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Effects of field and laboratory exposure to the toxic dinoflagellate *Karenia brevis* on the reproduction of the eastern oyster, *Crassostrea virginica*, and subsequent development of offspring

Anne Rolton [a,b], Julien Vignier [a,b], Aswani K. Volety [b,*], Richard H. Pierce [c], Michael Henry [c], Sandra E. Shumway [d], V. Monica Bricelj [e], Hélène Hégaret [a], Philippe Soudant [a]

[a] Université de Bretagne Occidentale—IUEM, LEMAR CNRS UMR 6539, Place Nicolas Copernic, Technopôle Brest Iroise, 29280 Plouzané, France
[b] Florida Gulf Coast University, College of Arts and Sciences, 10501 FGCU Blvd South, Fort Myers, FL 33965, United States
[c] The Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, United States
[d] Department of Marine Sciences, University of Connecticut, 1080 Shennecossett Road, Groton, CT 06340, United States
[e] Department of Marine and Coastal Sciences and Haskin Shellfish Research Laboratory, School of Environmental and Biological Sciences, Rutgers University, 6959 Miller Avenue, Port Norris, NJ 08349, United States

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ABSTRACT

Blooms of the brevetoxin-producing dinoflagellate, *Karenia brevis*, are a recurrent and sometimes devastating phenomenon in the Gulf of Mexico. The eastern oyster, *Crassostrea virginica*, is exposed regularly to these blooms, yet little is known about the impacts of *K. brevis* upon this important species. The present study considered the effects of exposure to both a natural bloom and cultured *K. brevis* on the reproductive development of *C. virginica*. Oysters had been exposed to a bloom of *K. brevis* that occurred in Lee County, Florida, from September 2012 through May 2013, during a period of gametogenesis and gamete ripening. Ripe adult oysters were collected from this bloom-exposed site and from a site 200 miles north which was not exposed to any bloom. In addition, responses to two 10-day laboratory exposures of either unripe or ripe adult oysters to whole cells of *K. brevis* at high bloom concentrations (1000 and 5000 cells mL^{-1}) were determined. Both field- and laboratory-exposed adult oysters accumulated PbTx (attaining $\sim 22 \times 10^3$ ng g^{-1} and 922 ng g^{-1} PbTx-3 equivalents in the laboratory and the field, respectively), and significant mucal, edematous, and inflammatory features, indicative of a defense response, were recorded in adult tissues in direct contact with *K. brevis* cells. Laboratory-exposed oysters also showed an increase in the total number of circulating hemocytes suggesting that: (1) new hemocytes may be moving to sites of tissue inflammation, or, (2) hemocytes are released into the circulatory system from inflamed tissues where they may be produced. The area of oyster tissue occupied by gonad (representative of reproductive effort) and reactive oxygen species production in the spermatozoa of oysters exposed to the natural bloom of *K. brevis* were significantly lower compared to oysters that were not exposed to *K. brevis*. Additionally, following 10-day exposure of ripe oysters, a significant, 46% reduction in the prevalence of individuals with ripe gametes was obtained in the 5000 cells mL^{-1} *K. brevis* treatment.

Brevetoxin (PbTx) was recorded within the spermatozoa and oocytes of naturally exposed oysters and was estimated to be 18 and 26% of the adult PbTx load, respectively. Larvae derived from gametes containing PbTx showed significantly higher mortalities and attained a smaller larval size for the first 6 days post-fertilization. These negative effects on larval development may be due to the presence of PbTx in the lipid droplets of the oocytes, which is mobilized by the larvae during embryonic and lecithotrophic larval development. Provision of a non-contaminated food source to larvae however, appeared to mitigate the early negative effects of this neonatal PbTx exposure.

Results herein show that adult eastern oysters and their offspring are susceptible to exposure to *K. brevis*. Caution should therefore be exercised when identifying oyster reef restoration areas and in efforts to establish aquaculture in areas prone to red tides.

* Corresponding author. Current address: University of North Carolina Wilmington, 601 S. College Rd, Wilmington, NC 28409, United States. Tel.: +1 910 962 7232.
E-mail address: voletya@uncw.edu (A.K. Volety).

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1. Introduction

The naked dinoflagellate, *Karenia brevis*, is the most common harmful algal bloom (HAB)-forming species in the Gulf of Mexico (Finucane, 1964; Rounsefell and Nelson, 1966; Tester and Steidinger, 1997; Magaña et al., 2003). Cells of *K. brevis* produce brevetoxins (PbTx), a group of intracellular, lipid-soluble biotoxins which bind to site 5 on the voltage-sensitive sodium channels of excitable cells, causing permanent activation of the channel and disruption of nervous transmission (Poli et al., 1986; Baden, 1989; Baden et al., 2005). In addition to PbTx, *K. brevis* produces other chemically characterized and uncharacterized, often unstable compounds that have been shown to have allelopathic, ichthyotoxic, and hemolytic effects (Baden et al., 2005; Kubanek et al., 2005, 2007; Neely and Campbell, 2006; Steidinger, 2009).

Blooms of *Karenia brevis* are initiated offshore before moving to inshore areas, where cells can reach high concentrations ($>10^5$ cells mL^{-1}), persist for long periods (>18 months), and cover large areas (>25,000 km^2) (Gunter et al., 1948; Rounsefell and Nelson, 1966; Tester and Steidinger, 1997; Hu et al., 2006; Brand and Compton, 2007; Steidinger et al., 2008; Steidinger, 2009; Heil and Steidinger, 2009; Florida Fish and Wildlife Conservation Commission, FWC, monitoring data). Once inshore, blooms can have substantial negative effects, including human health problems, caused by inhalation of aerosolized toxins and ingestion of toxic shellfish leading to neurotoxic shellfish poisoning (NSP) (Steidinger et al., 1998; Poli et al., 2000; Backer et al., 2003; Pierce and Henry, 2008; Pierce et al., 2011; Kirkpatrick et al., 2004; Fleming et al., 2005). Occasional catastrophic bloom events have also resulted in mass mortalities of marine mammals, birds, fish, crustaceans, bivalve molluscs and other marine invertebrates (Gunter et al., 1948; Finucane, 1964; Simon and Dauer, 1972; Summerson and Peterson, 1990; Tester and Fowler, 1990; Landsberg, 2002; Shumway et al., 2003; Stumpf et al., 2003; Vargo et al., 2004; Flewelling et al., 2005; Hu et al., 2006).

To date, the main interest in *Karenia brevis* blooms and bivalve molluscs such as the eastern oyster (*Crassostrea virginica*) has been driven by their public health significance as vectors of NSP. Shellfish exposed to concentrations as low as 5 cells mL^{-1} accumulate sufficient amounts of PbTx to cause NSP in humans (Baden et al., 1995) and, as a result, affected shellfish harvest areas in the United States are closed when this cell concentration is reached and when brevetoxin levels within shellfish tissues reach 20 mouse units (MU) 100 g^{-1} wet weight of shellfish meat [= 0.8 mg PbTx-2 equivalents (eq) kg^{-1}] (NSSP, 2011). This results in economic losses for fisheries, bivalve producers, and related industries (Baden et al., 1995; Pierce et al., 2004; Pierce and Henry, 2008). Eastern oysters also provide important ecosystem services in estuaries (Wells, 1961; Newell, 2004; Burkholder and Shumway, 2012) and may themselves be affected by exposure to *K. brevis*. Effects of exposure to toxic algae on bivalve survival, behavior, respiration, feeding and cellular and immune function have been well established (Shumway and Cucci, 1987; Shumway and Gainey, 1992; Wikfors and Smolowitz, 1995; Smolowitz and Shumway, 1997; Hégaret and Wikfors, 2005a,b; Hégaret et al., 2007a,b; Galimany et al., 2008a,b,c; MacQuarrie and Bricelj, 2008; Haberkorn et al., 2010a,b; Basti et al., 2011); however, studies on the effects on bivalve reproduction are very limited (Haberkorn et al., 2010a).

Previous studies have shown that bivalves such as *Crassostrea virginica* may be adversely affected by exposure to *Karenia brevis*. Gunter et al. (1948) suggested that field exposure to blooms of *K. brevis* may have resulted in mortalities of this species; however, subsequent short-term laboratory exposures of *C. virginica* to bloom concentrations of *K. brevis* (at >1400 and 9900 cells mL^{-1}) did not result in any mortalities or behavioral changes (Ray and Aldrich, 1967; Sievers, 1969). Possible toxic effects were shown when juvenile eastern oysters were exposed to a mixed suspension of *Isochrysis galbana* (2000 cells mL^{-1}) and whole cells of *K. brevis* (1000 cells mL^{-1}) for 1 h, which resulted in a 38% reduction of the clearance rate of *I. galbana* compared with control oysters fed unialgal *I. galbana* (Leverone et al., 2007). Deleterious effects were also demonstrated by Rolton et al. (2014, 2015) following exposure of the gametes and early life stages of *C. virginica* to bloom concentrations of whole cells, lysed cells, and culture filtrate treatments of *K. brevis* ($\geq$500 cells mL^{-1}).

There have been no reports on adverse effects of *Karenia brevis* or PbTx exposure on the gonadal development and reproductive effort of *Crassostrea virginica* or on any offspring produced by toxic adults. Summerson and Peterson (1990) did report near-total recruitment failure of bay scallops (*Argopecten irradians*) exposed to a *K. brevis* bloom, suggesting the toxic algae may have interfered with the spawning of adult scallops, survivorship of the planktonic larvae, larval settlement, or survivorship of newly settled scallops. As sessile inhabitants of shallow, nearshore coastal waters, eastern oysters in southwest Florida, FL, are frequently exposed to blooms of *K. brevis* (Brand and Compton, 2007; FWC, monitoring data). Furthermore, in the subtropical climate of this region, the reproductive periods of eastern oysters and blooms of *K. brevis* often overlap (Rounsefell and Nelson, 1966; Hu et al., 2006; Volety, 2008; Volety et al., 2009). Eastern oysters in Florida are found in spawning condition year-round with the main spawning period from March to November (Volety, 2008; Volety et al., 2009); however, periods of gamete maturation prior to spawning and during post-spawning periods of larval development and recruitment further extend this reproductive period. In the eastern Gulf of Mexico, blooms of *K. brevis* typically occur in late summer/autumn and may last several months (Rounsefell and Nelson, 1966; Stumpf et al., 2003; Hu et al., 2006). Indeed, the 2012–2013 bloom lasted from September 2012 through May 2013 and extended from Pinellas, FL, to the Florida Keys (FWC, monitoring data). Thus, adult eastern oysters may be exposed directly to bloom concentrations of *K. brevis* during gametogenesis, gamete maturation, spawning and larval recruitment. This may not only impact adults, but also any subsequently produced offspring that may be affected by maternal exposure to PbTx. It is unknown if PbTxs accumulate within the gametes of bivalves and are transferred directly to the offspring. Although prior studies confirmed the presence of PbTx in the embryos of mice and sharks, they did not consider the consequences of this transfer (Benson et al., 2006; Flewelling et al., 2010).

This study addresses the following questions: does exposure to bloom concentrations of *Karenia brevis* affect adult eastern oysters including their reproduction and gamete quality, and, does this adult exposure adversely affect subsequently produced offspring? The effects of different exposure scenarios of *K. brevis* in the field and laboratory on adult eastern oysters, *Crassostrea virginica*, were studied. Brevetoxin content, condition index (CI), gonadal stage (GS) and relative area, sex distribution, the prevalence of individuals with ripe gametes, histopathological features in oyster tissues, and changes in the cellular parameters of hemocytes were examined. Gametes were obtained from adult oysters exposed to *K. brevis*, and the PbTx content was assessed in stripped, filtered and concentrated gametes. Differences in the spermatozoa, fertilization success and larval development were assessed in offspring up to 11 days post-fertilization (PF).

2. Methods

The effects of field exposure of *Crassostrea virginica* to a natural bloom of *Karenia brevis* and two separate, 10-day laboratory