



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



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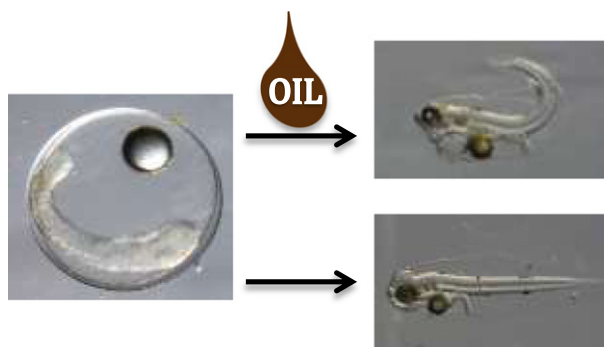
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HIGHLIGHTS

- Larval cardiac output is greatly impaired by oil exposure.
- Larval red drum is sensitive at low ppb PAH₅₀ 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) $\mu\text{g l}^{-1}$ 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 $\mu\text{g l}^{-1}$ 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.

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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 Bihan 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

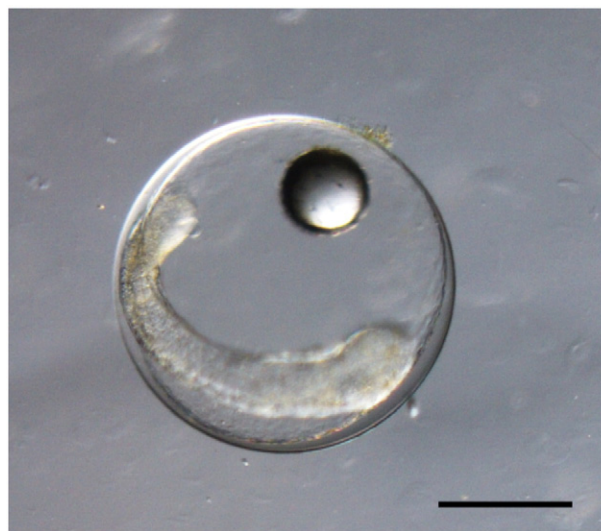


Fig. 1. A control 12 hpf red drum embryo. Note that this equates to the 4- to 8-somites stage, which precedes the development of bilateral cardiac primordia. Scale bar = 250 µm.

treatment consisted of four replicates. Test maintenance was as described above.

2.3. Morphological characteristics

Cardiotoxicity endpoints were assessed at 48 h post-exposure following an 80% hatching rate in controls. Test set-up and monitoring was performed as described above, with the exception that only 10 embryos were loaded per replicate. Yolk sac larvae were anesthetized using 250 mg l^{-1} of MS222 (buffered with 500 mg l^{-1} NaHCO_3). Preliminary experiments showed no impact of this concentration on the incidence of pericardial edema and heart rate (data not shown). Individuals were then mounted in left lateral view onto 3% methylcellulose in a Petri dish for image collection. All images and videos (12.4 fps) were collected using a Nikon SMZ800N microscope and Nikon Digital Sight DS U-3 and associated software. Pictures and videos of the heart were individually taken for each fish at 4 and 8 magnification respectively.

Still frames were imported into Image J (Schneider et al., 2012) and the pericardial area was quantified by outlining the pericardial cavity with freehand tools. These data was subsequently used to designate individual larvae as exhibiting a cardiotoxic or control phenotype. Specifically, the mean and standard deviation of the pericardial area of the control group was calculated, and any individual that exhibited a pericardial area greater than the control mean plus two standard deviations was designated as cardiotoxic. This method quantitatively scores pericardial edema, eliminating qualitative user scoring (Esbaugh et al., 2016b; Incardona et al., 2012; Mager et al., 2014).

Still images from 48 h post-exposure were assessed for craniofacial deformities and spinal curvature (Fig. 2). Pictures were randomized and blindly scored for the presence of a spinal curvature and craniofacial deformities, such as changes in the general shape of the head, jaw, and eyes. All scoring was based on qualitative assessment.

2.4. Cardiac function assessment

Techniques for this section were modified from previous studies (Hou and Burggren, 1995) (Bagatto and Burggren, 2006). From video sequences, the ventricular area and perimeter was determined by outlining the ventricle in Image J at the end of a systolic and diastolic event. This was performed for 3 systolic and diastolic events for each larvae and averaged to reduce experimental measurement error. Using the major (longitudinal) and minor (width) axis, area and perimeter were calculated. Previous studies have variously used a formula for either a prolate or ellipsoid sphere to represent the ventricle and calculate its volume. At 48 h of development in early fish larvae, the anteriorly located ventricle of larval fishes is relatively elongate compared to the larger and nearly spherical posteriorly located atrium. Later in development the ventricle grows to resemble the size and shape of the atrium. Thus, there may be some small differences in calculated cardiac output depending upon the formula used. For these red drum larvae at 48 h, we calculated the volume of the ventricle using the ellipsoid formula:

$$V = \frac{4}{3} \pi a b c$$

where a is the major semi-axis, b is the minor semi-axis, and c is the semi-height. In this study $b = c$. Stroke volume (SV) was determined as the difference between end-diastolic and end systolic volume of the ventricle. Heart rate (HR) was visually calculated from the acquired video. Using these two parameters, cardiac output (CO) could be calculated by multiplying SV and HR.

2.5. Statistics

All statistical tests were run on the biological average for each replicate to avoid pseudoreplication. LC_{50} and EC_{50} estimates and confidence

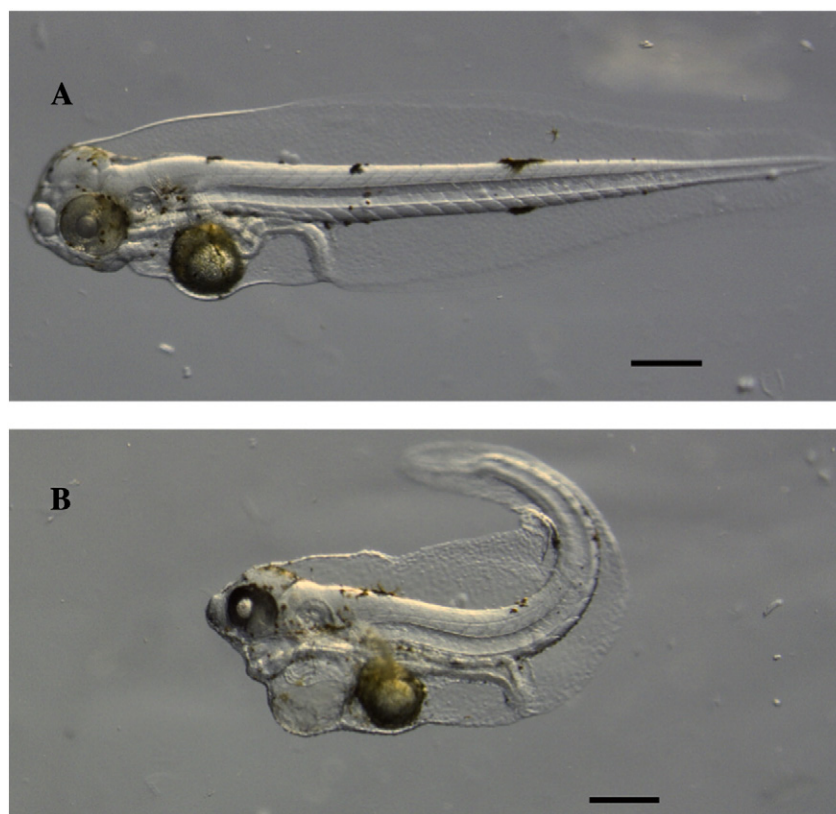


Fig. 2. Representative images depicting control (A) and OFS HEWAF exposed (B; $2.6 \mu\text{g l}^{-1}$ ΣPAH) embryonic red drum phenotypes at 48 hpf. Note the greatly increased pericardial area, spinal curvature and altered craniofacial shape between treatments. Scales = $250 \mu\text{m}$.

intervals were calculated using the U.S. Environmental Protection Agency's TRAP software package. In all cases, data were fit to a tolerance type Gaussian model with 3 parameters using log-transformation. The contribution of micro-droplets to survival was assessed using one-way ANOVA followed by a Holm-Sidak pairwise multiple comparisons test. Spinal curvature and craniofacial deformities were not normally distributed and therefore assessed using a one-way ANOVA run on ranks with a Kruskal-Wallis multiple comparisons test. Pericardial area was also not normally distributed and was analyzed using an ANOVA on ranks with a Dunn's post-hoc test against the control group. Cardiac output, stroke volume and heart rate were measured using one-way ANOVA followed by a Holm-Sidak pairwise multiple comparisons test.

3. Results

3.1. Chemical analysis of HEWAFs

The three HEWAF types showed the anticipated difference in relative PAH composition (Fig. 3). The non-weathered MASS HEWAF had the highest proportion of 2 ring PAHs (55%), while the unfiltered OFS HEWAF consisted of only 4% 2 ring PAHs. Conversely, the OFS HEWAF had the highest proportion of the purportedly more toxic 3 and 4 ring PAHs. Filtering resulted in further enrichment of 3 ring PAHs, which accounted for 88% Σ PAH; no detectable 4–5 ring PAHs were present in the filtered OFS HEWAF.

3.2. LC_{50} endpoints

Three distinct embryonic survival tests were performed to assess the influence of weathering and developmental stage on observed toxicity. All three tests exhibited an effective range in survival responses (Fig. 4), which allowed for robust LC_{50} estimates, as exhibited by the relatively narrow confidence intervals (Table 1). There was no difference in the sensitivity of embryos exposed to OFS HEWAF at 1 hpf and those exposed at 12 hpf, based on overlapping 95% confidence intervals. The effect of weathering was only assessed using 12 hpf embryos. No differences were observed between MASS and OFS HEWAFs despite the expected variation in PAH composition.

The influence of micro-droplets on survival was assessed using a standard filtering protocol. Identical nominal OFS HEWAF doses

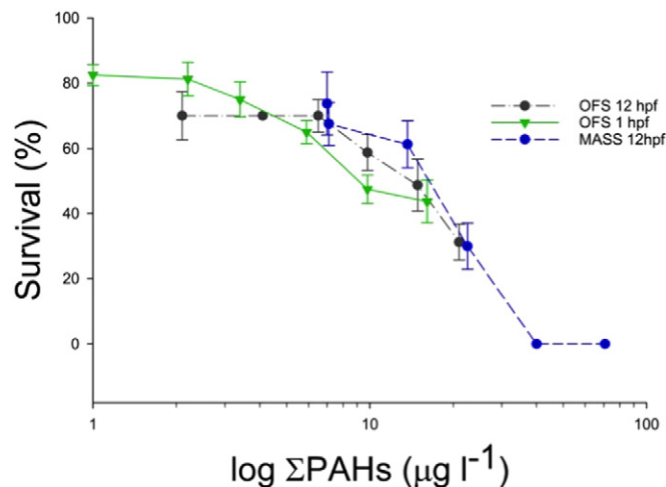


Fig. 4. Dose response effects of 72 h HEWAF exposure on red drum embryonic survival (mean \pm SEM; $N = 4$).

resulted in significant differences in observed toxicity when filtered, with the unfiltered sample exhibiting a significant drop in survival relative to control and filtered treatments (Fig. 5). Unfiltered PAHs accounted for only 1.1% of the total PAH load in the OFS HEWAF.

3.3. Craniofacial deformities and spinal curvature

All morphological endpoints are shown in Fig. 6A and B. Larvae were also scored for oil-induced deformities in spinal curvature and craniofacial deformity (Fig. 6A). Similar to the cardiotoxic phenotypes, both endpoints exhibited a dose dependent response to Σ PAH, with craniofacial deformity showing greater sensitivity than spinal curvature (Fig. 7). Notably, the craniofacial EC_{50} was similar to that observed for cardiac endpoints (Table 1).

3.4. Cardiotoxicity

All cardiotoxic endpoints showed a dose-dependent response to increasing Σ PAH. The lowest observable effect concentration for

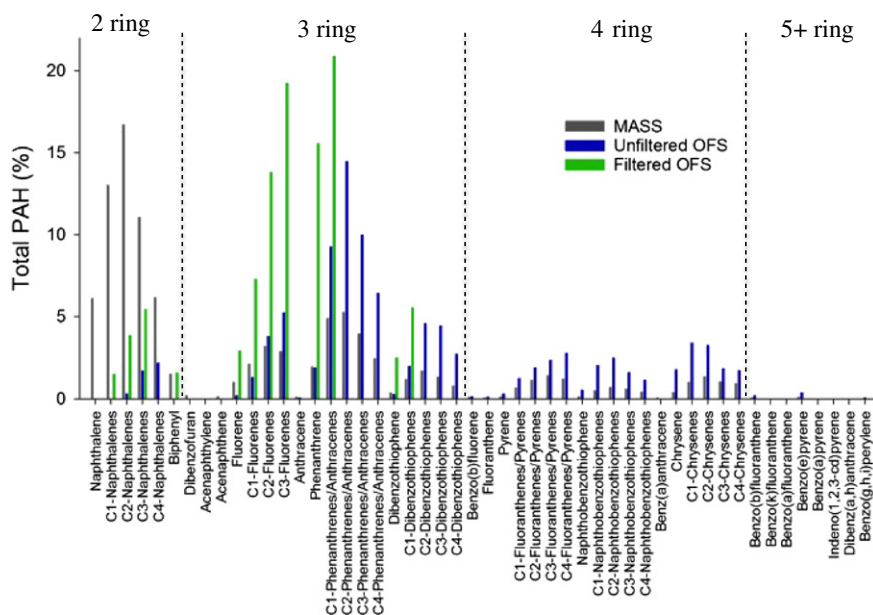


Fig. 3. Relative polycyclic aromatic hydrocarbon composition of the three utilized HEWAF types. 50 individual PAHs were measured and identified on the x axis. Generalized subclasses are divided by dotted lines and noted on the figure.

Table 1

Estimated 72 h LC₅₀ values for red drum embryos exposed to two oil types at different developmental times and 48 h EC₅₀ values for morphological endpoints after exposure to weathered oil. Values in parentheses represent the 95% confidence intervals.

Endpoint	Test	ΣPAH (μg l ⁻¹)
LC ₅₀	MASS 12 hpf	21.3 (19.1–23.8)
	OFS 1 hpf	14.6 (10.3–20.9)
	OFS 12 hpf	19.1 (16.2–23.4)
EC ₅₀ ^a	Pericardial edema	2.4 (2.1–2.7)
	Cardiac output	2.2 (2.1–2.3)
	Craniofacial deformities	2.2 (1.9–2.6)

^a All EC₅₀ tests were performed with weathered OFS HEWAF beginning at 12 hpf.

pericardial area was at 1.8 μg l⁻¹ (Fig. 6B) and the EC₅₀ for the occurrence of a cardiotoxic phenotype was estimated at 2.4 μg l⁻¹ (Table 1). The three measures of cardiac function all showed dose dependent decreases (Fig. 7). Stroke volume was significantly impacted at a lower dose than heart rate and was generally responsible for the observed reduction in total cardiac output (Fig. 7B and C). Nonetheless, the EC₅₀ for cardiac output was similar to pericardial edema and craniofacial deformities (Table 1). These estimates were also similar to that of the observed edema phenotype (Fig. 6B).

4. Discussion

The effects of oil on embryonic and larval fish have been intensely studied in regard to DWH and more broadly in other marine biota. These studies have shown consistent sensitivity of pelagic embryonic fish through the development of a cardiotoxic phenotype characterized by pericardial edema and reduced survival (Esbaugh et al., 2016b; Incardona et al., 2014). Here, we complement previous work through the study of a fast-growing, economically important coastal species with demonstrated tolerance to a wide array of environmental challenges (Ern and Esbaugh, 2016; Esbaugh et al., 2016a; Pan et al., 2016; Watson et al., 2014). More importantly, we provide the first thorough assessment of larval cardiovascular function following oil exposure in a marine fish species native to the Gulf of Mexico.

All LC₅₀ tests produced a dose-dependent response for lethality similar to values reported for other marine species (Esbaugh et al., 2016b; Incardona et al., 2014). LC₅₀ estimates ranged between 14.6 and 21.3 μg l⁻¹ ΣPAH for the various tests. Embryos were exposed at

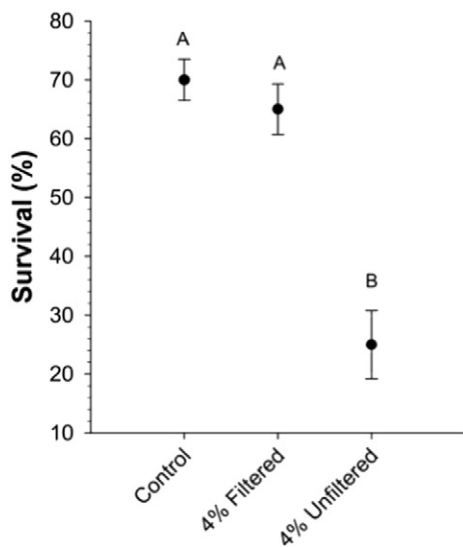


Fig. 5. The influence of micro-droplets on survival in 72 h static toxicity tests using OFS HEWAF. Letters indicate significant differences between the groups. (One-way ANOVA; N = 4 P < 0.05).

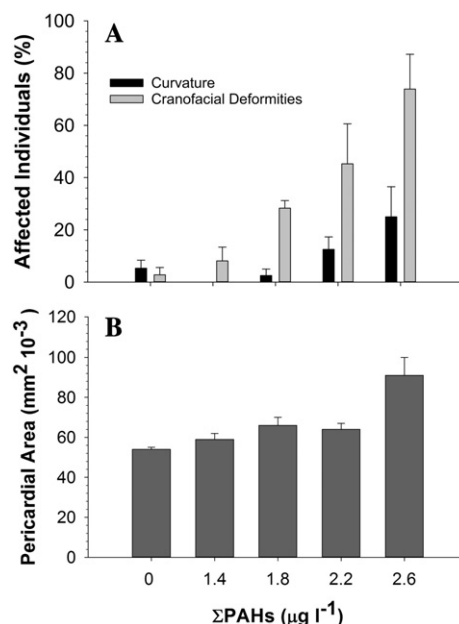


Fig. 6. Dose response of 48 h OFS HEWAF exposure on embryonic morphology; (A) mean pericardial area ± SEM (N = 4), (B) mean relative effect of spinal curvature and craniofacial deformities ± SEM (N = 4). Individuals were scored for the presence of deformities and means of each concentration are presented. Asterisks denote a significant difference from the control using a One Way ANOVA (P < 0.05).

different times in development (1 and 12 hpf) during the pre-hatching phase to explore potential influences on toxicity. There was no difference in LC₅₀ between these two time points, which is similar to previous work on zebrafish (Incardona et al., 2013). Overall, these data suggest that red drum are generally similar in sensitivity as the previously studied pelagic species (Esbaugh et al., 2016b). However, in contrast to previous work, red drum sensitivity was not influenced by oil weathering, no significant difference was observed between OFS and MASS oils. It is thought that toxicity in embryonic fish is caused by dissolved 3-ring PAHs (Incardona et al., 2004), which are enriched after weathering processes. Several comparisons between weathered and non-weathered oil toxicity in fish support this viewpoint (Carls et al., 1999; Esbaugh et al., 2016b; Heintz et al., 1998; Xu et al., 2016), with weathered oils typically showing greater toxicity on a ΣPAH basis. While an explanation for our results is not immediately clear, it is possible that the mechanisms of toxicity vary between species. A second important factor to note is that the current study used a surrogate source oil (MASS), as opposed to the true source oil used previously. While the MASS oil is an approved substitute for using DWH source oil, owing to its similar PAH profile, it is possible that an unknown non-PAH constituent may be impacting toxicity. Regardless, it is clear that our understanding of the factors pertaining to the effects of weathering on embryonic oil toxicity is incomplete.

The role of micro-droplets in observed embryonic toxicity is another point of debate (Carls and Meador, 2009; Jung et al., 2013; Redman et al., 2012). Micro-droplets refer to the components of ΣPAH that are not truly dissolved and thus can be removed through 0.3 μm filtration. Studies on zebrafish embryos suggest dissolved PAHs are responsible for toxicity (Carls et al., 2008). Similarly, research on mahi mahi indicated that differences in the relative dissolved to micro-droplet concentrations explained observed increased oil sensitivity when in the presence of the dispersant Corexit 9500 (Esbaugh et al., 2016b). Interestingly, when red drum embryos were exposed to the same percent volume of filtered and unfiltered OFS HEWAF, survival in the unfiltered treatment was significantly lower than the filtered treatment. This suggests that micro-droplets play some role in observed toxicity during static testing. It seems probable that micro-droplets act as a dissolved PAH reservoir

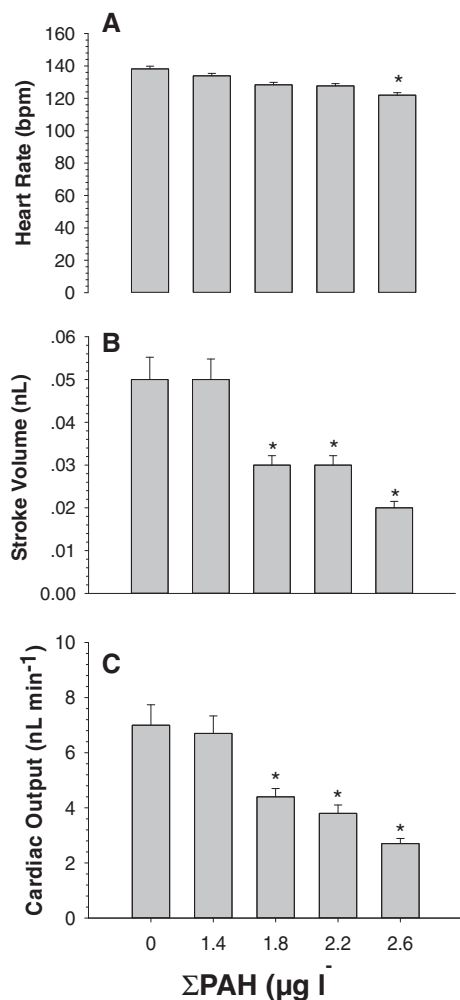


Fig. 7. Dose response effect of 48 h OFS HEWAF exposure on embryonic cardiac function; (A) mean heart rate \pm SEM ($N = 4$), (B) mean stroke volume \pm SEM ($N = 4$), and (C) mean cardiac output \pm SEM ($N = 4$). Individuals were scored and means for each concentration are presented. Asterisks denote a significant difference from the control using a One Way ANOVA ($P < 0.05$).

and maintain higher dissolved Σ PAH throughout the test. It is important to note that the dissolved Σ PAH fraction was only 1.1% of the total, and therefore micro-droplets represent a substantial reservoir.

As expected, embryonic red drum exhibited the typical cardiotoxic injury phenotype, which included pericardial edema, spinal curvature and cranial malformations. Red drum is similar in sensitivity to yellowfin tuna, and somewhat more sensitive than mahi mahi and amberjack with respect to occurrence of edema (Incardona et al., 2014). More importantly, cardiotoxicity was approximately 8-times more sensitive than survival in red drum. This may suggest that red drum can better tolerate reduced cardiac performance in early life; however, further study is required in this regard. Interestingly, craniofacial deformity was as sensitive as pericardial edema to HEWAF exposure. While craniofacial defects are commonly found after oil exposure, we are unaware of any studies that show this level of sensitivity. In fact, cardiotoxicity is commonly held as a singularly sensitive endpoint for oil exposure in larval fish (Incardona et al., 2011b). These data are not entirely surprising because both cardiac and craniofacial deformities are thought to be derived, in part, by early impacts on neural crest cell development (de Soysa et al., 2012). Importantly, craniofacial deformities may have severe later life fitness consequences related to proper jaw function and successful feeding. This is also supported by recent RNA sequencing studies that have shown non-cardiac endpoints,

including neurological and vision, general protein and amino acid metabolism, and urea and purine metabolism are targeted by oil exposure in early life marine fishes (Xu et al., 2016).

It has been suggested that cardiac function is reduced by oil exposure, and recent work with mahi-mahi attributed embryonic survival to reduced cardiac performance, as well as reduced swimming performance later in life, and survival in mark and recapture studies on pink salmon (Edmunds et al., 2015; Esbaugh et al., 2016b; Heintz et al., 2000; Mager et al., 2014; Perrichon et al., 2016). While these studies provided indirect evidence that cardiac function is impaired in concert with edema, to our knowledge this is the first study that has measured cardiac function directly through cardiac output in marine larval fish. Previous work has used heart rate, contractility or arrhythmia, but cardiac output is a more complete and physiologically relevant measure of routine cardiac performance. The importance of this distinction is obvious as the EC_{50} for cardiac output is well below the lowest observable effect concentration for heart rate. It is also notable that the method described here is easily replicable and uses the same data collection methods previously implemented for basic characterization of cardiac edema and heart rate. The reduction in cardiac output was proportional to the occurrence of pericardial edema, which suggests a relationship between the two variables. However, it is unclear whether the increased hydrostatic pressure from edema constrains stroke volume, or if poor cardiac performance initiates the development of edema. Further work is required to fully elucidate this question.

Regardless of the underlying cause, it is clear that larval red drum clearly show a dramatic decrease in cardiac output at low ppb concentrations. Yet this has no impact on survival in the short-term. This is true even with a 70% reduction in cardiac output. But it is important to consider the role of the circulatory system in early development. Generally, embryonic and larval fish use cutaneous gas exchange for oxygen delivery (Brauner and Rombough, 2012; Crossley et al., 2016; Fu et al., 2010; Wells and Pinder, 1996), which may reduce the importance of the cardiovascular system at these early stages (Pelster et al., 2010). Nonetheless, this level of cardiac impairment would certainly result in severely inhibited performance and mortality if it is persistent in life stages where convective flow and circulation are required for oxygen delivery (Bagatto and Burggren, 2006; Burggren, 2013; Gore and Burggren, 2012). These findings can also be used to re-evaluate previous work on the sensitivity of pelagic fish as these studies consistently demonstrated that indices of cardiac function – heart rate and contractility – were less sensitive than pericardial edema after exposure to HEWAFs. Our results clearly demonstrate that cardiac output is more sensitive than previous measures of cardiac function and is impacted in a very similar dose response pattern to pericardial edema. It should therefore be concluded that previous pericardial edema responses, which range from 0.8–21.6 $\mu\text{g l}^{-1}$ (Carls et al., 1999; Hicken et al., 2011; Incardona et al., 2014), will likely lead to greatly reduced performance and even mortality.

In conclusion, the results of this study address several key gaps in our knowledge of the impacts of the DWH oil spill on fish species in Gulf of Mexico, as well as providing novel insight into several more general areas of interest in the field of oil toxicology. Here we demonstrated that red drum – a fast growing, economically and ecologically important coastal species – show comparable sensitivity to pelagic species with similar developmental progressions, and that this sensitivity is not greatly influenced by weathering processes. More importantly, we have provided the first quantitative link between oil induced embryonic pericardial edema and reduced routine cardiac function, as determined through cardiac output. It is somewhat difficult to put these data within an environmental context, owing to the spatial and temporal variability in coastal oiling following the DWH spill, as well as the additive or synergistic influences of other environmental factors. Observed total PAH concentrations in the pelagic zones reached as high as 85 $\mu\text{g l}^{-1}$ (Bejarano et al., 2013; Diercks et al., 2010); however, dissolved PAH₃₃ concentrations in select coastal regions during the 2010 September

spawning season ranged only from 15 to 25 ng l⁻¹ (Allan et al., 2012). Other coastal areas maintained dissolved PAH₄₄ concentrations of approximately 80 ng l⁻¹ even a year after the spill (Hong et al., 2015). In both cases, the use of passive sampling devices that excluded microdroplet fractions, so these concentrations cannot be directly compared to our data. Nonetheless, the low µg l⁻¹ ΣPAH₅₀ sensitivity of red drum seems within an environmentally relevant range with respect to the DWH oil spill.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2016.11.026>.

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