Developmental and reproductive effects in grass shrimp (*Palaemon pugio*) following acute larval exposure to a thin oil sheen and ultraviolet light

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Abstract
Many early stages of estuarine species congregate at the surface or in the upper mixing layer making them prone to UV light exposure and oil sheens. Laboratory testing was used to assess UV-oil sheen interactions with grass shrimp (*Palaemon pugio*). Newly hatched grass shrimp larvae were exposed to a 1-µm thick oil sheen for 24 h with or without an 8-h pulse of UV light. Grass shrimp were then transferred to clean seawater and non-UV conditions to measure development, growth, and reproductive fitness. Minimal toxicity was observed after the initial exposure but larval development was significantly delayed in shrimp exposed to the UV enhanced sheen. After reaching sexual maturity, shrimp were paired to evaluate effects on reproduction. Shrimp initially exposed to the UV enhanced sheen as larvae had a significant reduction in fecundity compared to controls. This demonstrates the importance of examining interactions between UV light and oil since negative effects to aquatic organisms may be underestimated if based on standard laboratory fluorescent lighting. Acute exposures of early life stages to thin oil sheens and UV light may lead to long-term impacts to individuals and ultimately to grass shrimp populations.

Keywords
Oil sheen, grass shrimp, life cycle, ultraviolet light, photoenhanced toxicity

1. Introduction

A sheen is a very thin layer of oil (0.3 to 5 µm in thickness) floating on the water surface and is the most common form of oil seen in the later stages of an oil spill. Sheens can vary in color from rainbows, for the thicker layers, to silver and almost transparent for thinner layers (Garcia-Pineda et al. 2020). The toxicity of thin oil sheens to early life stages of estuarine species is important since early life stages of aquatic organisms may congregate at the water surface or in the upper mixing layers of the water column. For this research, we investigated how the effects of an oil sheen can be magnified by interaction with ultraviolet (UV) light especially in early life stages of the grass shrimp (*Palaemon* (*Palaemonetes*) *pugio*). Toxicity can be potentially enhanced by UV light in embryo and larval stages of aquatic organisms due to their translucence and occupation in the photic zone of the water column (Barron and Ka'aihue 2001). Even in relatively turbid estuarine waters, crustacean larvae are affected by UV light since they are positively phototactic (Wubben 2000). Finch and Stubblefield (2016) reviewed UV/chemical exposure studies and found that UV light can enhance PAH toxicity up to 54 times. Toyooka and Ibuki (2007) reviewed several studies citing the DNA damage after exposure to PAHs and UV light in a variety of organisms. Pelletier et al. (1999) found that phototoxicity of individual PAHs to a marine bivalve and a mysid species could be over 50,000 times that of PAH toxicity under non-UV light. Other studies with UV light and contaminant exposures have found significant effects on grass shrimp reproduction (Volz et al., 2002) and sea urchin embryo development (Steevens et al., 1999). More recently, research has concentrated on the changes in toxicity occurring in crude oil after exposure to UV light subsequent to the Deepwater Horizon oil spill incident in the Gulf of Mexico. This research focused on impacts to early life stage estuarine fish (Barron et al., 2003; Alloy et al., 2016; Alloy et al., 2017; Bridges et al., 2018) and crustaceans (Alloy et al. 2015;Wubben 2000; Finch and Stubblefield 2019). A review by Roberts et al. (2017) on phototoxicity stated that PAHs are the most well studied photoxicant in the realm of toxicology. However, these studies mainly dealt with exposure periods in days rather than hours.

For this present research, our organism of interest was the planktonic larvae of the grass shrimp. These shrimp inhabit estuaries from Nova Scotia to Texas, play a major role in nutrient cycling, can be prey for many recreationally and commercially valuable fishes, can often be the dominant macrofauna in estuarine creeks, and are used as a model crustacean in toxicity tests (Anderson, 1985; Key et al. 2006). The grass shrimp life cycle is approximately nine months long, and begins with females holding the eggs in their pleopods until hatching occurs. The larvae then swim in the upper water column molting several times until a final molt into a postlarval form (Anderson, 1985; Key et al. 2006). This present research focused on determining the long-term organismal health effects of a short-term exposure. The research previously mentioned has established that UV light can change the toxicity of oil and associated PAHs, but it has not established how it affects the organismal response in the long term after a short-term exposure.

To further investigate the above issues, newly hatched grass shrimp larvae were exposed for 24 h to an oil sheen with or without 8 hours of UV light and then raised to adult stage in clean seawater with the effects on reproduction observed.

2. Materials and Methods

Newly hatched grass shrimp (less than 24 h old) were obtained from gravid females collected from Leadenwah Creek (N 32° 38' 51.00"; W 080° 13' 18.05"), a tidal tributary of the North Edisto River, SC, USA. All seawater used for the exposures, grow out, and pairing was acquired from Charleston Harbor estuary (N 32° 45' 11.52''; W 79° 53' 58.31''), filtered to 5 µm, passed through activated carbon, UV sterilized, and then diluted with deionized water to adjust salinity to 20 ppt.

Acute 24 h exposures were conducted with or without a 1-µm oil sheen, and with or without UV light. The oil sheens were formed with fresh Louisiana Sweet Crude (LSC) oil. The four treatments were a seawater control exposed to 8 h UV light followed by 8 h non-UV light followed by 8 h darkness (Control UV); a 1-µm oil sheen under the same UV conditions (Sheen UV); a seawater control with 16 h non-UV fluorescent light followed by 8 h darkness (Control non-UV); and a 1-µm oil sheen under the same non-UV fluorescent light conditions (Sheen non-UV). We choose a 1-µm sheen to have close to 100% survival after a 24 h UV/non-UV exposure. Previous testing showed that near 100% survival was possible with a 1-µm sheen (data not shown). The 1-µm sheen also matched rainbow sheen concentrations recorded during oil spill scenarios in the Gulf of Mexico (Garcia-Pineda et al. 2020). The volume of oil needed to achieve the 1-µm sheen thickness was determined using the formula for the volume of a cylinder and the measured diameter of the 250-mL round glass exposure container. Thus, the 1-µm sheen was created by adding 5.67 µL of LSC oil to the seawater surface using a glass bore micropipette.

For those treatments under UV light, measured UV wavelengths were 1.6×10^{-3} W/cm^2 for UV-A as produced by a fixture holding two 54 watt F54T5HO UV-A Plus bulbs. The non-UV wavelengths of 1.8×10^{-6} W/cm^2 for UV-A were produced by a fixture holding two 17 watt F17T8 fluorescent bulbs (standard bulbs used in our toxicity tests). UV measurements were made using an ILT2400 light meter (International Light Technologies, Inc., Peabody, MA) placed at the top of the exposure container in the environmental chamber. The exposure containers were placed 30 cm below the light fixtures. For this research, only UV-A wavelengths were measured since the wavelength range of UV-A is the absorbance range for most PAHs. UV-B is only around 8% of total UV and is more effectively filtered from the water column (Ankley et al., 2003).

The larvae were exposed in 250-mL round glass containers that held 200 mL of either seawater with no oil sheen or seawater with a 1-µm oil sheen. There were 10 larvae per container with three replicates for all treatments. Treatments were conducted concurrently in two environmental chambers at 25°C - one for the UV exposures and one for the non-UV exposures. After 24 h, larvae were placed in clean seawater via pipettes then transferred to well plates. Pipettes that penetrated the sheen to retrieve the larvae did not come into contact with the clean seawater

vessel. Each well plate contained six wells. Each well held one larva with 10 mL of clean seawater. Larval development was monitored daily. Molts were recorded and removed, and the number of days to reach postlarval stage was determined. Each larva was fed 60 µL of newly hatched *Artemia* daily. Seawater in the well plates was changed every other day and water quality parameters (dissolved oxygen, salinity, pH, temperature) were measured at that time. This phase of the test was conducted in an environmental chamber at 25°C with 16 h non-UV light:8 h dark. When a larva molted to the postlarva stage, it was moved to a community 19-L aquarium fitted with mechanical filtration with water temperature at 25°C under a 16 h non-UV light:8 h dark cycle. The shrimp were kept together in their original exposure groups from the postlarval stage until sexed. The shrimp were fed daily with *Artemia* in excess. Dry weights of a random sample of 10 postlarvae from each exposure group were measured as a further indicator of growth.

As they matured, the shrimp were sexed according to Holthuis (1952), and the sexes were kept in separate aquaria until we paired one male with one female. The shrimp were paired within their replicates and exposure groups. Pairs were placed in Plexiglas cages with nylon mesh panels containing four compartments, one pair per compartment as based on Wirth et al. (2002). Two cages were placed in 76-L tanks for a total of eight mating pairs per tank. Each tank was setup with mechanical filtration with water temperature at 25°C under a 16 h non-UV light:8 h dark cycle. Water quality parameters (as listed above) were measured weekly. The shrimp were fed daily *ad libitum* with *Artemia*. As females became gravid, the number days to gravid was recorded, and gravid females were removed after the eggs reached the embryonic eye stage (6 to 9 days after fertilization). Gravid females were weighed, then eggs removed, weighed, and counted. After egg removal, the female was weighed again and length measured. The male shrimp was removed from the compartment and length and weight were also measured. Eggs were moved to 24-well plates, one egg per well in filtered 20 ppt seawater, and placed in an environmental chamber at 25°C with 16 h non-UV light:8 h dark to assess hatching. Hatching occurred approximately 4 days later. The reproduction portion of the test ended 60 days after pairing. This was considered a sufficient amount of time to mate since a typical *P. pugio* female will produce at least eight broods during a 180-day breeding season (Bauer and Abdalla 2000). All measured water quality parameters throughout the different phases of the test were within acceptable test conditions (temperature 24 - 26°C, dissolved oxygen 6.0 - 7.0 mg/L, salinity 20 ppt, and pH 7.8 - 8.1).

Water samples were collected to quantify the oil exposure beneath the sheens. Samples for chemical analysis were collected using separate thin oil sheen preparations where a standpipe (Teflon straw) was established in the glass container prior to water and oil additions. After 24h, the water beneath the sheen was collected from the standpipe using a siphon without disturbing the overlying oil layer. These samples were acidified to a pH of 2 and then transferred into solvent-rinsed 1-L separatory funnels to undergo liquid/liquid extraction. Samples were spiked with isotopically labeled internal standards and then solvent extracted three times with the following solvents, dichloromethane, 50:50 dichloromethane/hexane, and hexane. All solvent fractions were composited and then passed through GF/F paper containing anhydrous sodium sulfate and concentrated in a water bath (40°C) under a stream of nitrogen (14 psi) and solvent

exchanged into hexane. Final extracts were further prepared by passing the last hexane fraction through a silica solid phase extract column SPE and then reduced under nitrogen to a final volume of ~1 mL. This final extract was spiked with a recovery standard (p-terphenyl) prior to instrumental analysis on GC/MS. Extracts were then run on an Agilent 6890/5793N GC/MS with split/splitless injector containing an Agilent DB17ms analytical column (60 m × 0.25 mm × 0.25 μm). The mass spectrometer was operated in selected ion monitoring (SIM) mode. Fifty PAHs were analyzed, including both parent and alkylated PAHs (Table 1). These 50 PAHs, known as "total PAH50", represent a suite of target analytes in environmental petroleum samples (Boehm, 2006). Chemical analysis of 18 samples of the 1-μm LSC oil sheen measured 5.26 μg/L of total PAH50 (±4.21 μg/L standard deviation).

For statistical analysis, a two-factor nested design was used to test for differences between treatment groups. It accounted for subsampling within each replicate. The two factors were SHEEN (sheen or no sheen) and UV (UV or no UV). The TEST statement was used to apply the correct error term to the model where replicates were nested within the two factors [e=reps(SHEEN*UV)]. Model residuals were tested for goodness of fit to the normal distribution and homogeneity of variances. The residuals were found to be normally distributed and homogenous after performing data transformations. For number of molts to postlarvae, the reciprocal transformation (1/y) was used. For number of days to first molt, the reciprocal of the cube transformation (1/y3) was used. An all-pairwise TUKEY-KRAMER test was performed post-hoc to determine significant differences between treatments. An ANCOVA (PROC GLM) was used to model and compare the slopes of the relationship between the number of gravid females versus the number of days it took to become gravid after pairing. A multiple contrast was used post-hoc to determine significant differences in slopes between the SHEEN UV treatment and all other treatments. All statistical analysis was performed using SAS (SAS V.9.4, Cary, NC, USA). Alpha for all tests was set at 0.05.

3. Results

Average survival of the larvae after 24 h was similar across the four treatments ranging from 98% for the Sheen UV treatment to 100% for the Control non-UV treatment (Table 2). After all larvae reached postlarval status, average survival was at 85% for the Sheen UV treatment ranging up to 98% for the Control non-UV treatment (Table 2).

The average day for Control non-UV larvae to reach the postlarvae stage was around Day 21 (Figure 1). At Day 21, an average of 9% of Sheen UV larvae had become postlarvae while an average of 60% of Control non-UV were postlarvae (Table 2). Larval development was significantly delayed at Day 21 for the Sheen UV treatment as compared to both Controls and significantly delayed for the Sheen non-UV treatment as compared to the Control non-UV treatment (Table 2). After all the larvae had become postlarvae, there was still a significant difference between the Sheen UV treatment and the other three treatments (Figure 1). The Control non-UV, Control UV and Sheen non-UV shrimp were similar in average number of days to reach postlarval status ranging from 21 to 23 days. The Sheen UV shrimp took a significantly longer time to reach postlarvae – at an average of just over 27 days. The number of molts it took larvae to reach postlarval status were counted and a significant difference was observed as well

(Figure 2). It took an average of just under seven molts to become a postlarva for the Control non-UV shrimp while it took over eight molts for the Sheen UV shrimp. Larval dry weights ranged from an average of 390.8 µg (±12.2 µg) for Control non-UV up to 419.5 µg (±10.2 µg) for Sheen non-UV exposed shrimp. There was no statistically significant difference among the treatments.

The total number of males, females and male/female pairs from each treatment were consistent among the treatments (Figure 3). The total number of males (ranging from 38 for Sheen UV to 45 for Sheen non-UV) was higher than the total number of females (ranging from 28 for Sheen non-UV to 30 for Control UV), but this is similar to grass shrimp field data collected from estuarine creeks in South Carolina, USA (Leight et al., 2005). Even though there were similar numbers for males and females in total for each treatment, our experiment was limited to a maximum of eight pairs for each replicate. As females were the limiting factor, some replicates had less than eight females so our total number of pairs for each treatment ranged from 18 for the Sheen UV up to 22 for the Control UV. As soon as seven days after male/female pairing, and up to over 30 days later, females became gravid. There were several other parameters that were measured included male and female length, male and female weight, female weight with eggs, egg clutch weight, number of eggs, percent hatch, days to hatch. There was no statistically significant effect on these measured parameters from sheen or UV exposure (data not shown).

There were two other parameters measured from the adults where trends were evident. The first was the percent females to become gravid. While there was variability within each treatment, only 59% of the females in the Sheen UV treatment became gravid compared to up to 79% for the controls (Figure 4). The next measured parameter that showed a trend was the average number of days it took the females to become gravid after pairing (Figure 5). While this was not statistically significant, the average for the Sheen UV treatment was about a week later than the controls – 33 days for the Sheen UV and 26 days for the controls. Using this reproductive data, the cumulative number of gravid females versus the number of days it took to become gravid after pairing was plotted (Figure 6). Statistical analysis found that the slope for the Sheen UV gravid females was significantly less than the slope for the other treatments. Thus, it took longer for females that were exposed as larvae to the UV sheen to become gravid compared to the other treatments. For example, by Day 30, there were 11 gravid females in the Control non-UV, 12 in the Sheen non-UV, 14 in the Control UV, and only 5 in the Sheen UV. This gap continued until the test ended on day 60.

4. Discussion

After 8 h of UV exposure under a 1-µm sheen as newly hatched larvae, grass shrimp development was significantly delayed in the Sheen UV exposed treatment. Grass shrimp larval development has been extensively studied in earlier manuscripts (Sandifer and Smith, 1979; Buikema et al., 1980; Key et al., 1998; Key et al, 2003; Key et al., 2006). These papers point out that molting is one of the most important parameters to measure in crustacean larval life stages. While the normal molting period may be altered by contaminants, which may not affect overall survival, any extension of the larval life stage may lead to increased predation causing a reduction in recruitment (Sandifer and Smith, 1979; Buikema et al., 1980; Key et al., 1998; Key

et al, 2003; Key et al., 2006). Thus, more time spent as a larva may equate to more time spent in this planktonic stage, which may equate to more of a chance of becoming a prey item. In addition, the more molts a larva undergoes may cause greater stress on the larva leading to a greater chance of mortality (Anger, 2001; Key et al., 2003).

Identifying the exact components of oil that affect grass shrimp development was beyond the scope of this research, but it is well known that UV light can cause photo-enhanced toxicity of PAHs to invertebrates (Finch et al., 2017). For the grass shrimp larvae, the most likely scenario involved absorption of the phototoxic PAH compounds through the gill membrane. The phototoxic PAHs then form reactive oxygen species creating oxidative stress in the larvae. This in turn causes DNA damage, cell membrane damage, and damage to biomolecules (Finch and Stubblefield, 2016). Keitel-Groner et al. (2020) exposed Northern shrimp (*Pandalus borealis*) larvae to mechanically dispersed North Sea oil for 6 h followed by 30 days of recovery. It was determined that this short term exposure significantly affected long term larval fitness parameters and as these short exposures are seldom reported, the consequences are seldom known (Keitel-Groner et al., 2020). Researchers have pointed out that the aromatic compounds found in oil, especially heterocyclic aromatic compounds and alkyl-substituted PAHs, can contribute to toxicity in grass shrimp (Unger et al., 2008). Photolysis by UV light induces a multitude of reactions, which can produce an array of photochemical by-products. These products include acids, alcohols, esters, ketones, phenols and sulfoxides (Bobra 1992), among others, any of which could induce lethal and sub-lethal effects in grass shrimp. Most research available in the literature has dealt with individual PAH components of oil and their toxicity after UV exposure. Spehar et al. (1999) exposed fresh and saltwater aquatic organisms to fluoranthene and UV over a 96 h period and found that UV light increased acute toxicity by one to three orders of magnitude. When combined with 15 min of UV exposure, benzo[α]pyrene (BP) caused a high level of DNA lesions in grass shrimp embryos that were slowly repaired (Hook and Lee 2004). UV light is also known to oxidize PAH molecules, leading to increased cellular damage within an organism from oxidative stress (Barron and Ka'aihue, 2001). Of the total PAH50 measured in this research (Table 1), the parent anthracene, and the parent and alkylated pyrene and fluoranthene, are among those known to be phototoxic to marine invertebrates and fish (Finch et al., 2017).

Several measured parameters in adults were not significantly different from Control non-UV treatment or the Control UV treatment including growth, egg production, and egg hatching supporting the findings that the effect is based on the interaction of oil sheen and UV light and not a factor of UV light alone. As soon as a week later and up to over a month later, females became gravid. It was found that the rate at which gravid females were produced was significantly slower after 8h of UV exposure under a 1-µm oil sheen as newly hatched larvae. Others have documented delayed reproduction in female grass shrimp and molting delays in larval grass shrimp after chronic exposure with pesticides (Wirth et al. 2002; Key et al., 1998; McKenney et al., 1998; Key et al, 2003). Volz et al. (2002) exposed grass shrimp to UV light and the pesticide endosulfan and found that the percentage of gravid females was significantly lower compared to UV controls. However, these exposures were continuous for 50 days during the adult life stage unlike the present research exposures, which were only 24 h oil exposures

with 8 h of UV light in the newly hatched larval stage. Any reproductive delays in grass shrimp may affect population size and structure. The effect of a contaminant on sperm or egg quality in adult invertebrates has been previously studied by other researchers, especially in regards to the contaminant modifying the ability to reproduce (Erraud et al. 2019; Lewis and Ford 2012; Matozzo et al. 2008; Sharara et al. 1998). For this present study, it is difficult to determine if the effect of the oil sheen and UV exposure interaction was greater on male reproduction or female reproduction. The exposure did not significantly affect the number of males versus the number of females, but rather the ability to mate or to produce sperm or eggs was probably affected.

5. Conclusion

While many studies have shown the combined effects of oil or PAHs and UV light on aquatic organisms, few, if any, have shown an effect on adult organisms after just 24 h oil plus 8 h UV light exposure as larvae. To the best of our knowledge, this is the only full life cycle study of the interactive effects of short-term oil sheen and UV light exposures in grass shrimp. The sublethal effects demonstrated in this study occurred at environmentally relevant concentrations, within the range of oil sheen thickness, PAH concentration, and UV light intensity recorded during the Deepwater Horizon oil spill event (Alloy *et al.,* 2017; Diercks *et al.,* 2010; Bridges *et al.,* 2018). These experiments show that short-term exposures can have consequences on adult shrimp reproductive health, and points to the potential of these short-term interactive exposures having effects in other crustaceans and aquatic organisms as well. This research also demonstrates the importance of examining interactions between UV light and oil since negative effects to aquatic organisms may be underestimated if based on standard laboratory fluorescent lighting. Acute exposures of early life stages to thin oil sheens and UV light may lead to long-term impacts to individuals and ultimately to grass shrimp populations. The results of this study have logically led to other questions such as was the delay in larval development tied to the delay in gravid female production?, will any effects found in the F1 generation be carried over to the F2 generation?, and which PAH compounds from the UV sheen are responsible for developmental and reproductive effects? As the research in oil spill science progresses these and other questions will need to be answered. Characterization of the interactive effects of oil and UV light on grass shrimp populations will provide NOAA's Office of Response and Restoration with data that can be used to inform oil spill response and assessment.

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Disclaimer
The scientific results and conclusions, as well as any views or opinions expressed herein, are those of the authors and do not necessarily reflect the views of NOAA or the Department of Commerce.

Declaration of interests

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.

Author contributions

Peter B Key: Conceptualization; Data curation; Methodology; Project administration; Writing - original draft; Writing - review & editing. **Katy W Chung**: Conceptualization; Methodology; Writing - review & editing. **J. Blaine West**: Methodology. **Paul L. Pennington**: Formal analysis; Methodology; Writing - review & editing. **Marie E. DeLorenzo**: Conceptualization; Supervision; Writing - review & editing.

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Figure 1. The average number of days for grass shrimp to reach postlarval status in the four exposure groups. Error bars represent standard error of the mean. Treatments denoted by same letter were not statistically different from one another ($p<0.05$). Sample sizes were Control non-UV = 59, Control UV = 56, Sheen non-UV = 53, and Sheen UV = 51.

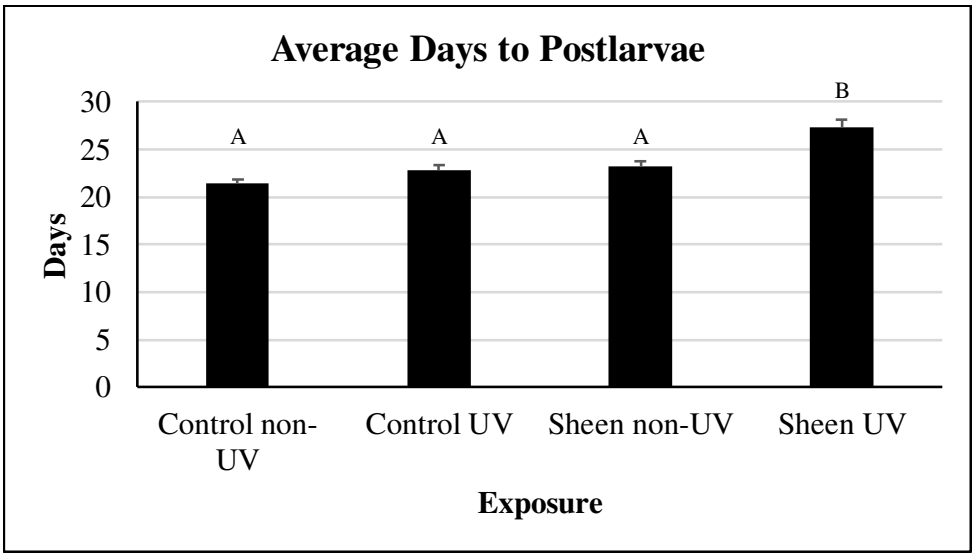

Figure 2. The average number molts for grass shrimp to reach postlarvae status in the four exposure groups. Error bars represent standard error of the mean. Treatments denoted by same letter were not statistically different from one another ($p<0.05$). Sample sizes were Control non-UV = 59, Control UV = 56, Sheen non-UV = 53, and Sheen UV = 51.

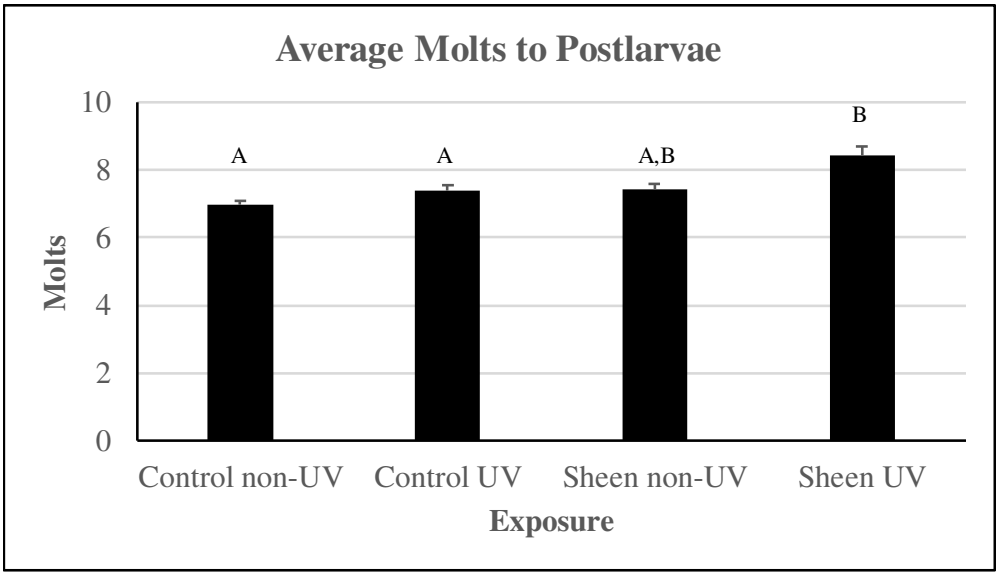

Figure 3. The total number of grass shrimp males, females, and pairs in the four exposure groups.

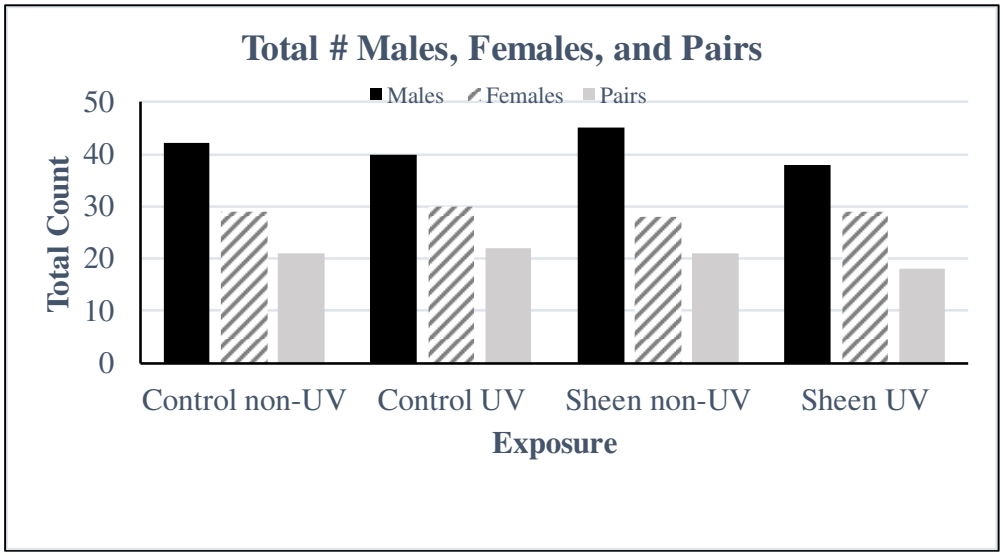

Figure 4. The average percent of female shrimp to become gravid in the four exposure groups. Error bars represent standard error of the mean. There were no statistical differences between the exposure groups.

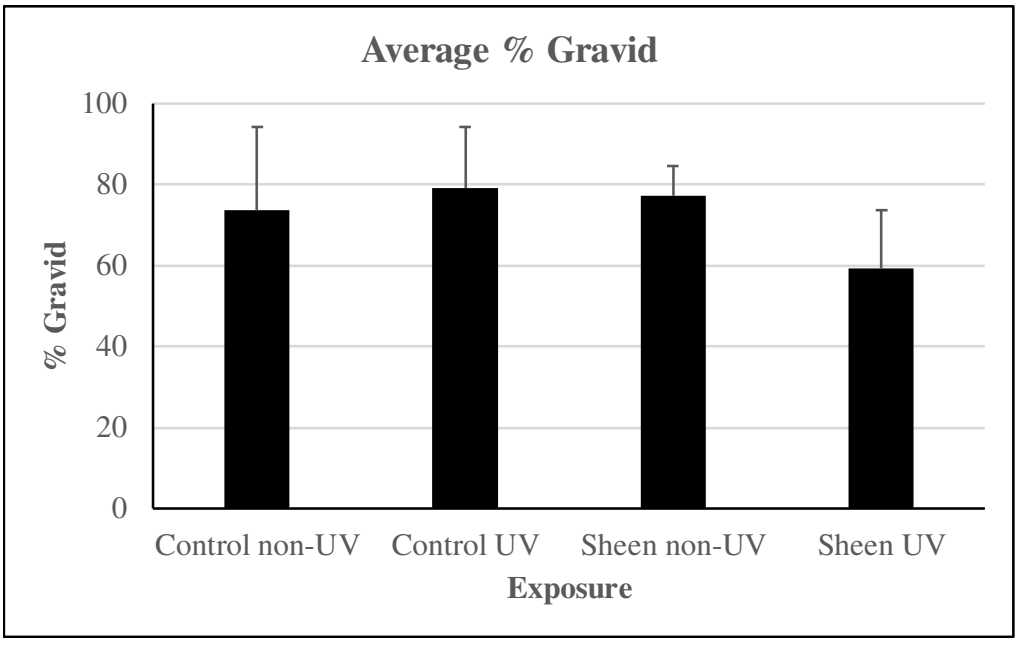

Figure 5. The average number of days it took the females to become gravid in the four exposure groups. Error bars represent standard error of the mean. There were no statistical differences between the exposure groups.

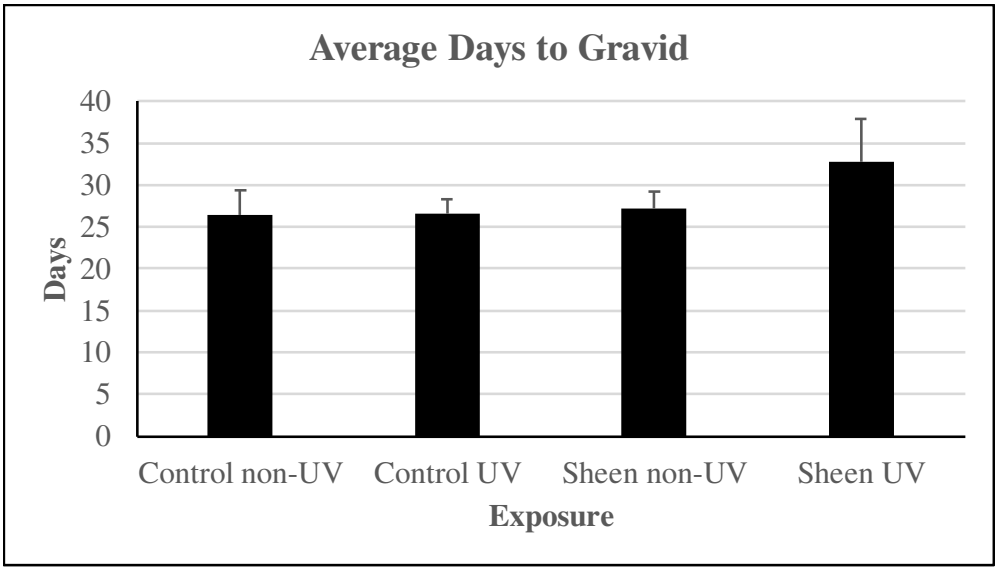

Figure 6. The cumulative number of gravid females versus the number of days it took to become gravid after pairing in the four exposure groups. The slope of the Sheen UV treatment was significantly lower than the slopes of the other treatments ($p<0.0001$).

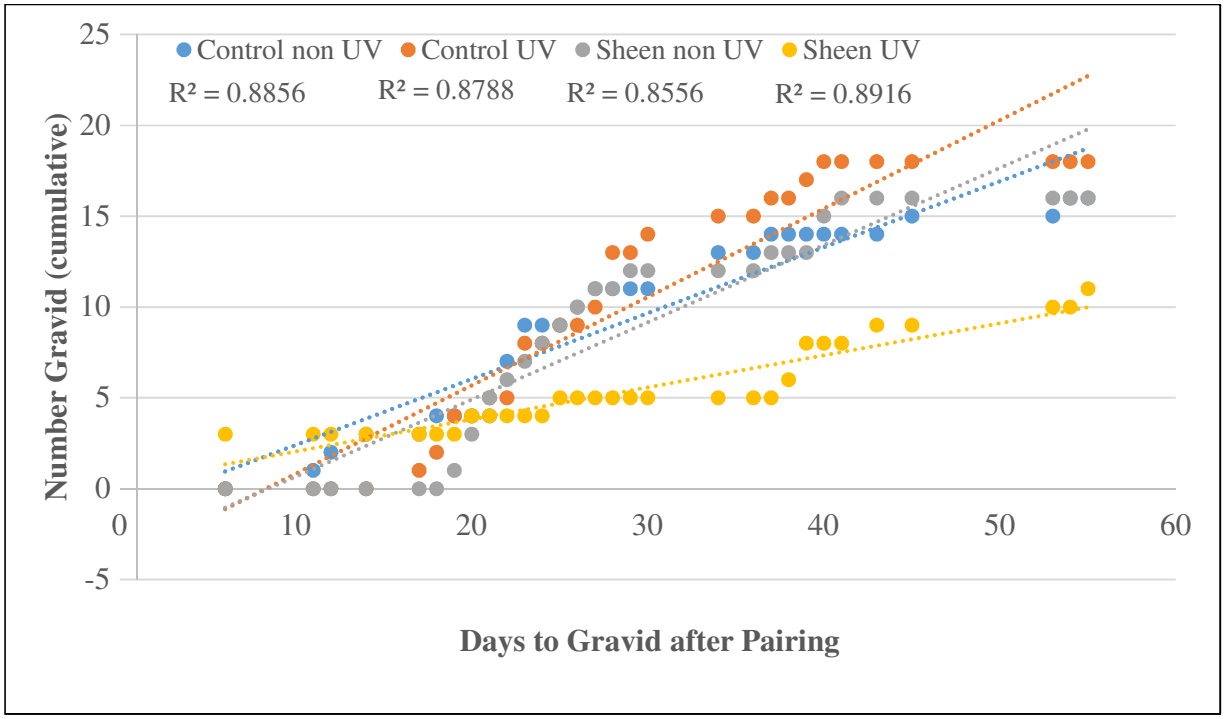

Table 1. The parent and alkylated PAH analytes measured in total PAH50 as defined by Boehm (2006).

PAH50 Analytes	
Parent PAH	**Alkylated PAH**
naphthalene	C1-naphthalenes
biphenyl	C2-naphthalenes
Acenaphthene	C3-naphthalenes
acenaphthylene	C4-naphthalenes
fluorene	C1-fluorenes
dibenzofuran	C2-fluorenes
dibenzothiophene	C3-fluorenes
phenanthrene	C1-dibenzothiophenes
anthracene	C2-dibenzothiophenes
fluoranthene	C3-dibenzothiophenes
pyrene	C4-dibenzothiophenes
benz(a)anthracene	C1-phenanthrenes/anthracenes
benzo(b)naphtho(2,1-d)thiophene	C2-phenanthrenes/anthracenes
chrysene+triphenylene	C3-phenanthrenes/anthracenes
benzo(a)fluoranthene	C4-phenanthrenes/anthracenes
benzo(b)fluoranthene	C1-fluoranthenes/pyrenes
benzo(j)fluoranthene	C2-fluoranthenes/pyrenes
benzo(k)fluoranthene	C3-fluoranthenes/pyrenes
benzo(a)pyrene	C4-fluoranthenes/pyrenes
benzo(e)pyrene	C1-chrysenes/benzanthracenes
dibenzo(a,h)anthracene	C2-chrysenes/benzanthracenes
indeno(1,2,3-c,d)pyrene	C3-chrysenes/benzanthracenes
benzo(g,h,i)perylene	C4-chrysenes/benzanthracenes
	C1-naphthobenzothiophenes
	C2-naphthobenzothiophenes
	C3-naphthobenzothiophenes
	C4-naphthobenzothiophenes

Table 2. The average percent (%) survival of larvae after 24-h exposure, average % survival of larvae to postlarval status, and average % of larvae becoming postlarvae by Day 21 all after 24-h exposure to four treatments. Standard error (SE) is in parentheses. Note the lag in development at Day 21 of larvae from the sheen treatments as compared to the controls.

Treatment	**% Larvae Survival after 24 h Exposure (SE)**	**% Survival to Postlarvae (SE)**	**% Larvae becoming Postlarvae by Day 21 (SE)**
Control non-UV	100 (0)	98.3 (1.7)	60 (3.6)
Control UV	100 (0)	93.3 (4.2)	41.7 (7.5)
Sheen non-UV	98.3 (1.7)	88.3 (3.1)	26.1* (6.4)
Sheen UV	98.3 (1.7)	85 (5.6)	9.3** (3.4)

* Significantly different from Control non-UV ($p=0.0016$)

**Significantly different from Control UV ($p=0.0026$) and Control non-UV ($p<0.0001$)