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Sublethal Effects of Crude Oil and Chemical Dispersant on the Eastern Oyster (Crassostrea virginica) at Multiple Life History Stages

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Sublethal Effects of Crude Oil and Chemical Dispersant on
the Eastern Oyster (*Crassostrea virginica*) at Multiple Life History Stages

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Biology
with a concentration in Ecology & Evolution
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DEDICATION

I dedicate this thesis to my loving parents, Joe and Irene Garcia. Their strong work ethic and unfailing ability to provide the best for their children will always motivate me. I will forever be grateful for their endless friendship and support.
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ABSTRACT

Oil spills in the marine environment can threaten vulnerable ecosystems that support ecologically and economically significant organisms, such as the eastern oyster (Crassostrea virginica), in coastal habitats. The use of chemical dispersant (Corexit 9500) was applied as a cleanup effort in response to the Deepwater Horizon blowout to minimize crude oil slicks, but also resulted in increased concentrations of polycyclic aromatic hydrocarbons in the water column. The effects of increased soluble fractions of crude oil and dispersant components may be harmful to marine organisms. This study aimed to investigate possible sublethal impacts to the eastern oyster at multiple life history stages in order to understand potential implications on performance at an organismal, population, and ecosystem levels. Specifically, this study addressed 1) veliger swimming, 2) pediveliger settlement rates, 3) pollutant induced larval inactivity and 4) adult clearance rates after acute exposures to relevant concentrations (10 – 100 µL L⁻¹) of water accommodated fractions of crude oil (WAF) and with a combination of chemical dispersant (CEWAF). No significant differences were observed in any tested swimming kinematics between controls and WAF or CEWAF treatments after 24 hour exposures for early staged veligers at concentrations up to 100 µL L⁻¹ WAF and CEWAF. However, settlements rates of competent pediveligers were significant decreased compared to control (52.1 % s.d. 1.66) rates at concentrations of 50 µL L⁻¹ WAF (30.9% s.d. 6.16) and 10 (41.2 % s.d. 0.857) and 50 (22.0% s.d. 1.23) µL L⁻¹ CEWAF. Later staged larvae also showed increased vulnerability to oil pollution given that a higher percentage of organisms were inactive (48.3% s.d. 4.80) compared to early
staged larvae (12.7% s.d. 7.68) after initial exposure at 50 µL L\(^{-1}\) CEWAF. Based on this result, we assumed effects of oil pollution were not manifested until the later larval life history stage evident by metamorphosis failure during the complex settlement transformation that results in reduced spat and eventually reduced adult oysters.

Adult oysters were also exposed to increasing concentrations of WAF and CEWAF for 24 hours and feeding experiments were conducted in both clean seawater and the same oiled seawater conditions as their initial exposure. Oysters fed in oiled seawater had decreased clearance rates, but oysters fed in clean water had increased clearance rates, suggesting feeding efficiency can be returned to control rates when moved to the presence of clean water. However, our long term study conducted in clean seawater suggested of the oysters exposed to crude oil only (9.31 L h\(^{-1}\) g\(^{-1}\) s.d. 2.04) are able to return to clearance rates comparable to controls (7.69 L h\(^{-1}\) g\(^{-1}\) s.d. 1.89) after the 33 day time period but oysters exposed to crude oil with a combination of chemical dispersant (2.12 L h\(^{-1}\) g\(^{-1}\) s.d. 1.08) were not. Decreased feeding efficiency can have negative impacts on water quality in estuarine ecosystems that support productive habitats. Understanding the impacts of crude oil, and crude oil with a combination of chemical dispersant on ecologically significant organisms can aid in future oil spill response decisions in order to minimize environmental impacts.
INTRODUCTION

Oil Spills and the Marine Environment

The drilling, transportation, and exploration of crude oil in the oceans pose a threat to marine ecosystems. An oil spill can cause long-term damage to a variety of marine habitats, and the organisms that inhabit them. Oil spills can threaten ecologically and economically significant coastal ecosystems, such as estuaries, sea grasses beds, and beaches, that support marine life in addition to nearby human communities that rely directly on coastal oceans for tourism, fisheries, and aquaculture.

Many oil spills have occurred historically, however, they have varied greatly in location, magnitude, and composition of species impacted. Consequently, response and cleanup efforts are created to address characteristics of specific spills. For example, one of the most recent and well-studied oil spills was the Deepwater Horizon (DWH) oil spill that occurred from April 20, 2010 to July 15, 2010. The National Oceanographic and Atmospheric Administration (NOAA) estimated that up to 100,000 liters of crude oil was spilled per day into the Gulf of Mexico impacting approximately 1,100 kilometers of coastline ranging from Texas, Louisiana, Alabama, Mississippi and Florida (Graham et al. 2011). Oil spills in the Gulf of Mexico pose an increased threat of shoreline oiling compared to other offshore drilling locations. This is due to semi-enclosed shape of the Gulf of Mexico, as well as the presence of a loop current that can distribute surface and subsurface oil rapidly over large distances (Graham et al. 2011).
In an attempt to prevent crude oil from reaching vulnerable coastlines, cleanup strategies such as the use of booms, skimmers, burning, and chemical dispersants were implemented during the DWH oil spill. Chemical dispersants are powerful surfactants that alter the chemical and physical components of oil, reducing the surface tension between oil and water, and allow the oil to be emulsified and dispersed in the water column more easily with sufficient wave energy (NRC 2005; Li et al. 2008). An estimated 7,200,000 L of chemical dispersant (Corexit 9500 and Corexit 9527) were applied at the surface and another 2,900,000 L at the submerged wellhead point of release of oil (Graham et al. 2011; Kujawinski et al. 2011). While these methods work to enhance the effectiveness of oil dispersal, the persistence of both polycyclic aromatic hydrocarbons (PAHs) and other potentially harmful dispersant ingredients increase in the water column (Kujawinski et al. 2011; NRC 2005; Reddy et al. 2012). Once emulsified, the small droplets are unable to recoalesce and this may increase interaction of micro droplets with planktonic organism or sessile organisms that feed on small particulates (NRC 2005).

The impacts of this dispersed crude oil to marine organisms, however, have not yet been completely explored or understood. Understanding the magnitude to which the use of chemical dispersants may impact marine ecosystems can provide insight on how to more effectively respond to future oil spills. As offshore drilling operations and large scale transport of crude oil continues, the impacts that oil spills and subsequent cleanup efforts have on marine organisms and coastal ecosystems need to be better understood.

*The Eastern Oyster*

The Eastern oyster is a bivalve species found in coastal estuaries along the Gulf of Mexico and the Atlantic Ocean (Harry 1985). They are a meroplankton species, which spend a portion of
their life as planktonic larvae prior to metamorphosing into a sessile organism. Oysters typically spawn in early spring and develop within the first few hours after fertilization into a motile trochophore. Within two days post fertilization (dpf), the trochophore metamorphoses into a planktotrophic veliger. As a motile larva, veligers rely on their swimming capabilities to orient themselves in the water column in order to seek available food sources and avoid predation. Approximately 14 - 21 dpf, the veliger develops into a pediveliger, which is competent to settle and seek out suitable habitats. Once appropriate cues are detected, pediveligers undergo an energetically costly metamorphic transition during settlement and develop into a juvenile oyster, commonly referred to as a spat. Within the first year, they reach sexual maturity (Andrews 1979).

Adult oysters are sessile benthic organisms that can live in shallow and low intertidal coastal zones and must be tolerant to withstand fluctuations in abiotic factors such as tides, salinity, temperature, and dissolved oxygen (Lenihan et al. 1999). Adult oysters are filter feeding organisms that remove plankton and suspended sediments from the water column. The filter feeding of oysters can improve water quality for submerged aquatic vegetation and may impact local phytoplankton populations (Newell and Koch 2004). Oysters are typically found in large, structurally complex reefs that can serve as wave energy dissipaters and protect inland estuarine communities from storms by stabilizing existing sediments along the shoreline (Grabowski et al. 2012; Meyer et al. 1997). The structural diversity of the reef provides habitat for many marine organisms, such as fish and crabs (Tews et al. 2004) and can also serve as a hard substrate for recruitment (Peterson et al. 2003). Adult oysters, and other bivalves, are an important link in estuarine food webs and are consumed by resident crab (Seed 1980), fish, and bird species (Coen et al. 1999; Baird et al. 2004).
In addition to the oyster’s ecological contributions, they are an important economic driver for coastal communities. Annual landings in the United States are worth roughly $17.3 million, with approximately 67% of the nation’s oyster landings coming from the state of Louisiana alone (VanderKooy 2012; Graham et al. 2011).

**Impacts of Crude Oil and Chemical Dispersant to Marine Organisms**

Oil pollution can threaten the development and survival of marine organisms at multiple life history stages. A variety of literature exists concerning lethal effects of crude oil and dispersants on marine organisms, however there is comparatively little information regarding the sublethal effects on larval staged marine organisms. Understanding sublethal impacts for larval organisms is essential as this is a crucial life history stage for growth and development. Consequently, many larval invertebrates (e.g. *Artermia salina*, *Crassostrea gigas*, and *Paracentrous lividus*) have demonstrated an increased sensitivity to pollutants, such as exposure to heavy metals or crude oil, compared to adults (Fichet et al. 1998; Saiz et al. 2009).

Negative impacts on the development of larval organisms have also been detected after exposure to crude oil and chemical dispersants. The heightened concentration of PAHs in the water column, due to the use of dispersants, have been found to cause a variety of physiological stressors, including narcosis, oxidative stress, and developmental impacts on larval and zooplankton species (Couillard et al. 2004; Webby et al. 2016). The addition of chemical dispersants to crude oil causes a synergistic effect to occur and can increase toxicity up to 52-fold (Rico-Martinez et al. 2013). As a result, decreased mobility, feeding, and growth rates have been observed in blue crabs (Pie and Mitchelmore 2015), copepods (Almeda et al. 2016; Saiz et al. 2009), and barnacle nauplii (Almeda et al. 2014) after exposure.
Similar effects on growth and development have been observed in larval oysters after crude oil and chemical dispersant pollution. Morris et al. (2015) observed a decrease in fertilization rates to eastern oyster gametes after exposure to crude oil in the water column. Embryos and veligers showed increased developmental abnormalities after exposure to crude oil, and these effects intensified with the addition of chemical dispersant (Laramore et al. 2014; Vignier et al. 2015).

Crude oil and dispersant exposure have been found to cause negative impacts on the membranes of larval organisms and inhibit successful metamorphosis in the process of embryogenesis (Vignier et al. 2015) and settlement (Vignier et al. 2016). Reduced ability for metamorphosis has also been observed in amphibians (Mahaney 1994), Pacific herring (Smith and Cameron 2011), lobsters (Capuzzo et al. 1984), zebrafish (Hicken et al. 2011), corals (Kushmaro et al. 1997) and crustacean (Katz 1973) species after petroleum contamination. It is important to recognize sources of mortality during larval stages, because it can signify a future bottleneck of the population (Williamson 1993; Carassou et al. 2014).

Marine zooplankton, such as copepods, have been observed to ingest crude oil droplets, which could introduce PAHs into higher trophic levels of the marine food web (Almeda et al. 2014). Comparatively, adult bivalves can filter feed on these potentially oiled plankton and it can possibly affect the organism’s ability filter water efficiently. Previous studies have detected degraded hydrocarbons in the tissues of bivalve species after exposure to oil that is present either in the sediment or water column (Blumer et al. 1970; Stagleman and Teal 1973; Boehm and Quinn 1976). Although, bivalves are able to metabolize PAHs internally, it is energetically costly and could result in low clearance rates. The organisms expend more energy towards the degradation process instead of feeding (Kim et al. 2007; Petushok et al. 2001).
**Research Objectives**

The purpose of this study was to determine the sublethal effects of the water accommodated fraction (WAF) of crude oil and the chemically enhanced water accommodated fraction (CEWAF) containing chemical dispersant on the eastern oyster (*Crassostrea virginica*) at multiple life history stages. Specifically, this study addressed: 1.) veliger swimming kinematics (cruising speed, sinking speed, and total distance traveled), 2.) pediveliger settlement rates, and 3.) adult clearance rates after acute exposure to ecologically relevant concentrations of WAF and CEWAF. Results from this study will aid in the understanding of the biological impacts associated with crude oil pollution on an ecologically and economically important species and in making crucial response decisions in the event of a future spill.
METHODS

Preparation of WAF and CEWAF

Louisiana Light Sweet Crude oil, a surrogate for the Macondo (MC252) crude oil released in the DWH oil spill, was used in all experiments. Corexit 9500A (Nalco/Exxon Energy Chemicals, L.P.) was used as the chemical dispersant, as it was the primary dispersant used during remediation operations during the DWH oil spill. It was applied at a 20:1 oil to dispersant ratio, as recommended by the manufacturer.

The water-accommodated fraction (WAF) treatments were created using a low energy mixing method (Tjeerdema et al. 2010). WAF treatments contain the soluble fraction of crude oil. Stock solutions were made in a 4 L beaker containing 3 L of artificial seawater (ASW). Water was circulated on a stir plate at 60 - 100 rpm, then a known amount of crude oil was inserted using a pipette. The mixture was allowed to stir for 21 hours, then left undisturbed for three hours to allow time for possible droplets to rise to the surface.

The chemically enhanced water-accommodated fraction (CEWAF) treatment were created using a high mixing energy method (Tjeerdema et al. 2010). Briefly, stock solutions of 3L of ASW were brought to a vortex of 25% water depth in a 4 L beaker on a stir plate mixed at 350rpm. Known amounts of crude oil and chemical dispersants were added and the solution mixed for 21 hours, then settled undisturbed for three hours to allow time for droplets to rise to the surface. For both WAF and CEWAF treatments, only the supernatant was used in experiments as per Tjeerdema et al. (2010).
The tested concentrations for this experiment range between 10 to 100 µL L\(^{-1}\) of crude oil. These values are within the recorded range of crude oil concentrations (24 – 710 µL L\(^{-1}\)) detected historically in the water column 24 hours after an oil spill (Law et al. 1997; Reddy et al. 2012). Additionally, these concentrations are considered sublethal because they are well below the lethal concentrations (215 - 1,300 µL L\(^{-1}\)) detected for pediveliger after 24 hours of exposure (Laramore et al. 2014).

**Veliger Swimming**

Oyster larvae were obtained from Southern Cross aquaculture facility in Cedar Key, Florida, USA and immediately transported back to the laboratory. Upon arrival, the stock was diluted into two 20 L aquaria, fed with *Isochrysis galbana*, and gently aerated. Larvae were examined under a stereo microscope to ensure mobility prior to use in experiments. Two hundred veligers of visually similar size were selected and transferred via soft pipette into 1 L glass bottles for experiments. Three replicates of each treatment were performed to ensure data robustness. A Student’s T-test was conducted between replicates of similar treatments in order to determine possible container effects and no effects were detected. The crude oil concentrations tested were 10, 50, and 100 µL L\(^{-1}\). The CEWAF treatments had crude oil at concentrations of 10, 50, and 100 µL L\(^{-1}\) and the addition of dispersant at concentrations of 0.5, 2.5, and 5 µL L\(^{-1}\), respectively. Veligers were exposed for 24 hours then collected from each bottle by gently filtering through 41 µm mesh sieve and placed in a petri dish for analysis under a stereo microscope.

The number of recovered veligers per bottle was recorded and each was designated as active or inactive. A veliger was counted as active if it was swimming and inactive if it appeared to be non-responsive as in Laramore et al. (2014).
Active veligers from each condition were transferred to a filming cuvette containing clean ASW. A high resolution camera (Edgartronic SC1) was used to record veliger swimming and illumination was provided by infrared (808 nm) LED lighting in an otherwise unlit room so as not to influence the swimming behavior of these phototactic organisms. Each experimental vessel was filmed for a total of 60 seconds at a frame rate of 60 frames per second.

Analysis of video recordings were performed using ImageJ software. The particle tracking feature was used to identify individual trajectories of swimming veligers to be analyzed. Mean cruising speed, sinking speed, and total displacement of identified trajectories were calculated using MATLAB software. As sinking occurs at higher speed than cruising, a cut-off speed of 2.00 mm s\(^{-1}\) was used in order to distinguish between cruising and sinking behavior. Differences in means between treatment groups were investigated using a One-way Analysis of Variance test (ANOVA) with Holm-Sidak Pairwise Test comparisons to determine where significant differences occurred. In cases where only differences between controls and experimental groups were considered, the results of the Holm-Sidak Pairwise test are presented. Significant differences were considered at \(\alpha = 0.05\). In all cases, data were tested for normality using Shapiro-Wilks test and equal variance using Brown-Forsyth test.

**Pediveliger Settlement**

Competent eyed pediveliger larvae were obtained from Research Aquaculture Inc. in Tequesta, Florida, USA and immediately transported back to the laboratory. Pediveligers were given 24 hours to acclimate prior to experimentation. Two hundred pediveligers of visually similar size were selected and transferred via soft pipette into 1 L glass bottles for experimental trials. Three replicates of each treatment were performed.
The crude oil concentrations tested were 10 and 50 µL L⁻¹. The CEWAF treatments included crude oil at concentrations of 10 and 50 µL L⁻¹ and the addition of dispersant at concentrations of 0.5 and 2.5 µL L⁻¹, respectively. Pediveligers were exposed for 24 hours then gently collected from each bottle using a 320 µm mesh sieve and placed in a petri dish for analysis under a stereo microscope to determine whether each was active or inactive. Active (swimming) individuals were then transferred to respective 20 L aquaria filled with 1.5 L of ASW. Ceramic tiles were placed on the bottom of the aquaria to allow a hard substrate for settlement. Larvae were gently aerated for 48 hours to provide time for settlement. *Isochrysis galbana* (approximately 200 cells ml⁻¹) was added daily as a food source. After 48 hours, pediveligers were filtered out with a 320 µm mesh sieve and analyzed. The number of settled spat on each surface was recorded, as well as the numbers of swimming and inactive individuals remaining in each trial. Differences in mean settlement success and swimming activity were compared using an ANOVA with Holm-Sidak Pairwise Test comparisons to determine where significant differences occurred. In cases where only differences between controls and experimental groups were considered, the results of the Holm-Sidak Pairwise test are presented. Significant differences were considered at $\alpha = 0.05$. In all cases, data was tested for normality using Shapiro-Wilks test and equal variance using Brown-Forsyth test.

**Adult Clearance Rate**

Adult oysters were collected from Upper Tampa Bay Park in Tampa, Florida, USA. Oysters were immediately transferred back to the laboratory and drip acclimated for 15 minutes to a salinity of 20, measured by a calibrated refractometer, to ensure all oysters were tested at the same salinity. Before experimentation, all algae, sediment, epiphytes, and fouling organisms (ex.
barnacles and other small oysters) were removed from the exterior of the shells. Oysters were
given 24 hours to acclimate to laboratory conditions before exposure to treatments.

For short term (24 hour) experiments, six oysters of visually similar shell length were
chosen for each treatment. The crude oil concentrations tested were 25, 50, and 100 µL L⁻¹. The
CEWAF treatments had crude oil at concentrations of 25, 50, and 100 µL L⁻¹ and the addition of
dispersant at concentrations of 1.25, 2.5 and 5 µL L⁻¹, respectively. Animals were exposed for 24
hours in 3 L of water with three oysters in each experimental treatment. A Student’s T-test was
conducted between replicates of similar treatments in order to determine possible container effects
and no effects were detected. After exposure, clearance rate tests were performed in either clean
seawater or in the same conditions as the 24 hour exposure treatments. Additionally, a longer term
study was conducted over 33 days after an acute 24 hour exposure to determine recover rates from
sublethal exposures. The crude oil concentration tested was 100 µL L⁻¹ and the addition of
chemical dispersant at 5 µL L⁻¹. Oysters were exposed for 24 hours, then maintained in clean
seawater within a 38 L aquaria, at a salinity of 20, with recirculating filtration over the duration of
the experiment. *Isochrysis galbana* (approximately 1,000 cells ml⁻¹) was added daily as a food
source. Clearance rate measurements were conducted in filtered sea water 1, 2, 3, 5, 12, 19, and
33 days after the initial exposure.

Clearance rate measurements were conducted in 1.5 L Carolina dishes with one oyster per
dish. Oysters were allowed to acclimate 30 minutes prior to the introduction of the microalgal food
as in Dupuy et al. (1999). *Isochrysis galbana* was introduced at approximately 1000 cells mL⁻¹ and
water aliquots of 5 mL were taken from each dish at 0 and 30 minutes. Aliquots were preserved in
a 5% Lugol’s Iodine solution for quantification. The shell length (mm) of each oyster was
measured using digital calipers and oysters were shucked and internal tissues were removed. Wet
tissue weight was recorded. Samples were transferred to a drying oven and allowed to dry for 24 hours at 180 °C. After the 24 hours, crucibles containing tissues were weighed and dry tissue weights were calculated.

Cell counts of preserved *Isochrysis galbana* samples were completed using a 1 mL Sedgewick Rafter counting chamber. The size independent clearance rate ($F_{NW}$, L h$^{-1}$) was calculated with the following equation:

$$F_{NW} = \frac{\ln C_0 - \ln C_30}{t - t_0} \times V$$

The initial ($C_0$) and final ($C_t$) concentration are recorded in number of cells per liter. The volume of suspension (V) for experiments was 1.3 L and the amount of elapsed time ($t-t_0$) was recorded in hours. Since the above clearance rate value ($F_{NW}$) does not standardize the rates based on oyster size or weight, an additional calculation was required. Using dry weight, $W$, and a standardized constant used for clearance rate calculations, $b = 0.73$ for the eastern oyster, a size specific clearance rate can be calculated (Dupuy et al. 1999).

$$F_{WW} = \frac{F_{NW}}{W^b}$$

Clearance rates ($F_{WW}$) were calculated for each condition at varying time points and treatment of water for feeding portion. Statistical comparisons of mean clearance rates were made using a one-way ANOVA with Holm-Sidak Pairwise Test comparisons to determine where significant differences occurred or in some cases, a Student’s t-test when only two groups were considered. In cases where only differences between controls and experimental groups were considered, the results of the Holm-Sidak Pairwise test are presented. Significant differences were considered at $\alpha = 0.05$. Normality of the dataset was confirmed using Shapiro-Wilks test and equal variance was confirmed using the Brown-Forsyth test.
RESULTS

*Veliger Swimming*

Kinematic measurements on early staged oyster larvae revealed that exposure to environmentally relevant concentrations of soluble fractions of crude oil and Corexit 9500 had little impact on swimming capabilities after 24 hours. Oyster veligers undergo an alternative swim-sink swimming pattern where these periods are distinctive from each other, differing greatly in speed. Sinking occurred at a magnitude approximately 6-fold higher than swimming. Mean cruising speed of the control group (n = 57, 0.467 mm s\(^{-1}\) s.d. 0.232) was not significantly different (ANOVA: F = 2.09, P = 0.055) than any of the other treatment groups (Figure 1). Likewise, there was no significant difference (ANOVA: F = 3.10, range: P = 0.281 – 0.944) observed in mean sinking speed of the control group (n = 46, 2.802 mm s\(^{-1}\) s.d. 0.704) compared to any of the other tested treatments (Figure 2). The percentage of time that veligers spend cruising versus sinking also did not vary significantly (ANOVA: F = 1.81, P = 0.102; ANOVA: F = 1.109, P = 0.361, respectively) between any of the experimental treatments. This resulted in no observed significant differences in total displacement of swimming larvae in any of the different treatments (ANOVA: F = 2.00, range: P = 0.066) (Figure 3).
**WAF/CEWAF Induced Inactivity**

Earlier staged veligers were less impacted with respect to inactivity compared to later staged pediveligers that were competent to settle when exposed to environmentally relevant concentrations of soluble fractions of crude oil and Corexit 9500 (Figure 4; Figure 5). The percentage of inactive early stage veligers was expectedly low in the control treatments at 1.10% (s.d. 1.00, n = 3), but the mean inactivity was nearly an order of magnitude higher in the pediveliger control treatments at 9.60% (s.d. 2.30, n = 2). Inactivity in both developmental stages increased with WAF concentration but the effect appeared to be more pronounced in late stage pediveligers. This was evident by the fact that we observed significantly higher number of inactive individuals at 10 µL L\(^{-1}\) WAF relative to the control treatments at the pediveliger stage (ANOVA: F = 21.67, range: P = 0.018) (Figure 5), but a significant difference in inactivity was not observed until 50 µL L\(^{-1}\) WAF with early stage veligers (ANOVA: F = 4.58, range: P = 0.041). Additionally, we observed no significant difference in inactivity (ANOVA: P = 0.173) between any of the WAF and corresponding CEWAF treatments of the same concentration. It should also be noted that all comparable treatments (control, 10 and 50 µL L\(^{-1}\) WAF and CEWAF), the inactivity of pediveligers was significantly higher than that of the early stage pediveligers (ANOVA: range: P = 0.003-0.028).

**Pediveliger Settlement**

We observed the highest percentage of settlement in control and 10 µL L\(^{-1}\) WAF treatments where mean settlement was 52.1% (s.d. 1.62, n = 2) and 51.4% (s.d. 5.56), respectively. While no significant differences (Student’s T-test: t = 0.174, P = 0.878) were found between the control
groups and the 10 uL L-1 WAF, all other treatment groups exhibited significantly (Student’s T-Test: range: t = 4.70 - 8.44, range: P = 0.014 - 0.042) lower settlement rates (Figure 6). It should also be noted that while not significantly different, the mean settlement was consistently lower when dispersants were added compared to oil only exposures at both 10 (Student’s T-test: t=2.57, P= 0.123) (WAF: 51.4% (s.d. 5.56, n = 2) vs. CEWAF: 41.2% (s.d. 0.85, n = 2) and 50 µL L^{-1} (Student’s T-test: t = 0.914, P = 0.457) (WAF: 30.9% (s.d. 6.16, n = 2) vs. CEWAF: 22.0% (s.d.12.3, n = 2).

**Short Term Adult Clearance Rates**

We generally observed a decrease in clearance rate for adult oyster when the feeding experiment was conducted in conditions that matched that of their initial 24 hour exposure. The clearance rate of WAF exposed oysters significantly decreased at 100 µL L^{-1} (Student’s t-test: t = 3.02 , P = 0.013) (n = 6, 2.01 L h^{-1} g^{-1} s.d. 0.56) relative to control rates (n = 6, 3.31 L h^{-1} g^{-1} s.d. 0.90). (Figure 7). Although not significant (Student’s t-test: t = 0.66, P = 0.524), clearance rate also decreased for organisms exposed 50 µL L^{-1} WAF (n = 6, 2.91 L h^{-1} g^{-1} s.d. 0.791) compared to control (n = 6, 3.53 L h^{-1} g^{-1} s.d. 1.24) rates. The only clearance rate that increased relative to control (n = 6, 2.73 L h^{-1} g^{-1} s.d. 0.678) when fed in oiled waters was at the lowest concentration of 25 µL L^{-1} WAF (n = 6, 3.51 L h^{-1} g^{-1} s.d. 0.781) and it was not a significant (Student’s t-test: t = -1.84, P = 0.095). When chemical dispersant was added, significant decreases in clearance rates were observed at lower concentrations than WAF. However, the clearance rates of WAF and CEWAF exposed oysters were not significantly different from each other at 25 (Student’s t-test: t = 1.98, P = 0.075), 50 (Student’s t-test: t = -1.06, P = 0.312), or 100 (Student’s t-test: t = 0.838, P = 0.421) µL L^{-1} in oiled waters. Significant declines in clearance rate compared to
controls were observed for oysters exposed to and fed at concentrations of 50 (Student’s t-test: \( t = 2.62, P = 0.025 \)) and 100 (Student’s t-test: \( t = 3.74, P = 0.004 \)) \( \mu \text{L L}^{-1} \) CEWAF (Figure 8).

Feeding experiments were also conducted in clean seawater to determine if the presence of oil components impacted organisms feeding efficiency. We generally observed increases in clearance rates for exposed organisms compared to controls when transferred to clean sea water. No significant differences in mean clearance rate was observed between control oysters and those exposed to soluble fractions of crude oil at the concentrations of 25 (Student’s t-test: \( t = -0.561, P = 0.587 \)), 50 (Student’s t-test: \( t = -1.54, P = 0.154 \)), or 100 (Student’s t-test: \( t =1.12, P = 0.287 \)) \( \mu \text{L L}^{-1} \) WAF exposed oysters (Figure 9). Although not significant, the only decrease in clearance rate occurred at the highest concentration of 100 \( \mu \text{L L}^{-1} \) WAF (\( n = 6, 2.67 \text{ L h}^{-1} \text{ g}^{-1} \) s.d. 1.75), relative to controls (\( n = 6, 3.56 \text{ L h}^{-1} \text{ g}^{-1} \) s.d. 0.824). When chemical dispersants were added, the clearance rates increased at all tested concentrations (Figure 10). Significant increases in clearance rates relative to control (\( n = 6, 3.17 \text{ L h}^{-1} \text{ g}^{-1} \) s.d. 0.889) rates was observed at 50 \( \mu \text{L L}^{-1} \) CEWAF (Student’s t-test: \( t = -3.425, P = 0.006 \)) (\( n = 6, 5.77 \text{ L h}^{-1} \text{ g}^{-1} \) s.d. 1.63).

**Long Term Adult Clearance Rates**

Given that clearance rates generally increased for WAF and CEWAF exposed oysters after 24 hour of exposure when fed in clean waters, we wanted to determine if high clearance rates were due to potential recovery or an initial response to the surrounding environment. An additional long term study was performed in clean water at the highest concentration of 100 \( \mu \text{L L}^{-1} \) WAF and CEWAF. Generally, the clearance rates of WAF exposed oysters did not significantly differ from those of control rates over the recovery period of 33 days (Student’s T-test: range: \( P = 0.086 \) -
0.208) (Figure 11). A significant decline in clearance rate was observed between controls (n = 6, 7.88 L h\(^{-1}\) g\(^{-1}\) s.d. 2.17) and those in 19 (Student’s T-test: t = 2.30 P = 0.044) (n = 6, 5.44 L h\(^{-1}\) g\(^{-1}\) s.d. 1.43) day treatments after initial exposure. Oysters initially exposed to treatments that included chemical dispersant exhibited clearance rates that were persistently lower than controls after five days during the recovery period (Figure 12). The clearance rates of CEWAF exposed oysters were significantly lower than those of controls after 12 (Student’s t-test: t = 3.74, P = 0.004), 19 (Student’s t-test: t = 4.83, P = < 0.001), and 33 (Student’s t-test: t = -6.26, P = < 0.001) days after exposure. Clearance rates of CEWAF exposed oysters were significantly lower than those of WAF exposed oysters on days 12 (Student’s t-test: t = 2.52, P = 0.030), 19 (Student’s t-test: t = -3.216, P = 0.009), and 33 (Student’s t-test: t = -6.26, P = < 0.001) post exposure (Figure 11; Figure 12).
DISCUSSION

Veliger Swimming

The ability to swim effectively is a necessity for larval organisms in order to find food, avoid predators, and seek suitable habitats. Many zooplankton species, such as copepods and barnacles, exhibit strong negative effects on swimming performance after exposure to oil pollution at concentrations much lower than those tested in this experiment (Almeda et al. 2014, Almeda et al. 2016; Donahue et al. 1977; Jiang et al. 2010; Jiang et al. 2012). For example, copepods exposed to dispersed crude oil at concentrations as low as 1 µL L⁻¹ for 48 hours experienced significant declines in growth and swimming speeds (Almeda et al. 2016) and showed signs of narcosis and loss of balance after WAF exposure (Cohen et al. 2014; Jiang et al. 2012). Reductions in phytoplankton consumption and fecal pellet production have been observed for copepods exposed to crude oil, likely due to reduced swimming ability (Hansen et al. 2012; Hansen et al. 2017). My study found no significant differences in swimming kinematics for early-staged oyster veligers as they appeared to be quite resistant to acute exposure of soluble fractions of crude oil and chemical dispersant (Figures 1; Figure 2; Figure 3). However, this appears to be somewhat consistent with other bivalve species which have been shown to be less vulnerable to sublethal concentrations of oil pollution (Steffansson et al. 2014). For planktotrophic larvae, effective swimming and other coordinated appendage movements are critical for growth and development. Given that the veligers in this experiment did not experience significant reductions in swimming kinematics or the percent of time spent cruising or sinking after 24 hour exposures to a wide array of crude oil
concentrations \((10 - 100 \mu L L^{-1})\), it could be assumed that they are able to successfully feed, maintain, and/or alter their position in the water column (Deksenieks et al. 1996).

Given that no significant impacts were detected between the cruising or sinking speeds between control and oil exposed veligers (Figure 1; Figure 2), it can be assumed that individuals able to withstand the initial exposure of WAF or CEWAF would likely be able to orient in the water column to feed normally and develop to competency. This is supported by a study where Laramore et al. (2014) exposed an eastern oyster veliger to a sublethal CEWAF concentration \((20 \mu L L^{-1})\) for 24 hours then monitored growth for three weeks in clean water. The results indicated that there were no severe impacts on development. However, some oyster veligers that have been exposed to pollutants have been observed to quickly retract their velum and close their valves, resulting in a drastic sinking behavior, preventing the organisms from being able to feed (Wisely and Blick 1967). In some cases, the valves shut before the velum had completely retracted and resulted in an exposed and protruding velum, resulting in a long term reduction in feeding efficiency (Wisely and Blick 1967; Vignier 2016). If an organism is unable to feed, it will not likely be able to survive.

In my study, I observed a lower percentage of inactive individuals after exposure to oil and oil with chemical dispersant at the veliger stages compared to the more developed pediveliger stages (Figure 4; Figure 5). These results differ from much of the previous literature where earlier staged organisms are generally found to be more vulnerable to pollutants compared to later staged organisms (Almeda et al. 2013; Saiz et al. 2009; George-Ares and Clark 2000; Goodbody-Gringley et al. 2013). Although no mortality was observed for either life history stage, inactive organism can be considered to be effectively dead because they are unable to swim, feed, and/or ultimately contribute to future populations. Based on this result, it is apparent the timing of oil
exposure relative to life history stage is critical in order to understand potential impacts on larval populations. Given that 50% of the exposed pediveligers were effectively dead after their settlement period (Figure 5), it could be expected that oil pollution may potentially induce a bottleneck effect on oyster populations.

It is important to note that although the organisms may not die from initial oil exposure, surviving pediveligers may have an enhanced risk of death due to predation by not metamorphosing and remaining in the planktonic stage for an extended period of time (Berias and His 1994). For example, sublethal effects such as decreased growth rates have been recorded for larval oysters that were exposed to oil pollution for longer time periods (48 - 96 hours) (Vignier et al. 2015; Vignier et al. 2016; Langdon et al. 2015; Salehi et al. 2017; Stefansson et al. 2016; Laramore et al. 2014; Jeong and Cho 2005). Although indicators of larval health can be observed by examining growth, development, or activity measurements, Langdon et al. (2016) argued settlement success was a better indicator of potential stress of meroplankton because it involves an energetically costly metamorphic change.

**Pediveliger Settlement**

The irreversible process of settlement for bivalves involves ending the planktonic life cycle phase and transitioning into a sessile adult. Functionality at the pediveliger stage is particularly important because they exhibit a dive-bombing, or rapid sinking, behavior in response to settlement cues in order to approach benthic substrate to seek habitats (Finelli and Wethey 2003). During the metamorphic period, both internal and external morphological alterations occur as crucial organs are rearranged in order to optimize the organism’s survival as an adult (Baker and
Mann 1994). Given the numerous and complex modifications that occur, crude oil pollution has the opportunity to induce adverse effects in pediveligers approaching competency. Competent pediveligers exposed to oil pollution for 24 hours in my study had significantly reduced settlement rates compared to control pediveligers. Decreased settlement has also been observed in pediveligers when exposed to WAF and CEWAF for 72 hours (Vignier et al. 2016). In the present study, increasing concentrations of crude oil caused a stronger negative effect on settlement success of pediveligers, but there was not an additional synergistic effect observed with the addition of chemical dispersant (Figure 6). This is opposite of what would be expected because CEWAF exposure has been observed to increase toxicity to larval organisms (Rico-Martinez et al. 2013).

Why do late stage oysters appear to be more sensitive to oil pollution than earlier developmental stages? Exposure to heavy metals and crude oil contaminated in the water column or sediments have been found to induce metamorphosis failure in bivalve species (Wisely and Blick 1967; Zhang 2002; Beiras and His 1994; His et al. 1997). A potential mechanism for metamorphosis failure is the destabilization of the phospholipid bilayer of an organism’s membrane by the presence of non-polar PAHs (Barron et al. 2001; Sikkema et al. 1995). Lipids stored within these cellular membranes are typically used as energy reserves in planktonic bivalve species during metamorphosis (Videla et al. 1998; Whyte et al. 1987; Chu et al. 1984; Farias et al. 1998; Haws et al. 1993; Rodriguez et al 1990; Garcia-Esquivel et al. 2001). Larval oysters must spend 5-7 days relying solely on reserves as an energy source, while appendages for feeding are being rearranged and formed. If an organism is unable to effectively utilize stored lipids and proteins needed for development and metabolism, it is unlikely that they will be able to undergo successful metamorphosis (Holland 1978; Capuzzo et al. 1984). It is possible the addition of
chemical dispersant intensified the inability to undergo metamorphosis given chemical dispersants have also been found to increase permeability of cellular membranes, allowing PAHs to enter and negatively impact fluidity and cellular functions (Wu et al. 2012). The proposed mechanism could also be responsible for decreased pediveliger settlement as observed in this experiment. Another potential inhibitor to settlement success in the natural environment could be the presence of an oil biofilm on oyster shells. Previous studies found that shells with oil coats were not suitable for pediveliger settlement and resulted in decreased spat abundance (Smith and Hackney 1989; Galtsoff 1964). Given that settlement rates of pediveligers decreases either when oil pollution is present as soluble fractions in the water column or as a biofilm slick, we can conclude that PAH presence can have negative effects on recruitment of competent oyster larvae. A large reduction in spat presence ultimately leads to a decreased adult populations.

**Adult Clearance Rates**

Adult oysters, like other sessile animals, are especially vulnerable to crude oil pollution since they are unable to move away from a toxicant. Consequently, their ability to effectively feed and be nourished can depend heavily on the conditions of their surrounding water. I found the clearance rates of oil exposed adults were decreased for both WAF and CEWAF treatments compared to controls after a brief 24 hour exposure, when feeding experiments were conducted in the same oiled conditions as the initial exposure (Figure 7; Figure 8). In order to determine if the decreased feeding response was due to the physical presence of oil WAF and CEWAF, adult oysters were also fed in clean seawater after an initial 24 hour exposure to soluble fractions of crude oil and chemical dispersant. This scenario represents a situation where an oil spill occurs then is rapidly diluted due to tidal and/or wind driven mixing. When oyster were
allowed to feed in filtered ASW conditions after their initial oil exposure, there was an observed increase in clearance rate for both WAF and CEWAF treatments after a 24 hour exposure (Figure 9; Figure 10). The increase in clearance rates was likely due to the organisms attempt to compensate for decreased feeding performed in the presence of oil (Kim et al. 2007). Bivalve species perform a variety of defense behaviors to prevent oil intrusion in order to reduce potential internal stress due to oil exposure.

Previous studies involving bivalve species have reported reduced feeding and selective ingestion when in the presence of a toxicant (Kim et al. 2007; Takayanagi et al. 2000). Another modified behavior that has been observed is altered valve gap movement. Mussels exposed to crude oil reduced the angle at which their valve gap opened and spent longer time periods closed compared to control mussels (Redmond et al. 2017). The presence of oil components inside adult oysters could impact clearance rates as a result of possible blockage of gills and pumping activity of lateral cilia, responsible for filtering (Axiax and George 1987). Bivalves are able to metabolize PAHs internally, but it is energetically costly and low clearance rates could be a result of selectively expending energy towards the degradation process instead of feeding (Kim et al. 2007; Petushok et al. 2001; Axiax and George 1987). These behaviors could be responsible for the observed decreases in clearance rate after 24 hours of exposure. Based on the result of the short term study (Figure 9; Figure 10), it would appear that adult oysters had the capacity to recover quickly and feed effectively after exposure to sublethal oil/dispersant concentrations once they were again in the presence of clean seawater. However, the results of the long term study suggested simply being transferred to clean seawater may not result in long term recovery of acutely exposed oysters. We observed that oysters exposed to a combination of crude oil and chemical dispersant are not able to recover to clearance rates comparable to
controls after 33 days, even when maintained continuously in clean seawater (Figure 11). Comparatively, oysters exposed to only crude oil were able to recover to clearance rates comparable to those of controls within the 33 day period when feeding in clean water (Figure 12). The results suggest that the addition of chemical dispersant was responsible for the organism’s inability to recover. There is a high probability that the organism is still contaminated after being transferred from oiled to clean seawater because PAHs can be retained in the lipid pools of internal tissues up to two months after exposure (Blummer et al. 1970; Soniat et al. 2011). One interesting result observed in the bivalve species of *Mya arenaria* and *Mytilis edulis* is that there were less PAHs present in the tissues of organisms exposed to both crude oil and chemical dispersant compared to crude oil alone (Gillifan et al. 1982). However, there was a heightened sublethal effect on enzymatic activity detected for organisms exposed to a combination of crude and chemical dispersant compared to crude oil only exposed organisms (Gillifan et al. 1982). The addition of chemical dispersant can disrupt chemical processes at a greater magnitude than the presence of crude oil alone in a variety of species at various life history stages (Almeda et al. 2014; Rico-Martinez et al. 2013). However, it has been proposed that although the effects on adult benthic invertebrates may be immediate and initially drastic, there is a possibility for complete depuration of accumulated hydrocarbons if given sufficient time to recover (Mageau et al. 1987). However, the aforementioned study did not measure feeding rates. Thus, it is not currently known if feeding rate of oysters exposed to crude oil and chemical dispersant are capable of full recovery in the long term (months to years). The ability to successfully metabolize PAHs may depend on the duration of initial exposure to soluble fractions of crude oil and chemical dispersant that in turn impact the magnitude and persistence of negative physiological and cellular responses (Widdows et al. 1981; Soniat et al. 2011).
Additional clearance rate studies may ultimately be required to determine if recovery is possible for oysters if given even longer time periods to recuperate.

**Environmental Consequences and Conclusions**

Adult oysters can be used as a bio-indicator for the health and recovery of estuarine ecosystems after an oil spill. Non-deleterious measurements such as clearance rates can help signify if adult oysters have recovered from oil pollution. Understanding potential impacts to adult populations can be indicative of the reproductive output they will be able to yield to support future generations.

After the DWH event, significant declines in the density of oyster spat and market sized adults were observed between pre (2006 - 2009) and post (2010 - 2012) spill observations from Louisiana to Florida. The cause of mortality for adult oysters after DWH was predicted to be persistent exposure to low salinities as a result of a freshwater diversion in order to prevent further oiling into the estuaries (Grabowski et al. 2017). Low densities observed at all life history stages imply reproduction and recruitment efforts have not been successful and presents a major challenge for oysters to recover naturally. In the presence of oil pollution, the energy budget of an adult oyster shifts towards metabolizing PAHs and allocates less energy towards growth and reproduction (Baas et al. 2010; Widdows and Donkin 1991). A decrease in fecundity, or reproductive output, per adult results in less gametes available to create future populations. Decreased fertilization rates have been observed for oyster gametes released as a result of oil exposure (Vignier et al. 2017; Morris et al. 2015). Decreases in settlement rates of oil and dispersant exposed pediveligers were observed (Figure 6) and we can assume there was a
disruption in metamorphic process after contact with a pollutant. The observed decrease in settlement ultimately can result in decreases in spat populations and eventually, adult oysters. Loss of adult oysters also leads to reduced shell cover and less available settlement locations for pediveligers (Powers et al. 2015).

Persistently low recruitment can lead to detrimental consequences for oyster populations and surrounding ecosystems. Estuaries will suffer given that oysters are keystone species in these habitats. In the absence of adult oysters, the water quality of the estuaries they inhabited would be negatively impacted. Results from this study suggest adult oysters exposed to a combination of crude oil and chemical dispersant can result in a long term reduction in clearance rates (Figure 12). As a result of decreased feeding, individuals exhibit slower growth rates and reductions in protein utilization (Axiax and George 1987). Deceased filtration rates could cause increased turbidity of water, causing less sunlight to be available to submerged aquatic vegetation, such as seagrass beds that support many juvenile marine organisms. A vital habitat for invertebrates and larval organisms is eliminated if oyster beds are no longer present, thus decreasing both species diversity and abundance (Crowe et al. 2004). Overall, oysters play an integral role in maintaining ecologically productive coastal ecosystems.

In conclusion, understanding the sublethal effects that occur at multiple life history stages is critical in determining potential impacts to oyster populations after an oil spill. No significant impacts to veliger swimming kinematics were observed. However, there was a significant decrease in settlement rates of pediveligers exposed to realistic concentrations of soluble fractions of crude oil and chemical dispersant. Given that oyster larvae at a later stage were observed to experience a greater impact from oil pollution compared to earlier staged larvae, it was predicted that effects of oil exposure may not be manifested until a later developmental stage, such as when
metamorphosis should occur. A reduction in later staged larvae can cause a bottleneck effect for future oyster populations. Reduced efficiency in clearance rate with the addition of chemical dispersants can cause negative impacts on the surrounding marine environment.

The findings from this study contribute to previous research conducted on the Eastern oyster (*Crassostrea virginica*) and exposure to oil pollution. Based on my study, only adults appeared to have an exasperated effect on performance due to the addition of chemical dispersants. Such results should be considered by emergency responders to oil spills in order to minimize impacts to both future adult oyster populations and the marine environment. The consequences of reduced oyster populations after an oil spill can severely impact economically and ecologically significant coastal ecosystems.
Figure 1. Mean cruise speed of veligers calculated after 24 hour exposure to varying concentrations of crude oil (WAF) and crude oil and addition of chemical dispersant (CEWAF). An organism was considered cruising if its instantaneous velocity was < 2.0 mm s\(^{-1}\). Error bars represent standard deviation.
Figure 2. Mean sinking speed of veligers calculated after 24 hour exposure to varying concentrations of crude oil (WAF) and crude oil and addition of chemical dispersant (CEWAF). An organism was considered cruising if its instantaneous velocity was $> 2.0 \text{ mm s}^{-1}$. Error bars represent standard deviation.
Figure 3. Mean total displacement traveled by veligers after 24 hour exposure to varying concentrations of crude oil (WAF) and crude oil and addition of chemical dispersant (CEWAF). Total displacement was calculated by determining the distance traveled in measured trajectory. Error bars represent standard error.
Figure 4. Veliger inactivity after 24 Hour Exposure. Mean percentage of inactive veligers observed after 24 hour exposure to varying concentrations of crude oil (WAF) and crude oil and addition of chemical dispersant (CEWAF). An organism was considered inactive if it did not appear to be swimming around, yet had an active internal heartbeat. Error bars represent standard deviation. Stars (*) denote significant differences between controls and treatment.
Figure 5. Pediveliger Inactivity. Percentage of inactive pediveligers observed after given 48 hour hours to settle after brief 24 hour exposure to varying concentrations of crude oil (WAF) and crude oil and addition of chemical dispersant (CEWAF). Individuals that did not settle were categorized as remained swimming or inactive. Error bars represent standard deviation. Stars (*) denote significant differences between controls and treatment for and inactivity.
Figure 6. Pediveliger Settlement. Pediveligers were given 48 hours to settle after 24 hour exposure to varying concentrations of crude oil (WAF) and crude oil and addition of chemical dispersant (CEWAF). Settlement is presented as a percentage of total organisms. Error bars represent standard deviation. Stars (*) denote significant differences between controls and treatment.
Figure 7. Clearance Rate of WAF Oysters in Oiled Water. Mean clearance rates observed after adult oysters exposed to crude oil (WAF) for 24 hours at increasing concentrations. Feeding experiment was conducted in oiled water at the same concentration of the exposure. Error bars represent standard deviation. Stars (*) denote significant differences between controls and treatment.
Figure 8. Clearance Rate of CEWAF Oysters in Oiled Water. Mean clearance rates observed after adult oysters exposed to crude oil and a combination of chemical dispersant (CEWAF) for 24 hours at increasing concentrations. Feeding experiment was conducted in oiled water at the same concentration of the exposure. Error bars represent standard deviation. Stars (*) denote significant differences between controls and treatment.
Figure 9. Clearance Rate of WAF Oysters in Clean Water. Mean clearance rates observed after adult oysters exposed to crude oil (WAF) for 24 hours at increasing concentrations. Feeding experiment was conducted in clean water. Error bars represent standard deviation. Stars (*) denote significant differences between controls and treatment.
Figure 10. Clearance Rate of CEWAF Oysters in Clean Water. Mean clearance rates observed after adult oysters exposed to crude oil and a combination of chemical dispersant (CEWAF) for 24 hours at increasing concentrations. Feeding experiment was conducted in clean water. Error bars represent standard deviation. Stars (*) denote significant differences between controls and treatment.
Figure 11. Long Term WAF Clearance Rate. Mean clearance rates observed of adult oysters after acute exposure to 100 µL L\(^{-1}\) crude oil (WAF) for 24 hours, and then returned to clean water for the duration of the experiment. Feeding experiments were conducted in clean water 1, 2, 3, 5, 12, 19, and 33 days after exposure. Error bars represent standard deviation. Stars (*) denote significant differences between controls and treatment.
Figure 12. Long Term CEWAF Clearance Rate. Mean clearance rates observed of adult oysters after acute exposure to 100 µL L⁻¹ crude oil and a combination of chemical dispersant (CEWAF) for 24 hours, and then returned to clean water for the duration of the experiment. Feeding experiments were conducted in clean water 1, 2,3,5,12,19, and 33 days after exposure. Error bars represent standard deviation. Stars (*) denote significant differences between controls and treatment.
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