Ecotoxicology and Environmental Safety 148 (2018) 473–479

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Toxicity of weathered *Deepwater Horizon* oil to bay anchovy (*Anchoa mitchilli*) embryos

Kathryn A. O'Shaughnessy[a,*], Heather Forth[b], Ryan Takeshita[b], Edward J. Chesney[a]

[a] Louisiana Universities Marine Consortium, 8124 Hwy 56, Chauvin, LA 70344, USA
[b] Abt Associates, 1881 Ninth Street, Suite 201, Boulder, CO 80302, USA

ARTICLE INFO

Keywords:
Bay anchovy
Anchoa mitchilli
Deepwater Horizon
Water accommodated fractions
Slick A
Slick B

ABSTRACT

The BP-contracted *Deepwater Horizon* Macondo well blowout occurred on 20 April 2010 and lasted nearly three months. The well released millions of barrels of crude oil into the northern Gulf of Mexico, causing extensive impacts on pelagic, benthic, and estuarine fish species. The bay anchovy (*Anchoa mitchilli*) is an important zooplanktivore in the Gulf, serving as an ecological link between lower trophic levels and pelagic predatory fish species. Bay anchovy spawn from May through November in shallow inshore and estuarine waters throughout the Gulf. Because their buoyant embryos are a dominant part of the inshore ichthyoplankton throughout the summer, it is likely bay anchovy embryos encountered oil in coastal estuaries during the summer and fall of 2010. Bay anchovy embryos were exposed to a range of concentrations of two field-collected *Deepwater Horizon* oils as high-energy and low-energy water accommodated fractions (HEWAFs and LEWAFs, respectively) for 48 h. The median lethal concentrations (LC_{50}) were lower in exposures with the more weathered oil (HEWAF, 1.48 μg/L TPAH50; LEWAF, 1.58 μg/L TPAH50) compared to the less weathered oil (HEWAF, 3.87 μg/L TPAH50; LEWAF, 4.28 μg/L TPAH50). To measure delayed mortality and life stage sensitivity between embryos and larvae, an additional 24 h acute HEWAF exposure using the more weathered oil was run followed by a 24 h grow-out period. Here the LC_{50} was 9.71 μg/L TPAH50 after the grow-out phase, suggesting a toxic effect of oil at the embryonic or hatching stage. We also found that exposures prepared with the more weathered Slick B oil produced lower LC_{50} values compared to the exposures prepared with Slick A oil. Our results demonstrate that even relatively acute environmental exposure times can have a detrimental effect on bay anchovy embryos.

1. Introduction

The bay anchovy, *Anchoa mitchilli* (Valenciennes, 1848), is an ecologically important, zooplanktivorous filter-feeder, and numerically the most abundant fish in the estuaries of the Gulf of Mexico (Hildebrand, 1943; Hildebrand and Schroeder, 1928; Lou and Brandt, 1993; Robinette, 1983). Bay anchovy are evening spawners, and spawn every 1.3–4 d during spawning season (Hildebrand and Cable, 1930; Lou and Musick, 1991). Bay anchovy embryos and larvae are also the dominant ichthyoplankter in Gulf estuaries throughout the summer. Overall average egg production is approximately 45,000 embryos per female per spawning season (Lou and Musick, 1991). They are a critical prey species in the diets of larger recreational and commercial fish species such as spotted seatrout (*Cynoscion nebulosus*), southern flounder (*Paralichthys lethostigma*), and Spanish mackerel (*Scomberomorus maculatus*; Hildebrand, 1943), and bay anchovy serve as an important trophic link between zooplankton and piscivores (Baird and Ulanowicz, 1989).

The largest marine oil spill in United States history occurred on 20 April 2010 when the BP-contracted *Deepwater Horizon* (DWH) Macondo well (Mississippi Canyon Block 252) exploded, killing 11 workers (Levy and Gopalakrishnan, 2010; McNutt et al., 2011; Operational Science Advisory Team, 2011). Even with multiple attempts to stop the leak, oil escaped continuously from the seafloor for nearly three months until it was sealed on 15 July 2010 (Azwell et al., 2011; Crone and Tolstoy, 2010). Despite unprecedented containment efforts, DWH oil washed ashore and affected more than 600 miles of Louisiana's coastal marshes and inlets (DWH NRDA Trustees, 2016; National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011; Silliman et al., 2012).

Bay anchovy are year-round residents of estuaries and inlets (Hildebrand, 1943; Springer and Woodburn, 1960); they spawn close to shore in water < 20 m deep (Jones et al., 1978; Robinette, 1983). Bay anchovy embryos float at the surface, and like oil, are aggregated by currents, tidal movements, and converging fronts (Hood et al., 1999; Jones et al., 1978; Peebles, 2002). The DWH spill occurred during the

* Correspondence to: School of Biological and Marine Sciences, Plymouth University, Plymouth PL4 8AA, UK.
E-mail address: kathryn.oshaughnessy@plymouth.ac.uk (K.A. O'Shaughnessy).

http://dx.doi.org/10.1016/j.ecoenv.2017.10.052
Received 20 June 2017; Received in revised form 17 October 2017; Accepted 24 October 2017

peak of bay anchovy spawning season (May–November) in the northern Gulf of Mexico (Jones et al., 1978), potentially exposing billions of ichthyoplankton, including bay anchovy embryos and larvae, to crude oil (DWH NRDA Trustees, 2016). Given the known toxic effects of oil exposure on developing early life stages of fish, it is important to understand how various oil mixture and aging of oil may affect fish embryos and larvae (Carls et al., 1999; Hoffman et al., 1990).

Crude oil is a complex mixture composed of tens of thousands of hydrocarbons, related hetero-compounds, and other trace constituents (McKenna et al., 2013). The polycyclic aromatic hydrocarbons (PAHs) and related polycyclic hetero-compounds, which are typically more persistent in the environment following an oil spill than their monocyclic counterparts, are seen as a key toxic fraction of the oil. Consequently, oil exposure in toxicity tests is often described in terms of PAH concentrations and the sum of the individual PAH concentrations (i.e., total PAH (TPAH) concentration) in the exposure media. In the environment, weathering processes such as volatilization/evaporation, biological and photochemical degradation, emulsification, dispersion, dissolution, and oxidation tend to preferentially remove lighter oil constituents. These processes affect both the chemical composition and the physical condition of the oil (Jordan and Payne, 1980; Neff and Anderson, 1981; NRC, 2003), which can influence the toxicity of the oil. Therefore, it is important to evaluate the weathering state when assessing the toxicity of oil, such as the DWH Macondo oil.

Although extensive research has been conducted on lethal and sublethal crude oil effects on various life stages of marine and estuarine predatory and forage fish species such as spotted seatrout (*C. nebulosus*; Brewton et al., 2013), mummichog (*Fundulus* spp.; Anderson et al., 1977; LaRoche et al., 1970), sheepshead minnow (*Cyprinodon variegatus*; Anderson et al., 1977; Dasgupta et al., 2015), and southern flounder (*P. lethostigma*; Brown-Peterson et al., 2015), effects on filter feeding and lower trophic level fish at the embryonic level are still largely understudied (but see Carls et al., 1999 for effects of weathered oil on Pacific herring embryos; and Duffy et al., 2016 for effects of whole oil on bay anchovy larvae). As an important component of coastal fish communities and a dominant part of ichthyoplankton, it is critical to understand how the DWH oil spill affected bay anchovy populations. The aim of the current study was to generate toxicity data on bay anchovy embryos using two different field-collected, weathered DWH oils (referred to as Slick A and Slick B) and two oil preparation methods (high-energy and low-energy water accommodated fractions). Many species were experimentally exposed as part of the Natural Resource Damage Assessment's (NRDA) effort to understand the effects of the *Deepwater Horizon* spill on fishes. These results with bay anchovy will contribute to a growing understanding of how spilled crude oil impacted coastal living resources during and after the 2010 spill.

2. Materials and methods

2.1. Bay anchovy broodstock

Bay anchovy embryos were produced by a captive population of broodstock housed at the Louisiana Universities Marine Consortium (LUMCON). The broodstock was established by collecting juvenile anchovy off the LUMCON dock (29°15'14.42"N, 90°39'49.64"W) using a neuston net (0.8 mm mesh) fitted with a solid glass codend to reduce the chance of damage to the juveniles, and the fish were then moved to a holding system where they were reared to adults. Collection of juveniles occurred during the summers of 2012–2015. Adult bay anchovy spawning populations were maintained in recirculating seawater systems (575-gallon, Red Ewald, Inc., Karnes City, TX, USA) fitted with aeration. The recirculating systems were maintained at a salinity of 25 ppt, a temperature of 26–27 °C and a summer photoperiod (15 L:9 D). Automatic feeders distributed dry commercial fish feed (size 0.3, Cargill®, Minneapolis, MN, USA) and a wet mixture of live hatched *Artemia* (Brine Shrimp Direct, Ogden, UT, USA), cultured rotifers, and phytoplankton *(Nannochloropsis oculata)* at 8 min intervals throughout daylight hours. An egg collection device positioned within the sump of the broodstock recirculating system caught newly spawned embryos overnight until they were collected the following morning for use in exposures. After spawning, embryos hatch into larvae in < 24 h. Time between spawning and exposure to oil was approximately 10 h, and exposure occurred when embryos reached the stage of development where the neurulation was complete. All embryos used for exposures were at the same stage of development. LUMCON's R/V Pelican collected and transported seawater from offshore Louisiana that was used to maintain broodstock fish and as makeup water in oil exposures. Because there may be low levels of background PAHs present within Louisiana's offshore waters, we refer to the collected water as ‘unoiled reference seawater’. Before use, all seawater was UV-sterilized, filtered using 5-µm filters (Whirlpool®, Benton Charter Township, MI, USA), and then diluted down to 25 ppt salinity with carbon filtered tap water.

2.2. Crude oil and test solutions

Two different weathered oils (Slick A and Slick B) were used in exposures, which were both collected from northern Gulf of Mexico surface water during the DWH spill. Slick A was collected by several skimming vessels near the Macondo well and then transported by holding barge CTC02404 to Port Fourchon, Louisiana on 29 July 2010. Slick B was collected by the U.S. Coast Guard skimming vessel “Jupiter” on 19 July 2010 (Forth et al., 2016). Both Slick A and Slick B were field-weathered oils with 68% and 85% depletion of the PAHs relative to hopane, respectively, which represents the mid to upper range of weathering states measured for oil samples collected by researchers throughout the northern Gulf of Mexico during the DWH spill (Aeppli et al., 2012; Forth et al., 2016; Stout, 2015). See Forth et al. (2016) for additional details on the chemistry and physical properties of these oils.

One of two oil preparation methods was used for oil exposures: (1) a mechanically dispersed high-energy water accommodated fraction (HEWAF) or (2) a low-energy water accommodated fraction (LEWAF) following the protocols described in Forth et al. (2016). Stocks of HEWAF were prepared within 24 h of test initiation in a decontaminated (acetone/hexane/dichloromethane rinse) stainless steel commercial Waring™ CB15 blender (Torrington, CT, USA) at an oil:seawater loading rate of 1 g:1 L. The water accommodated fraction (WAF) was then transferred to a decontaminated separatory funnel (ACE Glass, Inc., Vineland, NJ, USA) where it was allowed to separate for 1 h prior to use in exposures. From the separatory funnel, the bottom fraction of the HEWAF was pulled off for use in exposures. Stocks of LEWAF were prepared 24 h prior to exposure in a decontaminated aspirator bottle (Kimax®, Elmsford, NY, USA) at an oil:seawater loading rate of 1 g:1 L, and preparations were allowed to mix on a stir plate for 18–24 h with no vortex. To make dilutions for each treatment, the appropriate amount of 100% stock WAF was added to each test beaker (treatment-dependent) and then unoiled reference seawater was added to bring the volume up to 1 L.

2.3. Bay anchovy exposures

All exposures were run in 1 L glass beakers with the sides wrapped in black plastic to alleviate larvae swimming into the sides of the beakers. Test beakers were placed in a temperature-controlled water table that was maintained at 26–27 °C. The water table was equipped with hanging fluorescent lamps adjusted to a 14 L:10 D photoperiod to mimic summer spawning season conditions. Each test beaker was loaded with twenty-five embryos and exposed without renewal for 48 h. Because bay anchovy embryos hatch into larvae < 24 h after spawning, an additional exposure was run for 24 h followed by a 24 h grow-out period to assess delayed mortality and life stage sensitivity from an acute oil exposure. For this test, as much oil-contaminated water was exchanged with unoiled reference seawater as possible