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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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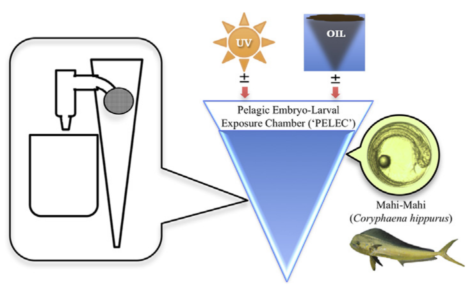
HIGHLIGHTS

- 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