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A novel system for embryo-larval toxicity testing of pelagic fish: Applications for impact assessment of *Deepwater Horizon* crude oil

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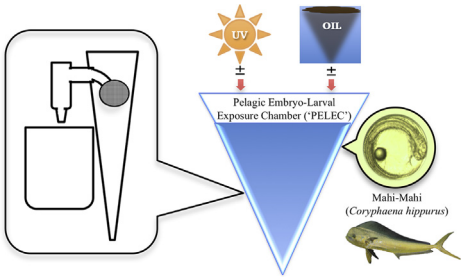
HIGHLIGHTS

- A novel exposure system for fish ELS toxicity testing (the PELEC) is reported.
- The method improves ELS toxicity testing of high-value pelagic fish species.
- Testing results indicate that mahi-mahi embryos are highly sensitive to PAHs.
- The PELEC also allows for testing of photo-induced crude oil toxicity.
- Natural sunlight co-exposure with *DWH* crude oil significantly increases toxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

Key differences in the developmental process of pelagic fish embryos, in comparison to embryos of standard test fish species, present challenges to obtaining sufficient control survival needed to successfully perform traditional toxicity testing bioassays. Many of these challenges relate to the change in buoyancy, from positive to negative, of pelagic fish embryos that occurs just prior to hatch. A novel exposure system, the pelagic embryo-larval exposure chamber (PELEC), has been developed to conduct successful bioassays on the early life stages (ELSs; embryos/larvae) of pelagic fish. Using this unique recirculating upwelling system, it was possible to significantly improve control survival in pelagic fish ELS bioassays compared to commonly used static exposure methods. Results demonstrate that control performance of mahi-mahi (*Coryphaena hippurus*) embryos in the PELEC system, measured as percent survival after 96-hrs, significantly outperformed agitated static exposure and static exposure systems. Similar significant improvements in 72-hr control survival were obtained with yellowfin tuna (*Thunnus albacares*). The PELEC system was subsequently used to test the effects of photo-induced toxicity of crude oil to mahi-mahi ELSs over the course of 96-hrs. Results indicate a greater than 9-fold increase in toxicity of *Deepwater Horizon* (*DWH*) crude oil during co-exposure to ambient sunlight compared to filtered

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ambient sunlight, revealing the importance of including natural sunlight in 96-hr *DWH* crude oil bioassays as well as the PELEC system's potential application in ecotoxicological assessments.

1. Introduction

Recent research following the BP *Deepwater Horizon* (*DWH*) oil spill of 2010 has revealed the potential for specific physiological impacts in fish, notably cardiac impairment and reductions in swim performance, resulting from *DWH* crude oil exposure (Brette et al., 2014; Esbaugh et al., 2016; Incardona et al., 2014; Mager et al., 2014; Stieglitz et al., 2016). Such findings are in agreement with previous studies examining the effects of crude oil exposure on teleost early life stages (ELSs; embryos/larvae) which reveal a host of common lethal and sub-lethal effects. Many of these effects appear to be the result of cardiotoxicity resulting from acute exposure to polycyclic aromatic hydrocarbons (PAHs) in crude oil during embryonic development (Carls et al., 2008, 1999; Couillard, 2002; Esbaugh et al., 2016; Heintz et al., 1999; Hicken et al., 2011; Incardona et al., 2004). Specifically, acute PAH exposure in teleost ELSs has been shown to induce defects in cardiac function, pericardial and yolk sac edema, neurodevelopmental abnormalities, jaw deformations, and other defects during morphogenesis (Carls et al., 2008; de Soysa et al., 2012; Esbaugh et al., 2016; Incardona et al., 2014, 2013, 2011, 2004; Irie et al., 2011). While teleost ELSs are putatively the life stages of fish most sensitive to crude oil exposure, the scientific literature suggests that effect thresholds vary significantly among species, crude oil compositions, and exposure conditions (Alloy et al., 2016; Esbaugh et al., 2016; Incardona et al., 2014). Due to these differences, it is imperative to conduct toxicity tests replicating the likely exposure scenarios encountered by native, pelagic teleost ELSs in the Gulf of Mexico (GoM) during the *DWH* spill event. One goal of the injury assessment has been to quantify the effect of the spill on economically and ecologically important teleost fish species in the GoM (McCrea-Strub et al., 2011; Sumaila et al., 2012). However, quantifying toxicity to high-value resident non-model species, such as tuna (*Thunnus* spp.) and mahi-mahi, or dolphin-fish, (*Coryphaena hippurus*) following such events using commonly accepted toxicity tests (96-hr bioassays) and endpoints (i.e. LC50) faces many logistical challenges. These challenges result primarily from the difficulty in obtaining and working with such species in a controlled environment.

Marine natural resource impact assessment research in the United States frequently utilizes traditional toxicological testing methods, such as those published by the American Society for Testing and Materials (ASTM) and the United States Environmental Protection Agency (USEPA) (ASTM - E47 Committee, 2005; USEPA, 2002). The use of these prescribed procedures facilitates comparisons among other relevant studies and allows for regulatory applications. This is particularly important for determination of the biological effects of an impact event, whereby acute toxicity tests provide an estimation of the contaminant's toxicity using a commonly accepted metric, such as the median lethal concentration (LC50). Of central importance to such tests is the ability to attain acceptably high levels of survival in control treatments in order to provide a baseline against which treatment effects can be accurately measured. Moreover, control survival of a test is one measure of test quality that provides evidence of organism vigor, physiological quality, suitability of the treatment chambers, and overall test conditions (ASTM - E47 Committee, 2005), particularly in the absence of published guidelines for a specific species and/or life stage (e.g., pelagic fish embryos). In combination with a necessity to maintain high control survival is a need for treatment chambers to contain sufficient numbers of test animals (*n*) to support high power statistical analyses. An additional benefit to a higher *n* per replicated treatment chamber is the ability to harvest sufficient numbers of test animals from replicates for use in post-hoc analyses such as morphometric imaging, immunohistochemical analysis, and ecotoxicogenomics (Snape et al., 2004). Accurate determination of the effects of acute and chronic environmental impact events on GoM representative species is aided by robust experimental design incorporating such analyses. In order to obtain high control survival during acute toxicological bioassays, a novel exposure system, the pelagic embryo-larval exposure chamber (PELEC), was designed and assessed for control survival using two different species of GoM-representative pelagic teleosts: mahi-mahi and yellowfin tuna (*Thunnus albacares*).

Aside from the documented oil contamination of GoM surface waters during the *DWH* incident (*Deepwater Horizon* Natural Resource Damage Assessment Trustees, 2016), the pelagic zone of the GoM receives significant ultraviolet (UV) radiation penetration, particularly in summer months (Alloy et al., 2016; Tedetti and Sempéré, 2006; Whitehead et al., 2000). The photo-induced toxicity of crude oil during co-exposure to UV-radiation dramatically decreases the LC50 of PAHs to many aquatic species (Barron et al., 2003; Little et al., 2000; Pelletier et al., 1997), including mahi-mahi embryos 48 h post fertilization (hpf) (Alloy et al., 2016). Given these results, it was hypothesized that existing 96-hr acute lethality estimates for mahi-mahi (Esbaugh et al., 2016) may be underestimated using traditional laboratory bioassays that do not incorporate exposure to levels of UV-radiation present in natural sunlight. Methods utilized for testing photo-induced toxicity of crude oil on 48-hpf mahi-mahi were unsuitable for obtaining 96-hr acute lethality estimates due to the challenges associated with maintaining high control survival over the course of this extended time period which encompasses the hatching and yolk-sac larval period. Consequently, the PELEC system was used to assess the effects of *DWH* crude oil exposure both with and without the addition of UV-radiation on 96-hr survival of mahi-mahi ELSs to determine potential photo-induced toxicity of *DWH* crude oil to an economically and ecologically valuable pelagic finfish species of the GoM over an extended time period (>48 hpf).

2. Materials and methods

2.1. Control testing of PELEC system

Mahi-mahi and yellowfin tuna (YFT) survival tests (96- and 72-hrs, respectively) were conducted to compare control performance using the two previously utilized methods (i.e., static and agitated beakers) and the newly developed PELEC system. Mahi-mahi embryos were obtained from captive volitionally spawning broodstock maintained in 80,000-L seawater tanks, at the University of Miami Experimental Hatchery (UMEH) on Virginia Key, Florida, USA. Embryos were collected the morning of a spawn and handled using methods described by Stieglitz et al. (2012). Following collection and handling, embryos were transferred to the University of Miami environmental chamber for bioassay set-up. A Leica Zoom2000

stereoscope at 45× magnification was used to assess embryo quality and for counting purposes. YFT embryos were obtained from captive volitionally spawning broodstock maintained at the Inter-American Tropical Tuna Commission's Achotines Laboratory in the Republic of Panama. Collection, handling, and bioassay set-up of the YFT embryos was conducted in a nearly identical manner to the mahi-mahi embryos, with bioassays conducted in a temperature controlled room on site at the Achotines Laboratory facility. Duration of the bioassays was limited to the period of time each species was able to survive off of endogenous yolk reserves prior to initiation of exogenous feeding, corresponding to 72-hrs for YFT and 96-hrs for mahi-mahi.

Four replicates were used in each of the control survival tests (static, agitated static, or PELEC system), and each mahi-mahi test was carried out three separate times using a new batch of embryos for each test (total *n* of 12 per exposure system). YFT tests had *n* values ranging from 6 to 12 depending on the treatment system. Given the sensitivity of YFT ELSs to bacterial contamination, a low dose (10 ppm) of oxytetracycline HCl was tested in two PELEC replicates to determine whether additional improvement in control survival could be obtained. 1 L glass beakers were used for the static and agitated static exposures. For the static agitation treatment, beakers were placed on a tray attached to a reciprocal shaker (Eberbach Model 6000 Mid Range Reciprocal Shaker) operated continuously at a low speed (~60 oscillations per minute).

The PELEC system is comprised of a custom designed conical glass vessel (Kimble Chase, Rockwood, TN), similar to an Imhoff cone used in water quality testing (American Public Health Association et al., 1994), coupled with a 1 L glass beaker. The total volume of each PELEC unit (cone + beaker) is 1.8 L and a schematic drawing of a PELEC unit is shown in Fig. 1, while detailed dimensions of the cone and attachments appears in Supplementary Data - Fig. S1. Each cone was outfitted with an overflow spout for draining into the 1 L glass beaker, and each had a polytetrafluoroethylene (PTFE) stopcock on the bottom. The test solution within one PELEC unit, in this case filtered and UV-sterilized seawater, was circulated between the cone and beaker of each respective unit using a peristaltic pump (4-Channel Peri-Star Pro™, World Precision Instruments, Inc.) and silicone tubing (size #17, 0.25 inches ID). Each 4-channel peristaltic pump supplied flow to 4 PELEC units simultaneously (i.e., one channel per independent PELEC unit). Pump flow for each unit was directed such that water was drawn from the glass beaker and delivered to the cone via the bottom stopcock at a low flow rate (~100 mL/min) to keep embryos gently suspended and circulating in the cone. Embryos/larvae were retained in the cone using a glass excluder attachment extending from the overflow drain with 300 μm nylon mesh fastened on both sides with silicone o-rings (Fig. 1 and Fig. S1). All silicone tubing and o-rings used in this study were new and had not been utilized in any previous trials, thereby limiting any potential effects of PAHs leaching from previously-used tubing or o-rings. Glass dishes were used to cover the tops of all cones and beakers within the different exposure systems to limit evaporation.

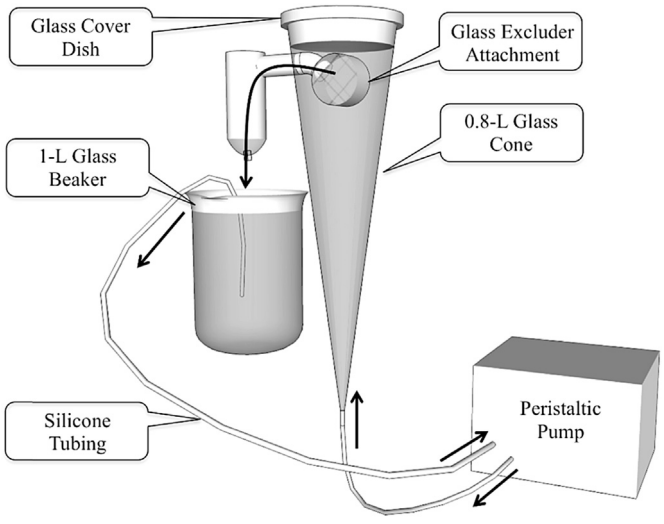

Fig. 1. Schematic drawing of the pelagic embryo-larval exposure chamber (PELEC). Coupled with the overflow beaker the total volume of the PELEC system is 1.8 L. Embryos/larvae are contained in the glass cone. Arrows indicate direction of water circulation.

All replicates were maintained within an environmental control chamber at 27 °C ambient temperature with a 16:8 light dark photoperiod. Embryos were randomly distributed into each exposure system unit at a density of 20 embryos per liter in the static and agitated beakers (1 L test solution volume each), and at a density of 40 embryos in the PELEC system units (1.8 L of test solution volume each) using a large-bore Pasteur pipette. Peak biomass loading in each exposure system did not exceed ~30–35 mg L^{-1}. Water quality parameters including temperature, pH, salinity, and dissolved oxygen (DO) in each exposure chamber were monitored daily. Survival was scored at the conclusion of the 72- or 96-hr period, for YFT and mahi-mahi respectively. Statistical differences in mean survival data between exposure systems were tested using analysis of variance (ANOVA) and a post-hoc multiple comparison test (Fisher's LSD). Outliers were detected using Grubb's outlier test. All statistical analyses were performed using XLSTAT (version 2014.3.02, Addinsoft™, USA).

2.2. Use of PELEC system for determining photo-induced toxicity of DWH crude oil over 96-hrs

In order to test the effects of *DWH* crude oil in combination with natural sunlight, the PELEC system was adapted for use in an outdoor environment by combining the bioassay system described above with a temperature controlled water bath in which the reservoir chambers (1 L beakers) for each independent PELEC unit were partially submerged to allow for accurate temperature control of all PELEC units Fifteen independent PELEC units were set up under the cover of clear, >95% UV-transmittance plastic sheeting (KNF CleanRoom Products, Tamaqua, PA, USA) and fifteen were set up alongside under clear, UV-opaque (~10% UV transmittance) plastic sheeting (GAM Products, Los Angeles, CA, USA) outdoors.

The crude oil used in this study (referred to as “Slick A”) was collected at the site of the *DWH* Oil Spill on July 29, 2010 and was obtained from barge number CTC02404. This barge received oil from a number of skimmer vessels (sample ID CTC02404-02) and oil samples from the barge were subsequently transferred under custody to the University of Miami. The oil solution was prepared as a high-energy water-accommodated fraction (HEWAF) of the oil within 24-hrs of the start of the exposure period, as described in the ‘Supplementary Data’ section. Nominal % WAF dilutions for the full UV exposed treatment group were: 0 (control), 0.03, 0.12, 0.5, 2.0 (% v/v), while dilutions for the limited UV exposed group were: 0 (control), 0.5, 2.0, 8.0, 32.0 (% v/v). Nominal dilutions for the full UV exposure treatments were lower than those used in the limited UV exposure treatments to account for the hypothesized photo-induced increase in toxicity. Each exposure dilution, including controls, was replicated in triplicate using a total of 30 independent PELEC units for the experiment.

Initial water samples for PAH analysis were obtained from each of the bulk nominal % WAF dilutions prior to addition to the PELEC units and final water samples were obtained at the conclusion of

the 96-hr static test. The water samples for PAH analysis were collected in 250 mL amber glass bottles, with 96-hr samples for each exposure concentration being comprised of an even mix of water samples from the three replicates in each treatment group. The 250 mL water samples were shipped on ice overnight to ALS Environmental (Kelso, WA) for analysis by gas chromatography/mass spectrometry – selective ion monitoring (GC/MS-SIM; based on EPA method 8270D). The GC/MS-SIM analytical procedure used had a detection limit rage of 1–5 ng L^{-1}. The reported ΣPAH[50] values represent the sum of 50 PAH analytes (Fig. 4, Supplementary Data - Table S3) in the two distinct PAH fractions within the HEWAF dilutions, both the micro-droplet fraction and the dissolved fraction.

UV-A (λ = 380 nm) was measured continuously during the daylight hours of the exposures using a BioSpherical PUV-2500 radiometer (BioSpherical Instruments, San Diego, CA, USA). Using a JAZ Radiometer (Ocean Optics, Dunedin, FL, USA), the glass of each PELEC unit was determined to be UV-transparent (λ = 380 nm). Total integrated ambient dose (λ = 380 nm) for the high UV treatment was 5664.71 mW s/cm^2 and 566.47 mW s/cm^2 for the low UV control. Total UV exposure time and mean UV intensities for several UV wavelengths and PAR are reported in Table 1. Dissolved oxygen and water temperature parameters were obtained using a ProODO handheld optical DO probe and meter (YSI, Inc., Yellow Springs, OH). Salinity was measured using a refractometer, and pH was measured using a PHM201 m (Radiometer, Copenhagen, Denmark) fitted with a glass electrode. All of the water chemistry measurements were taken on a daily basis, including at initial (0-hr) and final (96-hr) time points. Total ammonia was measured at the end of the 96-hr period using a colorimetric assay (Verdouw et al., 1978). Survival in each PELEC unit was scored at the conclusion of the 96-hr test.

Unless otherwise stated, all data are presented as mean ± standard error of the mean (SEM) and reported ΣPAH[50] concentrations represent geometric means of initial (0-hr) and final (96-hr) ΣPAH[50] concentrations, based on EPA recommended methods for quantifying exposure concentrations (Stephan et al., 1985). The LC50 values were estimated by fitting response data (mean survival at 96-hrs) and log transformed exposure concentrations (geometric mean of initial and final ΣPAH[50] measurements) to a tolerance type Gaussian model with two parameters using the Toxicity Relationship Analysis Program (TRAP; version 1.21a)(Erickson, 2013) freely available from the United States Environmental Protection Agency (USEPA).

3. Results

3.1. Control testing of PELEC system

Survival of mahi-mahi embryos after 96-hrs in the PELEC system (89.8% ± 2.12) was significantly greater than in the agitated static exposure (76.8% ± 4.49) and static exposure (67.5% ± 4.79) systems (Fisher's LSD test, $P < 0.05$)(Fig. 2). One mahi-mahi replicate with full mortality within the agitated static exposure was determined to be an outlier using Grubb's test ($P < 0.01$), and therefore mean survival data for this exposure system were based on an n of 11.

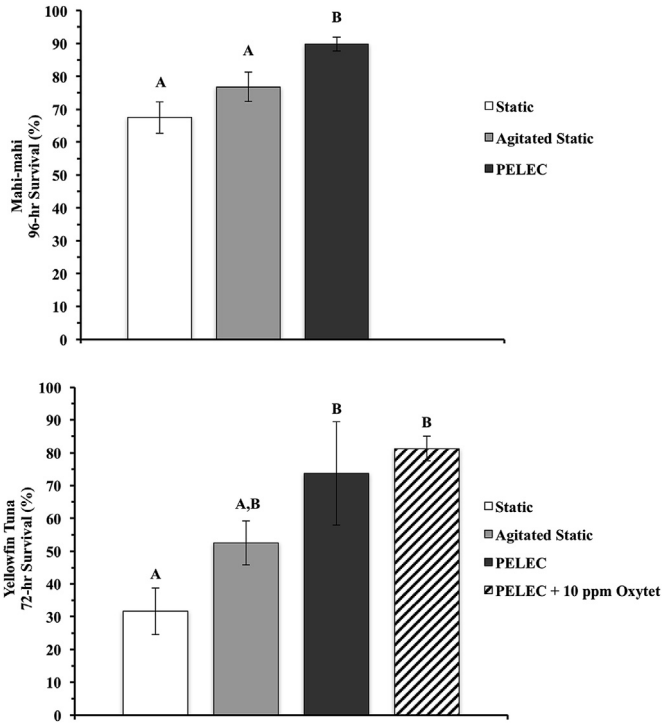

Fig. 2. Results of mahi-mahi and yellowfin tuna bioassays comparing different exposure systems. Control performance of embryos, measured as percent survival after 72-hrs (yellowfin tuna) or 96-hrs (mahi-mahi), was higher in the PELEC system than in the agitated or traditional static exposure system. Use of oxytetracyline HCL (10 ppm) in the PELEC resulted in marginal improvement in 72-hr survival of the yellowfin tuna embryos. Different letters represent significant differences between treatment groups ($P < 0.05$). Values expressed as mean ± SEM.

Survival of YFT embryos after 72-hrs in both the PELEC system (73.8% ± 15.76) and PELEC system with the addition of 10 ppm oxytetracycline HCl (81.3% ± 3.75) were greater than in the agitated static exposure system (52.5% ± 6.64) and significantly exceeded survival in the static exposure (31.7% ± 7.15) system (Fisher's LSD test, $P < 0.05$)(Fig. 2). Water quality parameters amongst all treatments were nearly identical within the mahi-mahi and, separately, within the YFT tests (supplementary data – Table S1).

3.2. Use of PELEC system for testing photo-induced toxicity of DWH crude oil

There was a greater than nine fold increase in toxicity of *DWH* crude oil to mahi-mahi maintained under full spectrum sunlight ('full UV exposure') in a natural diurnal pattern over the course of the 96-hr bioassay compared to organisms kept under the filtered sunlight treatment ('limited UV exposure'). Acute toxicity, measured as 96-hr LC50, of *DWH* Slick A HEWAF in combination with UV-radiation was 0.7 μg L^{-1} ΣPAH[50] (95% CI: 0.6–0.8 μg L^{-1} ΣPAH[50]), whereas acute toxicity of the same *DWH* Slick A HEWAF without UV-radiation exposure was 6.5 μg L^{-1} ΣPAH[50] (95% CI: 6.2–6.9 μg L^{-1} ΣPAH[50]) (Fig. 3). There were no significant

Table 1
Mean ambient intensities of PAR and six wavelengths of UV for the outdoor sunlight experiment. Reported values are for the full UV treatment. Limited UV treatment intensities are 10% of these values. Specific daily UV exposure and intensity data appear in Supplemental Data – Table S4.

UV Exposure Duration (hrs)	I = 305 nm (mW/(cm^2nm))	I = 313 nm (mW/(cm^2nm))	I = 320 nm (mW/(cm^2nm))	I = 340 nm (mW/(cm^2nm))	I = 380 nm (mW/(cm^2nm))	I = 395 nm (mW/(cm^2nm))	PAR (μE/cm^2sec))
Total 27:06:00	Mean 0.005	Mean 0.014	Mean 0.026	Mean 0.044	Mean 0.062	Mean 0.064	Mean 0.160

differences in water quality data between replicates (Supplementary Data – Table S2). Composition of the Slick A HEWAF dilutions used in this experiment (Supplementary Data - Table S3) were nearly identical in composition to the Slick A HEWAF dilutions used in other recent studies examining the effects of the *DWH* oil spill and importantly very similar to field collected samples from the active spill site (Esbaugh et al., 2016; Incardona et al., 2014; Mager et al., 2014; Stieglitz et al., 2016) providing an environmentally-relevant exposure scenario. The ΣPAH[50] concentrations in each exposure decreased over the course of the 96-hr experiment, with higher exposure concentrations within each treatment group exhibiting greater declines compared to lower exposure concentrations (Supplementary Data – Tables S2 and S3). Additionally, the decreases between the full and limited UV exposure treatments differed in both magnitude and in final ΣPAH[50] composition as might be expected given the likely increased photolytic breakdown of PAHs in the full UV exposure treatment (Fig. 4, Supplementary Data – Tables S2 and S3). Differences in ΣPAH[50] decreases found between the two treatments are most apparent in the overlapping 0.5% and 2% dilutions of the full UV exposed treatment compared to the limited UV exposed treatment (0.5%: 0.75 μg L^{-1} vs. 1.51 μg L^{-1} ΣPAH[50]; 2%: 2.50 μg L^{-1} vs. 3.78 μg L^{-1} ΣPAH[50], respectively)

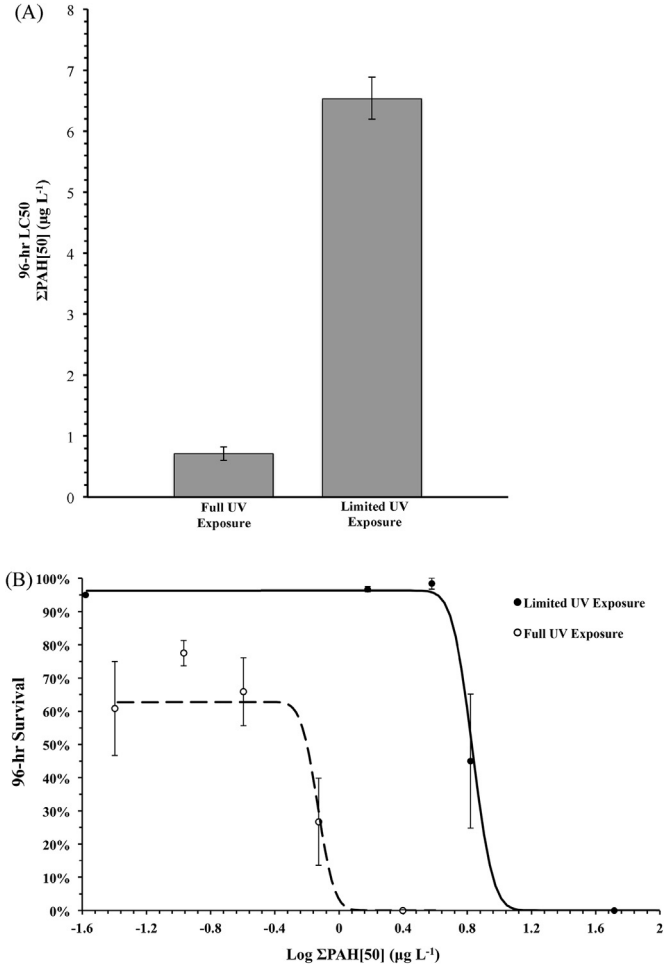

Fig. 3. Acute toxicity, quantified as 96-hr LC50, of Slick A HEWAF exposure with limited and full UV-radiation exposure (A). Values expressed in μg L^{-1} ΣPAH[50], with upper and lower 95% confidence intervals indicated by error bars (A). Graph of mahi-mahi ELS survival (mean ± SEM) at different crude oil exposure concentrations, expressed in Log μg L^{-1} ΣPAH[50], from both the limited and full UV-radiation exposure treatments (B). Reported ΣPAH[50] concentrations represent the geometric means of initial (0-hr) and final (96-hr) ΣPAH[50] concentrations.

(Supplementary Data - Tables S2 and S3). However, the decrease was not evenly proportional between individual PAHs. There was near total elimination of 4-ring PAHs in the full UV exposed treatment after 96 h. However, specific 4-ring PAHs, notably Benz(a) anthracene, Chrysene + Triphenylene, and Chrysenes (C-1 and C-2), within the limited UV exposed treatment increased in the amount of relative contribution (% ΣPAH[50]) between initial (0-hr) and final (96-hr) sampling (Fig. 4, Supplementary Data – Table S3).

4. Discussion

The present study demonstrates that for pelagic fish ELSs, the novel PELEC system significantly improves test performance, measured as survival of fish embryos and larvae under test conditions, and the system was employed to test for the impact of natural light on oil toxicity over an extended time period. Results show that co-exposure to UV-radiation increases the acute toxicity of *DWH* spill oil to a resident, GoM fish species by nearly an order of magnitude. These findings illustrate the importance of including natural sunlight in assessments of *DWH* spill oil as well as the PELEC system's potential application in ecotoxicological assessments of pelagic fish ELSs.

It was hypothesized that variability in control survival from static 96-hr bioassays could be attributed, in large part, to changes in embryo and larval buoyancy throughout the initial stages of development as well as a need for agitation of the micro-boundary layer of test solution proximal to the embryonic chorion. Different species of pelagic teleosts exhibit diverse life history strategies, such as changes in egg buoyancy during development, that allow for reproductive success despite numerous selective pressures in the open ocean (Ospina-Álvarez et al., 2012). Specifically, the change in buoyancy from positive to negative in the 2–4 h leading up to hatch has been documented not only in mahi-mahi but in other pelagic teleosts, such as yellowfin tuna (Margulies et al., 2007). The change in buoyancy likely results from the change in specific gravity that occurs as the osmotic permeability of the chorion increases and its structure softens prior to hatch (Blaxter, 1969; Margulies et al., 2007). While the pre-hatch onset of negative buoyancy likely confers a benefit to these organisms in the wild, serving to position embryos and yolk-sac larvae below the neuston layer (Margulies et al., 2007), this phenomenon may reduce survival and complicate testing for treatment effects in static beaker bioassays. Additionally, due to high rates of metabolism and rapid embryo and larval development of many sub-tropical pelagic marine fish, such as mahi-mahi (Benetti, 1992), suspension and movement may reduce the risk of hypoxia at the boundary layer of the chorion in the embryonic stage and the cutaneous layer in the larval stage. Theoretically, both the agitated static and PELEC systems should ameliorate any such hypoxia risk, though the latter system tends to allow for higher survival over 96-hrs than the former system. Increases in relative survival in the PELEC system suggest that an upwelling water movement may be more effective at reducing mortality caused by exposure systems than the mostly superficial lateral water movement provided by agitation. Additionally, the engineering of the PELEC system allows for full replacement of exposure media without physically touching test organisms, a key benefit for short-term window of exposure studies that may be conducted within a traditional 96-hr bioassay timeframe.

Use of the PELEC system to test the effects of crude oil in combination with exposure to ultraviolet radiation on ELSs of mahi-mahi reveals one potential application of this novel exposure bioassay system. The *DWH* incident of 2010 represents the largest marine oil spill in the United States, releasing over 3 million barrels (5.07×10^8 L) of crude oil into the GoM over the course of 87 days in

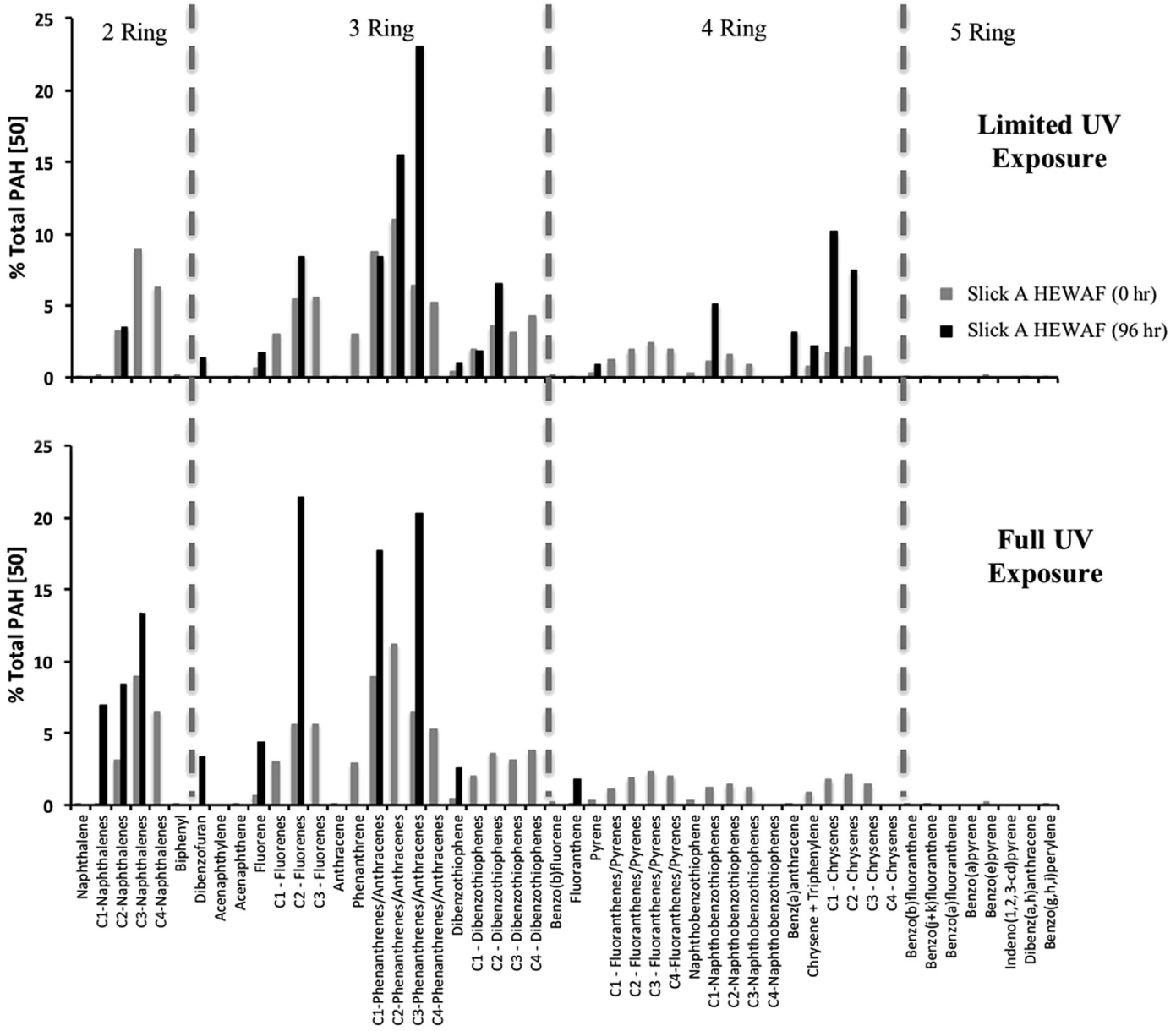

Fig. 4. Initial (0-hr) and final (96-hr) percent composition for 50 PAH analytes as determined by GC/MS-SIM for 2% dilutions of the Slick A HEWAF exposures, both in the full and limited UV-radiation exposed treatments.

the summer of 2010 (*Deepwater Horizon* Natural Resource Damage Assessment Trustees, 2016). The temporal and spatial aspects of the spill, as well as the accompanying ΣPAH concentrations in the water documented during the spill (Diercks et al., 2010; Incardona et al., 2014; Wade et al., 2011), are particularly significant for this study since the pelagic zone of the GoM represents an important spawning and feeding ground for top trophic level pelagic fish species, such as mahi-mahi and yellowfin tuna (Arocha et al., 2001; Lang et al., 1994; Palko et al., 1982; Rooker et al., 2012; Teo and Block, 2010). These economically- and ecologically-valuable gamefish species inhabit and spawn in the pelagic waters of the GoM during the time of year the *DWH* incident occurred (Arocha et al., 2001; Lang et al., 1994; Palko et al., 1982). Additionally, mahi-mahi and YFT ELSs are relatively transparent, and are commonly found in the surface and near-surface layers of the ocean, potentially increasing their exposure to waters in contact with or near surface oil slicks. Furthermore, residence in surface and near-surface waters increases the likelihood of exposure to significant levels of UV-radiation. Such exposure can be damaging to fish ELSs, as evident from the reduced survival of mahi-mahi larvae in the controls of the full-UV exposure treatment compared to the controls of the limited-UV exposure treatment (Fig. 3). Crude oil is a complex mixture composed of thousands of components and it is feasible that compounds not captured by the presented PAH measurements contribute to (photo-induced) toxicity. However, the presented data clearly demonstrates photo-induced oil toxicity and measurement and quantification of PAHs within crude oil allows for comparison between different studies since these compounds are well known to be primarily responsible for crude oil toxicity (Alloy et al., 2016; Carls et al., 2008, 1999; de Soysa et al., 2012; Heintz et al., 1999; Hicken et al., 2011; Incardona et al., 2014, 2013, 2004). *DWH* crude oil toxicity estimates from the current study are within the ranges found in other related studies on mahi-mahi embryos, where weathered *DWH*

surface slick oil had acute lethality at 8.8 μg L^{-1} ΣPAH (Esbaugh et al., 2016) and UV radiation was shown to increase toxicity of *DWH* crude oil (Alloy et al., 2016). Such findings are similar to the documented sub-lethal and lethal impacts of *DWH*-specific PAH exposure in the low (~1–15) μg L^{-1} ΣPAH range in laboratory settings (i.e. limited UV-radiation exposure) for other predatory pelagic fish species with similar life histories to mahi-mahi, such as tuna (*Thunnus* spp.) and amberjack (*Seriola* spp.) (Incardona et al., 2014). This range of ΣPAH exposure is relevant given that numerous upper pelagic zone water samples collected from the GoM during the active spill phase had ΣPAH concentrations in excess of 1 μg L^{-1}, including reported ΣPAH concentrations up to 85 μg L^{-1} (Diercks et al., 2010; Wade et al., 2011). Acute toxicity of the *DWH* crude oil in this study, quantified as 96-hr LC50, in the full UV exposed treatment (0.7 μg L^{-1} ΣPAH[50]) was nearly an order of magnitude greater than occurred under laboratory settings (6.5 μg L^{-1} ΣPAH[50]). The present study builds upon the results from short-term (48-hpf embryos) tests (Alloy et al., 2016) to reveal that 96-hr laboratory estimates that do not include UV exposure as a variable potentially underestimate ecological damage incurred by the *DWH* incident. Previous studies performed under fluorescent light report effect thresholds for resident GoM pelagic fish species as low as 1.2 μg L^{-1} ΣPAH[50] (reduced swim performance)(Mager et al., 2014) in juvenile mahi-mahi and 0.9 μg L^{-1} ΣPAH[50] (pericardial edema)(Incardona et al., 2014) in ELSs of Southern bluefin tuna (*Thunnus maccoyii*), a congeneric of the bluefin tuna species found in the GoM, the Atlantic bluefin tuna (*Thunnus thynnus*). Previously reported low effect thresholds (Esbaugh et al., 2016; Incardona et al., 2014; Mager et al., 2014; Pasparakis et al., 2016) may represent conservative estimates of *DWH* oil toxicity on these endpoints since they do not account for photo-induced toxicity. Such low effect thresholds add to the growing body of evidence that pelagic fish embryos are very sensitive to low level crude oil exposure concentrations (low μg L^{-1} range), with effect concentrations within, and frequently below, those which have been reported for other more commonly used test organisms in the wake of the *DWH* oil spill (*Deepwater Horizon* Natural Resource Damage Assessment Trustees, 2016).

Photo-induced toxicity of PAHs occurs through two distinct mechanisms: absorption of UV by a photosensitive PAH and eventual production of reactive oxygen species (ROS) or photo-oxidation resulting in the generation of toxic, modified photo-products (Lee, 2003). In the present study, it was not possible to discern between these two mechanisms. However, previous studies examining the photo-induced toxicity of PAHs on larval teleosts following the *Exxon Valdez* (Barron et al., 2003) and *Cosco Busan* (Incardona et al., 2012) oil spills indicate that the increased mortality observed in the full UV exposed treatment group likely resulted primarily from photosensitization. During such photo-sensitization absorbed PAHs in the embryonic mahi-mahi reacted with absorbed solar radiation to produce reactive oxygen species that in turn resulted in oxidative stress (Lee, 2003; Little et al., 2000). Given the differences noted between the final ΣPAH[50] compositions of the full UV exposure treatments compared to the limited UV exposure treatments, as well as the uneven pattern of degradation between the treatments, it is clear that there was a UV effect on chemistry, and this could be due to either photo-oxidation or photolysis (Fig. 4; Supplementary Data - Tables S2 and S3) (Lee, 2003). In the full UV exposure treatments, the proportion of 2- and 3-ring PAHs increased relative to the total. In the limited UV exposure treatment, however, 3- and 4-ring PAHs increased in proportion to the total (Fig. 4). The uneven pattern of proportional PAH decline provides insight on the effects of solar UV-radiation on oil-related compounds in the environment, while the increased mortality observed in the full UV exposure treatment reveals the toxic effects of this interaction. The declines in PAH exposure concentrations are accounted for in each treatment using geometric means of initial and final PAH concentrations though such declines combined with possible differences in sensitivity among life stages during the 96 h of exposure may reduce the precision of toxicity estimates. While there is little reason to suspect much variation in final PAH concentrations among replicate beakers, it should be noted that the LC50s were calculated using pooled final concentrations. Given that crude oil readily adsorbs to exposure chamber surfaces, the increased surface area of the PELEC bioassay system compared to other commonly used exposure vessels, such as glass beakers, may serve to increase this adsorption. Such adsorption may increase the rate of decline in PAH exposure concentrations over time and may reduce the proportion of droplet exposure when using unfiltered HEWAF dilutions in this exposure system. While a typical water chemistry sampling regime was utilized in this study, whereby initial and final samples are obtained, increased sampling frequency may serve to expand the resolution on the rate of ΣPAH[50] decline over time in each treatment. The present photo-induced crude oil toxicity study illustrates one way in which the PELEC bioassay system allows for successful quantification of the effects of environmental impact events, such as the *DWH* oil spill, on challenging pelagic fish ELSs using commonly accepted toxicological tests, metrics, and endpoints.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.07.069.

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