Marine Pollution Bulletin 133 (2018) 828–834

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Photo-enhanced toxicity of undispersed and dispersed weathered Macondo crude oil to Pacific (*Crassostrea gigas*) and eastern oyster (*Crassostrea virginica*) larvae

Bryson E. Finch[a], Emily S. Stefansson[b], Chris J. Langdon[b], Suzanne M. Pargee[b], William A. Stubblefield[a,*]

[a] Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, USA
[b] Hatfield Marine Science Center, Oregon State University, Newport, OR, USA

ARTICLE INFO

Keywords:
Phototoxicity
UV light
Oyster
Deepwater Horizon
Crude oil

ABSTRACT

During the Deepwater Horizon oil spill rapid natural weathering of Macondo crude oil occurred during the transport of oil to coastal areas. In response to the DWH incident, dispersant was applied to Macondo crude oil to reduce the movement of oil to coastal regions. This study aimed to assess the narcotic and phototoxicity of water-accommodated fractions (WAFs) of weathered Macondo crude oil, and chemically-enhanced WAFs of Corexit 9500 to Pacific (*Crassostrea gigas*) and eastern (*Crassostrea virginica*) oyster larvae. Phototoxic effects were observed for larval Pacific oysters exposed to combinations of oil and dispersant, but not for oil alone. Phototoxic effects were observed for larval eastern oysters exposed to oil alone and combinations of oil and dispersant. Corexit 9500 did not exhibit phototoxicity but resulted in significant narcotic toxicity for Pacific oysters. Oyster larvae may have experienced reduced survival and/or abnormal development if reproduction coincided with exposures to oil or dispersant.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a diverse class of compounds, each with unique chemical properties that dictate their toxicity and fate in the environment. PAHs have demonstrated abilities to absorb ultraviolet (UV) light, resulting in photo-excitation of outer valence electrons (Mekenyan et al., 1994; Veith et al., 1995). The majority of phototoxic PAHs have 3–5 rings that have both hydrophilic and lipophilic properties, resulting in increased bioavailability to aquatic organisms. PAHs UV absorbance capabilities are predicated on the energy gap between the highest occupied molecular orbital and lowest occupied molecular orbital (HOMO-LUMO; Mekenyan et al., 1994; Veith et al., 1995). The excitation of electrons by UV light (i.e. photo-excitation) can result in the production of oxyradicals that oxidize cell membranes, proteins, and DNA (Landrum et al., 1987). Lipid peroxidation of cell membranes can result in changes in cell membrane permeability, which may lead to inflammation and edema resulting in reduced diffusivity across the cell membrane (Weinstein et al., 1997).

Photo-enhanced toxicity occurs under environmental conditions conducive to high UV transmission through the atmosphere and hydrosphere. Longitude, seasonality, and climatic conditions are key variables that dictate the intensity and duration of UV exposure to aquatic organisms. Studies have demonstrated that UV light can penetrate to depths necessary for phototoxic risk (Sellin Jeffries et al., 2013); however, UV transmission is primarily dependent on water turbidity (Ireland et al., 1996) and dissolved organic content (Gensemer et al., 1998; Nikkila et al., 1999; Weinstein and Oris, 1999). Marine offshore waters tend to be more transparent than coastal waters (Tedetti and Sempéré, 2006). In coastal waters of the Gulf of Mexico, UV-A and UV-B transmissions were at 10% of incidence UV at 5 and 3 m, respectively (Wilhelm et al., 1998, 2002), whereas in offshore waters of the Gulf of Mexico, 10% of UV-A and UV-B incidence transmissions were measured at 37 and 13 m, respectively. UV-A's longer wavelengths enable deeper penetration into the water column compared to UV-B (Tedetti and Sempéré, 2006), implying that UV-A is a more important consideration in assessments of photo-enhanced toxicity in aquatic environments.

As organisms develop, sensitivity to photo-enhanced toxicity can change significantly (Finch and Stubblefield, 2015; Finch et al., 2016). Adaptive mechanisms afford some organisms protection against UV-induced toxicity and sensitive life stages can be identified, of which typically consist of embryonic or larval stages (Finch and Stubblefield, 2015; Finch et al., 2016). Physiology and life history traits, such as

* Corresponding author.
E-mail address: bill.stubblefield@oregonstate.edu (W.A. Stubblefield).

https://doi.org/10.1016/j.marpolbul.2018.05.065
Received 11 January 2018; Received in revised form 24 May 2018; Accepted 29 May 2018

mode of reproduction, development rate, and habitat preferences, often dictate sensitivity to phototoxicity. Broadcast spawners are of particular interest because of the potential for UV exposure during their early developmental stages, whereas benthic spawners are often afforded protection from UV light from macrophytes, overlying water, suspended materials and sediment. Broadcast spawners such as oysters, release free-floating gametes near the water's surface, resulting in the development of embryos and larvae near the water's surface. During oil spills, there is potential for free-floating embryos and larvae of broadcast spawners to be exposed to both hydrocarbons and UV light. Laboratory studies have demonstrated that larval oysters are sensitive to photo-enhanced toxicity (Finch et al., 2016). The toxicity of pyrene to embryos of the coot clam (*Mulinia lateralis*) was over 50,000-fold greater in the presence of UV light compared to light from fluorescent light (Pelletier et al., 1997). LC50s were 5000 times greater to juvenile coot clams exposed to pyrene under UV light compared with fluorescent light. Finch et al. (2016) found that a window of sensitivity exists, in which oyster early life stages are susceptible to photo-enhanced toxicity. Eastern oyster larvae were highly sensitive to the photo-enhanced toxicity of water-accommodated fractions (WAFs) of weathered Macondo crude oil, until shell development occurred at the veliger stage, at which point no phototoxicity was observed.

Several studies have reported increased toxicity of individual PAHs and fresh and weathered crude oil in the presence of UV light (Cleveland et al., 2000; Barron et al., 2003; Spehar et al., 1999; Oris and Giesy, 1987; Finch and Stubblefield, 2015; Finch et al., 2016; Finch et al., 2017), however few have examined effects to bivalves, while no research has examined the phototoxic potential of dispersed oil and dispersant alone to oyster larvae. Barron et al. (2003) reported phototoxic effects of both undispersed and dispersed Alaska North Slope crude oil to Pacific herring (*Clupea pallasi*) eggs and larvae; however, no significant differences were observed between the phototoxicity of dispersed and undispersed crude oil. Macondo crude oil WAFs and CEWAFs have demonstrated phototoxic effects to mahi-mahi (*Coryphaena hippurus*) embryos and blue crab (*Callinectes sapidus*; Alloy et al., 2015; Alloy et al., 2016). Further investigation is needed to characterize the photo-enhanced toxicity of dispersed oils to a wider range of species, especially invertebrate species whose early life stages may be susceptible to exposures to crude oil and dispersant.

During the Deepwater Horizon oil spill, approximately 3.19 million barrels of Macondo crude oil was released into the Gulf of Mexico 66 km offshore at a water depth of 1500 m (United States District Court for the Eastern District of Louisiana, 2015). In response to the oil spill, 2.1 million gallons of dispersant was applied to reduce the movement of crude oil to near-shore regions and promote biodegradation (Kujawinski et al., 2011). Fresh Macondo crude oil ascended 1500 m from the seafloor and was transported significant distances to other Gulf coastal regions. During transport, weathering processes such as evaporation, dissolution, biodegradation, wave and wind action, as well as photolysis likely affected the composition of Macondo crude oil; therefore, organisms residing in the near-shore were likely exposed to weathered crude oil. The current study evaluated the narcotic toxicity and phototoxicity of dispersed and undispersed weathered Macondo crude oil as well as dispersant alone on early life stages of Pacific (*Crassostrea gigas*) and eastern oyster (*Crassostrea virginica*) larvae.

2. Methods

2.1. Materials

Test oils used for bioassays were collected from the Gulf of Mexico during the Deepwater Horizon oil spill (BP Gulf Science Data, 2014). Naturally weathered CTC crude oil was collected from the Gulf of Mexico on the CTC 02404 barge on July 29, 2010. Oil was stored in a refrigerator at 4 °C in the dark prior to use. Corexit EC9500A was obtained from product manufactured by Nalco Company and stockpiled for emergency response. The stock tested was dispensed from totes of Corexit 9500A inventory obtained in the summer of 2010 from the Dispersants Operations staging area at Stennis Naval Air Station (Meridian, MS, USA), established as part of the DWH spill response. Corexit was stored in an amber glass jar at room temperature prior to use.

Eastern oyster were obtained from a commercial supplier in Washington State (Taylor Shellfish, Shelton, WA, USA). Pacific oyster broodstock were obtained from a local supplier in Yaquina Bay (Oregon Oyster, Newport, OR, USA). Broodstock were conditioned in the laboratory for 3 to 8 weeks, depending on reproductive condition and held in flowing 10 µm-filtered seawater from Yaquina Bay at 20 °C and a salinity of 30–32 g/L. They were fed a mixed algal diet of *Isochrysis galbana* and *Chaetoceros* sp. during the conditioning period. Spawning was induced by thermal shock to a maximum temperature of 30 °C. Test initiation for all studies was within 2 h post-fertilization (pf).

2.2. Experimental design

Methods used in the present study were similar to those reported by Finch et al. (2016) and Stefansson et al. (2016). Acute 48-h static toxicity tests were conducted with low energy WAFs of CTC weathered crude oil and CEWAFs of Corexit 9500 (dispersant), and a combination of weathered oil and Corexit 9500. Studies were conducted following slightly modified USEPA (1995a) and ASTM E724-98 (2004) standard methods. Methods for preparing WAFs and CEWAFs have been previously described (Singer et al., 2000, 2001a, 2001b; Barron and Ka'aihue, 2003). Filtered seawater (4 L) from Yaquina Bay was added to 4 L aspirator bottles with approximately 10% headspace. CTC oil was added to the 4 L of dilution seawater in the aspirator bottle at a loading rate of 1 g of oil per liter of water (wt/vol). For CEWAFs with oil, Corexit 9500 was added to aspirator bottles after the addition of the oil at a 1:20 dispersant to oil ratio (wt/wt; USEPA, 1995b). Corexit 9500 only CEWAFs contained equal concentrations of dispersant as in oil CEWAFs. Aspirator bottles were placed on individual stir plates (Corning PC-610D digital stir plates) operated at 60 rpm with no vortex and carried out in the dark at a temperature of 20 °C. WAFs and CEWAFs were mixed for 20 h, and then allowed to come to equilibrium for 4 h (settling period). Samples were then collected from the base of the aspirators bottles using the spout and subsequently used in toxicity tests.

Pacific and eastern oyster larvae were exposed to a five-dilution series of WAFs or CEWAFs at 100, 50, 25, 12.5, 6.25, and 0%(seawater control) WAF concentration in seawater, in combination with natural sunlight at the Oregon State University (OSU) Aquatic Toxicology Laboratory (Albany, Oregon) or UV-filtered natural light, using UV-filtering plexiglass (Acrylite OP-3 UV Filtering Museum Quality Plexiglass, EVONIK Industries, Parsippany, NJ, USA). The UV-filtering plexiglass was intended to eliminate exposure to UV light, while permitting exposure to visible light. The spectral transmittance of the UV-filtering plexiglass is presented in the Supplemental Data, Fig. S1. Test chambers consisted of 20 mL glass scintillation vials with high UV transmission (Kimble Chase, KG-33 borosilicate glass; see Supplemental Data, Fig. S2 for transmission efficiencies), with PTFE-lined screw caps. Tests were conducted with five replicate vials for each treatment, with each vial containing 10–15 larvae per mL of test solution (20 mL total). Vials were submerged in a water bath (25 °C) and oriented on their sides to ensure the opaque vial cap did not block UV light. Tests deviated from those of standard bivalve methods in that vials are usually oriented upright for the duration of the test. In order to account for differences in vial orientation, a control was included in each test, in which vials were kept upright in the dark.

Water quality was recorded at 0 (test initiation) and 48 h (test termination). Dissolved oxygen (DO), temperature, and pH were measured using a Hach meter (Model No. HQ40D, Loveland, CO, USA) and salinity was measured using an Oakton SALT 6+ salinity meter (Vernon Hills, IL, USA). At the end of the 48-hour test period, larvae were