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Photoenhanced Toxicity of Weathered Crude Oil in Sediment and Water to Larval Zebrafish

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Abstract

Solar radiation exposure can increase the toxicity of bioaccumulated oil compounds in a diversity of aquatic species. We investigated the photoenhanced toxicity of weathered South Louisiana crude oil in sediment and water accommodated fractions (WAF) to larval zebrafish. Larvae were first exposed for 24 hours to one of six treatments: no oil (sediment or water), 7.5 g oil/kg sediment, oil-only WAF, oil WAF plus the dispersant Corexit 9500A, or dispersant alone. Larvae were then exposed to high or low levels of sunlight in control water for 3 or 3.5 hours. Hydrocarbon concentrations were measured in exposure media, including alkanes, polycyclic aromatic compounds and total petroleum hydrocarbons. Significant phototoxicity was observed in larvae exposed to oiled sediment, oil-only WAF, and oil plus dispersant WAF. The results indicated that petroleum from the northern Gulf of Mexico can be phototoxic to larval fish exposed to oil in either the water column or sediment.

Keywords

Oil; Zebrafish; Photoenhanced Toxicity; Sediment

Ultraviolet radiation (UV) can increase the toxicity of polycyclic aromatic compounds (PACs) and petroleum products, known as photoenhanced toxicity, in the early life stages of a diversity of aquatic species (Barron 2017; Roberts et al. 2017). The primary mechanism of phototoxicity in aquatic invertebrates and fish is photosensitization, where UV activates bioaccumulated chemicals in translucent tissues resulting in a cascade of oxygen radical production and tissue damage (Barron 2017; Roberts et al. 2017). The degree of photoenhanced toxicity has been shown to depend upon multiple factors that modulate chemical, UV, and species-specific exposures (see reviews by Barron 2017; Roberts et al. 2017). The vast majority of phototoxicity studies have been laboratory water column exposures comparing PAC toxicity in UV exposed organisms to toxicity in low or no UV treatments. Although the potential was recognized decades ago (e.g., Davenport and Spacie 1991), there have been relatively few investigations of the phototoxicity of sediment-associated PACs. Previous phototoxicity studies have included sediments contaminated with PACs from creosote and petroleum refinery outfall, and amphipods collected from PAC

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Supplemental Information See file.

contaminated sediments (e.g., Boese et al. 2000; Diamond et al. 2003; Monson et al. 1995). However, there has been no previous studies of the influence of UV exposure on the toxicity of crude oil in sediment (Barron 2007; Barron 2017).

A unique aspect of petroleum spills in aquatic environments is the use of chemical agents to disperse the slick oil into the water column. Chemical dispersants are not phototoxic, but are known to enhance the toxicity of oil by solubilizing its phototoxic PACs (Barron et al. 2003; Barron 2017). There have been relatively few studies of the photoenhanced toxicity of chemically dispersed oil since the first report by Barron et al. (2003). Recently, studies with fish and invertebrate species native to the Gulf of Mexico have confirmed the phototoxicity of chemically dispersed oil from the Deepwater Horizon spill in laboratory water exposures (Alloy et al. 2017; Finch et al. 2017; Sweet et al. 2017).

The objective of the current study was to determine if South Louisiana crude (SLC), an oil representative of Deepwater Horizon and northern Gulf of Mexico petroleum formations, is phototoxic to larval zebrafish. Zebrafish have become a model species for investigating embryology and developmental toxicity of environmental chemicals and petroleum (e.g., Isogai et al. 2001; Jung et al. 2013), including photoenhanced toxicity of single compounds and mixtures of PACs that occur in oil (Willis and Oris 2014). Zebrafish larvae were exposed to oiled sediment, water accommodated fractions (WAF) of oil, or WAF chemically enhanced with the dispersant Corexit 9500A (CEWAF). The phototoxicity of WAFs and CEWAFs of Northern Gulf of Mexico oil has not been previously investigated in zebrafish, and the phototoxicity of crude oil in sediment has not been previously reported in any fish species (see reviews by Barron 2007; Barron 2017).

Materials and Methods

Two experiments were conducted to determine the photoenhanced toxicity of weathered SLC. In the first experiment, zebrafish larvae (*Danio rerio*) were exposed to control (no oil) and oiled sediment. In the second experiment, larvae were exposed in control water only (no oil), oil, dispersant-only, or oil plus dispersant in WAF (Table 1). For both experiments, a three step experimental design was used. First, zebrafish larvae were exposed in six replicates of each treatment for 24 hours. Second, larvae were then transferred to clean water and exposed to either 10% (3 of 6 replicates) or 100% (3 of 6 replicates) natural sunlight for 3.5 hours (sediment) or 3 hours (water). Lastly, larvae from all treatment replicates were removed from sunlight and monitored for delayed mortality and abnormalities for an additional 24 hours.

For both sediment and WAF experiments, the test oil was SLC weathered by nitrogen gas stripping and the dilution water was standardized zebrafish moderately hard freshwater (ISO 7346-3; Hollert et al. 2003; Raimondo et al. 2014). The sediment experiment included a no oil sediment control and a 7.5 g oil/kg sediment treatment; The level was selected based on preliminary phototoxicity results, and the absence of acute lethality without UV at this concentration (Raimondo et al. 2014). The control sediment was collected from uncontaminated sites in Choctawhatchee Bay, Florida, and had no detectable PACs (Raimondo et al. 2014). For the oiled sediment treatment, pneumonic stainless steel paint

can shakers were used to mix control sediment and oil as described by Raimondo et al. (2014). The second experiment consisted of a water control, two chemical dispersant controls (1:50 and 1:500 dilutions), two oil WAF treatments (1:2 and 1:20 dilutions), and two CEWAF treatments (1:50 and 1:500 dilutions; Table 1). WAF and CEWAF dilutions were determined in preliminary work based on observed phototoxicity, and dispersant controls matched CEWAF dilutions. WAF and CEWAF stock solutions were prepared using 25 g oil/L without or with Corexit 9500A (Nalco, Naperville, IL, USA) dispersant (1:10; dispersant: oil; v:v), respectively. WAF and CEWAF procedures followed traditional slow stir methods by mixing at an approximate 20% vortex for 18 hours followed by a 6 hour settling period (Barron and Kaaihue 2003). WAF, CEWAF, and dispersant control stocks were then diluted to create low and high exposure solutions for each treatment (Table 1).

Zebrafish larvae (24 hr post hatch) were obtained from in-house cultures (Gulf Ecology Division, Gulf Breeze, FL, USA) maintained at 26 ± 1 °C under flow-through freshwater chambers (Raimondo et al. 2014). The dilution water used in both sediment and water experiments was standardized zebrafish moderately hard freshwater (ISO 7346–3; Hollert et al. 2003; Raimondo et al. 2014). All experimental treatments were maintained at 26 ± 1 °C; all in-laboratory exposures were conducted under fluorescent lighting without UV. For the first phase of the sediment experiment, larvae were exposed to control or oiled sediment treatments in 24 well plates (0.5 g sediment, 1 mL water; 1 larva per well, 12 larvae per replicate; 6 replicates per treatment). For the first phase of the water experiments, larvae were exposed to water or dispersant controls, WAF, or CEWAF in 100 mL glass beakers containing 50 mL of treatment water (10 larvae per replicate beaker, 6 replicates per treatment). After 24 hours of sediment or water exposure, larvae were transferred to 100 mL beakers containing 50 mL clean dilution water for 3.5 hours (sediment) or 3 hours (water) of exposure to natural sunlight (1 beaker per replicate) (phase 2). Three replicates from each treatment were exposed to 10% sunlight and three replicates were exposed to 100% sunlight. Sunlight exposures were established by covering beakers with ultraviolet radiation attenuating plastics within a temperature controlled outdoor water bath maintained at 26°C (Fig. SI-1). Levels of incident solar radiation were quantified using a broad wavelength radiometer (Macam UV 203; Macam Photometrics, Livingston, Scotland) that had been factory calibrated and zeroed prior to each use. The total visible light and UV levels were determined from the measured intensity and duration of sunlight exposure (Table 2). For the third phase of the experiment, beakers were transferred to an incubation chamber (26°C) for 24 hours to determine any delayed mortality.

Sediment and water stock samples were collected prior to use in experiments and analyzed for PACs (57 analytes), alkanes (C9–C40), and total petroleum hydrocarbons (TPH; as total extractable hydrocarbons) as described in Raimondo et al. (2014). Known phototoxic PACs within the 57 analyte suite were identified from a review of the literature (e.g., Barron 2007; Barron 2017). Treatment levels of total PACs, total alkanes, TPH, and total phototoxic PACs were computed from concentrations in the stock solutions and the dilution level tested (Table 1). An enrichment factor of phototoxic PAC in CEWAF was computed from the CEWAF ratio (phototoxic PAC/total PAC) divided by the WAF ratio (phototoxic PAC/total PAC).

For each experiment, larval mortality was evaluated to detect impacts of control or oil treatments, direct UV exposure, delayed effects of UV exposure, and cumulative effects within UV and oil treatments. To evaluate the influence of UV, the proportion of larvae that died or demonstrated lethal abnormalities during the UV exposure (phase 2) was statistically compared between high and low UV within each treatment. This analysis was repeated to compare the proportion of larvae that died during the 24 h post-UV exposure period (phase 3) between high and low UV within each treatment. To determine if there were delayed effects of UV exposure, the proportion of larvae that died between phase 2 and 3 of the experiments were compared within each UV level (high/low) and treatment. The cumulative mortality that occurred from the initiation through end of the experiments was compared between high and low UV levels for each treatment. In both experiments, only one larva died in pre-UV exposure (phase 1), so mortality was not compared for any treatments during this phase of either experiment. Because the objective of this study was to assess photoenhanced toxicity, mortality was only compared between and within UV levels for each treatment and not compared across treatments. Additionally, the data were not normally distributed in any treatment using any type of data transformation, so the non-parametric Kruskal-Wallis test was used for all comparisons. Without the ability to normalize the data and analyze the treatments using a parametric mixed model, comparing mortality among treatments and UV levels would greatly increase the risks of Type 1 errors. The dose-response relationship between phototoxic PACs and larval mortality was determined graphically using data from all WAF and dispersed oil treatments.

Results and Discussion

The sunlight exposures were within two hours of solar noon under minimal cloud cover with a fully visible solar disk (Table 2), and the measured UV intensities were similar to those reported in a diversity of aquatic habitats (e.g., Barron et al. 2000; Roberts et al. 2017). Ultraviolet A (UVA) is considered to be the most important region of the sunlight spectrum for enhancing the toxicity of PACs, and UVA doses greater than 20 W*hr/m² can elicit significant phototoxicity in sensitive life stages of aquatic organisms (Barron 2017). In the current study, total UVA doses during solar radiation exposure were 4 W*hr/m² (10% sunlight) and 37 W*hr/m² (100% sunlight) in sediment treatments, and 5.6 W*hr/m² (10% sunlight) and 57 W*hr/m² (100% sunlight) in water treatments (Table 2). Thus, phototoxicity was expected in the sediment and water treatments in the 100% sunlight exposures, but not in the low solar radiation treatments.

Concentrations of petroleum hydrocarbons in oiled sediment were 22 mg PAC/kg and 1700 mg TPH/kg (Table 1). In the sediment experiment, mortality was significantly greater in the high UV compared to the low UV exposure during direct exposure to UV in the oiled sediment treatment (chi-square = 4.5; p-value = 0.03), but no difference was observed in the sediment control between the two UV treatments (chi-square = 1; p-value = 0.32; Fig. 1a). All larvae had died by the end of the exposure period in the high UV treatment (phase 2), whereas the low UV exposure and sediment control (both UV treatments) had a 100% survival rate throughout the duration of the experiment.

These results indicate that phototoxic oil components were bioaccumulated in fish from exposure to oiled sediment, which became activated under high UV (Table 2). This is the first demonstration of the photoenhanced toxicity of oiled sediment, which was considered likely because of prior research on individual PACs and environmental samples of PAC mixtures in sediment (Davenport and Spacie 1991; Barron 2007). In comparison, sediment oiled with the same weathered SLC caused developmental toxicity, but not mortality in zebrafish embryos after a 96 hour exposure to the same 7.5 g oil/kg without UV, and nearly complete mortality at levels four times higher (i.e., 30 g oil/kg; Raimondo et al. 2014). Thus, at sufficiently high UV levels, petroleum contaminated sediments can be phototoxic at similar or lower concentrations of oil (e.g., 20 mg/Kg; Table 1) than those that cause developmental toxicity in highly sensitive fish life stages (Raimondo et al. 2014).

Concentrations of petroleum hydrocarbons in the CEWAF stock solution were 30 to 50 fold higher (140 mg TPH/L, 14.4 mg PAC/L) than in oil-only WAF (2.8 mg TPH/Kg, 0.47 mg PAC/L). Hydrocarbon concentrations in each control, WAF, and CEWAF treatment are shown in Table 1. Phototoxic PAC concentrations were enriched 3.4 fold in CEWAF compared to oil-only WAF PAC levels. There was less than 10% mortality of zebrafish larvae in the water-only control and the two dispersant-only treatments, including the 100% sunlight exposures, indicating a lack of phototoxicity in the absence of oil. These results are consistent with other studies showing that dispersants alone are not phototoxic to aquatic organisms (Barron et al. 2003; Alloy et al. 2017; Finch et al. 2017; Sweet et al. 2017). There was 0% survival of fish exposed to 100% solar radiation in the oil WAF high and CEWAF high treatments, which were significantly different from the almost complete survival of larvae exposed to 10% solar radiation (chi-square 4.5; p-value 0.03; Fig 1). There was a median survival of 89% in the CEWAF low treatment when exposed to 100% solar radiation, that was not significantly different from the survival of larvae in the CEWAF low treatment exposed to 10% solar radiation (chi-square = 2.4; p-value = 0.12). During phase 3 of the exposure, the CEWAF low treatment exposed to 100% solar radiation had complete mortality, while larvae of this treatment exposed to 10% solar radiation had total survival (chi-square = 5; p-value = 0.03; Fig 1B). The highest tested concentration of CEWAF (2%; 1:50 dilution) caused complete mortality of larvae within 3 hours of 100% solar radiation exposure compared to almost complete survival in larvae of this treatment exposed to 10% solar radiation (chi-square = 5; p-value = 0.03), showing potential for substantial phototoxicity of chemically dispersed oil. These results show that under high sunlight exposure, CEWAF was phototoxic to zebrafish larvae even at the lowest dilution of 0.2% (1:500 dilution) CEWAF (Fig. 1). Mortality of larvae was directly related to the exposure levels of phototoxic PAC (Fig. SI-2). Overall, these results show the dose-dependence of phototoxicity with lower survival at with increasing levels of sunlight and oil exposure as has been demonstrated with multiple other petroleum products and in other aquatic species (reviewed by Barron 2017).

These experiments are the first demonstration of sediment-associated phototoxicity of oiled sediment to aquatic organisms and the first to show phototoxicity of chemically dispersed oil to zebrafish. Previous phototoxicity studies have included sediments contaminated with mixtures of pyrogenic and petrogenic PACs, but there have been no previous reports of the phototoxicity of sediments oiled with petroleum (e.g., Barron 2007, 2017; Boese et al. 2000;

Diamond et al. 2003; Monson et al. 1995). Although not an ecologically relevant species for marine oil spills, this research adds to the growing understanding of petroleum toxicology in this model species. The embryology and genomics of zebrafish are well characterized, and test organisms and testing protocols are available globally for comparison across oils and laboratories. Additionally, previous sediment testing allowed a direct comparison of the relative sensitivity of phototoxicity and developmental toxicity endpoints (Raimondo et al. 2014). Total PAC concentrations in water of 30 to 300 ug/L were phototoxic to zebrafish larvae following short-term SLC and sunlight exposure, similar to levels reported for species native to the Gulf of Mexico exposed to the compositionally similar Deepwater Horizon oil (Alloy et al. 2017; Finch et al. 2017; Sweet et al. 2017). Very low dilutions of CEWAF caused substantial phototoxicity and phototoxic PAC concentrations were enriched over 3 fold in CEWAF, suggesting that chemical dispersion of oil into the water column may increase risks to sensitive life stages exposed to sufficient levels of oil and sunlight. These results have implications for oil spill response, where dispersant application is a common mitigation approach, but phototoxicity risks are not typically considered (Barron 2017). Additionally, the results have implications for assessing the impacts and risks of oil spills and PAC contamination because adverse effects may occur at lower levels of PACs in the presence of sufficient UV exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Alloy M, Garner TR, Bridges K, Mansfield C, Carney M, Forth H, Krasnec M, Lay C, Takeshita R, Morris J, Bonnot S, Oris J, Roberts A (2017) Co-exposure to sunlight enhances the toxicity of naturally weathered Deepwater Horizon oil to early lifestage red drum (*Sciaenops ocellatus*) and speckled seatrout (*Cynoscion nebulosus*). *Environ Toxicol Chem* 36:780–785. [PubMed: 27868239]
- Barron MG, Little EE, Calfee RD, Diamond S (2000) Quantifying solar spectral irradiance in aquatic habitats for the assessment of photoenhanced toxicity. *Environ Toxicol Chem* 19: 920–925.
- Barron MG, Carls MG, Short JW, Rice SD (2003) Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to Pacific herring eggs and larvae. *Environ Toxicol Chem* 22:650–660. [PubMed: 12627655]
- Barron MG, Kaaihue L (2003) Critical evaluation of CROSERF test methods for oil dispersant toxicity testing under subarctic conditions. *Mar Pollut Bull* 46:1191–1199. [PubMed: 12932502]
- Barron MG (2007) Sediment-associated phototoxicity to Aquatic Organisms. *Hum Ecol Risk Assess* 13:317–321.
- Barron MG (2017) Photoenhanced toxicity of petroleum to aquatic invertebrates and fish. *Arch Environ Contam Toxicol* 73:40–46. [PubMed: 28695259]
- Boese BL, Ozretich RJ, Lamberson JO, Cole FA, Swartz RC, Ferraro SP (2000) Phototoxic evaluation of marine sediments collected from a PAH-contaminated site. *Arch Environ Contam Toxicol* 38:274–282. [PubMed: 10667924]

- Davenport R, Spacie A (1991) Acute phototoxicity of harbor and tributary sediments from lower Lake Michigan. *J Great Lakes Res* 17:51–56.
- Diamond SA, Milroy NJ, Mattson VR, Heinis LJ, Mount DR (2003) Photoactivated toxicity in amphipods collected from polycyclic aromatic hydrocarbon-contaminated sites. *Environ Toxicol Chem* 11:2752–2760.
- Finch BE, Marzocchi S, Di Toro DM, Stubblefield WA (2017) Phototoxic potential of undispersed and dispersed fresh and weathered Macondo crude oils to Gulf of Mexico marine organisms. *Environ Toxicol Chem* 36:2640–2650. [PubMed: 28418080]
- Hollert H, Keiter S, König N, Rudolf M, Ulrich M, Braunbeck T (2003) A new sediment contact test to assess particle-bound pollutants using zebrafish (*Danio rerio*) embryos. *J Soil Sed* 3:197–207.
- Isogai S, Horiguchi M, Weinstein BM (2001) The vascular anatomy of the developing zebrafish: an atlas of embryonic and early larval development. *Develop Biol* 230:278–301. [PubMed: 11161578]
- Jung J-H, Hicken CE, Boyd D, Anulacion BF, Carls MG, Shim WJ, Incardona JP (2013) Geologically distinct crude oils cause a common cardiotoxicity syndrome in developing zebrafish. *Chemosphere* 91:1146–1155. [PubMed: 23481301]
- Monson PD, Ankley GT, Kosian PA (1995) Phototoxic response of *Lumbriculus variegatus* to sediments contaminated by polycyclic aromatic hydrocarbons. *Environ Toxicol Chem* 14:891–894.
- Raimondo S, Jackson CR, Krzykwa J, Hemmer BL, Barron MG. 2014 Developmental toxicity of Louisiana crude oiled sediment to zebrafish. *Ecotoxicol Environ Safe* 108:258–264.
- Roberts AP, Alloy MM, Oris JT (2017) Review of the photo-induced toxicity of environmental contaminants. *Compara Biochem Physiol Part C: Toxicol Pharmacol* 191:160–167.
- Sweet LE, Magnuson J, Garner TR, Alloy MM, Stieglitz JD, Grosell M, Roberts AP (2017) Exposure to ultraviolet radiation late in development increases the toxicity of oil to mahi-mahi (*Coryphaena hippurus*) embryos. *Environ Toxicol* 36:1592–1598.
- Willis AM, Oris JT (2014) Acute photo-induced toxicity and toxicokinetics of single compounds and mixtures of polycyclic aromatic hydrocarbons in zebrafish. *Environ Toxicol Chem* 33:2018–2037.

Figure 1. Survival of zebrafish larvae (A) immediately following UV exposure and (B) 24 hours after UV exposure. See text for details.

Table 1.

Hydrocarbon concentrations (water: µg/L; sediment: mg/kg normalized to 1% organic carbon) in experimental treatments.

Exposure Media	Treatment	Concentration	Total PAC ²	Phototoxic PAC ²	Total Alkanes	TPH
Sediment ³	Control	0 g oil/kg	0	NR ⁴	NR	7.0
	Oil	7.5 g oil/kg	22	NR	NR	1724
Water	Water control	Water-only	NA ⁴	NA	NA	NA
	Dispersant control high	1:50 dilution	0.16	0.02	8.7	AE ⁴
	Dispersant control low	1:500 dilution	0.02	0.002	0.9	AE
	Oil WAF high	1:2 dilution	235	4.1	121	1400
	Oil WAF low	1:20 dilution	23.5	0.41	12.1	140
	CEWAF high	1:50 dilution	287	16.9	115	2800
	CEWAF low	1:500 dilution	28.7	1.69	11.5	280

¹ WAF: water accommodated fraction of oil; CEWAF: chemically enhanced WAF (oil + dispersant).

² Sum of polycyclic aromatic compounds (57 analytes); Sum of known phototoxic PAC analytes (see Table SI-1).

³ Hydrocarbon analyte results from Raimondo et al. (2014).

⁴ NR: not reported; NA: not analyzed; AE: analytical error.

Table 2.

Exposure duration, intensity of ultraviolet B (UVB), ultraviolet A (UVA), and visible light levels (W/m^2), and total dose¹ ($\text{W}^*\text{hr}/\text{m}^2$) during sunlight exposures (100% or 10%) to larval zebrafish initially exposed in sediment (7.5 g oil/kg) or water treatments.

Exposure Media	Exposure Duration (hr)	Sunlight Treatment	Visible		UVA		UVB	
			Intensity	Dose ¹	Intensity	Dose ¹	Intensity	Dose ¹
Sediment	3.5	10%	147–155	528	1.07–1.21	3.99	0.041–0.043	0.15
		100%	159–166	569	10.3–10.8	36.9	1.20–1.49	1.35
Water	3	10%	200 ²	600 ²	1.82–1.93	5.63	0.060–0.13	0.29
		100%	185–293	717	15.1–22.8	56.9	1.04–1.59	2.48

¹ Dose computed as product of sunlight exposure (hr) and average intensity (W/hr^2) at start and end of sunlight exposure.

² Estimated visible light due to instrument error.