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Ultraviolet Radiation Enhances the Toxicity of *Deepwater Horizon* Oil to Mahi-mahi (*Coryphaena hippurus*) Embryos

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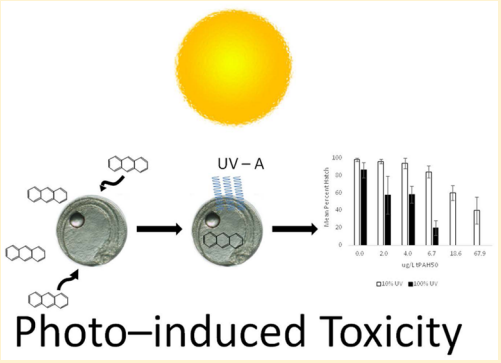
Supporting Information

ABSTRACT: The 2010 *Deepwater Horizon* oil spill resulted in the accidental release of millions barrels of crude oil into the Gulf of Mexico. Photoinduced toxicity following coexposure to ultraviolet (UV) radiation is one mechanism by which polycyclic aromatic hydrocarbons (PAHs) from oil spills may exert toxicity. Mahi-mahi (*Coryphaena hippurus*), an important fishery resource, have positively buoyant, transparent eggs. These characteristics may result in mahi−mahi embryos being at particular risk from photoinduced toxicity. The goal of this study was to determine whether exposure to ultraviolet radiation as natural sunlight enhances the toxicity of crude oil to embryonic mahi−mahi. Mahi-mahi embryos were exposed to several dilutions of water accommodated fractions (WAF) from slick oil collected during the 2010 spill and gradations of natural sunlight in a fully factorial design. Here, we report that coexposure to natural sunlight and WAF significantly reduced percent hatch in mahi−mahi embryos. Effect concentrations of PAH in WAF were within the range of surface PAH concentrations reported in the Gulf of Mexico during the *Deepwater Horizon* spill. These data suggest that laboratory toxicity tests that do not include UV may underestimate the toxicity of oil spills to early lifestage fish species.



INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic molecules composed of multiple benzene rings that vary widely in their chemical and toxicological properties.[1] As a group, PAHs are lipophilic and readily bioaccumulate.[2] PAHs exert toxicity through several mechanisms including photoinduced toxicity.[3−5] Photoinduced toxicity is a phenomenon in which a compound exhibits increased toxicity in the presence of certain wavelengths of light.[6] Photodynamic PAHs, including those found in crude oil,[7] have been shown to result in photoinduced toxicity in aquatic organisms.[5,8−10] Effects of PAH photoinduced toxicity include increased mortality,[8,11−14,1] reduced fecundity,[3] increased photoavoidance behavior,[15] and feeding inhibition.[16]

Beginning on the 20th of April, 2010, the Mobile offshore drilling unit *Deepwater Horizon* experienced a series of events that resulted in the sinking of the vessel and subsequent release of oil from the wellhead until it was sealed on July 15th, 2010.[17] We have previously shown that oil from the *Deepwater Horizon* spill is phototoxic to early lifestage blue crab at PAH concentrations within the range of those that occurred during the spill.[10] The timespan and scale of the oil release presented a hazard to both near-shore species such as the blue crab and open water species like the mahi−mahi. While photoinduced toxicity of PAHs to fishes has been reported,[5,11,15,18−20] little is known with respect to commercially and ecologically relevant pelagic fish species that likely resided in the Gulf of Mexico at the time of the spill.[21,22]

The mahi−mahi (*Coryphaena hippurus*) occurs in almost every ocean body lower than 30 degrees in latitude with local distributions sometimes ranging farther north and south with warmer currents.[23] It is found throughout the Gulf of Mexico and is an important recreational resource as well as a commercially fished species.[24] The mean combined annual mahi−mahi commercial and recreational harvest from the Gulf of Mexico from 2000 to 2012 was 980 t.[24] Reproductive activity in this species is primarily dictated by water temperature, with peak spawning activity occurring in warm oceanic waters.[25] The temperature-controlled recirculating aquaculture systems (RAS) allow for year-round spawning activity in captivity. Fertilized embryos typically hatch approximately 36−40 h post fertilization at water temperatures of 25−28 °C. Newly fertilized embryos are buoyant, yet in the hours leading up to

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hatch they become neutrally, and subsequently negatively, buoyant. Hatched larvae remain in the upper water column of the pelagic environment[26] where light levels and zooplankton concentrations are sufficient for feeding during the larval stage. In the course of a single reproductive event, mahi−mahi females release as many as 1.5 million buoyant embryos in offshore waters.[27] For the duration of their embryonic life stage the eggs remain buoyant in surface water where they are likely to encounter UV light.[27] Due to the relative transparency, and positive buoyancy of their embryos during early development, we hypothesized that mahi−mahi embryos would specifically be at risk to photoinduced toxicity following exposure to PAHs. To test this hypothesis, mahi−mahi embryos were exposed to a range of dilutions of water accommodated fractions (WAF) of artificially weathered source oil, or to slick oil. Exposures were carried out under gradations of UV achieved using natural sunlight filtered by plastics and mesh screening. Data from this study may be used as a component of the natural resource damage assessment (NRDA) following the DWH oil spill.

MATERIALS AND METHODS

Test Organism. Organisms were obtained from a wild cohort of mahi−mahi broodstock maintained at the University of Miami. Spawning occurred at dawn and embryos were collected in a purpose-built egg collection vessel that was attached to a recirculating broodstock maturation system. Broodstock was maintained in an outdoor facility under natural sunlight covered by shade cloth. Embryos were prepared using methods described in detail Stieglitz et al.,[28] whereby viable embryos were treated with a formalin (37% formaldehyde) solution at a concentration of 100 ppm for 1 h to remove parasites prior to use in toxicological tests. During the 1 h formalin soak supplemental oxygen was supplied to maintain dissolved oxygen concentration at or just above saturation. After the formalin soak the eggs were rinsed with sterilized, filtered seawater for an additional hour with continuous aeration.

Test Solutions. Two source oils were used in these experiments, a field collected oil and an artificially weathered oil. The field oil was obtained on July 29, 2010 from the hold of barge number CTC02404, which was receiving surface slick oil from various skimmer vessels near the Macondo Well. This slick oil is routinely used in NRDA testing for the *Deepwater Horizon* spill. The artificially weathered oil was obtained from the MC252 riser. Artificial weathering was accomplished using methods modified from Carls et al.[29] Oil was heated to approximately 98 °C and stirred lightly until its mass was reduced by 33%−38%. Filtered, UV-sterilized Atlantic seawater was used in control/dilution preparations. Water physicochemical parameters were within the following ranges, 30−35 ppt salinity, 49−55 mS conductivity, and 7.9−8.4 pH.

Stocks of high energy water accommodated fraction (HEWAF) were prepared prior to each test by mixing 1g of oil to one liter of water (in a Waring CB15 blender (Global Resources Inc., Winstead CT), on low power, for 30 seconds. The mixture was transferred to a separatory funnel and allowed to settle for 1 h. The separatory funnel was covered in aluminum foil so that settling could occur in darkness to avoid photo degradation of PAHs prior to test dilution preparation and chemical sampling. The bottom 50 mL was discarded and the middle portion used for PAH analysis and utilization in test dilutions. The top floating fraction was also discarded. Stocks of chemically enhanced water accommodated fractions (CEWAF) were prepared by adding known volume of water with a mass of slick oil (at a 1:1000 oil:water ratio) and dispersant (Corexit 9500) in a 10:1 oil:dispersant ratio. The solution was stirred by a Teflon bar at sufficient speed to achieve a 25% vortex in a 2L aspirator bottle. The bottle was covered in aluminum foil so that stirring could occur in darkness for 18−24 h prior to settling for 1 h. The solution was then sampled for PAH analysis and utilization in test dilutions. Test dilutions were prepared by serial dilution of the stock with control water to a desired percentage of HEWAF or CEWAF. All stocks were made to the same mass ratio of oil to water.

Samples of stocks and dilutions were taken with every preparation and shipped (4 °C) to Analytical Laboratory Services (Kelso, Washington) for analysis. PAHs were extracted from samples using EPA method 3541, automated Soxhlet. Whereby samples were mixed and dried with sodium sulfate, and then extracted with dichloromethane. Fifty specific PAH analytes were quantified using a method based on EPA method 8270D.[30] Quantification was performed using an Agilent 6890 gas chromatograph with an Agilent 5973 mass spectrometer in selected ion monitoring mode. The sum concentrations of these 50 PAH analytes are hereafter referred to as $tPAH_{50}$.

Toxicity Tests. The investigation consisted of four separate toxicity tests in which mahi−mahi embryos were exposed to either CEWAF derived from slick oil, CEWAF derived from artificially weathered source oil, HEWAF derived from slick oil, or HEWAF derived from artificially weathered source oil. This approach allowed the investigation into the possible differences in photoinduced toxicity brought about by dispersant based WAF preparation versus mechanical WAF preparation, and possible differences from slick oil or artificially weathered oil used in WAF preparations.

Mahi-mahi embryos were exposed to a range of PAH concentrations combined with two or three UV intensities in a factorial design for 48 h. Exposures were conducted in 250 mL borosilicate glass crystallizing dishes containing 10 embryos per dish. The exposure period included an approximately 17 h equilibration period, a 7 h solar exposure, an overnight recovery period (17 h), a second 7 h solar exposure, and an overnight recovery period after which percent hatch in each replicate was assessed. Each toxicity test had five to six PAH treatments with five replicate dishes per PAH treatment.

Replicate dishes were suspended in an outdoor, flow-through water bath to maintain temperature. Dishes were floated in polystyrene foam insulation board with holes cut to hold replicate dishes in contact with the temperature bath water across the underside, and the majority of the sidewall. Sunlight was used as the source of UV radiation. Screening materials were suspended over replicate dishes to achieve gradations of UV (λ = 380 nm). Percent transparency was also determined prior to test initiation under natural sunlight using the same radiometer that monitored the exposures. A specially formulated plastic sheet >90% transparent to UV was used for a full intensity (100% ambient) UV treatment (KNF Corporation, Tamaqua, PA). A metal mesh screen was added over the top of the full intensity plastic as an additional filter to achieve an approximately 50% ambient UV treatment. A different formulation of plastic sheet allowing transmission of <10% of ambient UV (Rosco Laboratories Inc., Stamford, CT) was used as a control. UV was measured continuously during the exposures using a Biospherical radiometer (BioSpherical Instruments, San Diego, CA).

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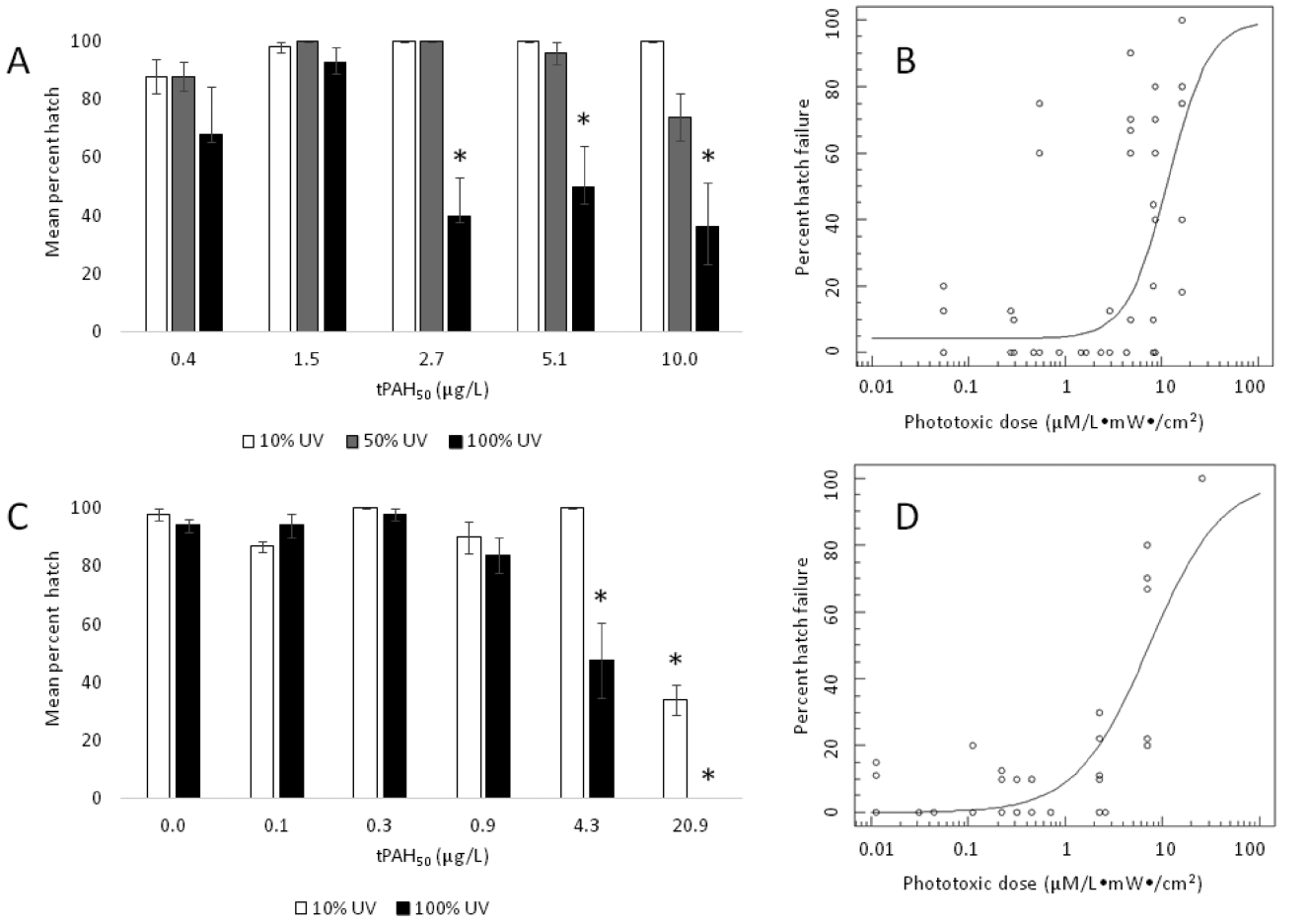

Figure 1. Hatching success in mahi-mahi embryos exposed to field collected slick oil (A) CEWAF by $tPAH_{50}$ concentration and UV treatment, (B) CEWAF by phototoxic dose. (C) HEWAF by $tPAH_{50}$ concentration and UV treatment, and (D) HEWAF by phototoxic dose. Errors bars represent 1 SE. Asterisks indicate treatments with mean percent hatch significantly different from controls.

Phototoxic Units. All tests were performed outdoors using ambient sunlight as the UV source. Tests performed on different days received different UV doses. To account for differences in UV coexposure, a phototoxic unit was calculated as described previously in Alloy et al.[10] using methods similar to Newsted and Giesy.[6] Concentrations of 14 known phototoxic PAHs were used in the calculations: anthracene, benzo[a]-anthracene, benzo[e]pyrene, benzo[g,h,i]perylene, chrysene, fluoranthene, fluorene (as well as C1 and 2), phenanthrene (as well as C1, 2, and 3), and pyrene. The aqueous concentration of each PAH was calculated as a molar value and multiplied by its relative photodynamic activity compared to anthracene (RPA). This produced the sum equivalent molar concentration of anthracene. This anthracene equivalent concentration was multiplied by the integration of the UV irradiance (λ = 380 nm) to produce the phototoxic dose. UV irradiances are reported as the integration of the test duration at a resolution of one second, expressed as mW·s/cm^2. Phototoxic units are expressed as μM/L·mW·s/cm^2.

Statistical Analyses. For each toxicity test, hatch data was arcsine transformed and a two factor analysis of variance (ANOVA) with a Dunnett's posthoc test was used to determine differences in percent hatch using the statistical software JMP (version 11, SAS Institute, Cary, NC). All statistical comparisons were made using α = 0.05. UV treatment and $tPAH_{50}$ concentration were used as factors in each ANOVA. Median effect concentrations (EC_{50}) for phototoxic dose were calculated using the *drc* package in R (version 3.1.2).[31]

A table of the anthracene equivalent molar concentrations required at a given UV dose to meet three effect concentrations was generated using the mean UV intensity of all tests, and the calculated effect concentrations for 20%, 50%, and 80%. The mean UV intensity was multiplied by an exposure duration of 14 h. This UV dose was used as the 100% UV exposure for the table, and was reduced accordingly for 75%, 50%, and 25% UV calculations.

RESULTS

Slick oil CEWAF dilutions contained 0.4, 1.5, 2.7, 5.1, and 10.0 μg/L $tPAH_{50}$. The total UV dose in each exposure was calculated by integrating the total irradiance received over the timecourse of the test during both solar exposure periods. The slick oil CEWAF test utilized three gradations of UV (100%, 50%, and 10%). Slick oil CEWAF 100% UV exposures received a total integrated dose of 3276 mW·s/cm^2 with a mean intensity (± 1SD) of 0.053 ± 0.023 mW/cm^2/s. Significant toxicity was observed at exposures ≥2.7 μg/L $tPAH_{50}$ in the 100% UV treatment only (p < 0.01) (Figure 1A). To account for differences in UV exposure between tests, EC_{50}s were calculated in phototoxic dose units for each test. The slick oil CEWAF phototoxic EC_{50} was 11.8 μM/L·mW•s/cm^2 (95% confidence interval = 7.84−15.7 μM/L·mW·s/cm^2) (Figure 1B).

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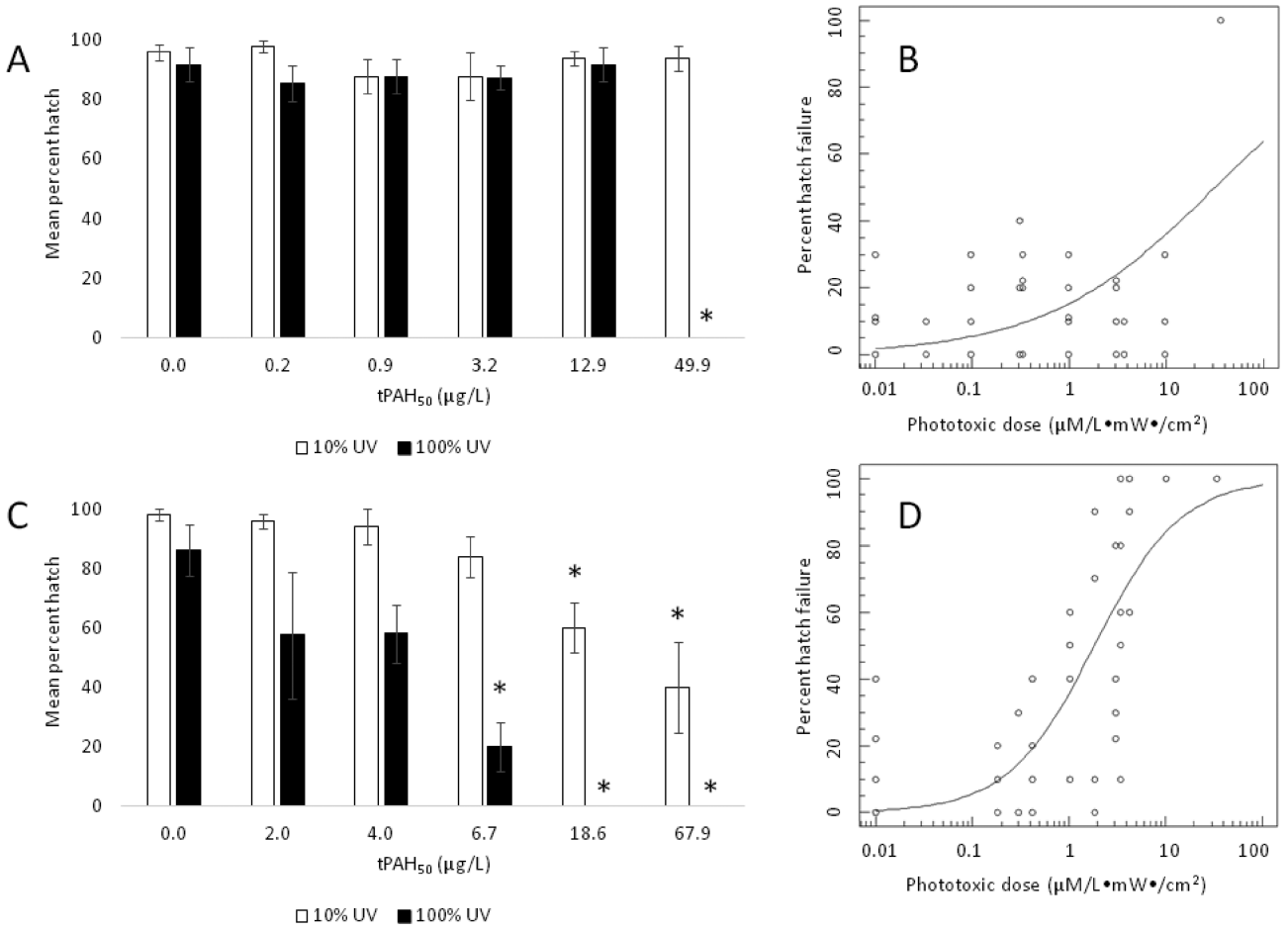

Figure 2. Hatching success in mahi-mahi embryos exposed to artificially weathered source oil (A) CEWAF by $tPAH_{50}$ concentration and UV treatment, (B) CEWAF by phototoxic dose. (C) HEWAF by $tPAH_{50}$ concentration and UV treatment, and (D) HEWAF by phototoxic dose. Errors bars represent 1 SE. Asterisks indicate treatments with mean percent hatch significantly different from controls.

Slick oil HEWAF dilutions contained 0, 0.1, 0.3, 0.9, 4.3, and 20.9 μg/L $tPAH_{50}$. The slick oil HEWAF test utilized two gradations of UV (100% and 10%) only. Slick oil HEWAF 100% UV exposures received an integrated dose of 3022 mW·s/cm^2 with a mean intensity of 0.044 ± 0.022 mW/cm^2/s. Significant toxicity was observed to occur at exposures ≥4.3 μg/L $tPAH_{50}$ in the 100% UV treatment, and at the 20.9 μg/L $tPAH_{50}$ exposures in the 10% UV treatment ($p < 0.01$) (Figure 1C). The slick oil HEWAF phototoxic EC_{50} was 6.77 μM/L·mW·s/cm^2 (5.91−7.64 μM/L·mW·s/cm^2) (Figure 1D).

Weathered source CEWAF dilutions contained 0, 0.2, 0.9, 3.2, 12.9, and 49.9 μg/L $tPAH_{50}$. Both weathered source oil tests utilized two UV gradations (100% and 10%). Weathered source CEWAF 100% UV exposures received an integrated dose of 2436 mW·s/cm^2 with a mean intensity of 0.040 ± 0.019 mW/cm^2/s. Significant toxicity was observed to occur only in the 49.9 μg/L $tPAH_{50}$ exposures in the 100% UV treatment ($p < 0.01$) (Figure 2A). The artificially weathered source oil CEWAF phototoxic EC_{50} was 14.7 μM/L·mW·s/cm^2 (No solution to 95% CI) (Figure 2B).

Weathered source HEWAF dilutions contained 0, 2.0, 4.0, 6.7, 18.6, 67.9 μg/L $tPAH_{50}$. Weathered source HEWAF 100% UV exposures received an integrated dose of 1754 mW·s/cm^2 with a mean intensity of 0.031 ± 0.014 mW/cm^2/s. Significant toxicity was observed at exposures ≥4.0 μg/L $tPAH_{50}$ in the 100% UV treatment, and ≥18.6 μg/L $tPAH_{50}$ in the 10% UV treatment ($p < 0.01$) (Figure 2C). The artificially weathered source oil HEWAF phototoxic EC_{50} was 2.16 μM/L·mW·s/cm^2 (1.52−2.79 μM/L·mW·s/cm^2) (Figure 2D).

DISCUSSION

In all four toxicity tests, toxicity, measured as decreased hatching success, occurred in a PAH and UV dependent manner. Normal hatching time for mahi−mahi at 25 °C is approximately 40 h post fertilization.[26] This suggests that embryos that did not hatch within a normal time frame (up to 48h in this study) are developmentally delayed following coexposure to PAH and solar radiation. This likely affects their potential for survival and recruitment compared to control embryos. Concentrations of $tPAH_{50}$ observed to result in photoinduced toxicity in these experiments are well within the range of concentrations (0−84.8 μg/L) reported in the spill area during the active phase of the 2010 *Deepwater Horizon* spill.[32] Measured $tPAH_{50}$ values in this study are based on samples taken immediately after WAF preparation, and do not account for loss of PAH over time in the exposure chambers. Exposure solutions were held static for the duration of the toxicity test. Thus, the toxicity values reported here are likely conservative and time-integrated effect values are likely lower.

Concentrations of PAH observed to result in toxicity in this study are also in agreement with other literature-reported values using oil and marine species. Barron and Ka'aihue[9] report an LC_{50} of 30 μg/L total PAH in a 96 h UV and PAH

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coexposure to pacific Herring embryos. Sellin-Jeffries et al.[19] induced significant mortality after 48 h at 15 μg/L anthracene in pacific herring young of the year in field UV exposure tests, and at 5 μg/L anthracene in laboratory tests using larvae and artificial UV. Duesterloh et al.[33] report a LC_{20} of approximately 2 μg/L PAH in a single 4 h UV and PAH coexposure to a marine calanoid copepod. Oil exposures without a UV component report much higher median effect values. Singer et al.[34] reported a range of LC_{50}s (16.3−40.2 mg/L) in a series of larval topsmelt exposures to crude oil. Pollino et al.[35] reported a range of LC_{50}s (1.28 mg/L WAF, 14.5 mg/L dispersant only, and 1.37 mg/L WAF and dispersant) in a series of WAF, dispersant, and WAF with dispersant exposures to day-of-hatch rainbowfish larvae. Interestingly, the range of PAH concentrations observed in this study to be acutely phototoxic under natural sunlight to embryonic mahi are similar to concentrations observed to result in sublethal effects in pelagic marine fish without UV. Mager et al.[21] reported significantly reduced critical swimming speed in juvenile mahi−mahi that were exposed once, as embryos, to concentrations as low as 1.8 μg/L $tPAH_{50}$. Incardona et al.[22] published similar findings, reporting pericardial edema EC_{50s} as low as 0.8 μg/L in tuna and 12.4 μg/L in amberjack.

Toxicity Associated with WAF Preparation Method. There was a significant reduction in photoinduced toxicity comparing CEWAF to HEWAF from the same source oil. Dilutions of CEWAF made using artificially weathered oil were approximately 7-fold less phototoxic than HEWAF counterparts. Similarly, CEWAF made from more weathered slick oil was approximately 3-fold less phototoxic than slick oil HEWAF. This implies that the reduction of photoinduced toxicity in CEWAFs could be related to the degree of weathering the oil has undergone prior to the addition of dispersant.

We have reported a similar finding in a previous study conducted using blue crab zoea coexposed to UV and WAF.[10] In that study, photoinduced toxicity was also reduced in CEWAF preparations compared to HEWAF preparations. Investigations into dispersed oil toxicity have reported preparations of oil and dispersant to be as toxic as oil alone,[36] to reduce toxicity compared to oil-only exposures,[37] or to exhibit greater toxicity.[38] Reports of increased bioaccumulation of PAHs with dispersant use would also be expected to increase photoinduced toxicity.[39,40] However, none of the cited studies investigated photoinduced toxicity specifically as a mechanism. Dispersants may mediate photoinduced toxicity by some other mechanism than altered bioavailability or bioaccumulation. Dispersants may have some unknown effect on irradiation by UV, thus reducing the number of photochemical reactions at the tissue level. Alternatively, dispersion may enhance photolysis and loss of PAH over time in the exposure systems. Further investigation into the mechanism by which dispersants mediate photoinduced toxicity is warranted as dispersants were widely utilized in the *Deepwater Horizon* incident, as well as many other accidental oil releases.

Toxicity Associated with Oil Source. Oil source significantly influenced toxicity in HEWAF preparations as indicated by lack of overlap in the phototoxic EC_{50} 95% confidence intervals. HEWAF prepared from artificially weathered source oil was more phototoxic (2-fold) than HEWAF prepared from Slick A source oil. This could be explained by analysis of the photodynamic PAH content of each preparation. HEWAF stock prepared with artificially weathered source oil was calculated to have more than double the anthracene equivalent of HEWAF stock prepared with slick oil, resulting in increased toxic effect (Table 1). Interestingly, this could not be used to explain the lack of difference in CEWAF. CEWAF stock prepared with artificially weathered source oil contained more than 20 times the concentration of photodynamic PAHs than the CEWAF stock prepared with slick oil. However, there was no difference in toxicity observed between the two. Furthermore, CEWAF reduced toxicity on a $tPAH_{50}$ basis when compared to HEWAF preparations of the same source oil. We have previously reported similar findings in photoinduced toxicity tests with blue crab zoea.[10] Taken together, these findings suggest that the mechanism by which dispersant mediates photoinduced toxicity may be independent of photodynamic PAH concentration.

Table 1. Calculated Anthracene Equivalent Concentrations from the Four Stock WAF Preparations. Concentrations Presented in Micromoles of Anthracene Equivalent Per Liter

WAF prep type	oil type	μM/L ANT equivalent
HEWAF	slick	0.79
HEWAF	artificially weathered source	1.75
CEWAF	slick	0.05
CEWAF	artificially weathered source	1.28

The positive buoyancy of mahi−mahi embryos removes significant UV attenuation by the water column as a factor in possible exposure scenarios. However, a variety of weather factors can reduce the amount of UV that reaches the surface. Table 2 illustrates how photochemical reciprocity shifts the concentration of PAH needed to produce the same phototoxic dose as UV is reduced. Even with the lowest UV given in the table—requiring 4-fold more PAH—the concentrations calculated to for the EC_{20}s range from 1.16 to 19.2 nM/L anthracene equivalent. The latter of which is slightly less than the photodynamic PAH concentration in the highest artificially weathered source HEWAF exposure in the present study. Thus, even under relatively low UV conditions significant hatch failure could occur at the higher PAH concentrations reported during the spill.

This study demonstrates that a UV component is important to a complete understanding of the toxicity of PAHs to aquatic organisms, or hazard may be greatly underestimated. Mahi-mahi and other pelagic fish species with similar life history traits may be particularly vulnerable to photoinduced toxic effects. Their embryos are buoyant and transparent, and newly hatched larvae remain close to the surface. This places them at risk for UV exposure during all early life stages. Toxicity tests using Deepwater Horizon oil, but without a UV component, have reported sublethal effects at low PAH concentrations (0.8−18.2 μg/L) in mahi−mahi and other pelagic predatory fishes.[21,22] Effect concentrations determined in the absence of UV may underestimate the toxicity of oil. It is possible that coexposure to UV may induce or exacerbate those sublethal effects at even lower concentrations of PAH.

Here, we have shown that coexposure to slick oil from the *Deepwater Horizon* spill and sunlight results in photoinduced toxicity in mahi−mahi embryos. Effects were observed well within the range of $tPAH_{50}$ concentrations reported during the spill and during relatively short exposure periods (48 h) using WAF preparations both with and without dispersants. This study demonstrates that photoinduced PAH toxicity likely

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Table 2. Calculated Anthracene Equivalent Concentrations at the EC_{20}, EC_{50}, and EC_{80} and Their 95% Confidence Intervals for Four UV Doses

	integrated UV dose mWs/cm^2	EC_{20} (ANT equivalents) nM/L (95% CI)		EC_{50} (ANT equivalents) nM/L (95% CI)		EC_{80} (ANT equivalents) nM/L (95% CI)	
Slick A	2423	1.68	(1.28−2.07)	2.79	(2.44−3.15)	4.66	(3.49−5.83)
HEWAF	1812	2.24	(1.72−2.77)	3.74	(3.26−4.22)	6.23	(4.66−7.79)
	1214	3.35	(2.56−4.13)	5.58	(4.87−6.29)	9.29	(6.96−11.6)
	607	6.70	(5.12−8.27)	11.2	(9.74−12.6)	18.6	(13.9−23.3)
WS	2423	0.289	(0.130−0.448)	0.891	(0.627−1.15)	2.74	(1.54−3.94)
HEWAF	1812	0.387	(0.174−0.600)	1.19	(0.839−1.54)	3.67	(2.06−5.27)
	1214	0.578	(0.259−0.895)	1.78	(1.25−2.30)	5.48	(3.08−7.87)
	607	1.16	(0.519−1.79)	3.56	(2.50−4.60)	11.0	(6.16−15.7)
Slick A	2423	1.95	(1.25−2.64)	4.87	(3.24−6.48)	12.1	(3.88−20.3)
CEWAF	1812	2.60	(1.67−3.53)	6.51	(4.33−8.66)	16.2	(5.19−27.1)
	1214	3.89	(2.50−5.27)	9.72	(6.46−12.9)	24.1	(7.75−40.5)
	607	7.78	(4.99−10.5)	19.4	(12.9−25.9)	48.3	(15.5−81.0)
WS	2423	4.81	(2.09−7.51)	6.06	(NA −13.7)	7.63	(NA-22.6)
CEWAF	1812	6.43	(2.80−10.0)	8.10	(NA-18.3)	10.2	(NA-30.2)
	1214	9.60	(4.18−15.0)	12.1	(NA-27.3)	15.2	(NA-45.1)
	607	19.2	(8.35−30.0)	24.2	(NA-54.5)	30.5	(NA-90.3)

contributed to overall toxic effects from the *Deepwater Horizon* oil spill on early lifestage fish.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b05356.

Table of 50 specific PAH concentrations in stock solutions. Table of the detection limits for each PAH (PDF)

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Notes

The authors declare no competing financial interest.

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