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Crude oil cardiotoxicity to red drum embryos is independent of oil dispersion energy

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HIGHLIGHTS

- Low and high energy water accommodated fractions of crude oil were compared.
- Toxic effects were assessed using cardiotoxicity endpoints in red drum embryos.
- The biological effects of both WAF types were virtually identical.
- Differences in toxic threshold levels based on ∑PAH measures were observed.

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ABSTRACT

The potential bioavailability of toxic chemicals from oil spills to water column organisms such as fish embryos may be influenced by physical dispersion along an energy gradient. For example, a surface slick with minimal wave action (low energy) could potentially produce different toxic effects from high energy situations such as pressurized discharge from a blown wellhead. Here we directly compared the toxicity of water accommodated fractions (WAFs) of oil prepared with low and high mixing energy (LEWAFs and HEWAFs, respectively) using surface oil samples collected during the 2010 *Deepwater Horizon* spill, and embryos of a representative nearshore species, red drum (*Sciaenops ocellatus*). Biological effects of each WAF type was quantified with several functional and morphological indices of developmental cardiotoxicity, providing additional insight into species-specific responses to oil exposure. Although the two WAF preparations yielded different profiles of polycyclic aromatic hydrocarbons (PAHs), cardiotoxic phenotypes were essentially identical. Based on benchmark thresholds for both morphological and functional cardiotoxicity, in general LEWAFs had lower thresholds for these phenotypes than HEWAFs based on total PAH measures. However, HEWAF and LEWAF toxicity thresholds were more similar when calculated based on estimates of dissolved PAHs only. Differences in thresholds were attributable to the weathering state of the oil samples.

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1. Introduction

The *Deepwater Horizon* (DWH) oil spill began on April 20, 2010. The damaged wellhead on the Northern Gulf of Mexico (GoM) seafloor subsequently released millions of barrels of crude oil into the ocean until the well was eventually capped on July 15, 2010 (Camilli et al., 2010; The Federal Interagency Solutions Group, 2010). The event was the largest marine oil spill in U.S. history. The spill was also unusual in that it originated in the deep ocean, under extreme pressure, with chemical dispersants used at both the wellhead and on the ocean surface. The result was chemically and mechanically dispersed petroleum compounds, including polycyclic aromatic hydrocarbons (PAHs), in a wide range of GoM environments that are essential for commercially and

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recreationally important fisheries (Ylitalo et al., 2012). Many species of fish were spawning during the active spill phase, or in the months after capping, in both nearshore and offshore nursery habitats.

The impacts of crude oil on fish early life stages are now fairly well known (Incardona et al., 2009, 2014; McIntosh et al., 2010; Dubansky et al., 2013; Mu et al., 2014; Jung et al., 2015; Madison et al., 2015). This focal line of research largely began in the aftermath of the 1989 Exxon Valdez oil spill, which extensively oiled coastal streams and shoreline spawning areas for pink salmon and Pacific herring, respectively, in Prince William Sound, Alaska (Peterson et al., 2003). Crude oil exposures cause a familiar syndrome of developmental defects in virtually all fish species tested, including fluid accumulation (edema) in the vicinity of the heart or the yolk sac, as well as craniofacial and body axis abnormalities (Incardona and Scholz, 2016). Mechanistic studies in zebrafish, an experimental model for development toxicity in fish and humans, revealed the heart to be the primary target organ for crude-oil derived PAHs, with visible extracardiac defects arising as secondary sequelae (Incardona and Scholz, 2016; Incardona, 2017). Certain PAHs – particularly those having three rings (tricyclics) – disrupt heart muscle cell repolarization and calcium cycling (Brette et al., 2014, 2017). This in turn disrupts the normal rhythm and contractility of the embryonic heart (Incardona et al., 2009, 2014; Jung et al., 2013; Sørhus et al., 2016). Cardiac morphogenesis depends on a functional heart, and perturbations in rhythm or output can lead to permanent and adverse changes in heart shape at later life stages (Hicken et al., 2011; Incardona et al., 2015; Incardona, 2017). Thus, relatively low crude oil exposure concentrations cause the developing heart to fail, leading to severe downstream anatomical defects and larval death. Fish may survive transient exposures to oil at even lower (trace) concentrations, but consequent changes in cardiac morphogenesis can cause lasting changes in heart shape that correspond to impaired swimming performance (Hicken et al., 2011; Incardona et al., 2015).

Whereas the majority of older studies focused on cold-water species impacted by the Exxon Valdez spill, the DWH disaster led to an expanded focus on a wider variety of species representing distinct ecophysiological niches. First, oil from the DWH-Macondo 252 (MC252) well has proven to be relatively conventional in terms of toxicity to fish early life stages. Crude oils from the MC252 well and the Alaska North Slope (Exxon Valdez) produced nearly identical injury phenotypes in zebrafish embryos and larvae (Incardona et al., 2013). However, studies on MC252 crude oil-induced cardiotoxicity in the early life stages of large pelagic predators such as bluefin (*Thunnus maccoyii*) and yellowfin tuna (*Thunnus albacares*) (Incardona et al., 2014), as well as mahi mahi (*Coryphaena hippurus*) (Edmunds et al., 2015; Esbaugh et al., 2016) demonstrated species-specific variation attributable to differences in developmental anatomy and ecophysiology (Incardona and Scholz, 2016). For those studies, a simple and readily reproducible method for producing high energy dispersions of oil droplets in the water column (high energy water-accommodated fractions, or HEWAFs (Incardona et al., 2013)) was developed to mimic exposure conditions that might have existed for open ocean pelagic species spawning in the vicinity of the damaged wellhead, where plumes of small oil droplets rose to the surface.

In addition to contaminating pelagic fish spawning habitats in the northern GoM, MC252 crude also came ashore (Nixon et al., 2016), thereby oiling embayments and marsh nursery habitats for red drum (*Sciaenops ocellatus*), speckled sea trout, and other economically important species that spawn in shallow coastal waters (Lowerre-Barbieri et al., 2016). Whereas visible oil disappeared in the upper surface waters of the pelagic zone relatively soon after the wellhead was capped, oil persisted in some shoreline habitats for up to two years (Michel et al., 2013). Besides impacts on fish actively spawning in the open ocean during the spill, this also suggests the possibility of lingering injury to early life stages of fish species such as red drum that spawn nearshore later in the year (i.e., late summer to early fall). However, the pathway for oil exposure for species such as red drum would have included lower energy mixing of surface slicks or oil stranded on marsh substrates.

Previous studies examined the effects of weathered MC252 oil on red drum embryos (Khursigara et al., 2017; Xu et al., 2017). While those studies used MC252 oil collected from surface slicks, exposures utilized HEWAFs, which are more representative of open ocean habitats closer to the wellhead rather than nearshore areas where red drum actually spawn. At the same time, some investigators have questioned the validity and environmental relevance of these simple HEWAF preparations (Echols et al., 2016; Sandoval et al., 2017). Thus, the primary objective of this study was to directly compare the toxic effects of standard HEWAF preparations to a low energy mixing method that has become an industry standard (Singer et al., 2000). In addition, we provide a more detailed analysis of red drum heart development and cardiotoxic effects that provide additional insight into how the ecophysiology of different fish species determines the precise responses to crude oil exposure during organogenesis.

2. Materials and methods

This study was conducted in support of the DWH Natural Resource Damage Assessment (NRDA). Detailed descriptions of the protocols and procedures developed and implemented for the NRDA, including the methods used in the current study, are provided elsewhere (Morris et al., 2015).

2.1. Facilities

Red drum husbandry and crude oil exposures were implemented in collaboration with the Texas Parks and Wildlife Department's Sea Center Texas marine hatchery in Lake Jackson, TX. The Sea Center uses filtered water from Galveston Bay as a source of natural seawater. The water for our experiments was obtained directly from the distribution lines for the Sea Center hatchery. Clean (control) seawater was stored in a 340-L carboy covered in dark plastic and maintained under aeration at ambient temperature. Samples were routinely collected from this carboy and analyzed for conventional water quality parameters (e.g., dissolved oxygen, pH, salinity) as well as background contamination (e.g., hydrocarbons, volatile organic compounds, pesticides, metals, major cations and anions, suspended and dissolved solids, turbidity, organic carbon, and chemical and biologic oxygen demand; Table S1). We also collected baseline (control) water samples at the beginning of each exposure. Analytical chemistry was conducted by ALS Environmental (Kelso, WA).

2.2. Red drum

The Sea Center maintains a broodstock of red drum under controlled temperature and diel lighting conditions. The adult fish spawn volitionally in the evening, at which time newly fertilized embryos were removed from egg collector troughs on the spawning tanks. For each spawning event, we visually assessed fertilization success by microscopy for a small subset of embryos. If the spawning event resulted in successful fertilization (>90%), we immediately loaded embryos into beakers containing different exposure solutions. The embryos were not treated with antibiotics in accordance with the conventional husbandry practices at the Sea Center. Animal care and experimental design were carried out with

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