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Impacts of *Deepwater Horizon* oil and associated dispersant on early development of the Eastern oyster *Crassostrea virginica*

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ABSTRACT

The explosion of the *Deepwater Horizon* (DWH) oil platform resulted in large amounts of crude oil and dispersant Corexit 9500A® released into the Gulf of Mexico and coincided with the spawning season of the oyster, *Crassostrea virginica*. The effects of exposing gametes and embryos of *C. virginica* to dispersant alone (Corexit), mechanically (HEWAF) and chemically dispersed (CEWAF) DWH oil were evaluated. Fertilization success and the morphological development, growth, and survival of larvae were assessed. Gamete exposure reduced fertilization (HEWAF: $EC20_{1\ h}$ = 1650 μg tPAH50 L^{-1}; CEWAF: $EC20_{1\ h}$ = 19.4 μg tPAH50 L^{-1}; Corexit: $EC20_{1\ h}$ = 6.9 mg L^{-1}). CEWAF and Corexit showed a similar toxicity on early life stages at equivalent nominal concentrations. Oysters exposed from gametes to CEWAF and Corexit experienced more deleterious effects than oysters exposed from embryos. Results suggest the presence of oil and dispersant during oyster spawning season may interfere with larval development and subsequent recruitment.

1. Introduction

The Eastern oyster, *Crassostrea virginica* (Gmelin, 1791), is one of the most commercially and ecologically important shellfish species propagating along the East Coast of the United States, from Maine to the Gulf of Mexico (Galtsoff, 1964; Volety et al., 2014). In 2012, total landings of *C. virginica* represented a value of $104 million in the United States from which $74 million originated in coastal regions of the northern Gulf of Mexico (National Marine Fisheries Service, 2012). In addition to its economic value, *C. virginica* is also an ecologically vital species. Oyster reefs, which have been built through successive reproduction and settlement of larvae onto existing reef structure, provide food, shelter, and habitat for many fish and shellfish species; improve water quality; stabilize bottom areas; and influence water circulation patterns within estuaries (Coen et al., 2007; Newell, 2004; Peterson et al., 2003; Volety et al., 2014; Wells, 1961). In the northern part of the Gulf of Mexico, the oyster spawning season typically occurs from mid-spring through late fall (Ingle, 1951). On April 20, 2010, the explosion of the *Deepwater Horizon* (DWH) oil platform in the Gulf of Mexico led to the release of millions of barrels of crude oil 80 km off the coast of Louisiana (McNutt et al., 2012). The oil leak was discovered two days after the incident at a depth of 1544 m. After almost three months and several attempts to stop the leak, the well was finally cemented on July 15, 2010 (Crone and Tolstoy, 2010). Approximately 7 million L of the chemical dispersant Corexit 9500A® was used directly at the wellhead and at the surface to disperse the oil slicks (Kujawinski et al., 2011). The DWH oil contaminated first the Louisiana coast and then the Mississippi, Alabama, and Florida coasts (Rosenbauer et al., 2010).

Petroleum hydrocarbon contaminants pose a severe ecological risk to marine organisms. They can affect organisms by physical action (light reduction, asphyxia), by modification of habitat [change in pH (Neff, 1987), decrease of dissolved oxygen, decrease in food availability], and by toxic effects. Crude oil constituents are of particular concern because of their high chemical stability, low degradation, and lipophilic nature. Most toxic effects of crude oil are typically attributed to the aromatic fraction, particularly polycyclic aromatic hydrocarbons (PAHs); PAHs are known to be persistent in the environment and are potentially mutagenic, genotoxic, and carcinogenic to organisms (Albers and Loughlin, 2003; Neff, 1985; Roesijadi et al., 1978).

Chemical dispersants are complex mixtures, primarily containing surfactants (dioctyl sodium sulfosuccinate, also known as DOSS) and solvents (propylene glycol), which reduce the interfacial tension at the oil–water interface, and therefore facilitate the mixing of oil into the water (Canevari, 1973; Li and Garrett, 1998). Therefore, oil slicks

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can be dispersed to concentrations below toxicity thresholds for most marine and benthic species (Lessard and DeMarco, 2000; Page et al., 2000) and become more accessible to hydrocarbon-degrading bacteria (Venosa and Zhu, 2003). Because of the higher exposure of aquatic organisms to petroleum compounds in coastal areas and estuaries, the manufacturer recommends using chemical dispersants at a minimum water depth of 20 m, with a current speed greater than 1 m s^{-1}, and at a minimum distance from the shore or from off-shore islands of 2 km (Ramachandran et al., 2004). According to manufacturers, new dispersants, such as Corexit 9500A®, are considered non-toxic and bio-degradable when used on their own and at recommended concentrations. However, aquatic organisms are likely to be exposed to both dispersant and oil in combination, which may alter the toxic effects (Almeda et al., 2013; Barron et al., 2003; Getter and Baca, 1984; Gulec et al., 1997; Ramachandran et al., 2004; Rico-Martinez et al., 2013). Moreover, little is known about the behavior and combined effects of oil and dispersants in the near-shore environment (Allen, 1984).

The prolonged turbulent mixing of crude oil by wind, currents, and waves results in the production of water accommodated fraction (WAF) (Barron et al., 1999; Rossi et al., 1976). WAF toxicity is commonly assessed by measuring early larval growth, survival, and morphological abnormality in marine organisms such as fish (Couillard et al., 2005), starfish (Davis et al., 1981), crustaceans (Cucci and Epifanio, 1979), or bivalves (Fucik, 1994; Saco-Alvarez et al., 2008). Early life stages are typically more sensitive than adult stages and represent a critical period in the life cycle (Chapman and Long, 1983; Connor, 1972; His and Beiras, 1999; Huffman Ringwood, 1991).

In an aquatic ecosystem, the physiological and ecological effects of environmental stress are numerous. Although the ultimate effect is mortality, sub-lethal effects include the alteration of normal physiological activities (Beiras and His, 1994; His and Robert, 1985) and increased expenditure of energy reserves affecting fecundity, as well as reduced fertilization success and larval viability and growth (Capuzzo, 1996; McDowell et al., 1999; Thompson et al., 1996). The *Deepwater Horizon* oil spill occurred at the beginning of the *C. virginica* spawning season. Sperm and eggs were therefore likely exposed to toxicants, as were adult and early life stage (ELS) of oysters. Research on the effects of Alaskan crude oil reported severe abnormalities in the developing embryos of Pacific oyster, *Crassostrea gigas* (Le Gore, 1974). PAHs, either alone or associated with sediments, as well as other organic chemicals, negatively affected *C. gigas* sperm motility, embryonic development, larval growth, and survival (Akcha et al., 2012; Geffard et al., 2002b, 2003; His and Robert, 1983; Laramore et al., 2014; Lyons et al., 2002; Pelletier et al., 2000; Renzoni, 1975). Moreover, the exposure of Pacific oysters to PAHs significantly reduced fertilization success and larval development (Jeong and Cho, 2005). Finally, ELS of *C. virginica* were adversely affected by acute exposure to dispersed oil (CEWAF), with decreased fertilization success (≥100 ppm CEWAF) and increased developmental abnormalities (≥100 ppm CEWAF) and mortalities ($LC50_{96h}$ = 24.8 ppm CEWAF) of D-larvae observed after incubation with a surrogate of *Macondo* oil dispersed with 1:10 of Corexit 9500A (Laramore et al., 2014). Therefore, fertilization success and subsequent embryogenesis and larval development of *C. virginica* might have been negatively affected by the exposure to oil, dispersed oil, and Corexit 9500A®, all of which were found in the Gulf of Mexico at the time of the spill. Although oysters have been used as a model organism in numerous ecotoxicological studies (Chapman and Long, 1983; Chapman, 1989; His et al., 1997; Thain, 1991, 1992; Woelke, 1972), little literature exists on the toxicity of dispersant and dispersed oil to *C. virginica* gametes and embryos; therefore, effects of these toxicants on fertilization success and early development need further investigation.

The purpose of this study was to examine the lethal and sub-lethal effects of acute exposure to surface-collected DWH oil (HEWAF), dispersant (Corexit 9500A®), or dispersed oil (CEWAF) on the two sensitive early life stages – gametes and embryos – of *C. virginica*. Fertilization success, morphological development, shell lengths, and survival were assessed at different time points (1 h, 24 h, and 96 h). In addition, lethal and sub-lethal concentration ranges were also determined. Research on the differences in gamete and embryo susceptibility to oil and dispersant may contribute to understanding the mechanisms of toxicity on sensitive early life stages of bivalves.

2. Material and methods

Separate preliminary range-finding tests were performed to establish the definitive test concentrations that cause lethal effects on oyster gametes and embryos, as well as sub-lethal effects, such as developmental abnormalities and reduced growth of gametes and embryos. Exposure designs were based on standardized protocols described in "U.S. EPA., 1996. *Ecological Effects Test Guidelines: OPPTS 850.1055: Bivalve Acute Toxicity Test (embryo larval)*."

2.1. Water accommodated fractions

Crude oil was obtained from Stratus Consulting under chain of custody during the *Deepwater Horizon* NRDA efforts. The DWH surface slick oil ("Slick A") was collected near the source on July 29, 2010, from the hold of barge number CTC02404, which received surface slick oil from various skimmer vessels near the *Macondo* well (sample CTC02404-02). The dispersant Corexit 9500A® (NALCO Environmental Solutions LLC, Sugarland, TX) was obtained from Stratus Consulting/Abt Associates. For all exposure solutions, we added contaminants to UV-sterilized and 0.1 μm-filtered seawater (FSW), maintained at a salinity of 20–25 PSU.

2.1.1. HEWAF

The oil-only exposure solutions or high energy water accommodated fractions (HEWAFs) were prepared at 25 °C under fluorescent lights to avoid photo-reactivity (Landrum et al., 1987). We artificially recreated the action of waves and currents by adding 2 L of filtered seawater (FSW) and 4 g of slick oil (with a gastight syringe) to a stainless steel blender pitcher (Waring™CB15, Waring Commercial, Torrington, CT). After 30 s at the lowest blending speed, the solution was transferred to a 2-L aspirator bottle and left to settle for at least 1 h to separate the residual floating oil (Incardona et al., 2013). The bottom layer of the mixture (or accommodated fraction) was then carefully drained from the aspirator bottle and FSW was added to this stock to prepare dilutions for exposure treatments. We did not filter preparations, so dilutions contained particulate oil in addition to dissolved PAHs.

2.1.2. CEWAF

The oil/dispersant mixtures or chemically enhanced water accommodated fractions (CEWAFs) were also prepared at 25 °C under fluorescent lights. Two grams of slick oil and 200 mg of dispersant (10:1 v:v) were added to an aspirator bottle filled with 2 L of FSW. Contaminants were added with a gastight syringe, and stirred at a vortex adjusted to 25% using a stirring rod and a magnetic stirrer for 18 h. To allow for the separation of the solution from the residual floating oil, the oil and dispersant mixture was left to stand for 3 h prior to use and the stock solution (or accommodated fraction) was carefully drained.

2.1.3. Corexit

Dispersant exposure solutions were prepared as described for CEWAF above, except that no oil was added and the mixture was not settled. The dispersant stock was collected by draining the aspirator bottle and, to obtain different exposure concentrations, the stock solution was diluted with FSW. Samples for DOSS concentrations became contaminated, so nominal dispersant concentrations were reported for these tests.

Nominal concentrations used for exposure to HEWAF, CEWAF, and dispersant, as well as corresponding tPAH50 contents, are listed in