. Geiger and J.M. Greenawalt. 2005. A novel approach to bay scallop (*Argopecten irradians*) restoration in Pine Island Sound, Florida: Release of competent larvae. J. Shellfish Res. 24: 326.

Lewitus, A.J., L.B. Schmidt, L.J. Mason, J.W. Kempton, S.B. Wilde, J.L. Wolny, B.J. Williams, K.C. Hayes, S.N. Hymel, C.J. Keppler and A.H. Ringwood. 2003. Harmful algal blooms in South Carolina residential and golf course ponds. Pop. Environ. 24: 387-413.

Li, S-C and W-X Wang. 2000. Ingestion and absorption efficiency of scallop (*Chlamys nobilis*) and clam (*Ruditapes philippinarum*) on a toxic dinoflagellate *Alexandrium tamarense*. Ninth International Conference on Harmful Algal Blooms. Tamsania, Australia. P. 165. (Unpublished).

Li, S-C and W-X Wang. 2001. Radiotracer studies on the feeding of two marine bivalves on the toxic and nontoxic dinoflagellate *Alexandrium tamarense*. J. Exp. Mar. Biol. Ecol. 263: 65-75.

Li, S-C, W-X Wang and D.P.H. Hsieh. 2002. Effects of toxic dinoflagellate *Alexandrium tamarense* on the energy budgets and growth of two marine bivalves. Mar. Envir. Res. 53: 145-160.

Lu, Y.T. and N.J. Blake. 1996. Optimum concentrations for *Isochrysis galbana* for growth of larval and juvenile bay scallops, *Argopecten irradians concentricus* (Say). J. Shellfish Res. 15: 635-643.

Luckenbach, M.W., K.G. Sellner, S.E. Shumway and K. Greene. 1993. Effects of two bloom-forming dinoflagellates, *Prorocentrum minimum* and *Gyrodinium uncatenum*, on the growth and survival of the eastern oyster, *Crassostrea virginica* (Gmelin 1791). J. Shellfish Res. 12: 411-415.

Machado, P.A. 1979. Dinoflagellate bloom on the Brazilian south Atlantic coast. Pp. 29-32 *In*: (D.L. Taylor and H.H. Seliger, eds.) Toxic Dinoflagellate Blooms. Elsevier, New York.

Mahoney, J.B. and F.W. Steimle, Jr. 1979. A mass mortality of marine animals associated with a bloom of *Ceratium tripos* in the New York Bight. Pp. 225-230 *In*: (D.L. Taylor and H.H. Seliger, eds.) Toxic Dinoflagellate Blooms. Elsevier, New York.

Matsuyama, Y., T. Uchida and T. Honjo. 1997. Toxic effects of the dinoflagellate *Heterocapsa circularisquama* on clearance rate of the blue mussel *Mytilus galloprovincialis*. Mar. Ecol. Prog. Ser. 146: 73-80.

Matsuyama, Y., H. Usuki, T. Uchida and Y. Kotani. 2001. Effects of harmful algae on the early planktonic larvae of the oyster, *Crassostrea gigas*. Pp. 411-414 *In*: (G. Hallegraeff, S. Blackburn, C. Bolch and R. Lewis, eds.), Harmful Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO.

Morohashi, A., M. Satake, K. Murata, H. Naoki, H.F. Kaspar and T. Yasumoto. 1995. Brevetoxin B3, a new brevetoxin analog isolated from the greenshell mussel *Perna canaliculus* involved in neurotoxic shellfish poisoning in New Zealand. Tetrahedron Lett. 36: 8995-8998.

Murata, K., M. Satake, H. Naoki, H.F. Kaspar and T. Yasumoto. 1998. Isolation and structure of a new brevetoxin analog, brevetoxin B2, from greenshell mussels from New Zealand. Tetrahedron Lett. 54: 735-742.

Nielsen, M.V. and T. Strømgren. 1991. Shell growth response of mussels (*Mytilus edulis*) exposed to toxic microalgae. Mar. Biol. 108: 263-267.

Nightengale,W.H. 1936. Red water organisms: their occurrence and influence upon marine aquatic animals, with special reference to shellfish in waters of the Pacific coast. Argus Press. Seattle, WA.

Padilla, D.K., M.H. Doall, C.J. Gobler, A. Hartson and K. O'Boyle. 2006. Brown tide alga, *Aureococcus anophagefferens*, can affect growth but not survivorship of *Mercenaria mercenaria* larvae. Harmful Algae 5: 736-748.

Pearce, I., J.H. Handlinger and G.M. Hallegraeff. 2005. Histopathology in Pacific oyster (*Crassostrea gigas*) spat caused by the dinoflagellate *Prorocentrum rhathymum*. Harm. Algae 4: 61-74.

Peters, E.C., P.P. Yevich, J.C. Harshbarger and G.E. Zaroogian. 1996. Comparative histopathology of gonadal neoplasms in marine bivalve molluscs. Dis. Aquat. Org. 20: 59-76.

Peterson, C.H. and H.C. Summerson. 1992. Basin-scale coherence of population dynamics of an exploited marine invertebrate, the bay scallop: implications of recruitment limitation. Mar. Ecol. Prog. Ser. 90: 257-272.

Pierce, R.H., M.S. Henry, L.S. Proffitt and P.A. Hasbrouck. 1990. Red tide toxin (brevetoxin) enrichment in marine aerosol. Pp. 397-402 *In*: (E. Graneli, B. Sundstrom, L. Elder and D. Anderson, eds.), Toxic Marine Phytoplankton. Elssevier, Amsterdam.

Pierce, R., M. Henry, P. Blum and S. Payne. 2001. *Gymnodinium breve* toxins without cells: Intra-cellular and extra-cellular toxins. Pp. 421-424 *In*: (G. Hallegraeff, S. Blackburn, C. Bolch and R. Lewis, eds.), Harmful Algal Blooms. Intergovernmental Oceanographic Commission of UNESCO.

Pierce, R.H., M. Henry, R. Dickey and S. Plakas. 2004. NSP (*Karenia brevis*) toxins and metabolites in oysters, clams, and whelks. Pp. 294-296 *In*: (K. A. Steiginger, J.H. Landsberg, C.R. Tomas, and G.A. Vargo, eds.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO.

Pierce, R.H., M.S. Henry, P.C. Blum, S.L. Hamel, B. Kirkpatrick, Y.S. Cheng, Y. Zhou, C.M. Irvin, J. Naar, A. Weidner, L.E. Fleming, L.C. Backer and D.G. Baden. 2005. Brevetoxin composition in water and marine aerosol along a Florida beach: Assessing potential human exposure to marine biotoxins. Harmful Algae 4: 965-972.

Plakas, S.M., K.R. El Said, E.L.E. Jester, H.R. Granade, S.M. Musser and R.W. Dickey. 2002. Confirmation of brevetoxin metabolism in the Eastern oyster (*Crassostrea virginica*) by controlled exposures to pure toxins and to *Karenia brevis* cultures. Toxicon 40: 721-729.

Poli, M.A., S.M. Musser, R.W. Dickey, P.P. Eilers and S. Hall. 2000. Neurotoxic shellfish poisoning and brevetoxin metabolites: a case study from Florida. Toxicon 38: 981-993.

Prakash, A., J.C. Medcof and A.D. Tennant. 1971. Paralytic shellfish poisoning in Eastern Canada. Fish. Res. Bd. Can. 177: 1-87.

Price, D.W., K.W. Kizer and K.H. Hansgen. 1991. California's paralytic shellfish poisoning prevention program, 1927-89. J. Shellfish Res. 10: 119-145.

Quayle, D.B. 1969. Paralytic shellfish poisoning in British Columbia. Fish. Res. Bd. Can. 168: 1-68.

Ray, S.M. and D.V. Aldrich. 1965. *Gymnodinium breve*: Induction of shellfish poisoning in chicks. Science 148: 1748-1749.

Ray, S.M. and D.V. Aldrich. 1967. Ecological interactions of toxic dinoflagellates and molluscs in the Gulf of Mexico. Pp. 75-83 *In*: (F. Russell and P. Saunders, eds.) Animal toxins. Pergamon, Oxford.

Roberts, B.S., G.E. Henderson and R.A. Medlyn. 1979. The effect of *Gymnodinium breve* toxins(s) on selected mollusks and crustaceans. Pp. 419-424 *In*: (Taylor and Seliger eds). Toxic Dinoflagellate Blooms. Elsevier, Amsterdam.

Sastry, A.N. 1965. The development and external morphology of pelagic larval and post-larval stages of the bay scallop, *Aequipecten irradians concentricus* Say, reared in the laboratory. *Bull. Mar. Sci*. 15, 417-435.

Savarese, M. S., G. Tolley and A.K. Volety. 2004. Using oyster reef communities in the design of estuarine restoration projects in South Florida. J. Shellfish Res. 23: 310-311.

Schwimmer, D. and M. Schwimmer. 1968. Medical aspects of phycology. Pp. 279-358 *In*: (D. Jackson, ed.) Algae, Man and the Environment. Syracuse University Press, Syracuse, N.Y.

Shumway, S.E. 1990. A review of the effects of algal blooms on shellfish and aquaculture. J. World Aquaculture Soc. 21: 65-104.

Shumway, S.E. 1995. Phycotoxin-related shellfish poisoning: Bivalve molluscs are not the only vectors. Rev. Fish. Sci. 3: 1-31.

Shumway, S.E. and T.L. Cucci. 1987. The effects of the toxic dinoflagellate *Protogonyaulax tamarensis* on the feeding and behavior of bivalve molluscs. Aquat. Tox. 10: 9-27.

Shumway, S.E. and A.D. Cembella. 1993. The impact of toxic algae on scallop culture and fisheries. Rev. Fish. Sci. 1: 121-150.

Shumway, S.E., T.M. Scott and J.M. Shick. 1983. The effects of anoxia and hydrogen sulphide on survival, activity and metabolic rate in the coot clam, *Mulinia lateralis* (Say). J. Exp. Mar. Biol. Ecol. 71: 135-146

Shumway, S.E., T.L. Cucci, L. Gainey and C.M. Yench. 1985. A preliminary study of the behavioral and physiological effects of Gonyaulax tamarensis on bivalve molluscs. Pp. 389-394 *In*: (D. Anderson, A. White and D. Baden, eds.), Toxic Dinoflagellates. Elsevier, Amsterdam.

Shumway, S.E., J. Barter and S. Sherman-Caswell. 1990. Auditing the impact of toxic algal blooms on oysters. Envir. Audit. 2: 41-56.

Sievers, A. 1969. Comparative toxicity of *Gonyaulax monilata* and *Gymnodinium breve* to annelids, crustaceans, molluscs and a fish. J. Protozool 16: 401-404.

Simon, J.L. and D.M. Dauer. 1972. A quantitative evaluation of red tide induced mass mortalities of benthic invertebrates in Tampa Bay, Florida. Environ. Lett. 3: 229-234.

Singer, M.M., D.L. Smalheer, R.S. Tjeerdema amd M. Martin. 1990. A simple continuous-flow toxicity test system for microscopic life stages of aquatic organisms. Water Res. 24: 899-903.

Smolowitz, R. and S.E. Shumway. 1997. Possible cytotoxic effects of the dinoflagellate, *Gyrodinium aureolum*, on juvenile bivalve molluscs. Aquacult. Int. 5: 291-300.

Sprague, V. 1970. Some protozoan parasites and hyperparasites in marine bivalve molluscs. Pp.511-526 *In*: (Sniesko, ed.). A Symposium on Diseases of Fishes and Shellfishes. Am. Fish. Soc., Wash., Spec. Publ. No. 5.

Steidinger, K.A., M.A. Burklew and R.M. Ingle. 1973. The effects of *Gymnodinium breve* toxin on estuarine animals. Pp. 179-202 *In*: (D.F. Martin and G.M. Padilla, eds.) Marine Pharmacognosy. Academis Press, New York.

Steidinger, K., B. Roberts and P. Tester. 1995. Florida red tides. Harmful Algae News 12/13: 1-3.

Steidinger, K.A., P. Carlson, D. Baden, C. Rodriguez and J. Seagle. 1998. Neurotoxic shellfish poisoning due to toxin retention in the clam *Chione cancellata*. Pp. 457-458. *In*: B. Reguera, J. Blanco, M.L. Fernandez and T Wyatt (eds.). Harmful Algae. Xunta de Galicia, IOC, Paris.

Summerson, H.C. and C.H. Peterson. 1990. Recruitment failure of the bay scallop, *Argopecten irradians concentricus*, during the first red tide, *Ptychodiscus brevis*, outbreak recorded in North Carolina. Estuaries 13: 322-331.

Tester, P.A. and F.K. Fowler. 1990. Brevetoxin contamination of *Mercenaria mercenaria* and *Crassostrea virginica*: a management issue. Pp. 499-503 *In*: (E.Granéli, B. Sundström, L. Edler and D.M. Anderson eds.) Toxic Marine Phytoplankton.

Proceedings of the Fourth International Conference on Toxic Marine Phytoplankton. Elsevier.

Tester, P.A., R.P. Stumpf, F.M. Vukovich, P.K. Fowler and J.T. Turner. 1991. An expatriate red tide bloom: transport, distribution, and persistence. Limnol. Oceanogr. 36: 1053-1061.

Tester, P.A. and K.A. Steidinger. 1997. *Gymnodinium breve* red tide blooms: Initiation, transport, and consequences of surface circulation. Limnol. Oceanogr. 42: 1039-1051.

Thompson, R.J., D.R. Livingstone and A. DeZwaan. 1980. Physiological and biochemical aspects of the valve snap and valve closure responses in the giant scallop *Placopecten magellanicus*. J. Comp. Physiol. 137: 97-104.

Tiffany, W.J. III and M.G. Heyl. 1978. Invertebrate mass mortality induced by a *Gymnodinium breve* red tide in Gulf of Mexico waters at Sarasota, Florida. J. Environ. Sci. Health A13: 653-662.

Todd, E.C. 1993. Domoic acid and amnesic shellfish poisoning: A review. J. Food Prot. 56: 69-83.

Twarog, B.M. and H. Yamaguchi. 1975. Resistance to paralytic shellfish toxins in bivalve molluscs. Pp. 381-393 *In*: (V.R. LoCicero, ed.) Proceedings of the First International Conference on Toxic Dinoflagellate Blooms. Mass. Sci. Tech. Fndn. Wakefield, MA.

Waller, T.R. 1981. Functional morphology and development of veliger larvae of the European oyster, *Ostrea edulis* Linne. *Smithsonian Contr. Zool*. 313, 1-70.

Wang, Z., S.M. Plakas, K.R. El Said, E.L.E. Jester, H.R. Granade and R.W. Dickey. 2004. LC/MS analysis of brevetoxin metabolites in the Eastern oyster (Crassostrea virginica). Toxicon 43: 455-465.

Wardle, W.J., S.M. Ray and A.S. Aldrich. 1975. Mortality of marine organisms associated with offshore summer blooms of the toxic dinoflagellate *Gonyaulax monilata* Howell at Galveston, Texas. Pp. 257-263 *In*: (V.R. LoCicero, ed.) Proceedings of the First International Conference on Toxic Dinoflagellate Blooms. Mass. Sci. Tech. Fndn. Wakefield, MA.

Watling, H.R. 1983. Comparative study of the effects of metals on the settlement of *Crassostrea gigas*. Bull. Environ. Contam. Toxicol. 31: 344-351.

Widdows, J and P. Salkeld. 1993. Practical procedures for the measurement of scope for growth. Phuket mar. biol. Cent Spec. Publ. 14: 43-53.

Widdows, J., N.M. Moore, D.M. Lowe and P.N. Salkeld. 1979. Some effects of a dinoflagellate bloom (*Gyrodinium aureolum*) on the mussel, *Mytilus edulis*. J. Mar. Biol. Ass. U.K. 59: 522-524.

Wikfors, G.H. and R.M. Smolowitz. 1993. Detrimental effects of a *Procentrum* isolate upon hard calms and bay scallops in laboratory feeding studies. Pp. 447-452 In: (T.J. Smayda and Y. Shimizu eds.) Toxic Phytoplankton in the Sea. Elsevier.

Wikfors, G.H. and R.M. Smolowitz. 1995. Experimental and histological studies of four life-history stages of the Eastern oyser, *Crassostrea virginica*, exposed to a cultured strain of the dinoflagellate *Prorocentrum minimum*. Bio. Bull. 188: 313-328.

Wilbur, A.E., S. Seyoum, T.M. Bert and W.S. Arnold. 2005. A genetic assessment of bay scallop (*Argopecten irradians*) restoration efforts in Florida's Gulf of Mexico Coastal Waters (USA). Conserv. Genet. 6: 111-122.

Yan, T., M. Zhou, M. Fu, Y. Wang, R. Yu and J. Li. 2001. Inhibition of egg hatching success and larval survival of the scallop, *Chlamys farreri*, associated with exposure to cells and cell fragments of the dinoflagellate *Alexandrium tamarense*. Toxicon 39: 1239-1244.

Yan, T., M. Zhou, M. Fu, R. Yu, Y. Wang and J. Li. 2003. Effects of the dinoflagellate *Alexandrium tamarense* on early development of the scallop *Argopecten irradians concentricus*. Aquaculture 217: 167-178.

Yomgjia, Z., B. Munday and J. Handlinger. 1995. Mass mortality of flat oysters (*Ostrea rivularis*) associated with a bloom of *Prorocentrum* sp. in the port of Zhanjiang, South China. Bull. Eur. Assoc. Fish Pathol. 15: 61-63.

Zar, J.H. 1996. Biostatistical Analysis. Prentis Hall. Upper Saddle River, NJ. 662 p.

About the Author

Jay Leverone received a B.A. in Biology in 1976 and a M.S. in Zoology in 1990 from the University of South Florida. He became a Staff Biologist at Mote Marine Laboratory in Sarasota in 1980 and has maintained his staff position during both his Masters and Doctoral Degrees. He entered the Ph. D. program at the University of South Florida in 1996.

While in the Ph.D. program, Mr. Leverone has been a student member of the National Shellfisheries Association and has presented portions of his doctoral research at the annual meetings. He also presented results from several shellfish restoration projects which he supervised while conducting his doctoral research. Mr. Leverone has made presentations at the International Conference on Shellfish Restoration and the International Pectinid Workshop.

Two manuscripts have been published on his doctoral research.

Leverone, Jay R., Norman J. Blake, Richard H. Pierce and Sandra E. Shumway. 2006. Effects of the dinoflagellate *Karenia brevis* on larval development in three species of bivalve mollusc from Florida. Toxicon 48: 75-84.

Leverone, Jay R., Norman J. Blake and Sandra E. Shumway. Comparative effect of the toxic dinoflagellate *Karenia brevis* on clearance rates in juveniles of four bivalve molluscs from Florida, USA. Toxicon (In press).

Mr. Leverone has been married to his wife, Barbara, for 23 years and has two wonderful children; a daughter, Donna and a son, Jason.