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Bioaccumulation and depuration of brevetoxins in the eastern oyster (*Crassostrea virginica*) and the northern quahog (= hard clam, *Mercenaria mercenaria*)

Andrew W. Griffith [a], Sandra E. Shumway [b], Aswani K. Volety [a,*]

[a] Florida Gulf Coast University, Coastal Watershed Institute, Vester Marine Field Station, College of Arts and Sciences, 10501 FGCU Blvd, Fort Myers, FL 33965, USA
[b] University of Connecticut, Department of Marine Sciences, University of Connecticut, 1080 Shennecossett Road, Groton, CT 06340, USA

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ABSTRACT

The eastern oyster (*Crassostrea virginica*) and northern quahog (= hard clam, *Mercenaria mercenaria*) are two species of economic and ecological significance in east coast waters of the United States and the Gulf of Mexico. Commercial industries for these species, especially within the state of Florida, are significant. The current study was undertaken to build upon the already established body of knowledge surrounding effects of the toxic dinoflagellate *Karenia brevis* on shellfish, to provide an understanding of the kinetics of brevetoxins within shellfish tissues, and to provide an estimate of brevetoxin retention times in these shellfish after a bloom event.

Individual clams and oysters were exposed to the toxic dinoflagellate, *K. brevis* at a bloom concentration of 5×10^5 cells·L^{-1} for eight days and then transferred to filtered water for depuration. Individuals were sampled periodically to determine depuration rates. Concentrations of brevetoxins (and/or their metabolites measured as PbTx-3 equivalent) in tissues were determined using an Enzyme Linked Immunosorbent Assay (ELISA). After five days of exposure, brevetoxin levels in tissues of both species reached concentrations well above the regulatory limit of 800 ng g^{-1} (Pb-TX3 equivalent). Averaged concentration of brevetoxins in clams was 1000 ng g^{-1}, while the oysters averaged 1986 ng g^{-1}. After two weeks of depuration, tissue concentrations in both species were below regulatory levels with clams averaging ~204 ng g^{-1} and oysters averaging ~437 ng g^{-1}. Toxins (or their metabolities) remained detectable in both clams (139 days) and oysters (82 days) for the duration of the experiment.

1. Introduction

Worldwide, there are approximately 300 species of microalgae that have adverse impacts within marine ecosystems (Hallegraeff et al., 1995; Smayda, 1997). Species-specific impacts vary, but range from sub-lethal effects on marine fauna, wide spread finfish and shellfish kills, and marine mammal and bird mortalities (Shumway and Cucci, 1987; Shumway, 1990; Landsberg, 2002; Shumway et al., 2003; Flewelling et al., 2005; Cohen et al., 2007). Over the past several decades bloom events of increasing intensity, frequency, and geographic distribution have been noted (Hallegraeff, 1993), due unquestionably to a number of factors including increased awareness, advances in detection techniques, the increased use of coastal areas for aquaculture, anthropogenic influences on nutrient loading and climate change, and possible introduction of resting cysts in the ballast water of transport ships (Hallegraeff, 1993; Hallegraeff et al., 1995; Hégaret et al., 2009).

* Corresponding author. Tel.: +1 239 590 7216.
E-mail addresses: avolety@fgcu.edu, avolety@yahoo.com (A.K. Volety).

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The harmful impacts of these algae are manifested in a variety of ways: 1) direct contact with associated toxins, i.e., the uptake of intact cells through ingestion, contact with whole cells, and exposure to extracellular toxins after lysis; 2) mechanical or physical damage to gill tissues of finfish or shellfish; and 3) anoxic conditions in the water column following dense blooms (Shumway, 1990; Hallegraeff et al., 1995; Landsberg, 2002). Vertebrates, including humans, may be exposed via inhalation of aerosolized toxins, e.g., toxins associated with *Karenia brevis* (brevetoxins) and *Pfiesteria* spp. are known to become aerosolized and subsequently inhaled by marine mammals and other air breathing organisms, causing respiratory distress (Pierce et al., 2005; Pierce and Henry, 2008; Glasgow et al., 1995). Brevetoxins can also become concentrated within the tissues of bivalve molluscs, rendering them toxic to humans and other vertebrate consumers, resulting in various forms of shellfish poisoning. Brevetoxins result in Neurotoxic Shellfish Poisoning (NSP) (Steidinger et al., 1998; Poli et al., 2000; Shumway, 1990; Hégaret et al., 2009) with symptoms ranging from mild gastrointestinal distress to bronchoconstriction posing a significant threat to both human health and aquaculture industries (Shumway, 1990; Hallegraeff et al., 1995; Matsuyama and Shumway, 2009).

Although several species of harmful algae exist naturally within the waters of the Gulf of Mexico, the most common species is the dinoflagellate *K. brevis* (Steidinger et al., 1999). While it can be present in other areas of the Gulf such as Louisiana, Mississippi, and Texas, it occurs most often on the west coast of Florida (Tester and Steidinger, 1997; Brand and Compton, 2007). There is some evidence that the intensity and/or duration of blooms within this region has been increasing, with documented blooms lasting as long as 13 months (Heil et al., 2006; Brand and Compton, 2007; Brand et al., 2012). Other reports of *Karenia* spp. have been documented in waters of the Caribbean, New Zealand, and the English Channel (Lackey, 1956; Nozawa et al., 2003; Llewellyn et al., 2005).

Toxins produced by *K. brevis* affect a wide range of organisms including zooplankton, bivalve molluscs, marine mammals, and humans (Shumway, 1990; Landsberg, 2002; Flewelling et al., 2005; Leverone et al., 2006; Cohen et al., 2007; Brand et al., 2012). The potential harm to shellfish aquaculture includes both economic and human health related impacts (Shumway, 1990; Matsuyama and Shumway, 2009). Shellfish species, via the consumption of *K. brevis*, become toxic to human consumers which in turn can have negative economic impacts on shellfish aquaculture (Cummins et al., 1971; McFarren et al., 1965). There is a large body of knowledge addressing the effects of *K. brevis* on marine organisms, yet we know little about the kinetics of brevetoxins within the tissues of bivalve molluscs.

Shumway et al. (1990) compiled data relating to toxin retention within species of shellfish and performed field experiments concerning the retention of toxins within species of oysters (*Crassostrea virginica* and *Ostrea edulis*) and mussels (*Mytilus edulis*). The depuration of shellfish toxins to below regulatory levels is dependent on a variety of factors, i.e., species of shellfish, time of exposure, and environmental factors (Shumway et al., 1990; Matsuyama and Shumway, 2009). Understanding the depuration of these toxins is of critical importance for management of monitoring programs and aquaculture facilities. Knowing when shellfish are close to quarantine levels may make regulatory monitoring more efficient (=less expensive) after intense bloom events and will provide information to the public as to when the harvesting of certain species of shellfish is safe.

Information regarding the retention of brevetoxins in shellfish and how long they pose a threat to human consumers is lacking. This research addresses the following questions: How long does it take for *Mercenaria mercenaria* and *C. virginica* to accumulate brevetoxins to levels in their tissues that pose a threat to human consumers? After intoxification, how long does it take for toxin concentrations within shellfish tissues to fall below safe limits for human consumption? Finally, how long will these toxins remain in tissues at concentrations below regulatory limits? The objective of this study is to examine the persistence of brevetoxins in oysters and clam tissues exposed to *K. brevis*. Results will have direct impacts on the regulatory practices regarding the closures of shellfish beds, provide regulators with information on how long different species of shellfish may remain toxic after exposure to *K. brevis*, and help to decrease monetary losses to local shellfish growers.

2. Materials and methods

2.1. Bivalve collection and maintenance

Adult eastern oysters (*C. virginica*) (average length 82.9 ± 3.1 mm) were collected in early June from Estero Bay, FL, with all epibionts removed. Northern quahogs (= hard clam, *M. mercenaria*) (average length 40.5 ± 3.1 mm, average width 45.7 ± 3.1 mm) were obtained from Cutthroat Clams, an aquaculture facility on Pine Island, FL. Both clams and oysters were kept in 25 L tanks with 25 individuals per tank in sterilized raw seawater at a salinity of 30. Each tank received 0.1 g Shellfish Diet© individual^{-1}day^{-1} (Chu and Volety, 1997).

2.2. Maintenance of cultures

The toxic dinoflagellate, *K. brevis* (CCMP #2229) was cultured in 8 L carboys in L1 medium (minus Si) at a salinity of 30 and at a temperature of 22–24 °C. Cultures were maintained on a 12:12 light dark cycle. Artificial seawater was passed through a 1 μm filter then autoclaved for 45 five minutes, cooled overnight, after which medium was added prior to inoculation. All cultures used for exposure were approximately 10–14 days old. Cell concentrations were determined by taking an aliquot from the main cultures, making ten-fold dilutions, and then counting them under a light microscope. Triplicate counts were made and averaged prior to dosing exposure tanks.

2.3. Experimental design

Twenty five individuals of each species were maintained in each tank with 25 L of raw sterilized seawater ($N = 5$ replicate tanks for each species and treatment) for