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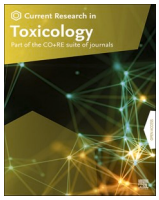

# Interactive effects of Louisiana Sweet Crude (LSC) thin oil sheens and ultraviolet light on mortality and swimming behavior of the larval Eastern oyster, *Crassostrea virginica*

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ABSTRACT

The Eastern oyster (*Crassostrea virginica)* is an important commercial bivalve species which also has numerous ecological roles including biogeochemical cycling, providing habitat for larval fish and crustaceans, and reducing the impacts of coastal storms. Oil may pose a threat to oyster larvae swimming in the water column, leading to potential negative effects on survival, growth, and development. Oil toxicity may be further enhanced by chemical changes in the presence of sunlight. This study determined the toxicity of thin oil sheens with and without ultraviolet (UV) light, then examined the latent effects of the short term exposure on longer term survival and swimming ability. Larval *C. virginica* were exposed to four different oil sheen thicknesses for 24 h with either no UV light or 2-h UV light. Following the exposure, larvae were transferred to clean seawater and no UV light for 96 h. The presence of a 2-h UV light exposure significantly increased oyster mortality, indicating photo-enhanced toxicity. The $LC_{50}$ for a 24-h oil sheen exposure without UV was 7.26 μm (23 μg/L $PAH_{50}$) while a 2 h-UV exposure lowered the sheen toxicity threshold to 2.67 μm (10 μg/L $PAH_{50}$). A previous 24-h oil sheen exposure (≥0.5 μm) led to latent effects on larval oyster survival, regardless of previous UV exposure. Sublethal impacts to larval oyster swimming behavior were also observed from the previous oil sheen exposure combined with UV exposure. This study provides new data for the toxicity of thin oil sheens to a sensitive early life stage of estuarine bivalve.

## Introduction

Louisiana Sweet Crude oil (LSC) is classified as a paraffinic-naphthenic light crude oil which has cyclic and acyclic alkanes with a low sulphur content (Killops and Killops, 1993; Byfield, 1998). Overall, when oil is released from a spill a thicker region forms which quickly dissipates into a larger and thinner surface oil sheen (Byfield, 1998). These sheens quickly spread through the surface water through gravitational and inertial forces which then give way to surface tension and viscous forces (Fay, 1971; Byfield, 1998). Oil sheens are normally defined as a duplex film (Adamson, 1982) which is when the film is thick enough for the air-film and film-water interfaces to be independent of each other (Byfield, 1998).

Thin oil sheens also form from currently permissible operational discharges of hydrocarbons (NRC, 2003; O'Hara and Morandin, 2010). Sheens (also known as "slicks") can also be produced by naturally occurring seeps which form thin sheens on the water's surface (NRC, 2003; MacDonald et al., 2011; MacDonald et al., 2015) or from vessel discharge or accidents (NRC, 2003). Oil sheens and slicks are defined by the Oil Slick Thickness Code (ASTM, 1996; Bonn Agreement, 2004) with (1) a silvery/grey sheen having an oil thickness of 0.004 to 0.30 μm, (2) a rainbow sheen has a thickness between 0.30 and 5.0 μm, and (3) a metallic sheen having a thickness of 5.0 to 50 μm (ASTM, 1996; Bonn Agreement, 2004; 2009; Leifer et al., 2012). Due to the proximity of oil drilling rigs and wells to coastlines of the U.S., oil sheens can travel to inshore and estuarine environments. Little research has been done on the toxicological effects of these oil sheens on marine organisms, especially on marine bivalves.

The Eastern oyster, *Crassostrea virginica*, is a common inshore and nearshore species found on the Atlantic Coast and Gulf of Mexico

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(Powers et al., 2017). On the Atlantic coast of the U.S., *C. virginica* aquaculture is growing and has provided a new stream for the economy in coastal communities with aquacultured oyster value in Rhode Island around $6.07 million in 2019 (Beutel, 2019) and around $14.54 million in 2018 in Virginia (Hudson, 2019). Furthermore, according to the National Oceanic and Atmospheric Administration (NOAA) Fisheries Landings Statistics, *C. virginica* in the U.S. was valued around $177,647,608, with Gulf of Mexico States (Alabama, Florida, Louisiana, and Texas) making up ~ 46% of this value (NOAA Fisheries Office of Science and Technology). Concurrently, *C. virginica* forms emergent reefs or hummocks near salt marsh edges (Powers et al., 2017). These oysters also serve as sanctuary areas for commercially and ecologically important fish and shellfish species which form spawning stock reserves (Powers et al., 2017). *C. virginica* serves numerous ecosystem roles from increasing water quality (Dame and Patten, 1981; Hoellein et al., 2015; Galimany et al., 2017) to forming habitat for benthic invertebrates, fishes, and mobile crustaceans (Meyer and Townsend, 2000; Peterson et al., 2003; Coen et al., 2007; Grabowski and Peterson, 2007). As of 2012, the commercial fish value of a hectare of oyster reef was calculated to be $4123 per year (Grabowski et al., 2012). Oyster reefs also serve as breakwaters, bulkheads, and jetties (Grabowski et al., 2012) to reduce wave energy which can cause shoreline erosion (Meyer et al., 1997; Peterson et al., 2003; Piazza et al., 2005; Stunz et al., 2010; Scyphers et al., 2011; Grabowski et al., 2012; Arkema et al., 2013; Lunt et al., 2017).

The effects of oil toxicity in the form of naturally weathered crude oil and chemically dispersed oil have been studied in *C. virginica* (Vignier et al., 2015; Vignier et al., 2016; Boulais et al., 2018; Jasperse et al., 2018; Finch et al., 2018, Vignier et al., 2019), yet less research has been done on oil sheens or in the presence of ultraviolet (UV) light. Polycyclic aromatic hydrocarbons (PAHs) are considered to be the primary toxic constituents of crude oil. UV light interacts with the photodynamic PAHs of oil, forming reactive oxygen species (Landrum et al., 1987; Ankley et al., 2003; Diamond, 2003; Sweet et al., 2017). These reactive oxygen species can then interact with proteins and fatty acids within an organism which can increase oil toxicity (Oris and Giesy, 1987; Holst and Giesy, 1989; Diamond, 2003; Alloy et al., 2017; Sweet et al., 2017). Studies have shown that UV light enhances oil toxicity in aquatic species whose eggs are positively buoyant and hatch near the water's surface (mahi-mahi: Alloy et al., 2016; Sweet et al., 2017; red drum and speckled seatrout: Alloy et al., 2017) and impacts marine invertebrate swimming larvae (Alloy et al., 2015). Oysters are broadcast spawners and their eggs are fertilized near the surface where the larvae then hatch (Sastry, 1979) and can therefore be affected by UV-induced toxicity.

The purpose of this study was to examine the effects of UV exposure on the toxicity of four different sheen thicknesses of LSC oil on larval oysters, *C. virginica*, and to further assess latent effects of the short term exposure to oil sheens with and without UV light on longer term survival and swimming ability.

## Materials and methods

### *Seawater & specimen collection*

Seawater was supplied to the NOAA/NOS laboratory from Charleston Harbor estuary (N 32° 45′ 11.52″; W 79° 53′ 58.31″): filtered (5 μm), ultraviolet sterilized, filtered with activated carbon, and diluted with deionized water to 30 psu. Seawater was further run through a 0.22 μm Corning 500 mL Bottle top filter and stored in a 4 °C refrigerator until use. *Isochrysis galbana* was purchased from Bay Shellfish Co. (Terra Ceia Island, FL), maintained in an incubator (25 °C, 24 h light cycle) and cultured in f/2 medium following the protocol of Guillard and Ryther (1962) and Guillard (1975). *I. galbana* was used since it was the primary microalgae used at the hatchery (Bay Shellfish Co.) and it has been used in previous larval oyster studies (Rhodes and Landers, 1973; Maciejewski et al., 2019).

*C. virginica* larvae were shipped overnight at five days old from Bay Shellfish Co. in 32 psu seawater. Larvae were gradually acclimated (over 24 h) to laboratory conditions (25 °C, 30 psu seawater, 16 h light/8h dark cycle) in 1000 mL glass bowls with aeration. Larvae were fed 100 cells/μL (10 mL) of *I. galbana* every 24 h.

### *Light conditions*

UV-A and UV-B intensity was measured using an ILT2400 light meter which was placed at the water surface level of the exposure vessels in each incubator. UV conditions were created within the laboratory using T5 AgroMax UV-A PLUS light bulbs. For UV conditions UV-A measured $2.4x10^{-3}$ W/cm$^2$ and UV-B measured $1.1x10^{-6}$ W/cm$^2$. Non -UV conditions were tested under cool light fluorescent bulbs with UV-A of $3.8x10^{-6}$ W/cm$^2$ while UV-B measured $1.2x10^{-6}$ W/cm$^2$. For comparison, the UV-A and UV-B intensity measured outside the Charleston laboratory in full sun during July 2019 was $1.8x10^{-3}$ W/cm$^2$ and $7.1x10^{-5}$ W/cm$^2$ respectively. The UV-B intensity in the UV treatment was comparable to that of the non-UV conditions and was also less than the full sunlight reading, thus the UV variable tested in this study was only UV-A. The duration of 2 h of UV exposure was selected for this study after preliminary studies determined that intensity/duration of UV alone did not impact oyster larval survival.

### *Chemical analysis*

Additional replicate exposure chambers without organisms were set up for analysis of PAHs in the water column under the sheen. A total of 50 PAHs ($PAH_{50}$) were analyzed, including both parent and alkylated PAHs, which represent a suite of target analytes in environmental petroleum samples (Boehm, 2006). We chose to express the results as $PAH_{50}$ to be comparable to previously published oil sheen studies (DeLorenzo et al., 2021; Key et al., 2020). In order to sample the water column without disturbing the sheen, a Teflon tube was taped to the side of each crystallizing dish prior to water and oil addition. All exposures took place at 25 °C and had no UV exposure. Following the 24 h exposure, 3 mL samples of seawater were removed from each dish (sheen treatment and the control) using a 10 mL syringe inserted in the Teflon tube. Replicate samples were combined across treatments yielding an 18 mL composite sample. Samples underwent analysis following the protocol of Key et al. (2020). Briefly, samples were acidified to a pH of 2 and transferred into solvent-rinsed 1-L separatory funnels and underwent a liquid/liquid extraction. The samples were spiked with an isotopically labeled internal standard and solvent extracted three times with dichloromethane, 50:50 dichloromethane/hexane, and hexane. Following extraction, the samples were passed through GF/F paper that contained anhydrous sodium sulfate and concentrated in a water bath (40 °C) under a nitrogen stream (14 psi). The extracts were prepared using a silica SPE which was spiked with a recovery standard before being analyzed by GC/MS Extracts ran on an Agilent 6890/5793 N GC/MS with a split/splitless injector containing a DB17ms analytical column.

### *24-hour LSC oil sheen exposure*

Larvae were randomly allocated to one of eight different light and oil sheen thickness treatments and a control (Table 1). The range of sheen thicknesses was selected to be representative of those characterized in oil spills (NOAA, 2016). For example, the 1-μm sheen would be considered a rainbow sheen based on measurements in the Gulf of Mexico (Garcia-Pineda et al., 2020). Each treatment had six replicates with 30 larvae per replicate. Larvae were exposed in 270 mL glass crystallizing dishes (90 × 50 mm, Kimax No. 23000) to which 200 mL of 30 psu seawater was added. Thirty free swimming larvae (approximately 11 days post hatch) were then added to each dish. Tests were run in two environmental chambers (Percival Intellus) – one for UV

**Table 1**
Experimental treatments for the 24 h LSC oil sheen exposure. Control treatments contained no oil. UV exposures: 2 h UV/ 14 h fluorescent/8h dark. No UV exposures: 16 h fluorescent/ 8 h dark.

| Sheen Thickness (μm) | Light Condition | Volume of Oil (μL) |
|---|---|---|
| Control (0) | No UV | 0 |
| Control (0) | UV | 0 |
| 0.25 | No UV | 1.42 |
| 0.25 | UV | 1.42 |
| 0.5 | No UV | 2.84 |
| 0.5 | UV | 2.84 |
| 1.0 | No UV | 5.67 |
| 1.0 | UV | 5.67 |
| 4.0 | No UV | 22.68 |
| 4.0 | UV | 22.68 |

exposures and one for non-UV exposures, both at 25 °C. The volume of oil per sheen thickness was calculated using the surface area of the crystallizing dish (DeLorenzo et al., 2021). Louisiana Sweet Crude Oil (LSC) was then pipetted onto the surface of the water to produce sheen thicknesses of 0.25 μm, 0.5 μm, 1.0 μm, and 4.0 μm (Table 1). Dishes were then covered with clear plastic 60x15 mm petri dishes (Falcon 351007) to reduce evaporation. These petri dishes were used because they do not impact UV penetration to the sample.

Both the UV and non-UV treatments were exposed for 24 h to the sheens. The non-UV treatment was incubated with no UV lighting for 24 h (16 h fluorescent light/8h dark). The UV treatment was incubated at 25 °C with a 2 h UV light period to determine UV enhanced photo toxicity (2 h UV/14 h fluorescent light/8h dark). The UV exposure occurred 2 h after addition of the oil slick to allow PAHs to partition into the water column. Following this 24 h test, the number of surviving larvae was counted. Larvae were considered dead if no detectable movement was observed under the dissecting microscope. The results of this test were used to determine a 24-h median lethal concentration for LSC oil sheens with and without UV light.

### *Post-exposure test for latent effects*

Following the 24-h oil sheen exposure, ten of the surviving larvae from each replicate of the four sheen treatments (Control 0.25, 0.5, and 4.0 μm), with and without UV exposure, were transferred to polystyrene well plates (Multiwell REF353046) containing 2 mL clean seawater per well, one larva per well, and placed in an incubator without UV light for 96 h. Every 24-h, surviving larvae were counted and their swimming behavior was determined. Swimming behavior was quantified as either swimming or not swimming. To determine swimming or non-swimming behavior, larvae were agitated with gentle flushing of clean seawater and then observed under a dissecting microscope for 30 s. Larvae were considered non-swimming if they were not moving in the water column but were moving their cilia. Larvae were considered dead if they were non-moving and did not move their cilia following gentle agitation and observing for 30 s. During the 96-h exposure, surviving larvae were transferred daily to clean seawater and fed 100 cells/μL of *I. galbana*.

### *Statistical methods*

Statistical analyses were conducted using Statistical Analysis Software (SAS V.9.4, Cary, NC, USA). Median lethal concentrations (24 h $LC_{50}$) with 95% confidence intervals (CI) were determined using Probit Analysis for oil sheen toxicity with and without UV. Significant differences ($p < 0.05$) between $LC_{50}$ values without and with a 2 h UV exposure were determined via a $LC_{50}$ ratio test (Wheeler et al., 2006). Two-Factor ANOVA was used to test for the interaction of oil and light exposure. One-Way ANOVA followed by a Dunnett's post hoc test to determine which oil sheen treatments were significantly different from the control under each light condition.

In the post-exposure for latent effects, mean percent non-swimming was calculated from the number of surviving larvae. Mean percent mortality and non-swimming behavior of larval oysters was analyzed via a One-Way ANOVA followed by a Dunnett's post hoc test to determine which oil sheen treatments were significantly different from the control.

## Results

### *Chemical analysis*

Quantification of the total PAH ($PAH_{50}$ (μg/L)) concentration in each sheen treatment revealed a dose-dependent increase in PAH content with sheen thickness (Table 2). These results are similar to $PAH_{50}$ concentrations measured under oil sheens in previous studies (DeLorenzo et al., 2021; Key et al., 2020).

### *24-hour LSC oil sheen exposure*

A 24 h LSC thin oil sheen exposure caused significant mortality in larval oysters with or without a 2 h UV light photoperiod. A two-way ANOVA indicated a significant effect of the oil treatment ($p = 0.0061$), a significant effect of the light treatment ($p < 0.0001$), and a significant interaction between the oil and light treatments ($p < 0.0001$). In the no UV exposure, significant mortality occurred at oil sheens $\geq$ 0.5 μm (one-way ANOVA $p < 0.0001$, Dunnett's test), and in the 2 h UV exposure, there was a significant difference in mortality compared to the control at oil sheen thicknesses $\geq$ 1.0 μm (one-way ANOVA $p < 0.0001$, Dunnett's test) (Fig. 1).

A 2 h UV light exposure significantly lowered the toxicity threshold for oyster larvae exposed to thin oil sheens (Fig. 2). The $LC_{50}$ for an LSC oil sheen for no UV exposure of 7.258 μm (95 % CI: 4.476–16.125) was significantly higher ($LC_{50}$ ratio test: $p = 0.0001$) than the $LC_{50}$ with a 2 h UV exposure of 2.657 μm (95% CI: 2.105–3.594), with oil sheen toxicity increasing 2.7 fold in the presence of 2 h of UV light (Table 3). The $LC_{50}$ values from the 24 h sheen exposure were compared using the measured PAH concentrations to provide an estimate of water column exposure. The $LC_{50}$ under no UV exposure of 7.258 μm sheen thickness would equate to 20.075 μg/L $PAH_{50}$ while the $LC_{50}$ with a 2 h UV exposure of 2.657 μm sheen thickness would equate to 11.683 μg/L $PAH_{50}$ (Table 3).

### *Post-exposure test for latent effects*

Previous short-term (24 h) exposure to thin oil sheens alone (ANOVA $p = 0.0039$) and in combination with a 2 h UV light exposure (ANOVA $p = 0.0007$) led to significant latent effects on larval oyster survival (Fig. 3). Both the previous UV and no UV conditions of 0.5 μm and 4 μm sheens led to significant mortality 96 h after transfer to clean seawater. In the short term exposure, a 0.5 μm sheen led to < 20% mortality, regardless of UV treatment. The surviving larvae of that oil sheen exposure experienced approximately 70% mortality after 96 h in clean seawater (Fig. 3). Of the oysters with a previous history of 4 μm sheen with 2 h UV exposure, only 8% survived after 96 h in clean seawater (Fig. 3).

Using the latent mortality at 96 h to calculate a post-exposure 96 h

**Table 2**
Mean $PAH_{50}$ (μg/L) per oil sheen thickness ± standard deviation of the mean.

| Oil Sheen Treatment (μm) | Mean $PAH_{50}$ (μg/L) |
|---|---|
| Control | 0 ± 0.00 |
| 0.25 | 3.44 ± 0.39 |
| 0.5 | 4.69 ± 1.65 |
| 1 | 7.16 ± 5.25 |
| 4 | 14.46 ± 9.42 |

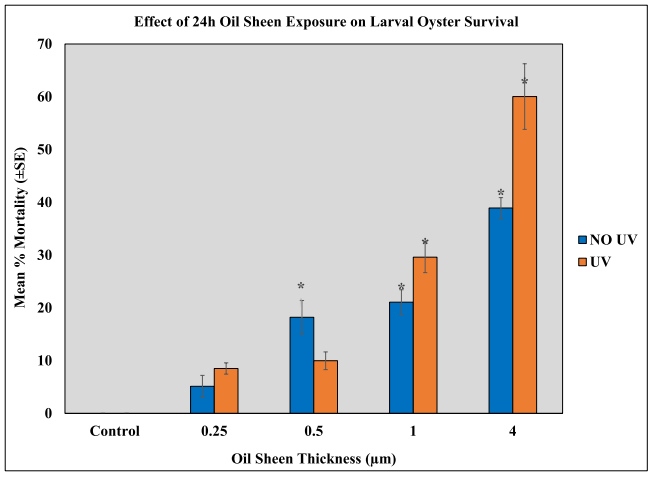


**Fig. 1.** Mean percent mortality of larval oysters following a 24-hour oil sheen exposure with no UV or with a 2 h UV exposure. Error bars represent the standard error of the mean. Asterisks indicate treatments that were significantly different from their respective light control.

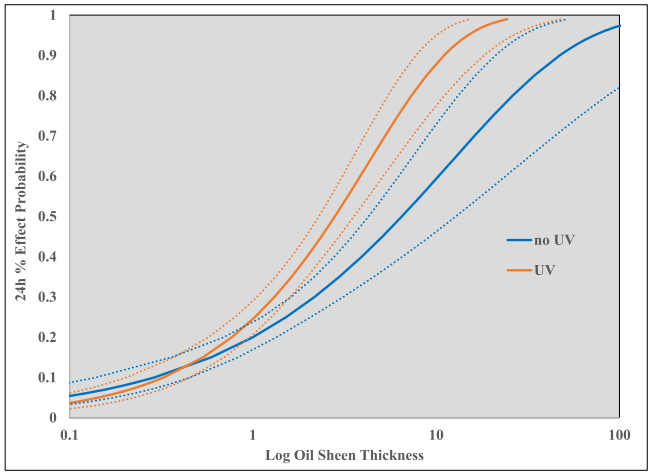


**Fig. 2.** Percent effect probability curves of 24 h mortality data from Probit analysis. Dashed lines are the 95% confidence intervals. The $LC_{50}$ ratio test indicated a significant difference between toxicity response, with the UV exposure significantly lowering the effects threshold.

LC50 value, the oil alone exposure resulted in an LC50 of 0.277 µm (95% CI: 0.001–0.712) based on sheen thickness, and 3.533 µg/L (95% CI: 0.069–5.866) based on estimated $PAH_{50}$ concentration in the water column (Table 3). The oyster larvae with previous oil and UV exposure had a latent LC50 value of 0.289 µm (95% CI: 0.013–0.614), or 3.620 µg/L $PAH_{50}$ (95% CI: 0.487–5.430) (Table 3). The addition of UV light during the 24 h oil exposure did not result in a significant difference in latent mortality 96 h post exposure (oil alone vs. oil + UV $LC_{50}$ ratio test: $p = 0.2611$).

The previous short-term (24 h) exposure to thin oil sheens without UV light did not lead to significant latent effects on larval oyster swimming behavior (ANOVA $p = 0.9205$), with 25–33% of the oysters not swimming across the treatments. However, the previous short-term (24 h) exposure to thin oil sheens in combination with a 2 h UV light exposure did lead to significant latent effects on larval oyster swimming behavior (ANOVA $p = 0.0046$) at the 4 µm sheen level (Fig. 4). All of the surviving oysters with a previous history of 4 µm sheen with 2 h UV exposure were immobile after 96 h in clean seawater. Of the surviving oysters with a previous history of 0.5 µm sheen with 2 h UV exposure, 58% were immobile after 96 h in clean seawater.

## Discussion

Previous studies have shown that marine bivalve larvae are sensitive to photo-enhanced toxicity of a single PAH, fluoranthene, (Weinstein, 2001), with younger life stages being more susceptible to this toxicity (Finch et al., 2016). In Finch et al., (2016) the authors identified a window in development where *C. virginica* larvae are more susceptible to photo-enhanced toxicity of fluoranthene before becoming less susceptible to UV following the formation of a shell. This reduction in photo-enhanced toxicity at older stages is hypothesized to be caused by the production of carbonate shells (Mitra, 1996; Pelletier et al., 1997) and or increased pigmentation (Finch and Stubblefield 2015). This study is one of the first to examine the impacts of thin oil sheens on *C. virginica* larvae, demonstrating that veliger *C. virginica* are susceptible to UV-enhanced photo-toxicity in the presence of LSC crude oil sheens. A 2 h UV exposure during a 24 h oil exposure significantly enhanced the toxicity of LSC sheen oil, with UV light exposure increasing toxicity 2.7 fold. The resulting 24 h $LC_{50}$ of 2.657 µm would be defined as a rainbow sheen (ASTM, 1996; NOAA, 2016). Previous studies (Diercks et al., 2010) that examined PAHs following Deepwater Horizon (DWH) Spill measured surface water concentrations up to 84.8 µg/L $PAH_{50}$, which is ~ 12 and 32 times higher than our calculated $LC_{50}$ thresholds for larval

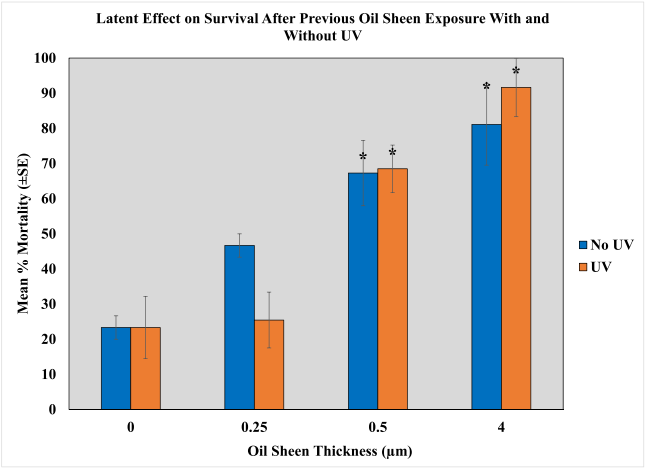


**Fig. 3.** Effect of previous short-term (24 h) exposure to thin oil sheens (with and without a 2 h UV light exposure) on larval oyster survival after being moved to clean seawater for 96 h. Asterisks indicate treatments that were significantly different from their respective light control (ANOVA, Dunnett's test).

**Table 3**
Larval oyster LC50 values (±95% confidence interval) expressed as sheen thickness (µm) and oil concentration under each test condition ($PAH_{50}$ (µg/L).

| | 24 h LC50 Oil + No UV | 24 h LC50 Oil + UV | Latent 96 h LC50 Oil + No UV | Latent 96 h LC50 Oil + UV |
|---|---|---|---|---|
| Sheen (µm) | 7.258 µm (4.476–16.125) | 2.657 µm (2.105–3.594) | 0.277 µm (0.001–0.712) | 0.289 µm (0.013–0.614) |
| $PAH_{50}$ (µg/L) | 20.075 µg/L (15.412–31.347) | 11.683 µg/L (10.424–13.492) | 3.533 µg/L (0.069–5.866) | 3.620 µg/L (0.487–5.430) |

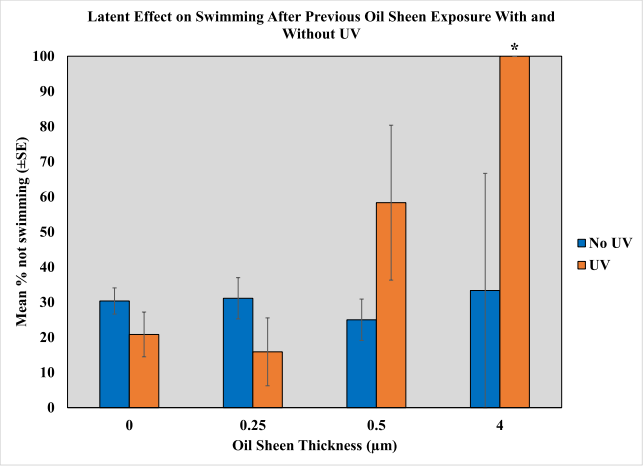


**Fig. 4.** Effect of previous short-term (24 h) exposure to thin oil sheens (with and without a 2 h UV light exposure) on larval oyster swimming ability after being moved to clean seawater for 96 h. Asterisks indicate treatments that were significantly different from their respective light control (ANOVA, Dunnett's test).

*C. virginica*. While the maximum $PAH_{50}$ concentration reported by Diercks et al., (2010) is possibly higher than concentrations in surface waters in coastal marshes immediately following DWH, the results of this study demonstrate that *C. virginica* larvae could be negatively impacted after an oil spill at environmentally relevant PAH concentrations due to their relatively low acute $LC_{50}$ values with and without added UV exposure (11.683 µg/L and 20.075 µg/L, respectively). The effect thresholds for *C. virginica* are similar to those found for other larval estuarine species (grass shrimp, mud snails, sheepshead minnow) in previous oil sheen testing with UV exposure (DeLorenzo et al., 2021).

Previous studies have shown that photo-enhanced oil toxicity is dependent on UV-light intensity (Ankley et al., 1995; Lyons et al., 2002; Finch and Stubblefield, 2015; Finch et al., 2016) and the subsequent toxicity varies with both dose and intensity. The 2 h UV light dose used in this study is of shorter duration than under natural conditions, and therefore may be a conservative estimate of UV-enhanced toxicity. Future studies with varying UV-light doses and intensities would further elucidate the intricate impacts of photo-enhanced toxicity on *C. virginica* larvae.

In addition to acute mortality, the combined thin oil sheen and UV light exposure led to prolonged effects on survival and fitness (swimming ability) post-exposure. Initial short term exposure to UV enhanced sheens has also been shown to significantly impact grass shrimp development and fecundity (Key et al., 2020). Larval oysters that were previously exposed to thin oil-sheens and UV light continued to be impacted four days after being moved to clean seawater. The increase in mortality after being moved to seawater indicates a continued adverse exposure–response and an inability for the oysters to recover from their previous exposure. The amount of latent mortality was not significantly different based on previous UV light exposure, suggesting that while the combination of oil sheen and UV light exacerbated toxicity in the 24 h exposure, it is previous oil exposure that is most influential in post-exposure oyster mortality. The combined oil sheen and UV light exposure did result in greater effect on latent swimming ability than oil alone. Exposure to other chemical stressors, such as insecticides, have also been shown to suppress larval oyster swimming activity, with effects noted at concentrations approximately tenfold lower than the corresponding $LC_{50}$ values (Garcia et al., 2014). This non-swimming behavior may cause larval oysters to be more susceptible to predation and or mortality due to their inability to find and catch food (Calabrese et al., 1973). Bivalve larvae that are less motile may also be limited in their ability to settle, thus leading to increased mortality rates and potential impacts to population recruitment and success (North et al., 2008).

This study did not examine the components of the oil sheens that impacted larval oyster mortality and swimming behavior, nor the photo-oxidized compounds that may have formed when interacting with UV-light (Sweet et al., 2017). These reactive oxygen species create oxidative stress which in turn causes DNA, cell membrane, and biomolecular damage (Barron and Ka'aihue, 2001; Finch and Stubblefield, 2016; 2018; Key et al., 2020). Further photo-enhancement of PAHs is known to produce toxic byproducts including acids, alcohols, esters, ketones, phenols, and sulfoxides (Bobra, 1992), which may cause lethal and sub-lethal impacts in Eastern oysters. This study is one of the first to analyze the impacts of a short-term oil or oil and UV exposure followed by a recovery period in clean seawater. Our study corroborated the results of previous studies on grass shrimp (Key et al., 2020) and northern prawns (Keitel-Groner et al., 2020) in which surviving a short-term toxic exposure will have lasting impacts on subsequent fitness and survival.

## Conclusions

This study found that short-term (24 h) exposure to thin oil sheens caused significant mortality in veliger *Crassostrea virginica* larvae (24 $LC_{50}$ of 7.258 µm (20.075 µg/L $PAH_{50}$). An additional 2-hours of UV light exposure decreased the toxicity threshold to 2.657 µm (11.683 µg/L $PAH_{50}$). Furthermore, following survival of a 24 h oil exposure and subsequent placement in clean seawater, larval survival and swimming behavior continued to be negatively affected by the previous oil sheen exposure. These findings are similar to previous studies that have found subsequent latent effects during recovery periods following short-term oil or oil and UV-light exposures (Key et al., 2020). While exposure to sheen oil may be short (24 h), there are long-term latent impacts which may affect larval bivalve ability to avoid predation and feed. Furthermore, these latent responses may cause delayed growth and settlement rates for the larvae.

Overall, depending on the timing of an oil spill, these subsequent oil sheens and photo-enhanced toxicity may significantly impact oyster population fitness and survival. Not only is photo-enhanced oil known to be detrimental to oyster sperm and younger larval stages (Finch et al., 2016), but may also negatively impact surviving larvae. To successfully model the impacts of oil spills on *C. virginica* larvae, additional studies should be conducted to assess other environmental factors (i.e., temperature, salinity, and pH), different forms of oil (i.e., oil types, weathered vs. non-weathered), and influence of chemical dispersants. The effects thresholds presented in this manuscript for thin oil sheens in an early life stage of the Eastern oyster characterize how toxicity changes with environmental conditions such as UV light and provides data on recovery from previous exposure that can be used to inform oil spill response and assessment.

## Disclaimer

The NOAA, National Ocean Service does not approve, recommend, or endorse any proprietary product or material mentioned in this publication.

## CRediT authorship contribution statement

**M.D. Zavell:** Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing. **K.W. Chung:** Conceptualization, Data curation, Methodology, Writing – review & editing. **P.B. Key:** Writing – review & editing. **P.L. Pennington:** Formal analysis, Methodology. **M.E. DeLorenzo:** Conceptualization, Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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