Environmental Toxicology and Chemistry—Volume 39, Number 12—pp. 2509–2515, 2020
Received: 3 July 2020 | Revised: 10 August 2020 | Accepted: 14 September 2020

Environmental Toxicology

Effects of Dissolved Organic Carbon, Ultraviolet Light and their Co-Exposure on *Deepwater Horizon* crude oil acute toxicity to larval red drum (*Sciaenops ocellatus*)

Fabrizio Bonatesta,* Rachel R Leads, Edwin R. Price, Aaron P. Roberts, and Edward M. Mager

Department of Biological Sciences and the Advanced Environmental Research Institute, University of North Texas, Denton, Texas, USA

Abstract: In the aquatic environment, ubiquitous natural factors such as ultraviolet light (UV) and dissolved organic carbon (DOC) are likely to influence crude oil toxicity. The present study examined the interactive effects of DOC, UV, and DOC–UV co-exposure on the acute toxicity of *Deepwater Horizon* crude oil in larval red drum (*Sciaenops ocellatus*). Although DOC alone did not influence crude oil toxicity, it mildly reduced UV photo-enhanced toxicity. *Environ Toxicol Chem* 2020;39:2509–2515. © 2020 SETAC

Keywords: Polycyclic aromatic hydrocarbons; Dissolved organic carbon; Photo-enhanced toxicity; Oil spills; Red drum; Gulf of Mexico

INTRODUCTION

In the 10 yr since the *Deepwater Horizon* oil spill, much has been learned regarding the toxicity of crude oil exposures to the early life stages (ELS) of teleost fishes (see Pasparakis, et al. 2019, for a recent review). This includes, for example, a better understanding of the effects of oil weathering and the relative toxicity of constituent polycyclic aromatic hydrocarbons (PAHs), the combined effects of oil and chemical dispersant application, and the influence of natural factors such as ultraviolet light (UV), temperature, and hypoxia on crude oil toxicity (Alloy et al. 2016, 2017; Langdon et al. 2016; Bridges et al. 2018a; Mager et al. 2018; Rodgers et al. 2018; Greer et al. 2019). Given its virtually ubiquitous presence in near-surface aquatic environments, UV has been shown to be among the most significant of these factors; it has greatly exacerbated crude oil toxicity to ELS fishes by up to approximately 10-fold (Alloy et al. 2016, 2017; Stieglitz et al. 2016). There are 2 mechanisms by which UV elicits photo-enhanced toxicity: photo-sensitization and photo-modification (Arfsten et al. 1996; Diamond et al. 2006; Roberts et al. 2017). Organisms that lack pigmentation, as in some fish embryos/larvae, are more sensitive to photo-sensitization where the UV penetrates the organism and interacts with accumulated PAHs. This interaction induces oxidative stress, oxidation of biomolecules, and tissue damage due to the generation of reactive oxygen species and free radicals. During photo-modification, UV interacts directly with PAHs in the aquatic (or external) environment by modifying them into more toxic compounds prior to bioaccumulation.

Whereas enhanced crude oil and PAH toxicity to marine organisms during UV exposure is well characterized, far less attention has been paid to the potential interactive effects of another ubiquitous factor in aquatic environments, dissolved organic carbon (DOC). From the limited number of studies investigating the topic, the majority have focused on freshwater environments using single PAHs and/or a single type of DOC (i.e., humic acid) as a surrogate, although both occur in the natural environment as complex mixtures. Some of these studies have shown a decrease in PAH toxicity in the presence of DOC (Haitzer et al. 1998; Weinstein and Oris 1999; Haitzer et al. 2001), although others have reported an increase in PAH bioaccumulation (Matsuo et al. 2006; Li et al. 2018) or no effect on PAH uptake (Akkanen et al. 2001). Because of this focus on freshwater environments, individual PAHs, and the use of humic acid as a surrogate for DOC, the potential mitigating or enhancing effects of DOC on the toxicity of complex crude oil mixtures to fish inhabiting marine environments, such as the Gulf of Mexico, remain largely unknown.

To complicate matters further, the influences of DOC and UV and their interactive effects on crude oil toxicity are likely influenced by the quality and quantity of DOC present (Haitzer et al. 1998, 1999). In terms of quality, DOC can be characterized as originating from primarily allochthonous or autochthonous

This article includes online-only Supplemental Data.
* Address correspondence to fabriziobonatesta@my.unt.edu
Published online 2 October 2020 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.4877

sources (Millero 2005). Allochthonous DOC originates from the breakdown of terrestrial detritus and is thus typical in freshwater, estuarine, and other near-shore environments (Millero 2005). On the other hand, autochthonous DOC originates from resident sources within the water and is thus more typical of pelagic marine environments (Millero 2005). In terms of DOC quantity, concentrations vary with salinity, depth, and temperature within the marine environment. Typical concentrations range between 0.3 and 2 mg/L (with an average of 1 mg/L) in shallow seawater and between 4 and 10 mg/L within estuaries (Thurman 2012). During UV exposure, DOC undergoes a variety of photochemical changes, including photochemical degradation of humic substances (Allard et al. 1994) and photochemical oxidation of chromophoric dissolved organic matter (CDOM; Powers and Miller 2015); both processes are significant in terms of the loss of DOC from the environment. In addition, DOC is an important component in the water column for attenuating UV penetration (Roberts et al. 2017), and the DOC attenuation capacity varies with its concentration and its physiochemical characteristics. Factors contributing to variability in DOC concentrations and its complexation properties with other chemicals could have important ramifications for the bioavailability and toxicity of crude oil constituents, and these effects likely vary among different aquatic environments.

Red drum (*Sciaenops ocellatus*) is an ecologically and economically important fish species of the Gulf of Mexico that is very sensitive to crude oil exposure, especially if exposed during the ELS (Alloy et al. 2017; Xu et al. 2017). They spawn along the Gulf coast and produce transparent, positively buoyant embryos that develop relatively quickly and hatch at approximately 1 d post fertilization (Wilson and Nieland 1994). Thus, larvae and juvenile red drum are mostly found in shallow near-shore waters and estuaries (Wilson and Nieland 1994) where there is high potential for UV exposure and allochthonous sources of DOC are likely to dominate. The present study investigated the effects of UV, DOC, and DOC–UV coexposure on the acute toxicity of *Deepwater Horizon* crude oil to ELS red drum using Suwannee River natural organic matter (NOM) as a representative source of allochthonous DOC. Characterizing the interactive effects of DOC, UV, and crude oil in this manner will facilitate a more context-dependent evaluation of potential short- and long-term outcomes of future oil spills and therefore aid in prioritizing response efforts.

MATERIALS AND METHODS

Test organisms

Red drum larvae were obtained from volitional spawns by a standing broodstock at the Texas Parks and Wildlife Department Fish Hatchery (Corpus Christi, TX, USA). All larvae were at approximately 24 h post fertilization (hpf) at the start of all tests.

Test solutions

The present study used slick oil (hereafter termed Slick A) collected from surface skimming operations in the Gulf of Mexico following the *Deepwater Horizon* oil spill. Slick A was collected on 29 July 2010 from the hold of barge CTC02404 and was previously used in photo-enhanced toxicity testing performed during the *Deepwater Horizon* Natural Resources Damage Assessment (Alloy et al. 2016, 2017). Suwannee River NOM isolate (2R101N; International Humic Substances Society) was used as a representative source of allochthonous DOC (Green et al. 2014) that contributes to the NOM in the Gulf of Mexico. Artificial seawater (31 ppt) made with Milli-Q water and Instant Ocean Sea Salt was used in all control solutions and in preparation of treatment dilutions. Test solutions were renewed daily.

Stock solutions of NOM were prepared daily at a nominal concentration of 10 mg/L by combining 100 mg of Suwannee River NOM with 10 L of artificial seawater (31 ppt) in a 10-L plastic carboy. The solution was mixed vigorously for approximately 24 h prior to testing using a magnetic stirrer. Samples (40 mL) of each NOM preparation were collected daily from the bulk solution prior to use in high-energy water accommodated fractions (HEWAF) dilutions and analyzed for DOC and total organic carbon (TOC). The DOC samples were filtered through a prewashed Whatman 0.45-µm glass microfiber membrane filter before analysis. Samples were acidified to pH <2 with sulfuric acid and held at 4 °C until analysis. Measurements of DOC and TOC were performed with a Teledyne–Tekmar meter (model TOC Fusion, S/N: US10165001; ALS Environmental) using US Environmental Protection Agency (USEPA) method 9060A (2004) and National Environmental Methods Index standard method 5310C (1998). Stock solutions of Slick A HEWAF were prepared daily with artificial seawater (31 ppt) at an oil loading rate of 1 g/L as previously described (Alloy et al. 2016). Separate stock solutions and dilutions of HEWAF were prepared with and without 10 mg/L NOM predissolved in seawater. For non-UV treatment groups, nominal test solutions of 0, 1.25, 2.5, 5, 10, and 20% HEWAF (with and without NOM) were prepared. Due to anticipated photo-enhanced PAH toxicity, lower nominal test solutions of 0, 0.063, 0.125, 0.25, 0.5, and 1% HEWAF (with and without NOM) were prepared for UV treatment groups. With each HEWAF preparation, a sample of the highest diluted sample from each series (i.e., 20 or 1%) was collected and analyzed for PAHs (Supplemental Data, Table S1). Samples were held at 4 °C until analysis. Extraction of PAHs was performed by ALS Environmental according to USEPA method 3510C (US Environmental Protection Agency 1996). The PAH analytes were quantified by ALS Environmental using gas chromatography–mass spectroscopy in single-ion monitoring mode according to USEPA method 8270D (US Environmental Protection Agency 2014). Reported ∑PAH concentrations represent the sum of 50 select PAH analytes (Supplemental Data, Table S2).

Toxicity test

Larvae were exposed to 1 of 5 HEWAF dilutions or control seawater in the presence or absence of NOM for 48 h while

TABLE 1: Water chemistry parameters measured for each test treatment expressed as the mean of day 1 and day 2 measurements ± standard error of the mean[a]

Test	Water temp. (°C)	pH (SU)	DO (mg/L)	Salinity (ppt)	TOC (mg/L)	DOC (mg/L)
–NOM, –UV	23.78 ± 0.09	8.21 ± 0.01	7.45 ± 0.03	31 ± 0	0.05 ± 0	ND
+NOM, –UV	23.85 ± 0.14	8.15 ± 0.01	7.36 ± 0.03	31 ± 0	1.47 ± 0.08	1.07 ± 0.03
–NOM, +UV	21.84 ± 0.14	8.14 ± 0.01	7.62 ± 0.02	31 ± 0	ND	ND
+NOM, +UV	21.85 ± 0.13	8.12 ± 0	7.62 ± 0.02	31 ± 0	0.42 ± 0.02	0.27 ± 0.02

[a]Except for the total organic carbon/dissolved organic carbon (TOC/DOC) measurements, which were collected from bulk natural organic matter (NOM) solutions prior to high-energy water accommodated fraction (HEWAF) preparation, all other water quality and polycyclic aromatic hydrocarbon (PAH) measurements were collected after HEWAF dilutions were prepared. –NOM represents the absence of added Suwanee River NOM, and +NOM represents the presence of added Suwanee River NOM, with nominal concentration = 10 mg/L. –UV represents the absence of artificial ultraviolet radiation, and +UV represents the presence of artificial ultraviolet radiation. The detection limit for TOC and DOC was 0.2 mg/L; concentrations measured below that limit are expressed as not detected (ND). Sample sizes are 30 except for TOC and DOC ($n = 4$ each).
SU = standard unit; DO = dissolved oxygen.

in the presence or absence of UV (8 h/d) for a total of 48 h. Non-UV treatment groups were exposed to a higher range of PAH concentrations (29.53 ± 0.19–475.92 ± 8.63 µg/L ∑PAHs) than UV treatment groups (1.55 ± 0.02–25.51 ± 1.66 µg/L ∑PAHs) due to the expected photo-enhanced PAH toxicity (Supplemental Data, Table S1). Exposures with or without UV were initiated on 2 different days (19 June 2019 and 26 October 2019, respectively) due to organism availability and to ensure adequate laboratory space and equipment availability. Larval quality was tested in control dishes during both experiments, by ensuring an average >90% mean survival after 24 h without UV, to ensure consistency between tests (Supplemental Data, Table S1). Exposures were conducted in 250-mL glass crystallizing dishes with 200 mL of test solution. Dishes were placed in an environmental chamber maintained at 25 °C with a 16:8-h light:dark photoperiod. The following water quality parameters were recorded daily from each test treatment replicate: temperature, pH, dissolved oxygen, and salinity (Table 1). Each experimental group contained 5 replicate dishes with 20 larvae/dish.

Larvae were held in test solutions for 2 h prior to UV exposure. Indoor UV exposures were conducted daily for 8 h under light banks containing UV-A bulbs (HTG Supply) as previously described by Bridges et al. (2018a). Irradiance (λ = 380 nm) was measured continuously throughout the UV exposures using a Biospherical Instruments BIC2104R radiometer. The average (mean ± standard deviation [SD]) intensity of the UV-A bulbs was 0.046 ± 0.002 mW/cm^2/s (Table 2). Non-UV groups were placed under fluorescent light bulbs emitting only visible light. Test solutions were renewed after 24 h. Survival was quantified at 24 and 48 h.

Tests of NOM solubility in different salinities

Because the measured DOC concentrations were much lower than expected in our test solutions, we decided to run a series of solubility tests for Suwannee River NOM at a range of concentrations (0, 2, 5, and 10 mg/L) and in a range of salinities (0, 20, and 35 ppt). Artificial seawaters (20 and 35 ppt) were prepared with Milli-Q water and Instant Ocean Sea Salt, and reconstituted hard water was used for the 0-ppt solution. First, 1-L solutions (in duplicate) were stirred for 24 h in a glass beaker using a magnetic stirrer. Samples (40 mL) of each NOM preparation were collected and shipped to ALS Environmental for DOC analysis as just described.

Statistical analysis

The 48-h median lethal concentrations (LC50s) and 95% confidence intervals (CIs) were calculated using the mean of the daily initial ∑PAH concentration of each dose. Data were generated with the USEPA TRAP software using a 2-parameter tolerance type Gaussian model.

RESULTS AND DISCUSSION

The aim of our study was to determine the influence of DOC on crude oil toxicity to ELS red drum, with or without co-exposure to UV light. To this end, 2 separate tests were performed in sequence, the first to examine the influence of DOC alone, and the second to examine the influence of DOC and UV. Considering the ubiquitous co-occurrence of these natural factors within the photic zones of aquatic environments, characterizing their interactive effects on crude oil toxicity is

TABLE 2: Integrated ultraviolet (UV; λ = 380 nm) dose and mean UV intensity of each exposure timepoint (24 and 48 h)[a]

Exposure timepoint (h)	Pre-UV exposure (h)	UV photoperiod (h)	Total integrated UV dose (mW s/cm^2)	Mean UV intensity ± SD (mW/cm^2/s)
24	2	8	1318.2	0.046 ± 0.002
48	2	8	1306.9	0.045 ± 0.002
Cumulative		16 total h	2625.2	0.046 ± 0.002

[a]Test chambers had a standardized 2-h pre-UV exposure duration. During each 24-h period, 8 consecutive h of UV-A radiation was delivered to test chambers. Incident UV_{380} was continuously measured throughout the exposure period.

essential to provide qualitative and quantitative information for the process of risk assessment.

To approximate a realistic scenario for coastal and estuarine areas affected by the *Deepwater Horizon* oil spill, Suwannee River NOM was used to assess the effects of DOC with and without UV on crude oil toxicity to red drum. We chose this source of NOM for 3 reasons: 1) the Suwannee River drains into the Gulf of Mexico (Light et al. 2007), thus contributing to the NOM therein, 2) this source likely provides a closer approximation of the complex mixture of DOC in natural environments over surrogates such as Aldrich humic acid, and 3) this NOM is commercially available and widely used in toxicity studies, thereby facilitating direct comparisons of the influence of NOM on crude oil and PAH toxicity. For the tests with UV, a total daily integrated UV-A of approximately 1300 mW s/cm^2 (Table 2) was delivered to the test chambers to emulate the solar radiation in the Gulf of Mexico estimated during the *Deepwater Horizon* oil spill (Bridges et al. 2018b; mean UV380 dose of 1550 ± 372 mW s/cm^2).

The results from the present study indicate that the presence of DOC at a measured concentration of 1.07 mg/L in the absence of UV does not elicit a significant effect on Slick A HEWAF toxicity to red drum larvae given there was no significant difference in 48-h LC50s (82.58 µg/L ∑PAHs; 95% CI = 71.09–94.07 µg/L ∑PAHs without DOC vs 65.94 µg/L ∑PAHs; 95% CI = 58.72–73.16 µg/L ∑PAHs with DOC; Figure 1A). The measured 1.07-mg/L DOC concentration is environmentally relevant, because it represents the average concentration found in shallow coastal waters (range of 0.3–2 mg/L), in which larval red drum can be found (Thurman 2012). Nevertheless, the measured concentration was lower than our target concentration of 5 mg/L based on a 50% estimate of carbon mass within NOM (nominal 10 mg/L; Millero 2005; Green et al. 2014). There are prior examples of solubility issues in high-salinity water (31 ppt) following NOM addition in the laboratory (Thurman 2012; Asmala et al. 2014). Indeed, this was supported by our tests of Suwannee River NOM solubility across a range of salinities that revealed a decrease in solubility with an increase in salinity (Supplemental Data, Table S3). However, the fact that full dissolution was not achieved even in freshwater indicates that factors other than salinity must also be at play. Although the NOM solubility limitations under laboratory conditions remain unclear, various factors might help explain the discrepancy with solubility observed under natural saltwater conditions, such as longer mixing times, seasonally higher temperatures, and pH.

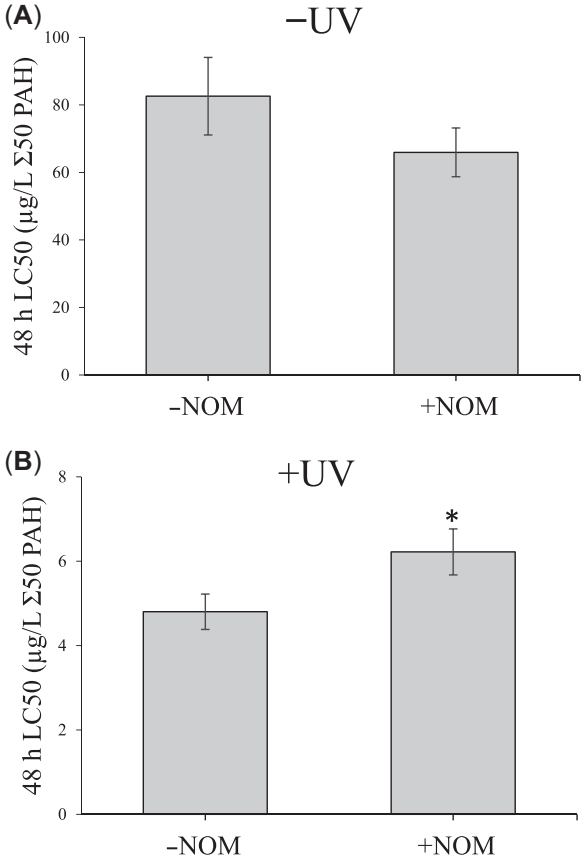

FIGURE 1: The 48-h ∑50 polycyclic aromatic hydrocarbon (PAH) median lethal concentrations (LC50s) of *Sciaenops ocellatus* exposed to slick A high-energy water accommodated fraction without (–natural organic matter [NOM]) or with (+NOM) Suwannee River NOM added (nominal concentration = 10 mg/L). Tests were performed from 24 to 72 hpf, with no ultraviolet (UV) exposure (**A**) and with artificial UV exposure (**B**). Error bars represent 95% confidence intervals (CIs), and * indicates no overlap between CIs.

Previous studies have shown seemingly inconsistent responses when the effects of DOC were analyzed during co-exposure to crude oil or individual PAHs in freshwater organisms, albeit they used endpoints different from those of the present study. For example, an increase in benzo[*a*]pyrene (BaP) bioaccumulation was observed in the bluegill sunfish (*Lepomis macrochirus*), whereas no effect was observed on naphthalene uptake with co-exposure to 2 mg/L of dissolved humic materials (McCarthy and Jimenez 1985b). Similarly, Akkanen et al. (2001) and Haitzer et al. (2001) demonstrated a reduced bioconcentration of BaP in *Daphnia magna* during co-exposure of various concentrations of DOC (as low as 1.4 mg/L), whereas the bioconcentration of atrazine and pyrene was not affected. In contrast to these studies, Li et al. (2018) demonstrated that gallic acid and tannic acid enhanced the bioconcentration of various isolated PAHs (phenanthrene, anthracene, fluoranthene, and pyrene) in adult zebrafish (*Danio rerio*) at concentrations ranging from 1 to 15 mg DOC/L. Furthermore, Matsuo et al. (2006) showed that juvenile tambaqui (*Colossoma macropomum*) co-exposed to Urucu oil with 22 mg C/L Aldrich humic acid increased the expression of liver cytochrome P450, relative to crude oil exposure alone.

Based on these studies, DOC appears to have the potential to increase or decrease the uptake and toxicity of PAHs. Such variance might reflect different interactions of individual PAHs with DOC or the types/concentrations of DOC used. For example, DOC from autochthonous and allochthonous origins has different physiochemical properties, which alter the sorption affinity for PAHs (McCarthy and Jimenez 1985a; Haitzer et al. 1999). Specifically, the efficiency of DOC complexation with PAHs and other organic compounds is correlated with molecular size, aromaticity, content of hydrophobic acids and neutral moieties, and polarity of the DOC (Haitzer et al. 1999).

Different concentrations of DOC may also influence PAH bioavailability and toxicity (McCarthy and Jimenez 1985a; Haitzer et al. 1999). Thus, an increased effect on PAH bioavailability and toxicity (either increase or decrease) is expected with an increase in the amount of PAHs bound to DOC (Black and McCarthy 1988), which should be achieved with higher amounts of DOC. It remains to be seen, however, whether DOC from different origins or higher DOC concentrations might alter the observed responses for red drum ELS.

Whereas the interactive effects of DOC and crude oil/PAHs are complex and not well characterized, the enhanced toxicity of PAHs with UV light has been well documented (Arfsten et al. 1996; Helbling and Zagarese 2007; Roberts et al. 2017). The toxicity of PAH is increased by photo-enhanced toxicity in a variety of aquatic organisms, including teleost fishes inhabiting the Gulf of Mexico (Alloy et al. 2016, 2017; Stieglitz et al. 2016; Bridges et al. 2018a), and this can occur via photosensitization and photo-modification. Embryonic and larval fishes, specifically those that are transparent and found in the upper water column, are likely at greater risk from photosensitization reactions. On the other hand, later life stages, as well as ELS fishes that exhibit UV behavioral avoidance, are putatively believed to be more susceptible to oxy-PAHs formed via photo-modification (Roberts et al. 2017). As expected, our results showed that UV light highly increased (>10-fold) the toxicity of Slick A HEWAF to ELS red drum regardless of whether NOM was added (Figure 1). A comparison of 48-h LC50s in the absence of added NOM revealed a 17-fold increase in toxicity with UV exposure (4.80 μg/L ∑PAHs; 95% CI = 4.38–5.22 μg/L ∑PAHs) than without (82.58 μg/L ∑PAHs; 95% CI = 71.09–94.07 μg/L ∑PAHs). In the presence of 10 mg/L nominal NOM, 48-h LC50s revealed an 11-fold increase in toxicity with UV exposure (6.22 μg/L ∑PAHs; 95% CI = 5.67–6.76 μg/L ∑PAHs) than without (65.94 μg/L ∑PAHs; 95% CI = 58.72–73.16 μg/L ∑PAHs). It should be noted, however, that while the nominal NOM concentrations were the same with and without UV, the measured DOC concentration was 4-fold less with UV than without (Table 1; discussed below). In addition, although the LC50s were obtained from 2 separate tests performed during different days, the 48-h survival rate of red drum larvae among the control groups (not exposed to UV and crude oil) was constant, supporting the idea that the results can be directly compared (Supplemental Data, Figure S1).

Consistent with the present results, most previous studies that have examined the influence of UV on crude oil toxicity to fish have demonstrated that UV highly increases crude oil lethality (Cho et al. 2003; Diamond et al. 2006; Alloy et al. 2016), especially if exposure occurs during the ELS. Notably, 2 studies have shown an increase in photo-enhanced crude oil toxicity in red drum larvae with exposure starting at the same life stage (post hatch, <48 hpf; Alloy et al. 2017; Bridges et al. 2018a). Alloy et al. (2017) demonstrated a significant increase in mortality in ELS red drum exposed to a total integrated UV-A (λ = 380 nm) dose of 705.79 mW s/cm^2 and *Deepwater Horizon* Slick A crude oil. The photo-enhanced toxicity was observed at ∑PAH concentrations as low as 3.13 μg/L after an exposure period of approximatively 20 h including an 8-h equilibration period (pre-UV PAH exposure), a 5- to 6-h photoperiod (UV exposure), and 5 h without UV. Similarly, Bridges et al. (2018a) examined the photo-enhanced toxicity of Slick A HEWAF by evaluating various photoperiods and equilibration periods. In summary, that study demonstrated a range of 15- to 75-fold increase in Slick A HEWAF toxicity to ELS red drum, measured as ∑PAHs LC50, by the addition of UV-A (λ = 380 nm) with a total integrated UV dose ranging from 1458 to 1680 mW s/cm^2. Although similar photo-enhanced toxicities were observed with our tests, a direct comparison cannot be made with the above-mentioned studies because different UV photoperiods, total exposure times, and total UV dose were applied. However, it can be concluded and confirmed that red drum are among the most sensitive fishes to crude oil and photo-enhanced crude oil toxicity tested to date (Alloy et al. 2017; Xu et al. 2017).

Despite its potential importance, the effect of DOC on photo-enhanced crude oil toxicity to teleost fishes has received little to no attention. To our knowledge, ours is the first study that has examined the effect of DOC, as Suwannee River NOM, on the photo-enhanced toxicity of crude oil on an ELS teleost fish in a marine environment. Our results demonstrated that DOC significantly reduced crude oil toxicity when UV light was present, although this apparent protective effect was mild (Figure 1B). The Slick A HEWAF 48-h LC50 for red drum was 4.80 μg/L ∑PAHs (95% CI = 4.38–5.22 μg/L ∑PAHs), and with the DOC co-exposure it was 6.22 μg/L ∑PAHs (95% CI = 5.67–6.76 μg/L ∑PAHs).

Although not directly comparable because single PAHs and freshwater organisms were used, 2 other studies have shown a similar decrease in photo-enhanced toxicity during DOC exposure (Oris et al. 1990; Weinstein and Oris 1999). Specifically, these studies revealed that DOC concentrations ranging from 0.5 to 6 mg C/L (modified by addition of Aldrich humic acid) decreased anthracene and fluoranthene photo-enhanced toxicity (expressed as LT50) to fathead minnows (*Pimephales promelas*) and *D. magna*.

There are several possible mechanisms by which DOC might serve a protective role against UV-induced phototoxicity (Oris et al. 1990; Roberts et al. 2017). For one, UV exposure might photo-modify DOC and/or PAHs (and other crude oil constituents) such that binding affinity increases between DOC and these toxic agents, rendering them less bioavailable and reducing toxicity. Alternatively, PAHs might be taken up while complexed with DOC, potentially rendering them less susceptible to photo-sensitization. Finally, DOC contributes to the attenuation of UV radiation, especially in the upper water column, but this effect is likely negligible at the DOC concentrations and the depth of the exposure chambers we used. In any event, the exact mechanism of DOC protection against UV-induced crude oil phototoxicity remains unclear at this time and warrants further investigation.

The measured DOC concentration we used is more typical of pelagic marine environments where autochthonous sources dominate (Thurman 2012). Red drum larvae share similar characteristics with other pelagic teleost fish larvae found in the Gulf of Mexico (e.g., mahi mahi, *Coryphaena hippurus*), such as high transparency and positive buoyancy (Incardona and

Scholz 2016). Therefore, it is likely that DOC has similar, albeit mild, protective effects on UV-enhanced crude oil phototoxicity to ELS teleost fishes in the pelagic environment. However, DOC derived from such areas (autochthonous origin) of the Gulf of Mexico should be investigated, because DOC from different origins might have different efficacies or effects in terms of UV-enhanced phototoxicity.

Although the same nominal concentration of NOM was used as in the no-UV experiment (10 mg/L), the measured DOC concentration with UV was much less at 0.27 mg/L compared to 1.07 mg/L without UV. The reason for this discrepancy in measured DOC concentrations despite the same nominal additions of NOM across experiments is unclear, but may reflect the heterogeneity of DOC solubility in salt water. Nevertheless, this mild protective effect of DOC is conservative considering that an even greater protective effect would be expected at higher DOC concentrations (Haitzer et al. 1999), more typical of estuarine environments.

The results of our study confirm the high sensitivity of ELS red drum to UV-induced crude oil photo-toxicity to environmentally relevant ∑PAH concentrations, even with DOC co-exposure. The photo-enhanced ∑PAH LC50s measured in our study with or without added NOM (4.80 and 6.22 µg/L, respectively) are well within the lower range of ∑PAH concentrations measured during the *Deepwater Horizon* spill. Moreover, considering that only initial values were measured and that loss of PAHs over 24 h can be considerable (Forth et al. 2017), our LC50s very likely underestimate the true acute toxicity. Following the accident, ∑PAH concentration ranged between 29.4 to 189 µg/L in the pelagic area around the spill (Diercks et al. 2010). Even coastal areas and estuaries reported similarly high concentrations, which seemed to persist over time. Specifically, water samples analyzed from Barataria Bay, Louisiana (USA), where red drum are known to spawn, revealed ∑PAH concentrations >200 µg/L at the beginning of May, which decreased over time to as high as 22 µg/L by the end of June (Whitehead et al. 2012). Although only mortality was assessed as a measure for acute toxicity in the present study, it should be noted that crude oil induces other sublethal effects (e.g., cardiac and ocular function and development) to developing red drum at much lower concentrations (Khursigara et al. 2017; Xu et al. 2017; Magnuson et al. 2018). Most likely these negative effects have later repercussions to the developing fish and subsequently jeopardize their survival in the natural environment.

CONCLUSIONS

Our study revealed that DOC at a concentration as high as 1.07 mg/L does not affect *Deepwater Horizon* crude oil toxicity to red drum ELS. Moreover, our results confirmed that *Deepwater Horizon* crude oil toxicity to ELS red drum is greatly enhanced during UV-A co-exposure (>10-fold increase). Perhaps most importantly, our results revealed that a low concentration of DOC (0.27 mg/L) mildly reduced the observed acute photo-enhanced toxicity of crude oil to ELS red drum. Considering that DOC concentrations in estuarine and other near-shore environments can typically reach concentrations as high as 5 mg/L, the protective effect of DOC might be even greater in these natural environments.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4877.

Acknowledgment—The authors thank C. Emadi, C. Green, K. Roark, K. Buschang, C. Benedict, D. Porter, and A. Chapman for their technical assistance and the Texas Parks and Wildlife Department Sea Center Texas for providing test organisms. The present study was supported by a grant from the Gulf of Mexico Research Initiative (grant SA-1520 to the RECOVER consortium [Relationship of effects of cardiac outcomes in fish for validation of ecological risk]).

Author Contributions Statement—E. Mager and A.P. Roberts conceived and designed the experiments. F. Bonatesta, R.R. Leads, and E.R. Price performed the experiments. F. Bonatesta performed the statistical analysis. F. Bonatesta, R.R. Leads, and E. Mager wrote the manuscript. E.R. Price and A.P. Roberts provided technical and editorial assistance.

Data Availability Statement—Data are publicly available through the Gulf of Mexico Research Initiative Information and Data Cooperative (GRIIDC), at https://data.gulfresearchinitiative.org (https://doi.org/10.7266/n7-tsr7-hs05). Data, associated metadata, and calculation tools are also available from the corresponding author (fabriziobonatesta@my.unt.edu).

REFERENCES

Akkanen J, Penttinen S, Haitzer M, Kukkonen JVK. 2001. Bioavailability of atrazine, pyrene and benzo[a]pyrene in European river waters. *Chemosphere* 45:453–462.

Allard B, Borén H, Pettersson C, Zhang G. 1994. Degradation of humic substances by UV irradiation. *Environ Int* 20:97–101.

Alloy M, Baxter D, Stieglitz J, Mager E, Hoenig R, Benetti D, Grosell M, Oris J, Roberts A. 2016. Ultraviolet radiation enhances the toxicity of *Deepwater Horizon* oil to mahi-mahi (*Coryphaena hippurus*) embryos. *Environ Sci Technol* 50:2011–2017.

Alloy M, Garner TR, Bridges K, Mansfield C, Carney M, Forth H, Krasnec M, Lay C, Takeshita R, Morris J. 2017. Co-exposure to sunlight enhances the toxicity of naturally weathered *Deepwater Horizon* oil to early life-stage red drum (*Sciaenops ocellatus*) and speckled seatrout (*Cynoscion nebulosus*): Photo-induced toxicity to red drum and speckled seatrout. *Environ Toxicol Chem* 36:780–785.

Arfsten DP, Schaeffer DJ, Mulveny DC. 1996. The effects of near ultraviolet radiation on the toxic effects of polycyclic aromatic hydrocarbons in animals and plants: A review. *Ecotoxicol Environ Saf* 33:1–24.

Asmala E, Bowers DG, Autio R, Kaartokallio H, Thomas DN. 2014. Qualitative changes of riverine dissolved organic matter at low salinities due to flocculation. *J Geophys Res Biogeosci* 119:1919–1933.

Black MC, McCarthy JF. 1988. Dissolved organic macromolecules reduce the uptake of hydrophobic organic contaminants by the gills of rainbow trout (*Salmo gairdneri*). *Environ Toxicol Chem* 7:593–600.

Bridges KN, Krasnec MO, Magnuson JT, Morris JM, Gielazyn ML, Chavez JR, Roberts AP. 2018a. Influence of variable ultraviolet radiation and oil exposure duration on survival of red drum (*Sciaenops ocellatus*) larvae. *Environ Toxicol Chem* 37:2372–2379.

Bridges KN, Lay CR, Alloy MM, Gielazyn ML, Morris JM, Forth HP, Takeshita R, Travers CL, Oris JT, Roberts AP. 2018b. Estimating incident ultraviolet radiation exposure in the northern Gulf of Mexico during the *Deepwater Horizon* oil spill. *Environ Toxicol Chem* 37:1679–1687.

Cho E, Bailer AJ, Oris JT. 2003. Effect of methyl tert-butyl ether on the bioconcentration and photoinduced toxicity of fluoranthene in fathead minnow larvae (*Pimephales promelas*). *Environ Sci Technol* 37:1306–1310.

Diamond SA, Mount DR, Mattson VR, Heinis LJ, Highland TL, Adams AD, Simcik MF. 2006. Photoactivated polycyclic aromatic hydrocarbon toxicity in medaka (*Oryzias latipes*) embryos: Relevance to environmental risk in contaminated sites. *Environ Toxicol Chem* 25:3015–3023.

Diercks A-R, Highsmith RC, Asper VL, Joung D, Zhou Z, Guo L, Shiller AM, Joye SB, Teske AP, Guinasso N. 2010. Characterization of subsurface polycyclic aromatic hydrocarbons at the *Deepwater Horizon* site: Subsurface PAH at *Deepwater Horizon* site. *Geophys Res Lett* 37:L20602.

Forth HP, Mitchelmore CL, Morris JM, Lay CR, Lipton J. 2017. Characterization of dissolved and particulate phases of water accommodated fractions used to conduct aquatic toxicity testing in support of the *Deepwater Horizon* natural resource damage assessment. *Environ Toxicol Chem* 36:1460–1472.

Green NW, McInnis D, Hertkorn N, Maurice PA, Perdue EM. 2014. Suwannee River natural organic matter: Isolation of the 2R101N reference sample by reverse osmosis. *Environ Eng Sci* 32:38–44.

Greer JB, Pasparakis C, Stieglitz JD, Benetti D, Grosell M, Schlenk D. 2019. Effects of corexit 9500A and Corexit-crude oil mixtures on transcriptomic pathways and developmental toxicity in early life stage mahi-mahi (*Coryphaena hippurus*). *Aquat Toxicol* 212:233–240.

Haitzer M, Abbt-Braun G, Traunspurger W, Steinberg CEW. 1999. Effects of humic substances on the bioconcentration of polycyclic aromatic hydrocarbons: Correlations with spectroscopic and chemical properties of humic substances. *Environ Toxicol Chem* 18:2782–2788.

Haitzer M, Akkanen J, Steinberg C, Kukkonen JVK. 2001. No enhancement in bioconcentration of organic contaminants by low levels of DOM. *Chemosphere* 44:165–171.

Haitzer M, Höss S, Traunspurger W, Steinberg C. 1998. Effects of dissolved organic matter (DOM) on the bioconcentration of organic chemicals in aquatic organisms—A review. *Chemosphere* 37:1335–1362.

Helbling EW, Zagarese H. 2007. *UV Effects in Aquatic Organisms and Ecosystems*. Royal Society of Chemistry, Cambridge, UK.

Incardona JP, Scholz NL. 2016. The influence of heart developmental anatomy on cardiotoxicity-based adverse outcome pathways in fish. *Aquat Toxicol* 177:515–525.

Khursigara AJ, Perrichon P, Martinez Bautista N, Burggren WW, Esbaugh AJ. 2017. Cardiac function and survival are affected by crude oil in larval red drum, *Sciaenops ocellatus*. *Sci Total Environ* 579:797–804.

Li Y, Wang H, Xia X, Zhai Y, Lin H, Wen W, Wang Z. 2018. Dissolved organic matter affects both bioconcentration kinetics and steady-state concentrations of polycyclic aromatic hydrocarbons in zebrafish (*Danio rerio*). *Sci Total Environ* 639:648–656.

Light HM, Darst MR, Mattson RA. 2007. Ecological characteristics of tidal freshwater forests along the Lower Suwannee River, Florida. In Conner WH, Doyle TW, Krauss KW, eds, *Ecology of Tidal Freshwater Forested Wetlands of the Southeastern United States*. Springer, Dordrecht, The Netherlands, pp 291–320.

Langdon CJ, Stefansson ES, Pargee SM, Blunt SM, Gage SJ, Stubblefield WA. 2016. Chronic effects of non-weathered and weathered crude oil and dispersant associated with the *Deepwater Horizon* incident on development of larvae of the eastern oyster, *Crassostrea virginica*. *Environ Toxicol Chem* 35:2029–2040.

Mager EM, Pasparakis C, Stieglitz JD, Hoenig R, Morris JM, Benetti DD, Grosell M. 2018. Combined effects of hypoxia or elevated temperature and *Deepwater Horizon* crude oil exposure on juvenile mahi-mahi swimming performance. *Mar Environ Res* 139:129–135.

Magnuson JT, Khursigara AJ, Allmon EB, Esbaugh AJ, Roberts AP. 2018. Effects of *Deepwater Horizon* crude oil on ocular development in two estuarine fish species, red drum (*Sciaenops ocellatus*) and sheepshead minnow (*Cyprinodon variegatus*). *Ecotoxicol Environ Saf* 166:186–191.

Matsuo AYO, Woodin BR, Reddy CM, Val AL, Stegeman JJ. 2006. humic substances and crude oil induce cytochrome P450 1A expression in the Amazonian fish species *Colossoma macropomum* (Tambaqui). *Environ Sci Technol* 40:2851–2858.

McCarthy JF, Jimenez BD. 1985a. Interactions between polycyclic aromatic hydrocarbons and dissolved humic material: Binding and dissociation. *Environ Sci Technol* 19:1072–1076.

McCarthy JF, Jimenez BD. 1985b. Reduction in bioavailability to bluegills of polycyclic aromatic hydrocarbons bound to dissolved humic material. *Environ Toxicol Chem* 4:511–521.

Millero FJ. 2005. *Chemical Oceanography*, 3rd ed. CRC, Boca Raton, FL, USA.

National Environmental Methods Index. 1998. Standard Methods: 5310C: Total organic carbon by persulfate-UV or heated-persulfate oxidation method. Washington, DC.

Oris JT, Hall AT, Tylka JD. 1990. Humic acids reduce the photo-induced toxicity of anthracene to fish and daphnia. *Environ Toxicol Chem* 9(5): 575–583.

Pasparakis C, Esbaugh AJ, Burggren W, Grosell M. 2019. Impacts of *Deepwater Horizon* oil on fish. *Comp Biochem Physiol C Toxicol Pharmacol* 224:108558.

Powers LC, Miller WL. 2015. Photochemical production of CO and CO_2 in the Northern Gulf of Mexico: Estimates and challenges for quantifying the impact of photochemistry on carbon cycles. *J Mar Chem* 171: 94055584.

Roberts AP, Alloy MM, Oris JT. 2017. Review of the photo-induced toxicity of environmental contaminants. *Comp Biochem Physiol C Toxicol Pharmacol* 191:160–167.

Rodgers ML, Jones ER, Klinkhamer C, Mahapatra CT, Serafin J, Bosker T, Perkins C, Griffitt RJ, De Guise S, Sepúlveda MS. 2018. Combined effects of *Deepwater Horizon* crude oil and environmental stressors on *Fundulus grandis* embryos. *Environ Toxicol Chem* 37:1916–1925.

Stieglitz JD, Mager EM, Hoenig RH, Alloy M, Esbaugh EJ, Bodinier C, Benetti DD, Roberts AP, Grosell M. 2016. A novel system for embryo-larval toxicity testing a pelgaic fish: Applications for impact assessment of *Deepwater Horizon* crude oil. *Chemosphere* 162:261–268.

Thurman EM. 2012. *Organic Geochemistry of Natural Waters*. Springer Science & Business Media, New York, NY, USA.

US Environmental Protection Agency. 1996. Method 3510C: Separatory funnel liquid-liquid extraction. Washington, DC.

US Environmental Protection Agency. 2004. Method 9060A: Total organic carbon. Washington, DC.

US Environmental Protection Agency. 2014. Method 8270D: Semivolatile organic compounds by gas chromatography/mass spectrometry. Washington, DC.

Weinstein JE, Oris JT. 1999. Humic acids reduce the bioaccumulation and photoinduced toxicity of fluoranthene to fish. *Environ Toxicol Chem* 18:2087–2094.

Whitehead A, Dubansky B, Bodinier C, Garcia TI, Miles S, Pilley C, Raghunathan V, Roach JL, Walker N, Walter RB, Rice CD, Galvez F. 2012. Genomic and physiological footprint of the *Deepwater Horizon* oil spill on resident marsh fishes. *Proc Natl Acad Sci USA* 109: 20298–20302.

Wilson CA, Nieland DL. 1994. Reproductive biology of red drum, *Sciaenaps acellatus*, from the neritic waters of the northern Gulf of Mexico. *Fish Bull* 92:841–850.

Xu EG, Khursigara AJ, Magnuson J, Hazard ES, Hardiman G, Esbaugh AJ, Roberts AP, Schlenk D. 2017. Larval red drum (*Sciaenops ocellatus*) sublethal exposure to weathered *Deepwater Horizon* crude oil: Developmental and transcriptomic consequences. *Environ Sci Technol* 51:10162–10172.

