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Cardiac function and survival are affected by crude oil in larval red drum, *Sciaenops ocellatus*

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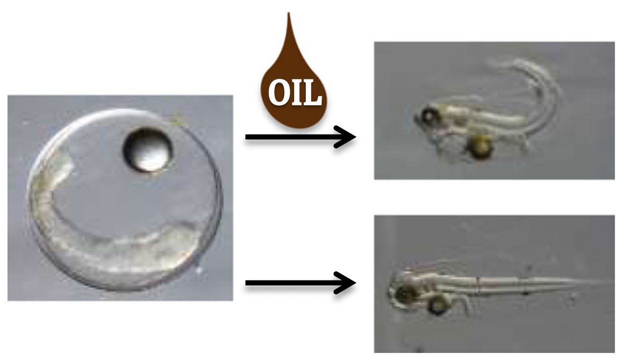
HIGHLIGHTS

- Larval cardiac output is greatly impaired by oil exposure.
- Larval red drum is sensitive at low ppb PAH_{50} concentrations.
- Weathering does not influence survival sensitivity.

GRAPHICAL ABSTRACT



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ABSTRACT

Following exposure to weathered and non-weathered oil, lethal and sub-lethal impacts on red drum larvae were assessed using survival, morphological, and cardiotoxicity assays. The LC50 for red drum ranged from 14.6 (10.3–20.9) to 21.3 (19.1–23.8) $\mu g\ l^{-1}$ ΣPAH with no effect of exposure timing during the pre-hatch window or oil weathering. Similarly, morphological deformities showed dose responses in the low ppb range. Cardiac output showed similar sensitivity resulting in a major 70% reduction after exposure to 2.6 $\mu g\ l^{-1}$ ΣPAH. This cardiac failure was driven by reduced stroke volume rather than bradycardia, meaning that in some species, cardiac function is more sensitive than previously thought. After the *Deepwater Horizon* oil spill, much of this type of work has primarily focused on pelagic species with little known about fast developing estuarine species. These results demonstrate similarity sensitivity of the red drum as their pelagic counter parts, and more importantly, that cardiac function is dramatically reduced in concert with pericardial edema.

1. Introduction

The *Deepwater Horizon* (*DWH*) oil spill of 2010 released approximately 700 million l of crude oil into the northern part of the Gulf of Mexico (Camilli et al., 2012; Crone and Tolstoy, 2010). This coincided with the spawning season of many commercially and ecologically

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important fish species, the larvae of which are known to exhibit a suite of defects following oil exposure (Incardona et al., 2011a). This includes fin-fold deformities (Jung et al., 2013), lack of fin ray precursors (Incardona et al., 2014), spinal curvature(Collier et al., 2014), craniofacial deformities (de Soysa et al., 2012), reduced growth (Heintz et al., 1998) pericardial edema (Carls et al., 2008; Incardona et al., 2008; Le Bihanic et al., 2014), as well as bradycardia and reduced cardiac contractility (Edmunds et al., 2015; Incardona et al., 2011a; Mager et al., 2014). Cardiac impairment in particular has been suggested as the underlying cause of reduced survival at later stages (Heintz et al., 2000; Muhling et al., 2012; Perrichon et al., 2016).

Despite the abundance of oil toxicity research on larval fish, there remain key unexplored areas. First among these is the physiological significance of cardiotoxicity on cardiovascular function in marine larvae. Previous studies related to *DWH* used heart rate, contractility, and arrhythmia as indicators of cardiac performance (Incardona et al., 2008). While arrhythmia can be indicative of cardiac failure, changes in heart rate and contractility are more difficult to interpret as many fish actively manipulate stroke volume to control cardiac output (Anttila et al., 2013; Crossley et al., 2016.; Webber et al., 1998). Cardiac output is therefore the most functionally significant measure of cardiovascular function; however, effects on this endpoint have curiously been overlooked with respect to marine fish larvae and *DWH*.

A second important knowledge gap pertains to our limited understanding of potentially sensitive coastal species in the Gulf. The majority of work related to the *DWH* oil spill has been performed on pelagic species (Esbaugh et al., 2016b; Incardona et al., 2014) or slow developing coastal species (Dubansky et al., 2013; Hedgpeth and Griffitt, 2016). Coastal species are often more tolerant of environmental stress owing to the highly variable nature of their environments, which may extend to anthropogenic stresses. This can also be extended to our understanding of the differential sensitivities to weathered and non-weathered oils. Weathering is believed to increase toxicity by concentrating 3-ring polycyclic aromatic hydrocarbons (PAHs) (Heintz et al., 2000), while eliminating less toxic low molecular weight PAHs. This is particularly important for coastal species of the Gulf of Mexico, as these areas were likely to be exposed to heavily weathered oil.

The current study sought to address these knowledge gaps using the economically and ecologically important red drum, *Sciaenops ocellatus*. Found in estuaries throughout the Gulf of Mexico, red drum exhibit a similar developmental time course to the previously studied pelagic species (Incardona et al., 2014), but develops much quicker than studied estuarine species (Dubansky et al., 2013; Hedgpeth and Griffitt, 2016). Furthermore, they are known to be tolerant of a wide array of environmental perturbation (Ern and Esbaugh, 2016; Esbaugh et al., 2016a; Pan et al., 2016; Watson et al., 2014). Our purpose was to determine the lethal and sub-lethal sensitivity of early life stages to crude oil, as well as the influence of oil weathering on sensitivity. Most importantly, we sought to provide a more comprehensive assessment of the impacts of exposure on cardiac function in relation to the *DWH* oil spill by examining cardiac output.

2. Methods

2.1. Animal care

All experiments were approved by the institutional animal care and use committee (IACUC) at the University of Texas at Austin (AUP-2014-00375). Embryonic red drum (*Sciaenops ocellatus*) were collected from brood stock tanks at the Texas Parks and Wildlife – CCA Marine Development Center in Corpus Christi, Texas, USA and transported under constant aeration to the University of Texas Marine Science Institute. Embryos were subsequently treated with formalin (1 ppt) for 1 h with aeration to remove any bacteria. Embryos were then rinsed with sterilized seawater and checked for buoyancy to assess viability and coloration using a Nikon SMZ800N microscope for egg quality. Spawns with low fertilization rates or poor egg quality were not used.

2.2. Toxicity testing

Oil exposures were generated according to standard protocols for high energy water accommodated fractions (HEWAF), as previously described (Esbaugh et al., 2016b; Incardona et al., 2013). This protocol is intended to isolate the effects of dissolved and micro-droplet constituents that enter the water column under high energy conditions. Oil loading rate was 1 g per 1 l of seawater (35 ppt). Testing was performed using two different oil types. The first was a naturally weathered oil collected from a slick in the Gulf of Mexico on June 29th, 2010 from the hold of barge number CT02404 (referred to as OFS). The second was a non-weathered source oil (referred to as MASS) from a Massachusetts pipeline recognized as a suitable surrogate for the DWH source oil. Both oils were delivered to the University of Texas Marine Science Institute through proper chain of custody and stored at 4 °C until used.

The testing protocol has been described previously(Esbaugh et al., 2016b; Incardona et al., 2013) with the exception that the test duration was only 72 h. This modification to the protocol was required because red drum larvae exhaust their yolk sac between 72 h and 96 hpf, which results in first feeding-related mortality. Each test consisted of 6–7 concentrations with 4 replicates (1 l) per concentration. A water sample (250 ml) was collected from each concentration for PAH analysis, which was performed commercially (ALS Environmental) under complete quality compliance and assurance standard operating procedures. Each replicate contained 20 embryos aged 1 hpf or 12 hpf (Fig. 1). Water quality parameters, including pH, dissolved oxygen, salinity, and temperature were measured in one replicate of each concentration each day of the test (Supplemental Table 1). The test was performed in an environmental control chamber set at 25 °C with a 14:10 h light: dark photoperiod. Survival was assessed daily. A minimum of 70% hatching success at 24 h and 80% subsequent survival was required for the test to be valid.

A second test was used to assess the role of micro-droplets in determining observed toxicity. A freshly made OFS HEWAF was divided in half, and one half was filtered through a 0.3 μm filter to remove micro-droplets. The test consisted of nominal 4% concentrations of the filtered and unfiltered OFS HEWAFs, as well as a no HEWAF control where embryos were placed solely in sterilized sea water. Each

Fig. 1. A control 12 hpf red drum embryo. Note that this equates to the 4- to 8-somites stage, which precedes the development of bilateral cardiac primordia. Scale bar = 250 μm.