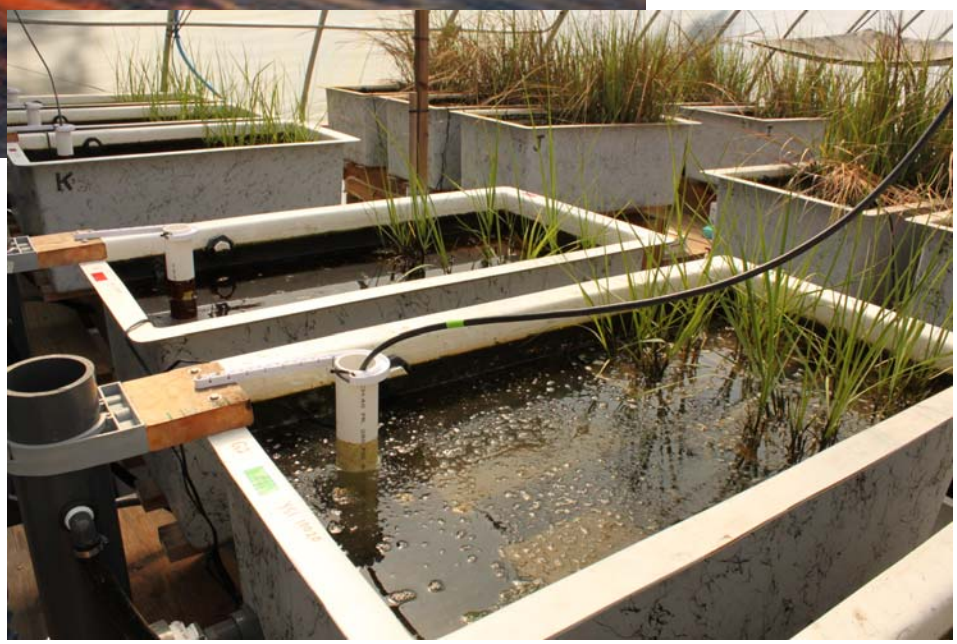
Assessment of Crude Oil and a Dispersant in a Simulated *Spartina alterniflora* Salt Marsh Ecosystem



NOAA Technical Memorandum NOS NCCOS 186

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List of Abbreviations

% Sat: percent saturation
5-OH-Cyt: 5-hydroxycytosine
5-OH-5-MeHyd: 5-hydroxy-5-methylhydantoin
8-OH-Ade: 8-hydroxyadenine
8-OH-Gua: 8-hydroxyguanine
ANOVA: Analysis of Variance
B-IBI: Benthic Index of Biotic Integrity
CI: confidence interval
ddH_2O: distilled, deionized water
DEG: differentially expressed genes
DNA: Deoxyribonucleic acid
DOSS: dioctyl sodium sulfosuccinate
DPM: disintegrations per minute
DTT: dithiothreitol
EDTA: ethylenediaminetetraacetic acid
EROD: ethoxyresorufin-O-deethylase
ESI: Environmental Sensitivity Index
FapyAde: 4,6-diamino-5-formamidopyrimidine
FapyGua: 2,6-diamino-4-hydroxy-5-formamidopyrimidine
GC/FID: gas chromatography/flame ionization detection
GC/MS: gas chromatography/mass spectrometry
HPLC: high-performance liquid chromatography
ISTDs: internal standards
LC-MS/MS: liquid chromatography tandem mass spectrometry
NADPH: nicotinamide adenine dinucleotide phosphate
NC: not calculated
PAH: polycyclic aromatic hydrocarbon
PVC: polyvinyl chloride
RNA: ribonucleic acid
SE: standard error
TBAA: tetrabutyl ammonium acetate
TEH: total extractable hydrocarbon
TEM: total extractable matter
TG: thymine glycol
USEPA: United States Environmental Protection Agency

ABSTRACT

NOAA's Environmental Sensitivity Index (ESI) lists saltmarshes along the Atlantic and Gulf Coasts as the most vulnerable habitat to oil spills. As a result of the *Deepwater Horizon* offshore drilling rig accident, more that 200 million gallons of crude oil were released into the Gulf of Mexico, and more than 1.8 million gallons of dispersant were applied in the response effort. While previous studies have characterized the toxicity of crude oil and dispersants in aquatic species, there is a need to better understand potential impacts of both crude oil and dispersants on sensitive saltmarsh communities. The purpose of this project was to assess acute and chronic impacts on various biological constituents of a simulated (mesocosm) saltmarsh community. The project was conducted in two phases, with Phase I focused on simulating the impacts of a single incoming crude oil slick. Treatments included crude oil only, the dispersant Corexit only and a crude oil+Corexit mixture. Phase II simulated multiple re-oiling events with incoming tides and included the same treatments as in Phase I. Results from Phase I showed that oil and Corexit concentrations in water were significantly elevated at 12 h to 24 h in the oil only and oil+Corexit treatments, but measured concentrations declined rapidly thereafter. Oil concentrations in sediments were slightly elevated at 30 d and 60 d in the oil only and oil+Corexit treatments. No treatment-related effects were observed on the survival of fish, molluscs, or crustaceans. Likewise, no significant effects were observed in the benthic community or on the growth of marsh grass. Phase II results showed that waterborne oil concentrations were significantly elevated at 7 d in all oil and oil+Corexit treatments. Oil concentrations were significantly elevated in sediments for the oil only and oil+Corexit treatments. Survival of clams was significantly reduced in all oil and Corexit treatments. Growth of marsh grass was reduced in the oil only and oil+Corexit treatments. A trend toward decreased benthic community diversity and total densities in the oil only and Corexit only treatments was observed after 7 d. No significant effects on the survival of fish or grass shrimp were observed; however, ethoxyresorufin-O-deethylase (EROD) activity (a biomarker of polycyclic aromatic hydrocarbon [PAH] exposure and uptake) was induced in the fish in both the oil only and oil+Corexit treatments at 7 d. Reductions in dissolved oxygen were observed throughout most of the exposure (~ 20 d) in the oil only and oil+Corexit treatments and were most

extreme in the oil+Corexit treatment. The results of this study highlight the utility of a mesocosm approach to identify impacts associated with a complex environmental exposure scenario, like a crude oil spill with and without a chemical dispersant, in various compartments of estuarine ecosystems.

INTRODUCTION

Providing state-of the art science to protect our nation's valuable natural resources directly supports NOAA's responsibilities under the Oil Pollution Act of 1990, National Oil and Hazardous Substances Pollution Contingency Plan and Emergency Support Function 10 of the National Response Framework (http://www.ecfr.gov/cgi-bin/text-idx?tpl=/ecfrbrowse/Title40/40cfr300_main_02.tpl). NOAA's Environmental Sensitivity Index (ESI) Maps, used to protect shorelines in the US from oil spill impacts, list salt marshes as the most vulnerable habitat within the Gulf of Mexico (http://response.restoration.noaa.gov/maps-and-spatial-data/environmental-sensitivity-index-esi-maps.html). Between 20 April and 15 July 2010, more than 200 million gallons (4.9 million barrels) of crude oil were released from the *Deepwater Horizon* offshore drilling rig into the Gulf of Mexico (Ramseur, 2011). In response to this spill, approximately 1.8 million gallons of dispersants were applied both at the surface and sub-surface which dispersed about 32 million gallons of oil (Ramseur, 2011). The use of chemical dispersants as an oil spill countermeasure in the United States has long been controversial. According to the National Research Council's "Oil Spill Dispersants: Efficacy and Effects" (2005), oil spill dispersants do not actually reduce the total amount of oil entering the environment. Instead, dispersants alter the chemical and physical properties of oil thus affecting the oil's transport, fate, and potential bioeffects.

The objective of dispersant use is to enhance the amount of oil that physically mixes into the water column, reducing the potential that a surface slick will contaminate shoreline habitats or come into contact with surface dwelling aquatic organisms. Conversely, by promoting dispersion of oil into the water column, dispersants increase the potential exposure of water-column and benthic biota to spilled oil. Dispersant application thus increases hydrocarbon load (resulting from a spill) on one component of the ecosystem (e.g., the water column) while reducing the load on others (e.g., coastal wetland, beaches).

There is a considerable amount of research data available regarding the effects of oil on aquatic species (Moles, 1998; DeLorenzo et al., 2012). The reason why additional oil studies are required hinges on the fact that oil is a very diverse substance, with the various components present in specific oils greatly affecting their toxicity. In addition,

the conditions upon which oil enters the environment (e.g., depth, distance from shore, type of shoreline, currents, tidal activity, season, weather events, use of dispersants) all dramatically alter the potential for ecological effects.

A salt-marsh mesocosm can be used as an integral part of assessing natural resource damage that may be caused by dispersants and dispersed oil. This salt marsh mesocosm simulation assessment will further support the ESI approach used by NOAA and will provide additional comparisons between salt marsh vulnerabilities to oil, dispersants and mixtures of both oil and dispersant. The data generated will enhance our knowledge and our ability to respond to and mitigate environmental impacts.

The purpose of this project was to determine the acute and chronic effects of a crude oil, a crude oil dispersant and dispersed crude oil on a simulated estuarine salt marsh community. The test species used were grass shrimp (*Palaemonetes pugio*), hard clams (*Mercenaria mercenaria*), mud minnow (*Fundulus heteroclitus*), sheepshead minnow (*Cyprinodon variegatus*), bacteria, phytoplankton, marsh grass (*Spartina alterniflora*), and the benthic sediment community. The organisms selected for study are common to both the Atlantic and Gulf coast estuaries and are vital natural resources which serve as food to commercially and recreationally important fish and shellfish species.

Objectives

The project objectives were as follows:

1. Characterize the chemical fate of dispersed oil in estuarine surface water, sediment, and the tissues of aquatic plants and animals in a simulated salt-marsh system.
2. Compare the acute and chronic effects of dispersed oil on a simulated estuarine saltmarsh community, including benthic, pelagic, and microbial components.
3. Characterize the toxicity of the dispersed oil over time to evaluate the potential for ecosystem recovery.
4. Improve NOAA's capacity to respond to major oil spills by increasing scientific knowledge of the impacts of oil, with and without chemical dispersant, on estuarine salt marsh ecosystems.

The endpoints included survival, enzyme induction, contaminant uptake and bioaccumulation, changes in community structure, and environmental partitioning (water, sediments, biota). Additional sublethal biomarkers included nutrient cycling; bacterial productivity; growth rate and primary productivity in phytoplankton; phytoplankton community composition (HPLC pigment analysis); measurement of detoxification enzyme activity in fish; cellular DNA damage in shrimp; and growth of clams, fish, and marsh grass. Responses at multiple time points were examined, including acute (48h, 7d and 14d) and chronic (30d and 60d), to assess the extent of ecological impact from crude oil and a dispersant on salt marsh tidal creek species and to characterize the potential recovery of affected estuarine biota. The purpose of this NOAA Technical Memorandum was to report on the design and results of the crude oil and dispersant research as carried out in our mesocosm systems. Discussion of the results will follow in other publications.

This project improves NOAA's ability to respond to future oil or hazardous material spills by providing accurate data on oil spill chemical fate and effects in estuarine salt marshes. The project will also help area planners and may be used to assist response decisions on dispersant use. The toxicology data generated from this study will allow NOAA products to be expanded and integrated into additional hazards planning, response and recovery activities at the local and regional level. Data collected could be used to evaluate possible exposure effects of aquatic organisms to compounds specific to an oil spill including dispersants. Alterations in aquatic organism health can have profound effects on population numbers. This project will serve to supplement the dispersant work summarized in National Research Council's "Understanding Oil Spill Dispersants: Efficacy and Effects" (2005) and expand upon the current acute toxicity work (presented by USEPA http://epa.gov/bpspill/dispersants-testing.html) by providing an ecosystem level assessment.

MATERIALS AND METHODS PHASE I

Mesocosm Setup

Mesocosm tanks

The mesocosm system, located at NOAA CCEHBR in Charleston, SC, consisted of two tanks, one upper and one lower in accordance with procedures outlined in NOAA Technical Memorandum NOS NCCOS 62 (Pennington et al, 2007). The 12 systems used in this study were held in a greenhouse which incorporated natural light and temperature conditions. The lower tank, or sump, provided tidal water to the upper tank via a pump set to a timer. The tide was semi-diurnal so twice daily seawater was pumped into the upper tank (mesocosm) from the lower tank (sump) to simulate a flood tide (Figure 1).

The systems were further modified for this study to accommodate working with oil slicks. To the upper tank of each mesocosm, a PVC pipe (52 cm high by 10 cm diameter) was attached to the outside of the tank below the low tide water level. This allowed water to flow from the upper tank with the tidal cycle to the sump without any surface oil slick being lost to the sump. It also set the high tide volume in the upper tank. A PVC pipe (52 cm high by 5 cm diameter) was placed vertically inside each upper tank and used to collect water samples without having to penetrate any surface layer oil slick. The lower tank was divided with a 0.5 cm plastic mesh screen to separate the grass shrimp from the mud minnows. The screen was placed inside across the width of the tank, top to bottom, at 31.5 cm from the tank outside edge. A similar screen covered the top of this section. This outer section held mud minnows (*Fundulus heteroclitus*) while the inner section held grass shrimp (*Palaemonetes pugio*).

Eight months prior to dosing, seawater for the experiment was collected from the Hollings Marine Laboratory (HML) polished seawater system. Briefly, the HML seawater (usually 28‰ to 34‰) is drawn from Charleston Harbor at high tide (+/- 2 h) and allowed to settle for at least 4 d in one of four 284,000 L tanks. Once settled, the seawater is pumped into a 19,000 L secondary holding tank where the seawater is polished via sand filtration, UV sterilization and 5 micron nominal filtration. The polished seawater was collected from the 19,000 L tank and diluted to 20‰ and dispensed into the 12 mesocosm systems (443 L each). The seawater was then cycled

Figure 1. Phase I setup showing upper tanks (mesocosms) with their respective lower tanks (sumps) in top picture. Middle picture shows close-up of a mesocosm tank at low tide. Bottom picture shows close-up of a sump tank.

through the fully functioning systems for stabilization prior to the addition of sediments and test organisms.

Four tanks (one in each treatment) were monitored continuously with a YSI 5200A Continuous Aquaculture Monitor for water quality parameters (temperature, pH, dissolved oxygen, salinity). The YSI probes were placed in the same PVC pipe used to collect water samples so no surface layer oil slick could contact the probes. These parameters varied diurnally in accordance with daytime heating and photosynthetic activity; however, these differences were within the established norms for this system (Pennington et al, 2007).

Sediment collection

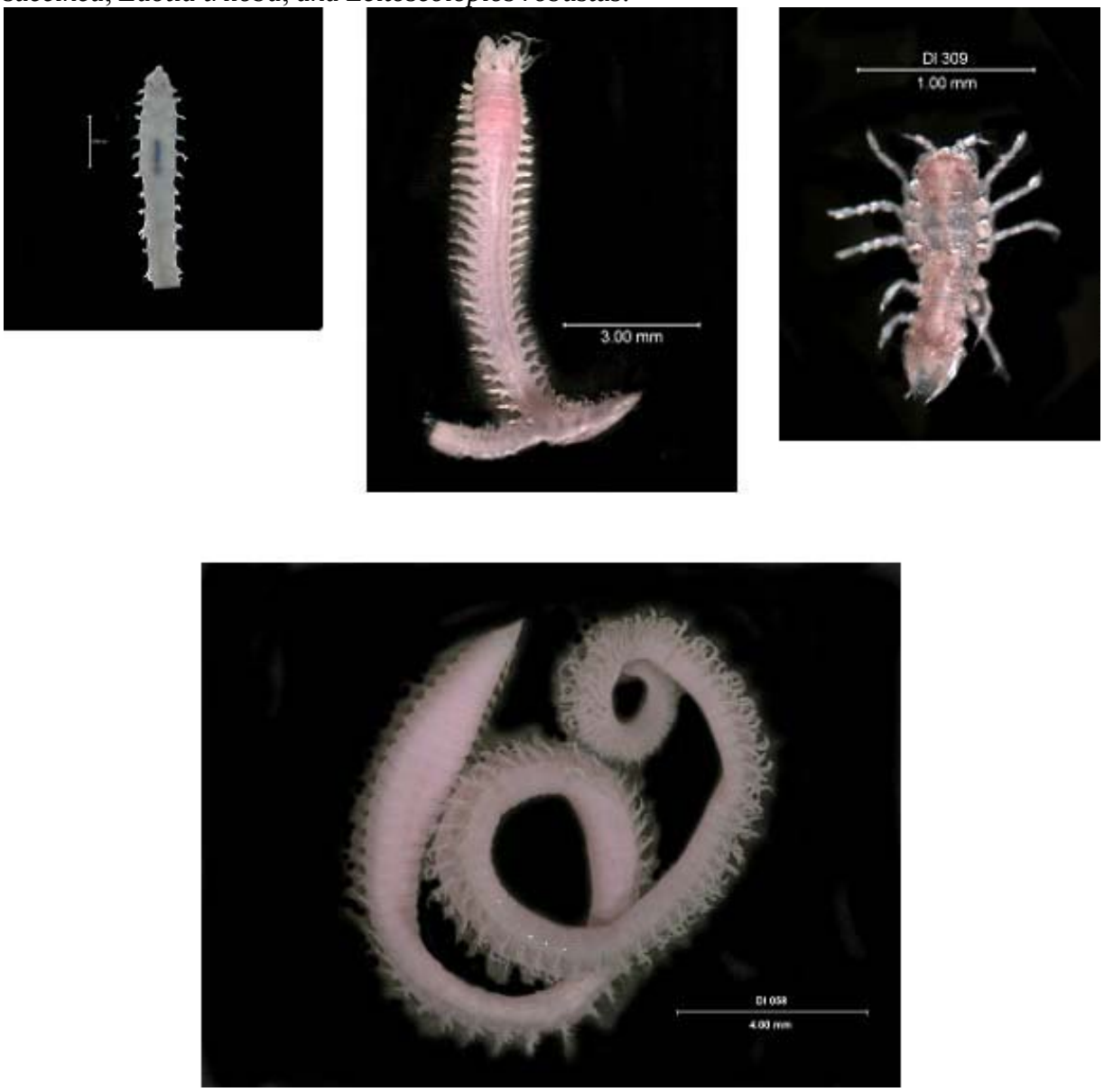
Eight weeks prior to dosing, intertidal sediments were collected for each mesocosm from a site at Leadenwah Creek (32º 38.848' N, 080º 13.283' W), Wadmalaw Island, SC. Specifically, the sediments were collected from the mud flat at low-tide within 2-3 m of the lower edge of the creek adjacent to *Spartina* stands. Using a shovel, the top 2-4 cm of sediment from the mud flat were removed and placed into plastic buckets. The buckets containing the sediments were transported back to the greenhouse at NOAA CCEHBR. The sediments were scooped out of the buckets and placed directly into the mesocosm sediment trays (20 cm x 20 cm x 20 cm depth) until slightly overflowing (approximately 12.75 kg of mud per tray). A total of 48 sediment trays were filled and then placed randomly into each of the 12 mesocosm systems (4 trays per system). These sediment trays were underwater at high tide and allowed to drain from the bottom at low tide to simulate tidal pumping and sediment drainage.

Benthic infaunal community sediments were collected during the same week and from the same creek as the *Spartina* sediments. The sediments were collected as square, intact plots (20 cm x 20 cm x 11 cm depth) from the exposed mud flat at low tide. The plots were transferred directly into benthic sediment trays of the same dimensions and then placed into tote bins. At the greenhouse, the benthic trays (48) were removed from the bins and placed randomly into each of the 12 mesocosm units (4 trays per unit). Any large fauna (>1 cm) such as crabs, mussels, and snails were removed. A sample of the

organisms found in the Leadenwah Creek benthic infaunal community is seen in Figure 2.

Sediment for clams was press-sieved through a 1-mm mesh screen, homogenized, and aliquotted into Nalgene trays measuring 270 mm x 270 mm x 27 mm. Sediment depth was set at 20 mm for each tray. After the sediment was added to the trays, they were placed into mesocosm tanks eight weeks prior to dosing. Clam sediment trays were not exposed to air or the surface oil slick during low tide.

Figure 2. Some benthic infaunal community organisms found in sediments collected from Leadenwah Creek, SC. Clockwise from top left: *Streblospio benedicti*, *Neanthes succinea*, *Edotia triloba*, *and Leitoscoloplos robustus*.



Test species

Test species included crustaceans (grass shrimp, *Palaemonetes pugio*), molluscs (hard clams, *Mercenaria mercenaria*), fish (mud minnow, *Fundulus heteroclitus*; sheepshead minnow, *Cyprinodon variegatus*), phytoplankton, and marsh grass (*Spartina alterniflora*) and benthos (Figures 2 and 3).

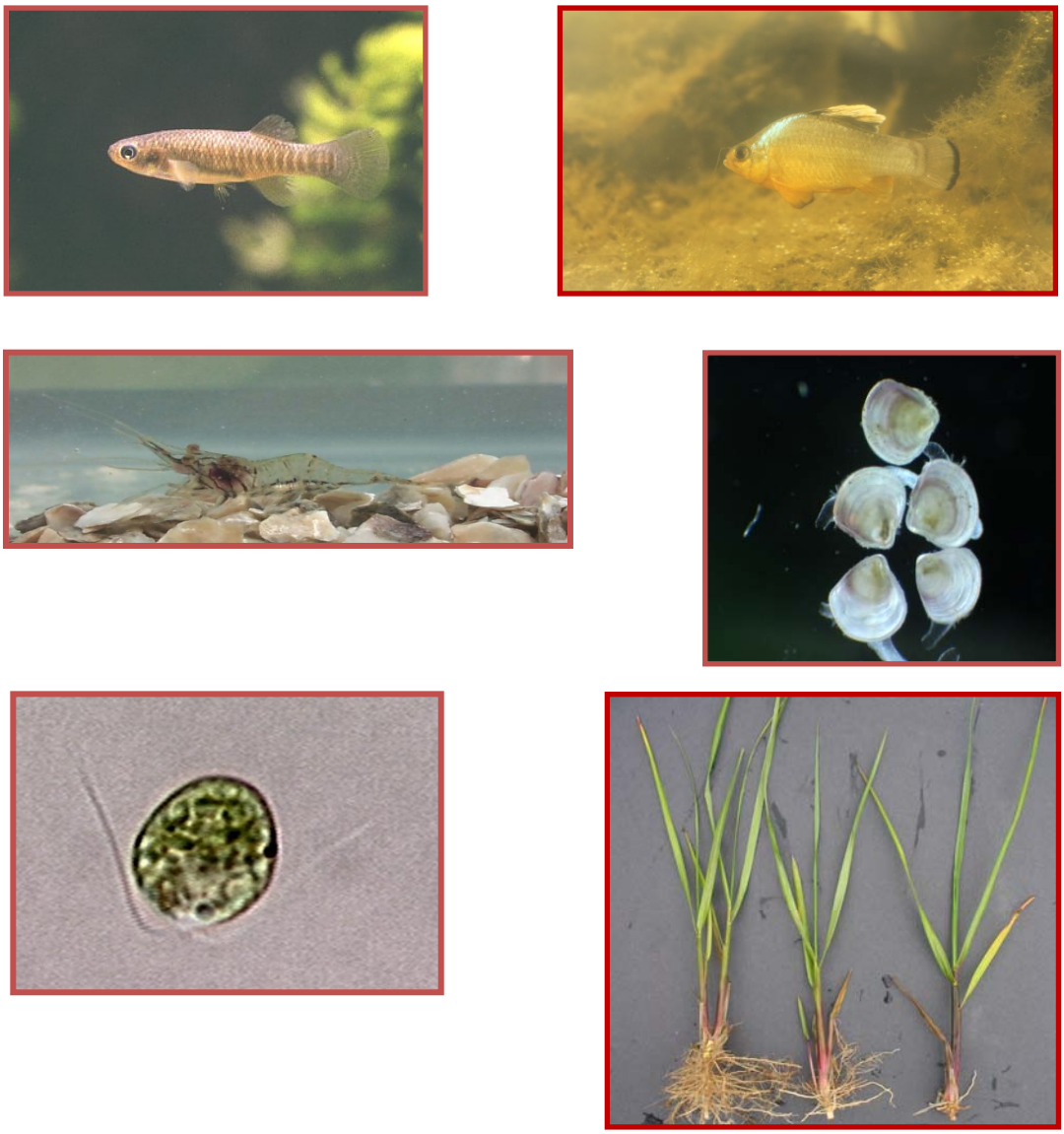
The week following the sediment collections, marsh grass plugs (5 cm x 5 cm) were obtained commercially from the Nursery at Environmental Concern, Inc. (St. Michaels, MD). Four plugs were placed into each of the 48 *Spartina* sediment trays. *Spartina* was allowed to grow in the tank system 45 d before the addition of other species.

Juvenile hard clams of approximately 1-mm were acquired from Bay Shellfish Company, a commercial aquaculture facility located in Terra Ceia, FL on May 3, 2011. Clams were acclimated at 20‰ salinity for 48 h in 5-L precleaned rectangular glass jars with a clear plastic lid in the greenhouse. A 1-mL glass pipette was inserted and attached to an airline for gentle aeration. Partial water changes (approximately 2/3 of the total volume) were conducted daily. Clams were fed 200 mL daily of *Isochrysis galbana* (6 million cells/mL) initially obtained from Bay Shellfish Company. One hundred and fifty clams were added to each clam sediment tray which remained in place for 59 d. Each upper tank contained one sediment tray for clams. At the termination of the test, clam trays were carefully lifted out of the tanks at low tide. Clams were retrieved by re-sieving the water and sediment in the test trays through a 1-mm sieve and placing the clams in polystyrene petri dishes for endpoint evaluation. Clams were determined to be dead if they exhibited gaping shells, lack of response to stimuli, and/or shell closure for more than 5 min. Dead clams were excluded from sublethal endpoint analysis.

Adult grass shrimp, 25 mm to 30 mm in length, were collected from a site at Leadenwah Creek (32º38.850’ N; 080º13.301’ W), a relatively pristine tidal creek tributary of the North Edisto River, SC. Shrimp were acclimated in 76-L tanks at 25ºC, 20‰ salinity and 16-h light: 8-h dark cycle and fed TetraMin Fish Flakes. Seven days prior to the start of the test, shrimp were added to the mesocosms. In the upper tank of each mesocosm, 50 shrimp were added and allowed to swim freely. In the lower tank of each mesocosm, 80 shrimp were added to the inner section of the divided tank. Shrimp

in the upper tank were not fed during the course of the test due to the availability of naturally occurring food in the mesocosm system. Shrimp in the lower tank were fed 400 mg of TetraMin Fish Flakes per day.

Figure 3. Animal and plant test species used in Phase I and Phase II. Clockwise from top left: *Fundulus heteroclitus, Cyprinodon variegatus, Mercenaria mercenaria, Spartina alterniflora*, Phytoplankton (*Dunaliella tertiolecta*), *Palaemonetes pugio*.



Mud minnows, 55 mm to 65 mm in length, were obtained from Cherry Point Creek (32°36.072'N; 080°11.117'W), a tidal creek of the North Edisto River, SC. These fish were acclimated and fed as stated for grass shrimp. Juvenile sheepshead minnows, 10 mm to 20 mm in length, were obtained from Aquatic Biosystems (Fort Collins, CO) and were acclimated and fed as stated for grass shrimp. Seven days prior to the start of the test, fish were added to the mesocosms. In the upper tank of each mesocosm, 25 sheepshead minnows were added and allowed to swim freely. In the lower tank of each mesocosm, 40 mud minnows were added to the outer section of the divided tank. Fish in the upper tank were not fed during the course of the test due to the availability of naturally occurring food in the mesocosm system. Fish in the lower tank were fed 1200 mg of TetraMin Fish Flakes per day.

Dosing procedure

For this project, four treatments with three replicates each were used: control, oil, dispersant, oil with dispersant added (Figure 4). The oil concentrations tested were based on the results of preliminary laboratory studies with the oil:dispersant mixture at a 10:1 ratio. Table 1 shows the dosing scheme for Phase 1. The maximum dose applied was equivalent to a 50-µm thick oil slick. This required 74 mL of oil per tank or a total of 444 mL oil for 6 tanks. This was equivalent to a 145 mg/L exposure per dosed tank if all the oil went into solution. Since a significant portion of the oil was expected to swiftly volatilize, the precise dose that remained in the mesocosms could not be predicted.

The dosing procedure as occurred on 9 May 2011 was as follows:

1. At high tide, 74 mL of oil, (MC252 Source Oil Q-4000), was poured slowly into the open channel of the mesocosm upper tank. The three oil+Corexit tanks were dosed first.
2. This was repeated for the three oil-only tanks.
3. After 30 min, the dispersant, Corexit 9500 (Corexit; Nalco Energy Services, Sugar Land, TX), was added to the three oil+Corexit tanks using a multi-channel pipettor. With the pipettor set at 100 µL and using six tips, the pipettor was moved in a snaking transect of four columns, beginning in the bottom right corner of the mesocosm upper tank and dispensing three times per column, for a total of 12 times. Four tips were then pulled off

the pipettor and Corexit was dispensed one more time with just 2 tips for a total of 7.4 mL.

4. This was repeated for the three Corexit-only tanks.

5. Nothing was added to the three control mesocosms.

Figure 4. Diagram of experimental mesocosm set-up (12 mesocosms= 4 treatments x 3 replicates) in a randomized block design.

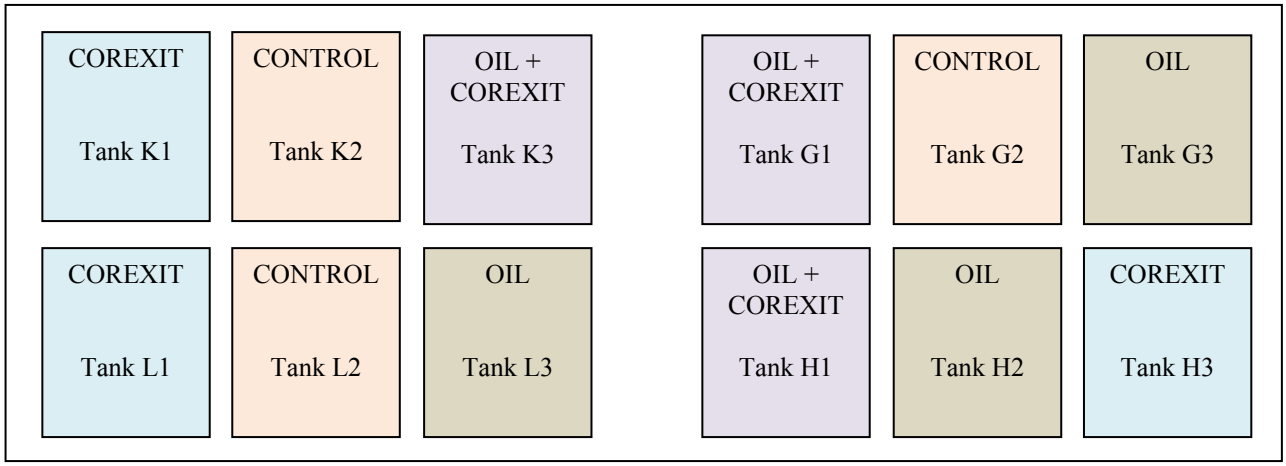


Table 1. Dosing scheme for mesocosm Phase I which consisted of three treatments and a control.

TREATMENT (# replicates)	**Amount of Oil Added**	**Amount of Corexit Added**	**Oil* Conc.[1]**	**Corexit* Conc.[2]**	**Oil Slick Thickness**
Control (3)	–	–	–	–	–
Oil (3)	74 mL	–	145.3 mg/L	–	50 µm
Corexit (3)	–	7.4 mL	–	15.87 mg/L	–
Oil+Corexit (3)	74 mL	7.4 mL	145.3 mg/L	15.87 mg/L	50 µm

* Assuming a total system volume of 443 L and that all of the dosed material goes completely into solution.

1. The density of the oil is 0.87g/mL.
2. The density of the Corexit is 0.95 g/mL.

Sample collection

The following pre-dose samples and measurements were taken at least 24h before the addition of any oil and dispersant (see also Table 2):

1. Marsh grass stem density and shoot height was measured.
2. One benthic tray was removed for processing.
3. Water samples (250 mL) for primary productivity, heterotrophic productivity, and chlorophyll were removed and processed.
4. Three replicates of 50 clams were removed from holding tanks for baseline measurements and used for initial dry masses.
5. Samples for water and sediment chemistry were removed.
6. Mud minnow and grass shrimp replicates were removed from holding tanks for EROD, DNA and microarray analysis.

Following dosing, sample collections in the mesocosms occurred at 24 h, 48 h, 7 d (168 h), 14 d (336 h), 30 d (720 h) and 59 d (1416 h; end of test). Water samples were collected from the sampling pipe of each upper tank and pipetted into glass amber bottles with Teflon-lined lids.

The collection schedule was as follows (see also Table 2):

24 h: Collected 250 mL and 15 mL aliquots of water for chemical analysis.

48 h: Collected 250 mL and 15 mL aliquots of water for chemical analysis. Collected 250 mL water for phytoplankton and nutrient analyses. Two mud minnows from each lower tank were netted and placed in labeled jar filled with tank water then livers were dissected for EROD activity. Three shrimp were collected from each lower tank and processed for RNA assessment. Two more shrimp from each lower tank were collected and processed for DNA assessment.

7 d: Repeated as for the 48 h sampling plus collected one benthic sediment tray from each mesocosm for assessment. Collected 7 g to 10 g of sediment for chemical analysis from one sediment tray using a Teflon scoop and placed in a 4-oz jar then sealed with a Teflon-lined lid. Collected same for Microtox analysis. Mud minnows were collected as before for EROD analysis. Shrimp were collected as before for RNA and DNA assessment.

14 d: Repeated as for the 48 h sampling. Mud minnows were collected as before for future EROD analysis. Shrimp were collected for DNA assessment and future RNA assessment.

30 d: Repeated as for 14 d sampling. Marsh grass stem density and shoot height was measured.

59 d (last day of test): Repeated as for 7 d sampling. All sheepshead minnows and clams were collected for growth and mortality assessments. All remaining mud minnows and shrimp were collected for future analysis. Marsh grass stem density and shoot height was measured.

Table 2. Sample collection times for mesocosm Phase I. "X" denotes matrix was collected.

Sample Time	Matrix Collected						
	Water	**Sediment**	**Benthic Tray**	***F. heteroclitus***	***P. pugio***	***M. mercenaria***	***C. variegatus***
Pre-dose	X	X	X	X	X	X	
24 h	X						
48 h	X			X	X		
7 d	X	X	X	X	X		
14 d	X			X	X		
30 d	X	X	X	X	X		
59 d	X	X	X	X	X	X	X

C. variegatus Assessment

At the end of the test, all sheepshead minnows were removed from the mesocosm tanks and immediately weighed and measured for growth assessment.

M. mercenaria Assessment

At the end of the test, clams were weighed, dried overnight at 68°C, and weighed again to determine dry mass (mg). Dry mass was measured using an OHAUS Analytical Plus (Model AP250D) five-place balance (OHAUS Corp., Florham Park, NJ). The clams were weighed in batches by treatment. The batch dry masses were then divided by the number of clams per batch to obtain mean per clam dry mass estimates. Shell length and

area measurements were determined using a dissection microscope. Images were captured and analyzed for shell area (mm^2), major axis length (mm), and minor axis length (mm) using digital imaging software (Image Pro Plus, Version 6.3). Major axis length was defined as the length from the umbo to the shell edge. Minor axis length was defined as the width from shell edge to shell edge. Lengths were measured for individual clams and mean values were calculated for each replicate. A condition index was determined using the following ratio (Walne, 1970; Crosby and Gale, 1990): dry mass (mg)/shell volume (mm^3). Shell volume (mm^3) was estimated by multiplying major shell axis length^2 by minor shell axis length.

S. alterniflora Assessment

Marsh grass stem density and shoot height were assessed pre-dose, 30 d post-dose and 60 d post-dose. Stems were considered to be the bundle of foliage arising from the soil. Each stem contained shoots. The shoots were considered to be an individual foliage blade. Plant stem density was measured by directly counting the number of stems in each mesocosm. Shoot height was determined by measuring each shoot with a ruler to the nearest 0.1 cm.

Benthic Infauna Assessment

Samples for identification and enumeration of benthic macroinfauna were collected during Phase I sampling just prior to dosing, 7 d after dosing, 30 d after dosing, and 59 d after dosing. A sample consisted of one randomly selected tray removed from each system while mesocosm water level was drawn down below the top edge of the sediment tray. The entire contents of the sediment tray (4,129 cm^3) were sieved through a 0.5-mm mesh screen and preserved in 10% buffered formalin with rose bengal. All infaunal samples were transferred to 70% ethanol in the laboratory. Animals were sorted from sample debris under a dissecting microscope and identified to the lowest practical taxon, usually to species.

Samples for sediment characterization and TOC analysis were collected by scraping the top 2-3 cm of sediment from the intertidal saltmarsh trays at the end of Phase I. TOC analysis followed USEPA Method 9060. A minimum of 5 g (wet weight) of

sediment was initially dried for 48 h. Weighed subsamples were ground to fine consistency and acidified to remove sources of inorganic carbon (e.g., shell fragments). The acidified samples were ignited at 950ºC and the carbon dioxide evolved was measured with an infrared gas analyzer. Silt-clay samples were prepared by sieve separation followed by timed pipette extractions as described in Plumb (1981).

Microtox Solid Phase Assessment

Sediment samples were collected in pre-cleaned 4-oz glass jars and stored at 4ºC until analysis. Microtox assays were conducted according to the standardized solid-phase protocols with the Microtox Model 500 analyzer (SDIX, Newark, DE). All materials and reagents were purchased from SDI. Sediment was homogenized and a 7.0-g to 7.1-g sediment sample was used to make a series of sediment dilutions with 3.5% NaCl diluent. Test samples were placed in a 15ºC water bath for 10 min incubation. Luminescent bacteria (*Vibrio fisheri*) were then added to the test concentrations for 20 min incubation. At the end of the incubation period a column filter was used to separate the liquid phase from the sediment phase, and bacterial post-exposure light output was then measured using Microtox Omni Software. An EC50 (the sediment concentration that reduces light output of luminescent bacteria by 50% relative to the controls) value was calculated for each sample. Triplicate samples were analyzed simultaneously and the mean EC50 was reported.

***F. heteroclitus* EROD Activity Assessment**

After 2 d and 7 d, two fish were netted from each lower tank, placed in a labeled jar filled with the tank water, and transported to the laboratory where the fish were decapitated and the livers removed. The two fish livers from each mesocosm were then pooled into a 2-mL centrifuge tube, flash frozen in liquid nitrogen, and stored at -80°C. Fish livers were weighed and homogenized on ice in 2.5 mL buffer (50 mM Tris HCl, 0.15 M KCl, pH 7.4 to7.5) using 8 manual passes with a Potter-Elvehjem micro-tissue Teflon grinder. The homogenate was transferred to a 15 mL centrifuge tube, and an additional 1.5 mL of buffer was used to rinse the tissue grinder into the centrifuge tube for a total volume of 4 mL. Samples were centrifuged (IEC Multi RF, Thermo Electron

Corporation, Needham Heights, MA) for 10 min at 7,000 *g* and 4°C to sediment nuclei and debris and then without transferring for 10 more min at 12,000 *g* to sediment mitochondria. The supernatant was then transferred to an ultracentrifuge tube and centrifuged (Sorvall MX Micro-Ultracentrifuge, Thermo Scientific, Waltham, MA) at 66,000 *g* and 4°C for 3 h. The supernatant (cytosolic fraction) was removed and 1 mL of resuspension buffer (50 mM Tris HCl, 1 mM EDTA, 1 mM DTT, 20% v/v glycerol, pH 7.4-7.5) was added to each pellet. The tube was vortexed to get the pellet off of the side and a small Teflon homogenizer was used to resuspend the pellet while keeping the tube on ice. The microsomal sample was transferred to chilled cryovials which were then stored in liquid nitrogen.

Induction of cytochrome P4501A, using the 7-ethoxyresorufin-*O*-deethylase (EROD) assay was measured according to Kennedy and Jones (1994), whereby the protein content of each sample is simultaneously assessed in a fluorescent plate reader. Sodium phosphate buffer (150 µL; 50 mM, pH 8.0) was added to each well of a 48-well plate. Microsomal suspensions (10 µL) followed by ethoxyresorufin (final concentration 10 µM) were then added to the wells. After 10 min incubation at room temperature, sample reactions were started with the addition of NADPH (final concentration 1 mM), whereas blank wells received an equivalent volume of sodium phosphate buffer. Reactions were stopped with acetonitrile (100 µL) that contained fluorescamine (300 µg/mL). After 15 min incubation at room temperature, fluorescence of the samples was read at 530-nm excitation/590-nm emission for resorufin, and at 400-nm excitation/460-nm emission for proteins. EROD and protein concentrations of the samples were determined using standard curves for resorufin (0.0125, 0.025, 0.075, 0.25, 0.5 µmol/L) and BSA (7.5, 45, 75, 105, 150 µg/mL).

P. pugio RNA Assessment

Three shrimp from all experimental treatments and control were collected at the 48 h time point. Whole shrimp were sampled individually and placed in 10 mL plastic tubes containing 1 mL of Trizol solution and homogenized for 15 s using a Pro200 handheld tissue shredder (Pro Scientific, Oxford, CT). Samples were allowed to incubate for a minimum for 5 min before adding 200 µL of chloroform to the homogenate.

Samples were then immediately shaken by hand vigorously for 10 s and the resulting slurries transferred to 1.5 mL microcentrifuge tubes. Tubes were then centrifuged at 4°C at 10 *g* for 15 min in a Fisher accuSpin R1 high speed centrifuge. Sample supernatant was removed and transferred to a new microcentrifuge tube and the remaining fraction was frozen at -80°C. Five hundred µL of 100% ethanol (EtOH) was added to the supernatant and the mixture incubated at room temperate for 5 min. After incubation, samples were centrifuged again at 4°C at 10 *g* for 15 min to precipitate RNA. The resulting RNA pellet was washed once with 70% EtOH, briefly allowed to dry and resuspended in 200 µL of nuclease-free water. Re-suspended RNA was then split into two 100 µL fractions, with one archived at -80°C and the other taken through column purification. RNA was column purified using Qiagen RNeasy (Qiagen, Inc., Valencia, CA) spin columns following the manufacturer's protocol for RNA clean-up. Cleaned RNA was eluted in 50 µL of nuclease-free water. Quantity of the purified RNA was determined by UV-Vis spectrophotometer, NanoDrop 1000 (Thermo Scientific, Waltham MA) and sample quality was evaluated by capillary electrophoresis using a 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA).

Grass shrimp microarray

A total of 253.6 Mb of sequence data generated from a 454 GS FLX (Roche Diagnostics Corporation, Branford, CT) pyrosequencing project of *P. pugio* conducted by the CCEHBR lab was used as a starting point for array construction. Sequence data was assembled using the Newbler software package. A total of 11,090 consensus regions (contigs) with a minimum length of 100bp were constructed from Newbler assembly. Sequence homology of the resulting contigs was investigated using the BlastX search algorithm against NCBI database as implemented in the computer program Blast2GO. BlastX searches revealed a high number of contigs with no homology (73%) versus those with identified homology (27%) using an e-value cut-off of 10^{-3}.

Assembled contigs and the associated homology information were uploaded to the Agilent Technologies eArray website for probe design and microarray construction. Probes (60-mer) were successfully designed for 10,876 of the 11,090 contigs. Arrays followed an 8 x 15k design (8 arrays containing 15,000 features per glass slide) and

contained Agilent standards in addition to 15 probes from contigs of known identity printed at 10x to 15x to serve as experimental internal standards. The remaining space on the array was filled randomly from the original probe data. The resulting custom *P. pugio* 8 x15k array was manufactured by Agilent Technologies (Santa Clara, CA).

RNA labeling and microarray hybridization

One hundred ng of sample RNA were labeled with cyanine 3-CTP fluorescent dye using a one-color Quick Amp Labeling Kit (Agilent Technologies, Santa Clara, CA) following manufacturer's protocol. Quantity and dye incorporation of labeled products was determined by spectrophotometer. Fragmentation of each reaction consisted of 300 µg of the labeled cRNA added to 5 µL of blocking buffer and 1 µL of fragment buffer. Reaction volume was adjusted to 25 µL with nuclease-free water and incubated for 30 min at 60°C. Following fragmentation incubation, 25 µL of 2X hybridization buffer was added to the labeled product. Of this, 40 µL was used to hybridize to individual custom DNA microarrays at 65°C for 17h. The following day, arrays were washed and immediately scanned using an Agilent DNA Microarray Scanner. Hybridization intensity was collected and quality checked using Feature Extraction Software (Agilent Technologies, Santa Clara, CA) using protocol GE1-v5_95_Feb07 for one-color gene expression. Rosetta Resolver (version 7.2 Rosetta Inpharmatics LLC, WA) was used to analyze extracted hybridization intensities. Normalization of arrays consisted of removal of both control and QC flagged features. Data was then trimmed by removing data from the top and bottom 5% of intensity data and then scaled to mean signal intensity. Treatment group (control, oil, Corexit, and mixture) arrays were combined and the intensity of the features averaged in Rosetta Resolver using an error model for Agilent data. From these data, ratios were built between the groups and used to identify differentially expressed features using a p-value of >0.001 and 2 fold difference in absolute expression.

P. pugio DNA Assessment

Two shrimp were removed from each tank at each sample collection time point. The shrimp were weighed and then their cephalothoraxes were removed. Shrimp were

placed in a plastic tube and ground with 180 µL Qiagen Buffer ATL (Qiagen, Inc., Valencia, CA) using a sterile tissue grinder for each tube. Next, 20 µL of Qiagen proteinase K was added and mixed thoroughly by vortexing, then incubated at 56°C until the tissue was completely lysed (overnight). Samples were vortexed occasionally during incubation to disperse the sample. Next, samples were vortexed for 15 s then 200 µL of Qiagen Buffer AL was added and mixed thoroughly by vortexing again. Then 200 µL ethanol (96–100%) was added and vortexed. The mixture was pipetted (including any precipitate) into a Qiagen DNeasy Mini spin column, placed in a 2 mL collection tube then centrifuged at 6000 *g* for 1 min. The DNeasy Mini spin column was placed in a new 2 mL collection tube to which 500 µL Buffer AW1 was added, and centrifuged for 1 min at 6000 *g*. The DNeasy Mini spin column was again placed in a new 2 mL collection tube to which 500 µL Qiagen Buffer AW2 was added and centrifuged for 3 min at 20,000 *g* to dry the DNeasy membrane. The DNeasy Mini spin column was placed in a clean 1.5 mL or 2 mL microcentrifuge tube into which 200 µL of Qiagen Buffer AE was pipetted directly onto the DNeasy membrane, incubated at room temperature for 1 min, and then centrifuged for 1 min at 6000 *g* to elute. The samples were then sodium chloride-ethanol precipitated and washed with 70% EtOH after precipitation to remove salts. Samples were centrifuged and the pellet was frozen in ethanol.

Isotope-dilution GC/MS/MS determination of oxidatively modified DNA bases (DNA lesions) in shrimp DNA extracts using multiple reaction monitoring (MRM) mode quantification was conducted based on modifications to the selected ion monitoring (SIM) mode gas chromatography/mass spectrometry (GC/MS) (Dizdaroglu, 1984, Dizdaroglu, 1985, Dizdaroglu et al., 2002). In the current MRM mode methodology, specific reaction transitions for seven lesions [4,6-diamino-5-formamidopyrimidine (FapyAde), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), 8-hydroxyguanine (8-OH-Gua), 8-hydroxyadenine (8-OH-Ade), 5-hydroxycytosine (5-OH-Cyt), thymine glycol (TG), 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd)], as well as for their stable isotopically-labeled analogues (FapyAde-$^{13}C,^{15}N_2$, FapyGua-$^{13}C,^{15}N_2$, 8-OH-Gua-$^{15}N_5$, 8-OH-Ade-$^{13}C,^{15}N_2$, 5-OH-Cyt-$^{13}C,^{15}N_2$, TG-d_4, and 5-OH-5-MeHyd-$^{13}C,^{15}N_2$) were identified and optimized on the basis of the original SIM ions. The isotopically-labeled lesion analogues functioned as internal standards (ISTDs) for lesion

quantification. Brief enzymatic hydrolysis and analysis details for the extracted DNA pellets follow (all analyses were conducted using independent samples): DNA pellets were re-dissolved in distilled, deionized water (ddH_2O) and the DNA concentrations were determined spectrophotometrically. Sample aliquots containing approximately 30 μg DNA were prepared and the seven lesion ISTDs were added to each sample. The samples were dried under vacuum and then stored at 4°C prior to enzymatic digestion. Subsequent to enzymatic digestion, samples were dissolved in a buffer consisting of 50 mM sodium phosphate, 100 mmol/L potassium chloride, 1 mM EDTA and 100 μM dithiothreitol (pH 7.4). To this solution, 2 μg each of *E. coli* formamidopyrimidine DNA glycosylase - Fpg (Trevigen Inc., Gaithersburg, MD) and *E. coli* endonuclease (III) - EndoIII (Trevigen) were added and each sample was digested at 37°C for 1 h. Hydrolysis using these enzymes prevents artifactual formation of DNA lesions because it only releases modified bases; consequently, there is neither intact DNA nor unmodified base present during the trimethylsilylation step. The digestion was terminated with the addition of ice-cold absolute ethanol in combination with sample storage at –20°C. Samples were centrifuged at 14 000 g for 30 min, supernatant fractions containing the excised DNA lesions were transferred to glass vials and the solvent was removed under Savant SpeedVac Concentrator (Thermo Scientific, Waltham, MA). Samples were solubilized in ddH_2O, lyophilized, and then trimethylsilylated using bis (trimethylsilyl) trifluoroacetamide) / 1% trimethylchlorosilane in pyridine (120°C for 30 min). Following derivatization, samples were analyzed by GC/MS/MS and final results were reported in terms of the number of lesions quantified / 10^6 DNA bases.

Mass spectrometry analyses were performed on an Agilent 7000 series triple quadrupole GC/MS/MS system (Agilent Technologies, Santa Clara, CA) operated in positive ion mode with electron ionization. The modular system consisted of a 7693 autosampler, a 7890A GC oven and a 7000 triple quadrupole (MS/MS) mass analyzer set to widest resolution for MS1 and MS2. The GC column was an HP-Ultra 2 high-resolution fused silica capillary column (12.5 m x 0.2 mm i.d.) coated with cross-linked 5% phenylmethylsilicone gum phase (film thickness, 0.33 μm) (Agilent Technologies). Gas chromatography was performed with a temperature programmed ramp from 130°C to 300°C (130°C, 2 min hold, 8°C per min to 207°C, 0 min hold; 10°C

per min to 300°C, 4 min hold). Trimethylsilyl derivatives of DNA lesions and their stable isotope-labeled analogues were detected in multiple reactions monitoring (MRM) mode. The following instrumental parameters were used for detection and quantification of the DNA lesions in MRM mode: dwell time = 44 ms, collision gas = nitrogen (1.5 mL/min), quench gas = helium (2.25 mL/min), inject mode = split, split ratio = 10:1, split flow = 7.8584 mL/min, inlet temperature = 250°C, inlet pressure = 9.428 psi (6.500 x 10^1 kPa), MS/MS source temperature = 130°C, repeller voltage = 15.4 V, extractor voltage = 6.3 V, MS1 quad DC = 11.4 V, MS2 quad DC = 0.4 V, collision cell hexapole RF = 400 V, collision cell hexapole DC = 11.4 V, collision cell entrance = 12.4 V, collision cell exit 5.4 V, electron energy = - 70 V, delta EMV = 800 V, injection volume = 5 µL, run time = 24.75 min. The relevant MRM mass transitions were: *m/z* 369 → *m/z* 354 and *m/z* 372 →*m/z* 357 for FapyAde / FapyAde-$^{13}C,^{15}N_2$; *m/z* 457 →*m/z* 442 and *m/z* 460 →*m/z* 445 for FapyGua / FapyGua-$^{13}C,^{15}N_2$; *m/z* 455 →*m/z* 440 and *m/z* 460 →*m/z* 445 for 8-OH-Gua / 8-OH-Gua-$^{15}N_5$; *m/z* 367 →*m/z* 352 and *m/z* 370 →*m/z* 355 for 8-OH-Ade / 8-OH-Ade-$^{13}C,^{15}N_2$; *m/z* 343 →*m/z* 328 and *m/z* 346 →*m/z* 331 for 5-OH-Cyt / 5-OH-Cyt-$^{13}C,^{15}N_2$; *m/z* 448 →*m/z* 259 and *m/z* 452 →*m/z* 262 for TG / TG-d_4; *m/z* 331 →*m/z* 331 and *m/z* 334 →*m/z* 334 for 5-OH-5-MeHyd / 5-OH-5-MeHyd-$^{13}C,^{15}N_2$. MRM chromatograms were integrated using MassHunter software (Agilent Technologies) and the measured lesion levels were determined using the MRM area ratios from the modified DNA base of interest and its labeled analogue in conjunction with the known amount of the labeled analogue spiked into each sample. Note: 5-hydroxycytosine could not be reliably detected nor quantified in any of the shrimp extracts.

Chlorophyll, Productivity, Phytopigment, and Nutrients Assessments

Chlorophyll and productivity

Pre-dose and 48 h, 7 d, 14 d, 30 d, and 60 d post-dose water samples (250 mL) were collected and analyzed for chlorophyll concentration, bacterial productivity, phytoplankton productivity, phytoplankton pigment concentrations, and nutrient concentrations.

Chlorophyll *a* was quantified using a fluorometric method (Welschmeyer 1994). At each sampling time, water samples (40 mL) were filtered onto Whatman glass

microfiber Grade GF/F filters. The filters were placed in 20 mL scintillation vials, 1 mL of saturated magnesium carbonate solution was added, and the vials were frozen (-20ºC) until analysis. For chlorophyll extraction, 9 mL of 100% acetone was added to each sample (90% acetone final concentration), and the samples were refrigerated (4ºC) for 24 h. The samples were then vortexed for 1 min and again refrigerated (4ºC) for 24 h (total extraction period of 48 h). Fluorescence was measured using a Turner Model TD-700 fluorometer (Turner Designs, Sunnyvale, CA). Chlorophyll *a* concentration (µg/L) was determined using the instrument standard curve.

Heterotrophic bacterial productivity was measured by incorporation of tritiated leucine (Kirchman, 1993). A 5 mL water sample was added to a scintillation vial and spiked with 10 nM (final concentration) 3H-leucine (40 to 60 Ci/mmol/L). To create a negative control, 0.5 mL of 50% trichloroacetic acid (TCA) was added to a separate 5 mL sample from each mesocosm. The samples were incubated at 26ºC (to approximate mesocosm *in situ* temperature) in the dark for 60 min. The incubation was stopped by adding 0.5 mL of 50% TCA. The samples were extracted in a water bath (80°C) for 15 min. After cooling, samples were filtered under low vacuum onto 0.45 µm cellulose nitrate filters. The filters were rinsed twice with 3 mL cold 5% TCA, then twice with 2 mL cold 80% ethanol. The filter towers were then removed and the filters were gently rinsed with 1 mL cold 80% ethanol. The filters were placed in scintillation vials and dried under the hood, after which 0.5 mL of ethyl acetate was added to dissolve the filter. Scintillation cocktail (4.5 mL) was then added to the vials and activity (CPM over a 1-min period) was measured using a Wallac liquid scintillation counter. Activity of the negative controls was subtracted from each sample's activity.

Primary productivity was estimated via photosynthetic carbon assimilation (Li et al. 1980). A 5 mL water sample was added to a scintillation vial and spiked with 0.05 mL of 1 µCi/mL $NaH^{14}CO_3$. Scintillation vials were placed in an incubator set at 26ºC and on a 16 h light:8 h dark photoperiod for 24 h to approximately simulate average mesocosm *in situ* light and temperature conditions. Each mesocosm also had a dark sample to correct for heterotrophic activity. Dark samples were created by covering a separate ^{14}C-spiked sample from each mesocosm in aluminum foil during the incubation. Samples were then filtered onto 0.45 µm cellulose nitrate membrane filters and rinsed

with 0.2 µm filtered 20‰ seawater. Filters were placed back in the scintillation vials and 0.5 mL of 10% HCl was added. Samples were dried overnight in the dark. The next day, 4.5 mL of scintillation fluid was added to each vial. After a 48 h stabilization period, disintegrations per minute (DPM) were measured (2-min counts) using a Wallac liquid scintillation counter. Activity of the dark samples was subtracted from each sample's activity.

Phytopigments

For phytoplankton, up to 80 mL of sample water was filtered and 40 mL of sample water for chlorophyll *a* was filtered. Filters were stored frozen at -80ºC until analysis by HPLC, targeting nineteen phytopigments with known taxonomic importance for phytoplankton identification. Under subdued lighting, filters were removed from foil packets and placed into pre-labeled 2.5-mL microfuge tubes using methanol-rinsed forceps. To each was added 1 mL HPLC-grade acetone (99+%; Burdick and Jackson, Muskegon, MI). Samples were kept cold during processing. Samples were vortexed (5 s) and extracted in the dark for 18 h at 4ºC. Extracts were decanted to 3 mL polypropylene luer-slip syringes (Microliter, Suwanee, GA) and filtered through 13 mm 0.2 µm PTFE membrane syringe filters (Pall Life Sciences, Port Washington, NY) into pre-labeled borosilicate glass automated sampler vials (Microliter).

Sample buffer and elution solvents were prepared from 28 mM tetrabutyl ammonium acetate (TBAA) solution adjusted to pH 6.5 and methanol (MeOH). Injection buffer was 95:5 (volume fraction) TBAA:MeOH. Solvent A was 30:70 (volume fraction) TBAA:MeOH. Solvent B was 100% MeOH. All reagents were 0.2 µm vacuum-filtered HPLC-grade (B&J) with the exception of TBAA stock.

An Agilent 1100 Series HPLC with refrigerated autoinjection system, quaternary pump, and thermally-controlled column compartment was used to inject 150 µL of each sample onto an XDB-C8 monomeric reverse-phase ZORBAX Eclipse column (150 mm x 4.6 mm x 3.5 µm; Agilent Technologies, Santa Clara, CA). Injection protocol followed the Van Heukelem and Thomas (2001) method with slight modification. In brief, extracts were mixed with injection buffer and eluted at 1 mL/min for 27 min using a linear gradient from 5% to 100% solvent B. The autosampler compartment was kept at

4ºC and covered completely throughout each batch to prevent thermo- and photodegradation of pigments. To prevent temperature gradients within the column, solvent lines were preheated to match the column temperature of 60ºC.

Chromatograms were generated using a photodiode array detector monitoring at 436 nm, 450 nm, and 665 nm. Peaks were identified using spectral libraries for absorbance profile matching and retention times from a technical mixture of phytopigments (DHI, Hørsholm, Denmark). Retention times and spectral absorbance were verified manually in each sample to assure correct identification. Quantification was performed at 450 nm using ChemStation for LC 3D Systems software [Rev B.01.01] (Agilent) using a 5-point calibration curve.

Nutrients

Water from each replicate sample was filtered through a pre-combusted 0.7-µm GF/F, and filtrate was analyzed within 24 h of sample collection for orthophosphate (PO_4^{3-}), dissolved inorganic nitrogen (DIN) as nitrite/nitrate (NO_{2-}/NO_{3-}), and ammonium (NH_{4+}). Filtrate was also collected and frozen at -20°C then analyzed (within 28 d) for dissolved nitrogen and phosphorus (TDN, TDP), using a Lachat Quick-Chem 8000 nutrient auto-analyzer (Hach Company, Loveland, CO) and standard laboratory methodologies (Grasshoff 1983; Johnson and Petty 1983; Zimmerman and Keefe 1991).

Chemical Assessment

Pre-dose, 12 h, 24 h, 48 h, 7 d, and 14 d post-dose water samples were collected and hydrocarbon (total extractable material [TEM; 250 mL]) and dispersant (dioctyl sodium sulfosuccinate [DOSS; 15 mL]) concentrations were quantified. Sediment samples (7 g to10 g) were collected for total extractable hydrocarbon (TEH) and PAH analyses at 7 d, 30 d and 60 d. Biota samples (fish and grass shrimp) were collected at 60 d for DOSS analysis.

TEM/TEH analysis

TEM (total extractable matter) and TEH (total extractable hydrocarbons), representing the total aromatic and aliphatic hydrocarbon content of sample extracts, were

quantified in water and sediments using gas chromatography/flame ionization detection (GC/FID) methods as described in detail in the Mississippi Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment Analytical Quality Assurance Plan, Version 2.2 (now updated to Version 3.1; http://gulfsciencedata.bp.com). Briefly, for TEH, sediment sample extracts were cleaned up by silica gel prior to analysis by GC/FID. The result was reported as based on integration of the FID signal over the entire hydrocarbon range from n-C9 to n-C44 and calibrated against the average alkane hydrocarbon response factor. The water sample extracts did not receive any clean-up so the result was reported as Total Extractable Matter (TEM) because the extract may have contained non-hydrocarbon compounds. This work was performed by NOAA's Northwest Fisheries Science Center, Seattle, WA.

DOSS analysis

Analysis for the Corexit compound dioctyl sodium sulfosuccinate (DOSS) in water and tissue samples was conducted using a QuEChERS (i.e., quick, easy, cheap, effective, rugged and safe) sample preparation procedure. Briefly, each sample was weighed into a 50 mL QuEChERS tube (Restek U.S., Bellefonte, PA), 10 mL of distilled water was added and each sample was spiked with a surrogate standard (d34 DOSS; 10 µg). Each sample extraction tube was mixed on a shaker for 5 min. After mixing, 15 mL of acetonitrile was added to each sample and the extract was mixed on the shaker for 5 min. Next, 6 g of magnesium sulfate and 1.5 g of sodium acetate were added and the tube contents were mixed for 5 minutes on the tube shaker. An internal standard (d25 SDS; 10 µg) was added to each sample tube and the tube contents were vortexed for 5 s to ensure adequate mixing. The sample extracts were centrifuged at 3400 *g* for 10 min to separate the solvent layers and the acetonitrile layer (upper) containing the DOSS was filtered through a 0.20 µm PFTE filter and the filtered extract was diluted by a factor of 15 and then analyzed for DOSS using liquid chromatography tandem mass spectrometry (LC-MS/MS) (11, 12). The analytes and standards were separated on an Agilent ZORBAX SB-C18 Rapid Resolution HD 2.1x50mm 1.8µm LC column. Prior to analysis, the mass spectrometer was tuned in negative ion mode using infusions of DOSS, d34-DOSS and d25-SDS, each at about 5 µg/mL. The instrument was calibrated using sets of

up to seven multi-level calibration standards of known concentrations. Quality assurance samples (a method blank, spiked blank and oyster control material) were analyzed with each sample batch. This work was performed by Columbia Analytical Services, Inc. (now known as ALS Environmental), Houston, TX 77099

Sediment PAH analysis

Frozen sediments were thawed prior to extraction. Samples were homogenized and 2 g to 5 g aliquots of sediment were transferred to a pre-weighed aluminum pan for dry fraction determination. Dry fraction sediments were placed in a drying oven (100°C) for at least 24 h. Dried sediments were weighed and dry fractions calculated. PAHs were extracted by grinding approximately 10 g of wet sediment mixed with 23 g to 25 g anhydrous sodium sulfate in a solvent-washed glass mortar bowls. When dry, the sample was transferred to 33 mL Accelerated Solvent Extraction cells (ASE, Dionex, Inc.) for extraction. Matched carbon and deuterated internal standards were added to each cell and the samples were extracted in acetone and dichloromethane (50/50 v/v) using a Dionex ASE200. Solvent extracts were passed through Whatman phase separation filter paper (1PS) and additional anhydrous sodium sulfate to remove residual water. Solvent volumes were reduced under nitrogen and samples were solvent exchanged into 2 mL dichloromethane. Additional sample clean up included gel permeation chromatography (size exclusion, SX-3 in DCM) and solid phase extraction (activated alumina) for a final 0.5 mL sample in hexane. A recovery standard (p-terphenyl) was added to each sample prior to instrumental analysis. PAHs were identified and quantified using GC/MS (Varian 4000 GCMS) with a Varian FactorFour VF-Xms capillary column (30 m x 0.25 mm x 0.25 μm). A series of blanks, reagent spikes, matrix spikes and standard reference materials were also analyzed to assure data quality.

RESULTS PHASE I

C. variegatus Assessment

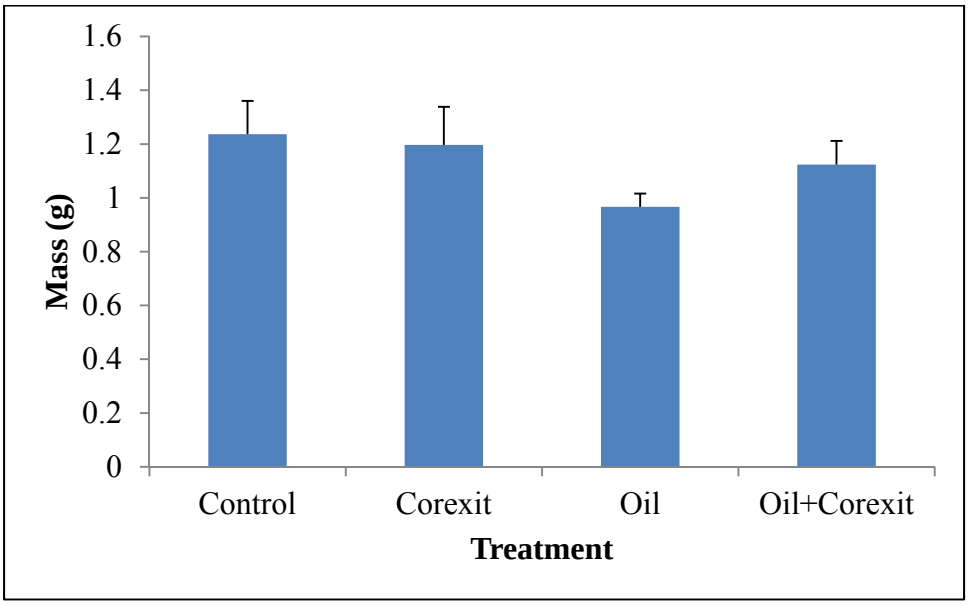
The results for survival of *C. variegatus* at the end of Phase I are shown in Table 3. The percent recovery ranged from 60% in Tank L1 to 100% in Tank H1.

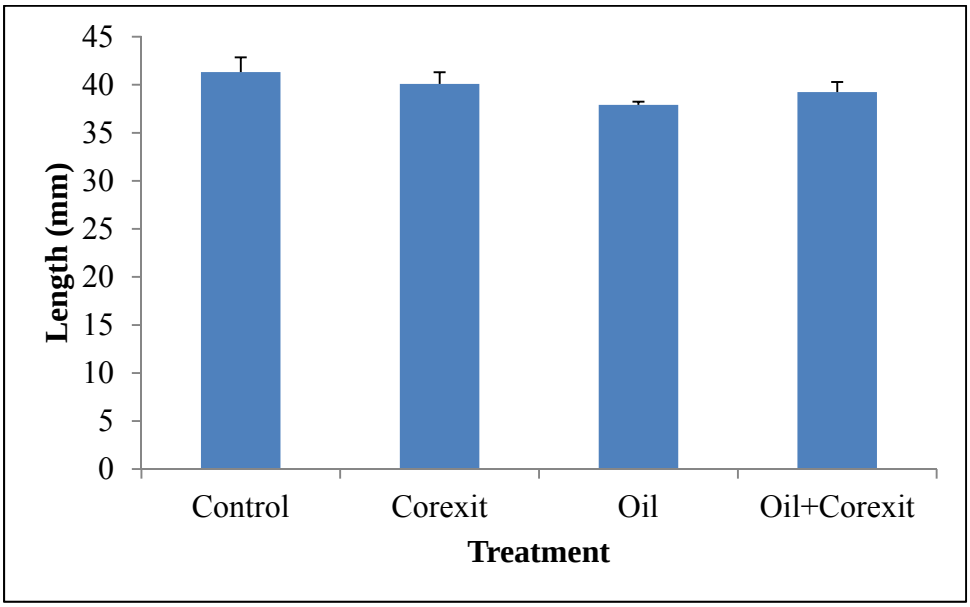
Table 3. Number of surviving *C. variegatus* in each mesocosm tank at the end of Phase I.

Treatment and Tank #		sex			Total
		Female	Male	Unknown	
Control	**G2**	9	14	.	23 (92%)
	K2	10	8	.	18 (72%)
	L2	10	11	.	21 (84%)
Corexit	**H3**	16	8	.	24 (96%)
	K1	17	7	.	24 (96%)
	L1	6	9	.	15 (60%)
Oil	**G3**	12	10	.	22 (88%)
	H2	14	8	.	22 (88%)
	L3	13	7	.	20 (80%)
Oil + Corexit	**G1**	10	10	.	20 (80%)
	H1	16	9	.	25 (100%)
	K3	10	10	1	21 (84%)

Mean mass and length are shown in Figure 5. Samples sizes were as listed in Table 3. There were no treatment-related differences in either length or mass (one way ANOVA: $p = 0.26$ length; $p = 0.36$ mass).

Figure 5. *C. variegatus* mean mass and length at the end of Phase I. Error bars indicate standard error.





***M. mercenaria* Assessment**

A large number of clams were discovered missing in each tank at the end of Phase I. It is believed this was due to the design of the clam containers used in the tanks. Clam survival ranged from 11.3% in oil Tank H2 up to 82% in control Tank K2 (Table 3). The lowest survival occurred in the oil-only tanks and mortality was highly variable within

treatments (Table 4). The dry masses of the surviving clams were lowest in the Corexit treatments at 2.23 mg and highest in the oil+Corexit treatments at 40.98 mg (Table 5). Condition indices of the surviving clams were fairly consistent across all treatments (Table 5). The lowest total shell area occurred in Corexit tanks while the highest total shell area was found in the oil+Corexit mixture tanks (4.58 mm^2 and 35.2 mm^2, respectively; Table 6). The results for major and minor axis lengths followed that of the total area determination (Table 6).

Table 4. *M. mercenaria* survival at the end of Phase I.

Tank #	Treatment	# Clams Alive	# Clams Dead	# Clams Missing
G2	Control	47 (31.3%)	8 (5.3%)	95 (63.3%)
L2	Control	95 (63.3%)	13 (8.6%)	42 (28%)
K2	Control	123 (82%)	7 (4.6%)	20 (13.3%)
G3	Oil	56 (37.3%)	4 (2.7%)	90 (60%)
L3	Oil	23 (15.3%)	109 (72.7%)	18 (12%)
H2	Oil	17 (11.3%)	107 (71.3%)	26 (17.3%)
H3	Corexit	107 (71.3%)	14 (9.3%)	29 (19.3%)
K1	Corexit	103 (68.7%)	12 (8%)	35 (23.3%)
L1	Corexit	27 (18%)	82 (54.7%)	41 (27.3%)
G1	Oil+Corexit	74 (49.3%)	12 (8%)	64 (42.7%)
H1	Oil+Corexit	108 (72%)	12 (8%)	30 (20%)
K3	Oil+Corexit	95 (63.3%)	16 (10.7%)	39 (26%)

Table 5. *M. mercenaria* dry mass and condition index (CI) at pre-dose and the end of Phase I. SE is standard error of the mean.

Treatment	Tank or Replicate	Pre-Dose		59 d	
		Dry Mass (mg)	CI	Dry Mass (mg)	CI
Baseline	1	7.196	1.311	-	-
	2	7.106	1.249	-	-
	3	7.121	1.252	-	-
	Mean	7.141	1.271		
	SE	0.027	0.020		
Control	G2	-	-	2.560	0.122
	K2	-	-	7.335	0.163
	L2	-	-	32.328	0.134
	Mean			14.075	0.139
	SE			9.230	0.012
Oil	G3	-	-	3.317	0.139
	L3	-	-	7.492	0.135
	H2	-	-	3.702	0.120
	Mean			4.837	0.131
	SE			1.332	0.0057
Corexit	H3	-	-	2.783	0.151
	K1	-	-	3.122	0.158
	L1	-	-	0.797	0.1198
	Mean			2.234	0.143
	SE			0.725	0.0116
Oil+Corexit	G1	-	-	28.007	0.125
	H1	-	-	48.444	0.142
	K3	-	-	46.494	0.121
	Mean			40.982	0.129
	SE			6.512	0.0066

Table 6. *M. mercenaria* shell area, major axis length and minor axis length at pre-dose and the end of Phase I. SE is standard error of the mean.

Treatment	Tank or Replicate	Pre-Dose			59 d		
		Shell Area (mm^2)	Major Axis (mm)	Minor Axis (mm)	Shell Area (mm^2)	Major Axis (mm)	Minor Axis (mm)
Baseline	1	2.359	1.836	1.629	-	-	-
	2	2.349	1.904	1.569	-	-	-
	3	2.359	1.904	1.568	-	-	-
	Mean	2.356	1.881	1.589			
	SE	0.0032	0.0228	0.0201			
Control	G2	-	-	-	5.658	2.954	2.4067
	L2	-	-	-	10.324	3.808	3.101
	K2	-	-	-	30.286	6.745	5.319
	Mean				18.76	5.02	4.01
	SE				1.028	0.146	0.109
Oil	G3	-	-	-	6.368	3.055	2.556
	L3	-	-	-	11.159	4.089	3.318
	H2	-	-	-	7.360	3.339	2.762
	Mean				7.68	3.35	2.77
	SE				0.408	0.0870	0.0684
Corexit	H3	-	-	-	5.266	2.818	2.318
	K1	-	-	-	5.701	2.881	2.387
	L1	-	-	-	2.632	2.0014	1.661
	Mean				5.15	2.75	2.27
	SE				0.1799	0.044	0.0365
Oil+Corexit	G1	-	-	-	27.863	6.567	5.194
	H1	-	-	-	37.611	7.747	5.664
	K3	-	-	-	40.237	7.937	6.103
	Mean				35.91	7.50	5.69
	SE				1.167	0.142	0.0955

S. alterniflora Assessment

Results for the number of marsh grass stems counted in each mesocosm tank before and after exposure are given in Table 7. An increase in stem density (Table 7) and shoot height (Table 8) was seen in all tanks from Day 0 to the end of the test. There was no statistical difference between the control and treatments (one way ANOVA: $p = 0.71$ at Day 0; $p = 0.57$ at Day 30; $p = 0.55$ at Day 60), but the Corexit tanks tended to have a lower stem count at 30 d and 60 d compared to control and the other treatments. The average shoot height of *Spartina* in each mesocosm tank was also measured (Table 8). There was no statistical difference between the control and treatments and no apparent trend (one way ANOVA: $p = 0.65$ at Day 0; $p = 0.30$ at Day 30; $p = 0.33$ at Day 60).

Table 7. *Spartina* stem density in each mesocosm tank before and after exposure to four treatments.

Treatment and Tank #		**Test duration (d)**		
		0	**30**	**60**
Control	**G2**	55	96	151
	K2	49	77	111
	L2	38	66	90
Corexit	**H3**	41	73	105
	K1	58	77	115
	L1	45	66	84
Oil	**G3**	52	89	129
	H2	50	85	121
	L3	44	77	115
Oil+Corexit	**G1**	27	68	109
	H1	49	91	127
	K3	47	86	122

Table 8. Average shoot height of *Spartina* and total number of shoots in each tank. SE is standard error of the mean.

Treatment and Tank #		Test duration (d)								
		0			30			60		
		Mean	SE	n	Mean	SE	n	Mean	SE	n
Control	**G2**	25.9	0.904	213	34.2	0.951	471	44.5	1.048	675
	K2	26.7	1.020	164	39.8	1.179	275	49.0	1.302	472
	L2	28.0	1.338	128	36.2	1.390	270	46.1	1.234	451
Corexit	**H3**	27.7	1.095	132	37.9	1.258	238	40.6	1.207	487
	K1	27.7	1.086	176	44.2	1.281	288	50.3	1.413	491
	L1	27.2	1.145	134	36.9	1.351	221	40.9	1.265	366
Oil	**G3**	26.5	0.974	179	35.5	1.114	397	44.7	1.218	568
	H2	27.1	0.941	169	36.6	1.170	299	36.7	1.162	548
	L3	28.0	1.037	163	37.0	1.294	292	41.7	1.123	541
Oil+Corexit	**G1**	27.6	1.344	80	29.9	1.229	277	43.4	1.180	521
	H1	28.2	0.948	177	38.3	1.106	334	41.3	1.080	660
	K3	26.8	0.927	175	34.4	1.126	301	41.0	1.196	507

Benthic Infauna Assessment

Benthic infauna community characteristics (density, number of taxa, diversity, and B-IBI [Benthic Index of Biotic Integrity]) were similar across all treatments (Table 9, Figures 6 and 7). While no significant effects related to treatment were detected by two-way ANOVA, Figure 3 shows a slight reduction in the number of taxa within the first week of exposure to the oil+Corexit. This then recovers to conditions similar to the other treatments (including the control) between 1-2 months. Time was found to have a significant effect for both diversity and B-IBI (Table 10). Both diversity and the mean B-IBI increased in value from the pre-dose sampling period in May to the one month sampling period in June (Figure 6). Between the one month and two month sampling period the mean B-IBI held relatively steady while diversity declined slightly.

Separate one-way ANOVAs among treatments at each time point were completed to further investigate the possibility of a treatment effect. No significant treatment effects were detected (Table 11). Sediment characteristics were similar for all treatments (Figure 7). Separate one-way ANOVAs among treatments were completed for percent silt-clay and TOC. No significant differences were detected for silt-clay or TOC (Table 12).

Table 9. Benthic community characteristics by phase, treatment and time in Phase I.

Time	Treatment	Number of Taxa	Diversity (H')	Density (m^{-2})	B-IBI[a]
Pre-dose	Control	9	1.62	4575	2.7
	Corexit	8	1.48	4408	2.3
	Oil	10	2.22	3533	2.8
	Oil+Corexit	6	0.81	4042	1.7
One week post-dose	Control	9	1.82	2592	2.3
	Corexit	9	1.74	3375	2.7
	Oil	12	2.59	4133	3.2
	Oil+Corexit	7	1.67	4308	2.3
One month post-dose	Control	9	1.91	3700	2.8
	Corexit	10	2.66	1542	3.3
	Oil	9	1.99	2125	2.7
	Oil+Corexit	8	2.42	1300	3.0
Two months post-dose	Control	9	1.32	5683	2.5
	Corexit	9	1.23	8942	2.7
	Oil	9	1.66	12025	2.5
	Oil+Corexit	9	1.98	5108	2.8

[a] Following Van Dolah et al. (1999)

Table 10. Results of two-way ANOVA of benthic infaunal data Phase I.

Model	*df*	*F*	*Pr > F*	Effect	*df*	*F*	*Pr > F*
H' = treatment + time + (treatment x time)	15	1.65	0.1143	Treatment	3	1.07	0.3746
				Time	3	**3.17**	**0.0377**
				Treatment x time	9	1.34	0.2562
# Taxa = treatment + time + (treatment x time)	15	0.98	0.4988	Treatment	3	2.40	0.0865
				Time	3	0.68	0.5700
				Treatment x time	9	0.60	0.7844
Density= treatment + time + (treatment x time)	15	0.66	0.7986	Treatment	3	0.21	0.8917
				Time	3	2.21	0.1066
				Treatment x time	9	0.30	0.9684
B-IBI= treatment + time + (treatment x time)	15	**2.18**	**0.0316**	Treatment	3	1.37	0.2706
				Time	3	**3.30**	**0.0327**
				Treatment x time	9	2.08	0.0622
				Treatment x time	6	0.95	0.4801
B-IBI= treatment + time + (treatment x time)	11	0.77	0.6692	Treatment	3	1.40	0.2661
				Time	2	1.68	0.2068
				Treatment x time	6	0.14	0.9893

Table 11. Results of separate one-way ANOVAs by time of sampling and benthic infaunal data.

Effect	Time	*df*	*F*	*Pr > F*	Significant Differences
H'	Pre-dose	3	1.71	0.2411	
	One week post-dose	3	2.92	0.1003	
	One month post-dose	3	2.51	0.1329	
	Two months post-dose	3	0.40	0.7542	
# Taxa	Pre-dose	3	2.70	0.1160	
	One week post-dose	3	2.37	0.1467	
	One month post-dose	3	0.27	0.8435	
	Two months post-dose	3	0.02	0.9960	
Density	Pre-dose	3	0.13	0.9411	
	One week post-dose	3	0.15	0.9272	
	One month post-dose	3	1.15	0.2881	
	Two months post-dose	3	0.28	0.8412	
B-IBI	Pre-dose	3	3.83	0.0571	
	One week post-dose	3	3.19	0.0842	
	One month post-dose	3	0.65	0.6058	
	Two months post-dose	3	0.73	0.5607	

Table 12. Results of separate one-way ANOVAs of sediment characteristics data.

Effect	Time	*df*	*F*	*Pr > F*
Percent Silt-Clay	Two month post-dose	3	1.15	0.3848
TOC	Two month post-dose	3	1.53	0.2799

Figure 6. Benthic infauna community characteristics for four sampling periods during Phase I.

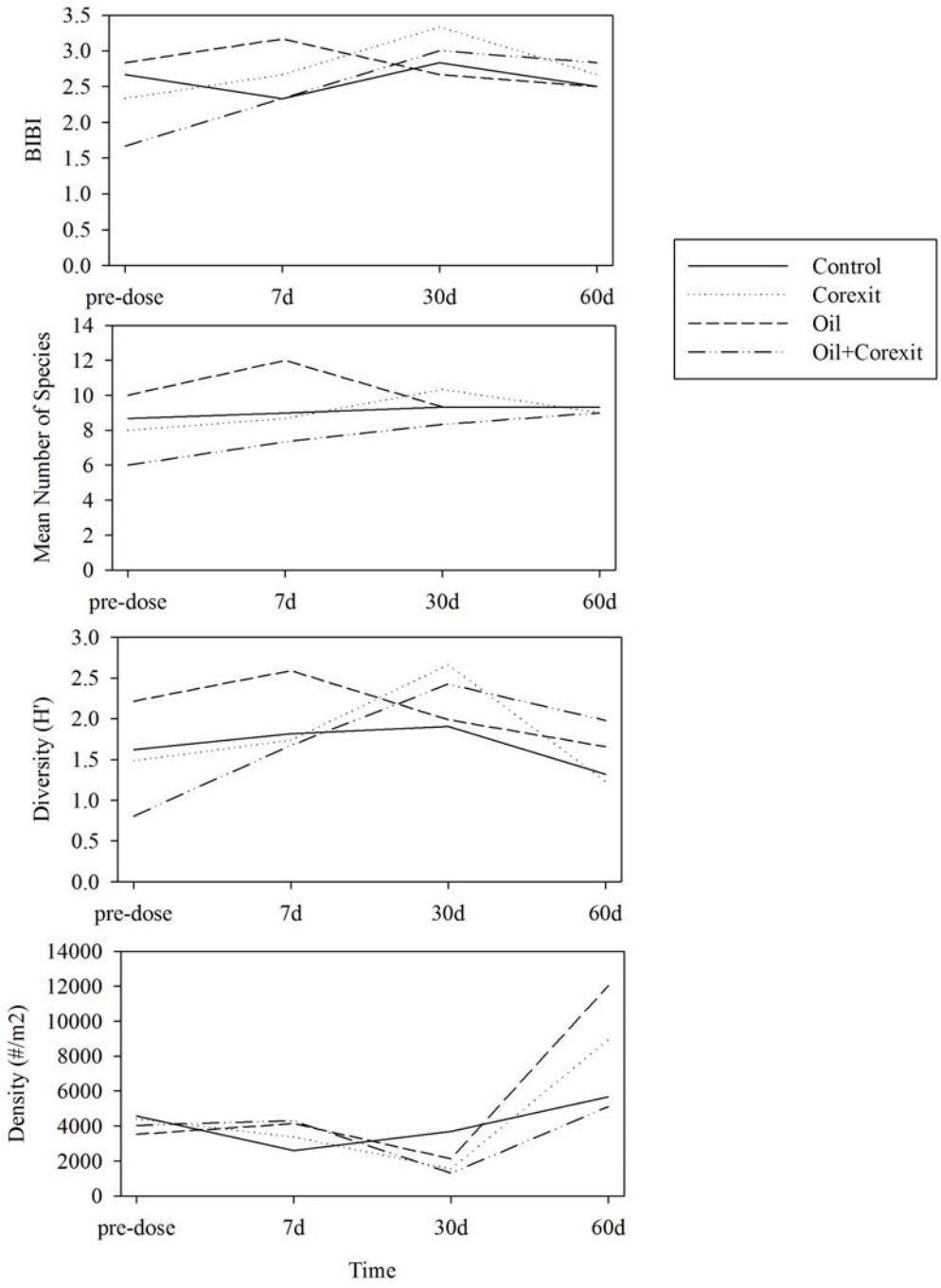
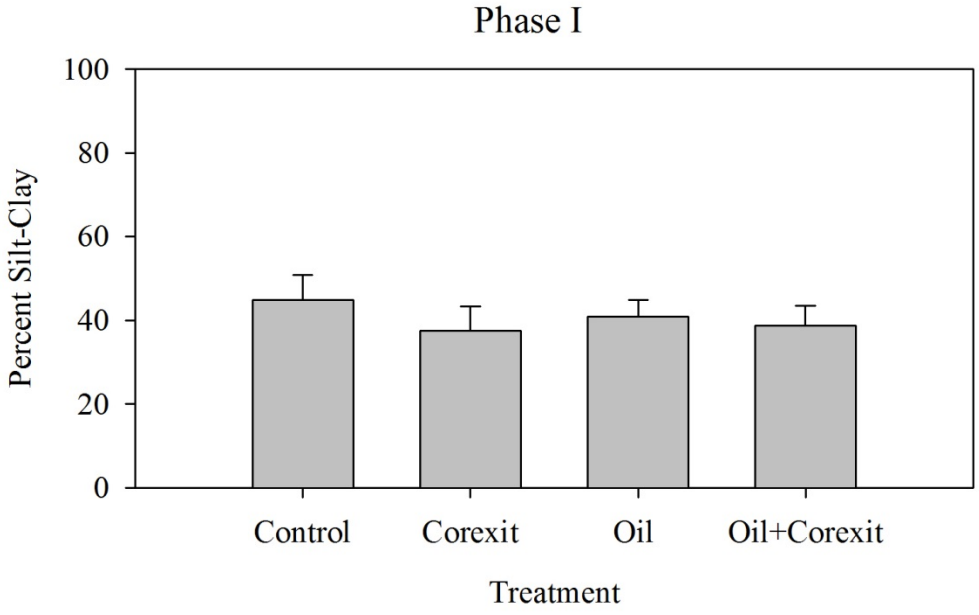
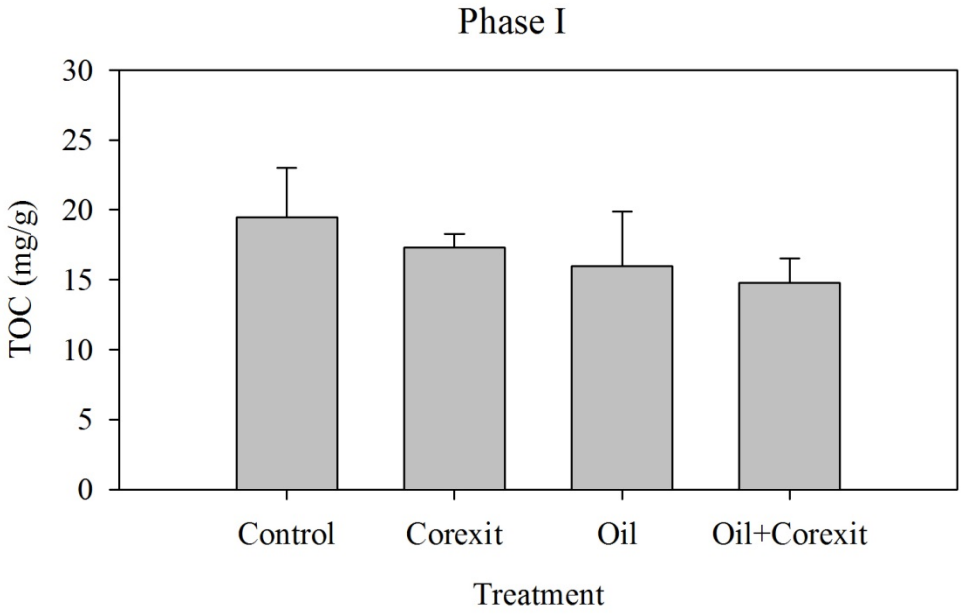


Figure 7. Benthic sediment characteristics for each treatment during Phase I. Error bars indicate standard error.

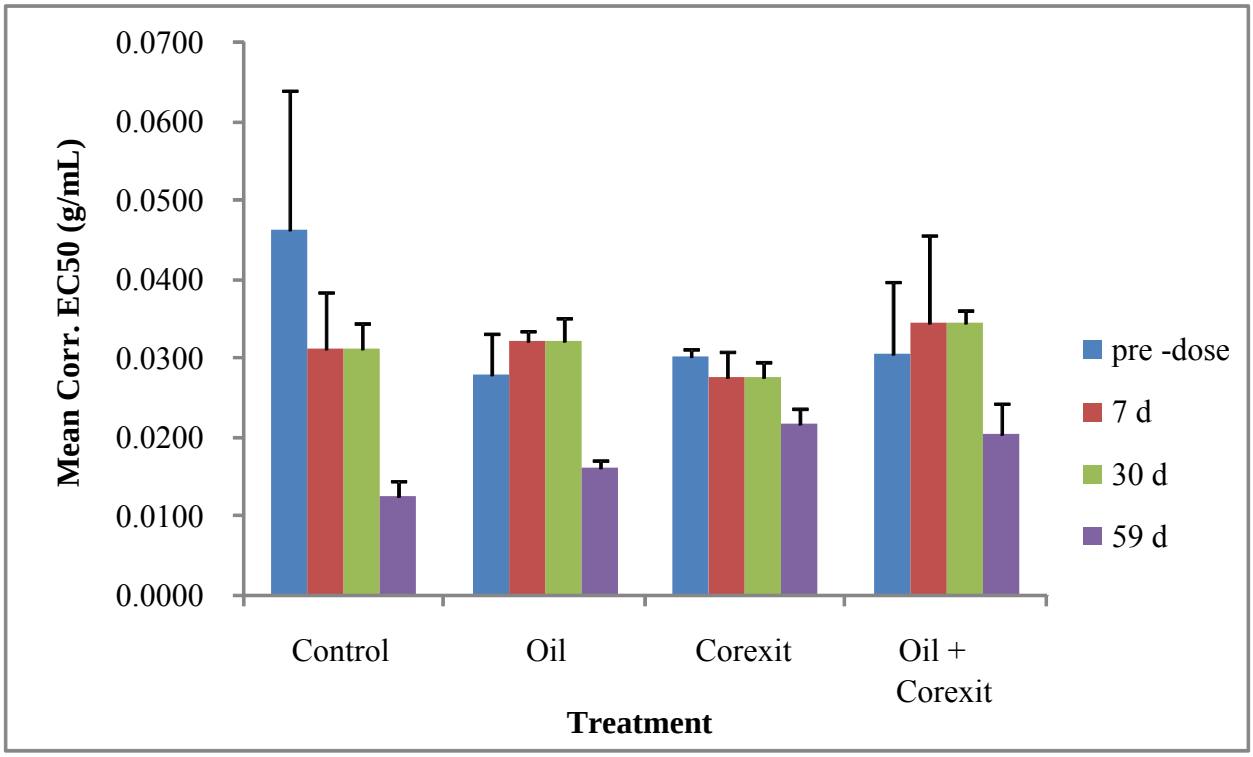




Microtox Solid Phase Assessment

There were no significant effects of treatment on Microtox toxicity. The results of the Microtox assays showed variable EC50 values from pre-dose to the end of the test (Figure 8). The 59 d EC50s trended lower for all treatments.

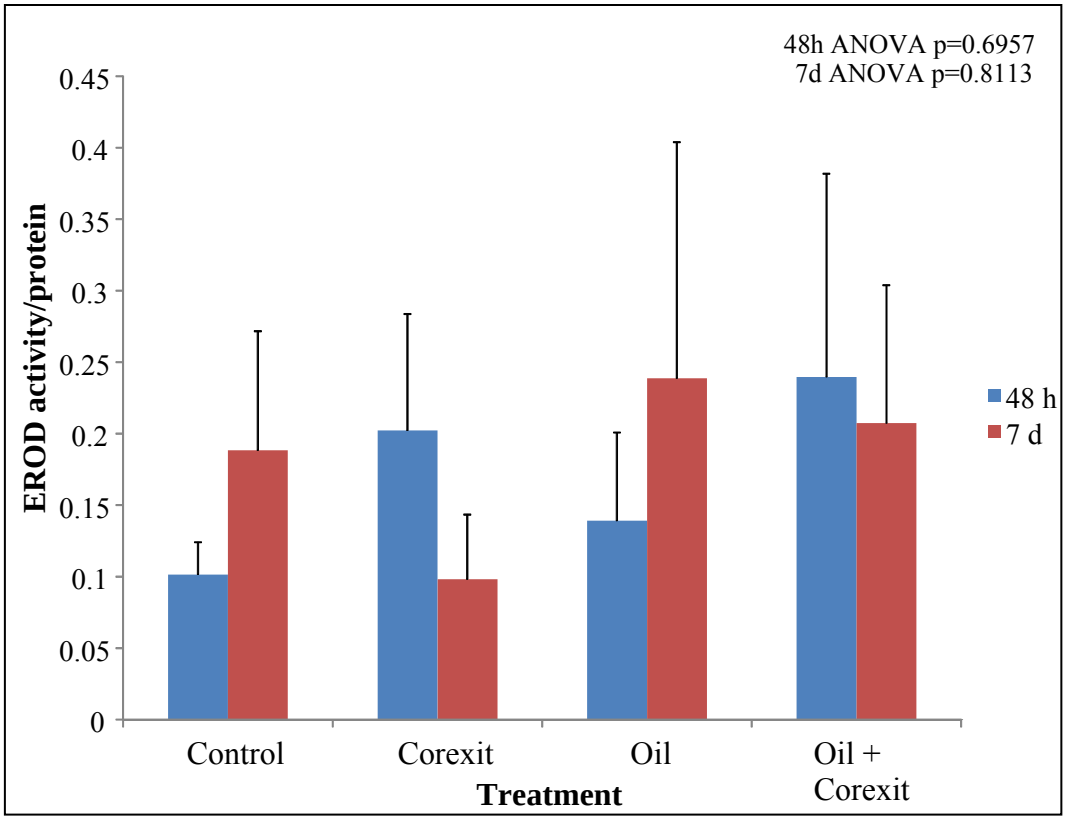
Figure 8. Results of Microtox sediment assays expressed as Mean Corrected EC50s (g/mL) assessed at four time periods. Each bar represents the mean of three samples. Error bars are the standard error of the mean.



F. heteroclitus EROD Activity Assessment

EROD activity was measured in mud minnows exposed for 48 h and 7 d (Figure 9). Activity between control and treatments was not significantly different but there was an increasing trend in EROD activity at 7 d in the oil and oil+Corexit treatments compared to control. There was also no significant difference for each treatment between the 48 h and 7 d values (p = 0.9237).

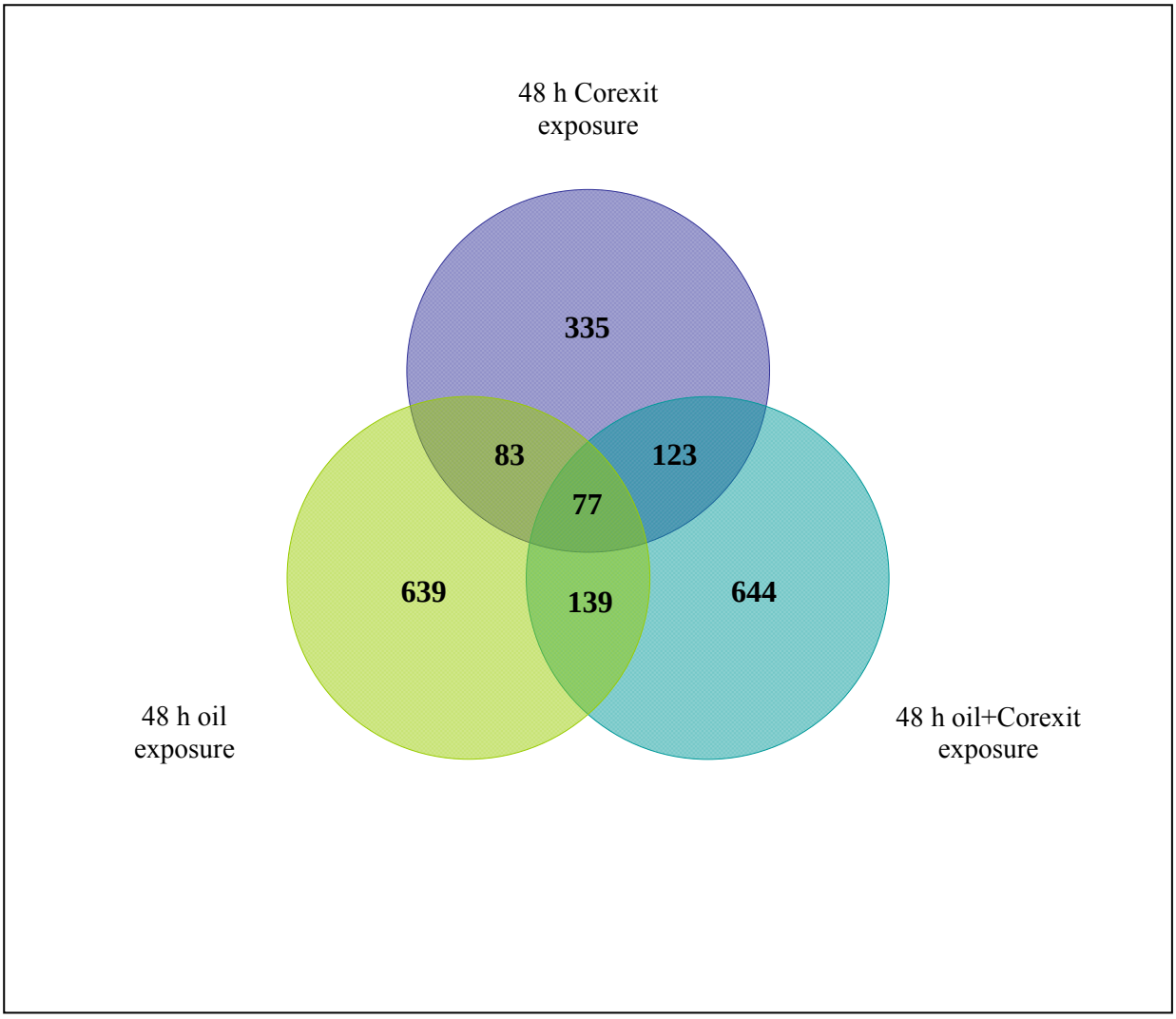
Figure 9. Results of Phase I fish liver EROD activity. Bars represent the mean of six samples. Error bars represent the standard error of the mean.



P. pugio RNA Assessment

Comparisons between treatment groups and control identified 938, 618 and 983 significantly differentially expressed genes (DEG) ($p \leq 0.001$; absolute fold change ≥ 1.5) for oil, Corexit and mixture, respectively. For these, 77 were observed in all treatments, 83 were shared between oil and Corexit, 139 between oil and the mixture and 123 between Corexit and the mixture (Figure 10). The number of DEG unique to a specific treatment group ranged from a high of 644 for the mixture group to a low of 335 for the Corexit group. For the oil treatment group, 639 DEG were identified. It appears that both generic biological responses to xenobiotic exposure were detected in features that were shared across the various treatment groups while a large number of DEG were unique for each of the three treatment groups. Taken together, these data show promise in developing biomarkers for different levels of xenobiotic exposure ranging from universal stress responses to compound specific expression profiles. Further analysis of the biological pathway to which the identified DEG are involved will likely improve the success of biomarker development.

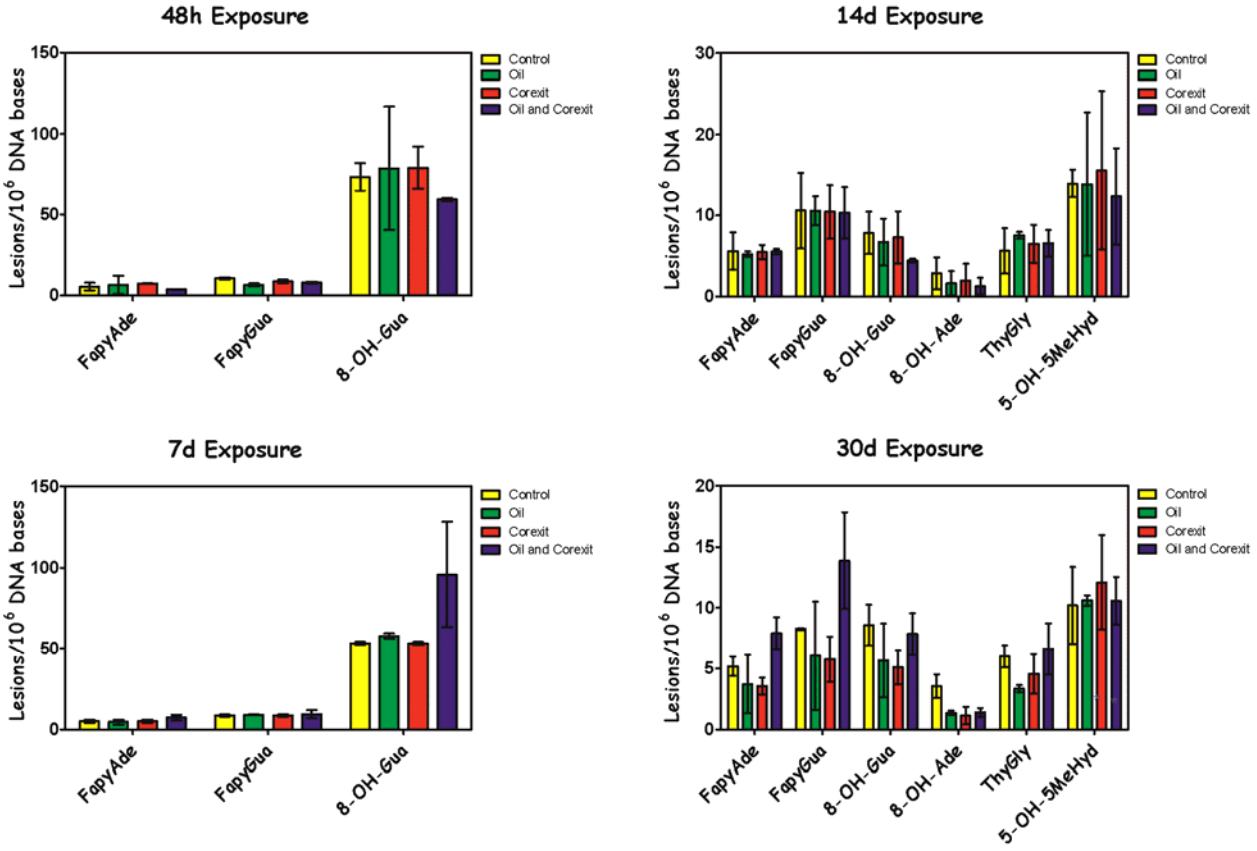
Figure 10. *P. pugio* RNA comparison between treatments**.**



P. pugio DNA Assessment

Measurements of oxidatively-induced DNA damage to grass shrimp were compared among the mesocosm treatments (Figure 11). There was not a statistically significant increase from the oil, Corexit, or oil+Corexit treatment at any of the time points (ANOVA analysis with Dunnett's multiple comparison post hoc test comparing the treatment conditions against the control). Thymine glycol (ThyGly), 8-OH-Adenine (8-OH-Ade) and 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd) for the 48 h and 7 d exposure were not readily quantifiable and thus not included.

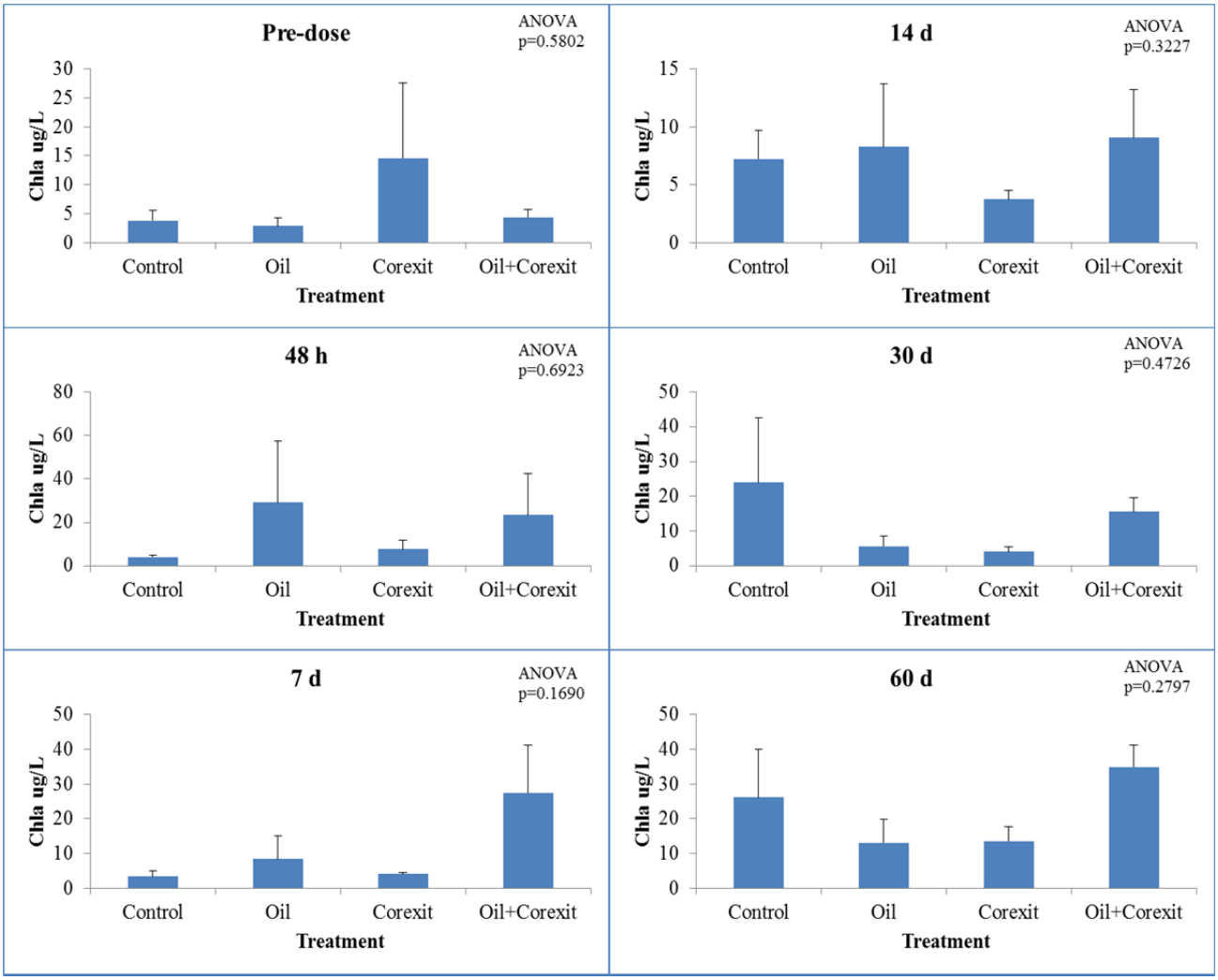
Figure 11. Oxidatively-induced DNA damage to grass shrimp after exposure at four time points. Data represent the means of multiple independent samples (n ≥ 2) and error bars indicate the standard deviations.



Chlorophyll, Productivity, Phytopigments and Nutrients Assessment

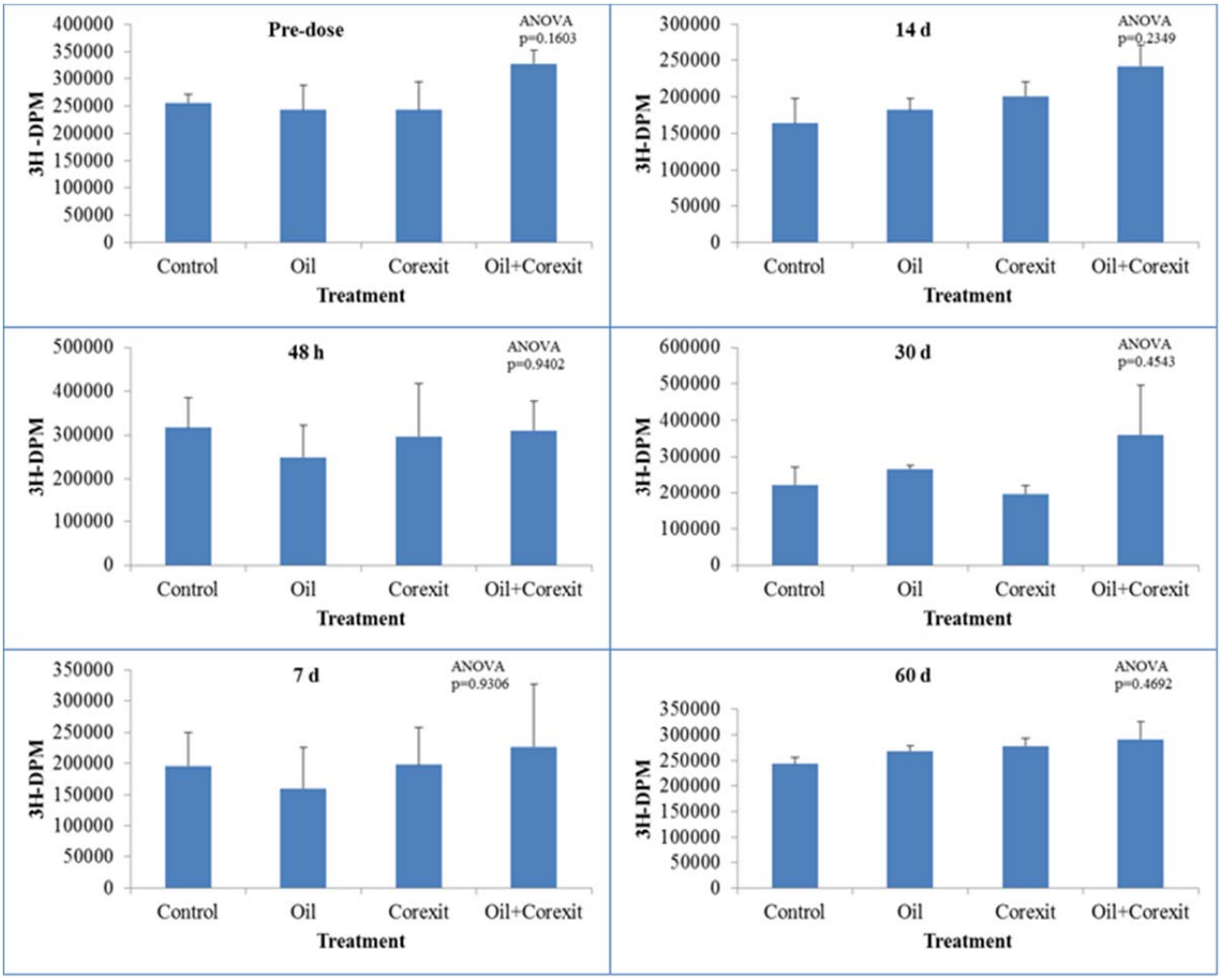
Chlorophyll concentrations were variable throughout the experiment, but did tend to be elevated in the oil+Corexit treatments after the pre-dose measurement (Figure 12). There was a significant effect of time on chlorophyll concentrations overall (repeated measures ANOVA, $p = 0.0002$) but no significant treatment effects at the time points measured.

Figure 12. Phase I chlorophyll concentrations at six time points. Bars represent the mean of three samples. Error bars represent the standard error of the means.



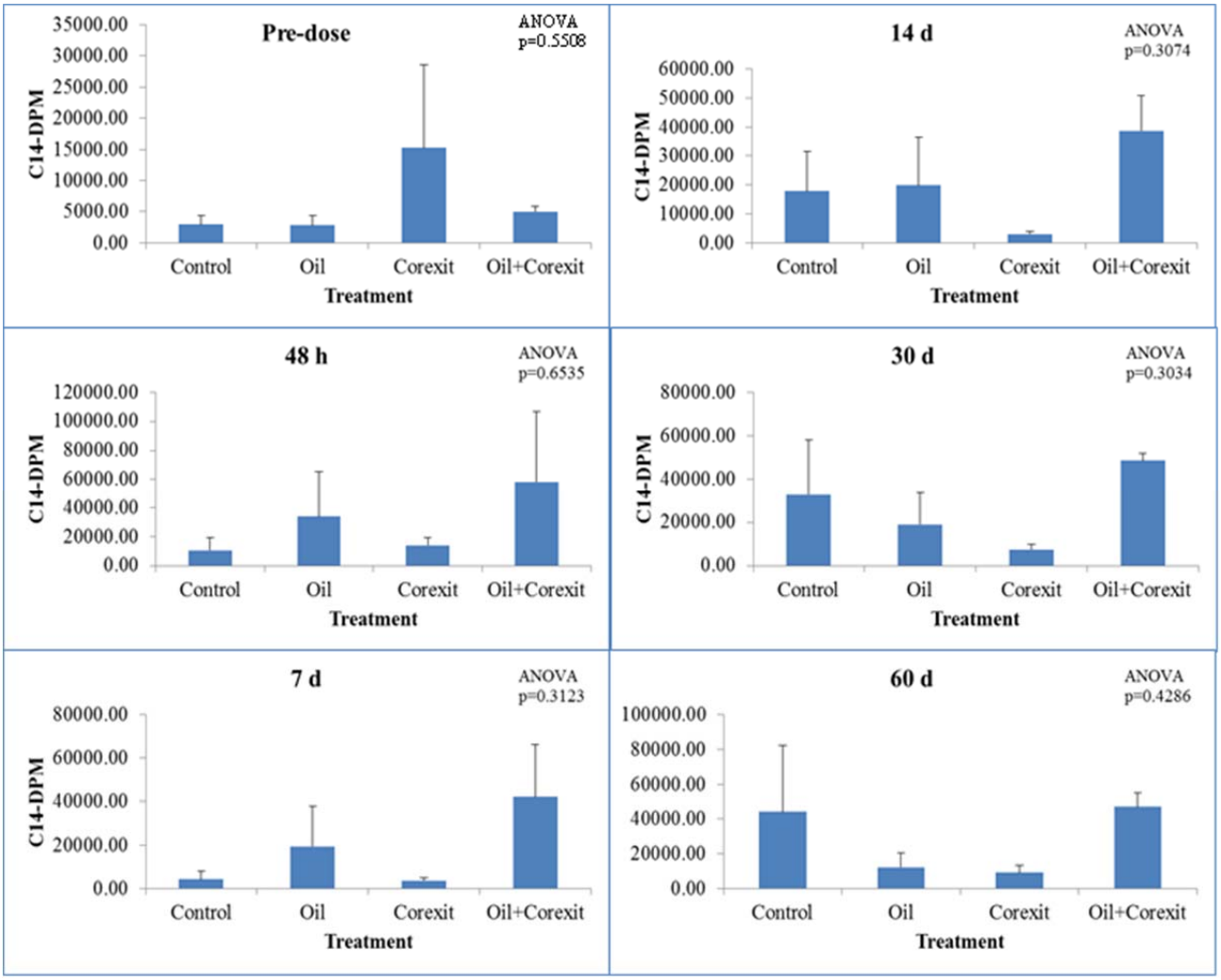
Bacterial productivity for the experiment is given in Figure 13. While there were no significant differences between treatments at the time points measured, productivity tended to be elevated in the oil+Corexit treatments.

Figure 13. Phase I bacterial productivity measured at six time points. Bars represent the mean of three samples. Error bars represent the standard error of the means.



Primary productivity is given in Figure 14. The results were variable throughout the experiment at the time points measured. There were no significant effects, however primary productivity tended to be elevated in the oil+Corexit treatments after the pre-dose measurement.

Figure 14. Phase I primary productivity measured at six time points. Bars represent the mean of three samples. Error bars represent the standard error of the means.



Nutrient data, including total dissolved phosphorus, orthophosphate, total dissolved nitrogen, ammonium and nitrate/nitrite levels are shown in Figure 15. There were no significant differences (one way ANOVA, $p > 0.05$) in any of the nutrient concentrations compared to control levels at any of the time points assessed. Total dissolved nitrogen and nitrate-nitrite levels generally increased throughout the

experiment. The 30 d and 60 d Corexit exhibited a trend toward higher nitrate + nitrite levels. Ammonium levels increased up to 7 d then decreased.

Figure 15. Nutrient levels sampled at four time periods during the experiment. Bars represent the mean of three samples. Error bars represent the standard error of the means.

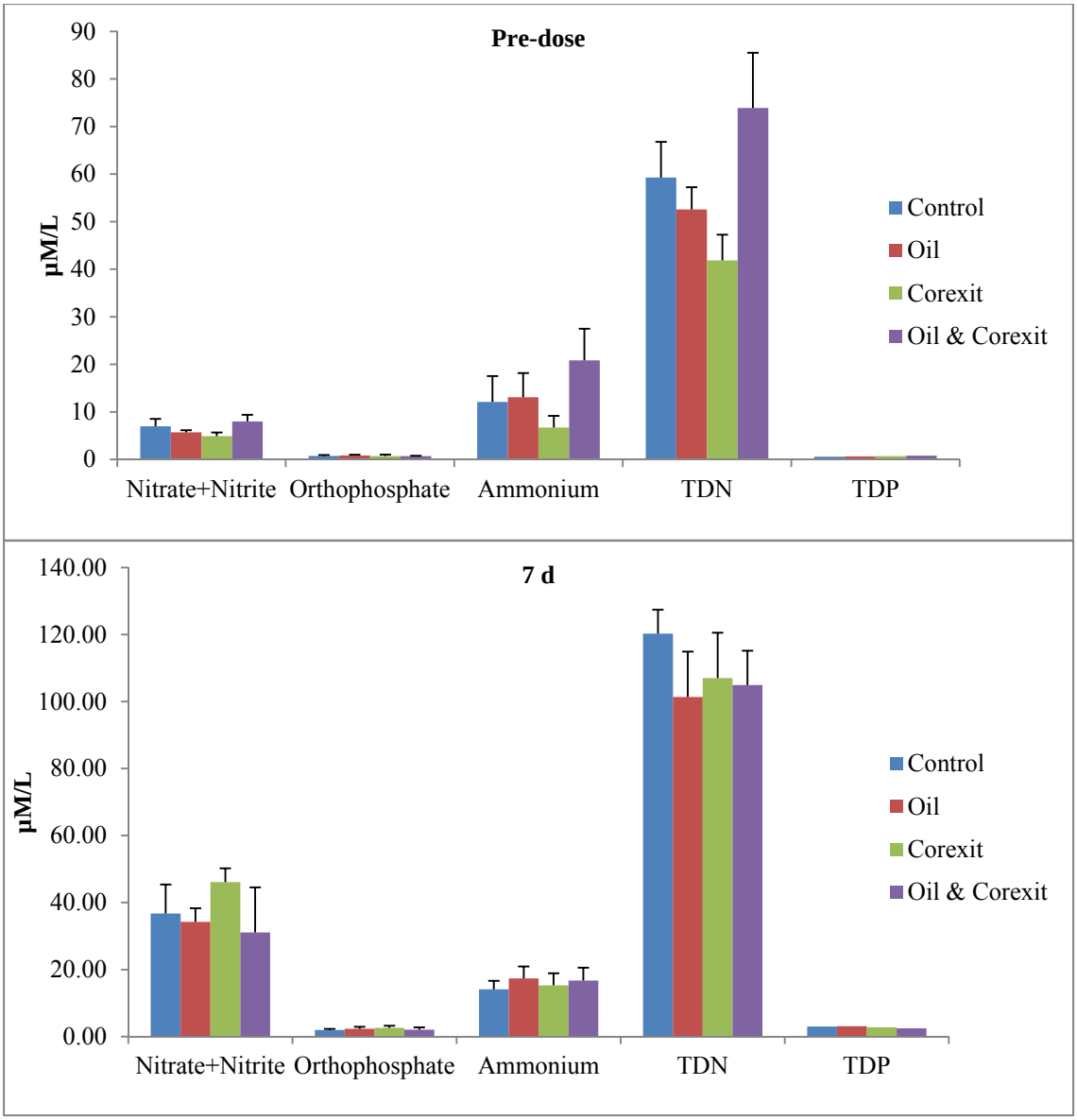
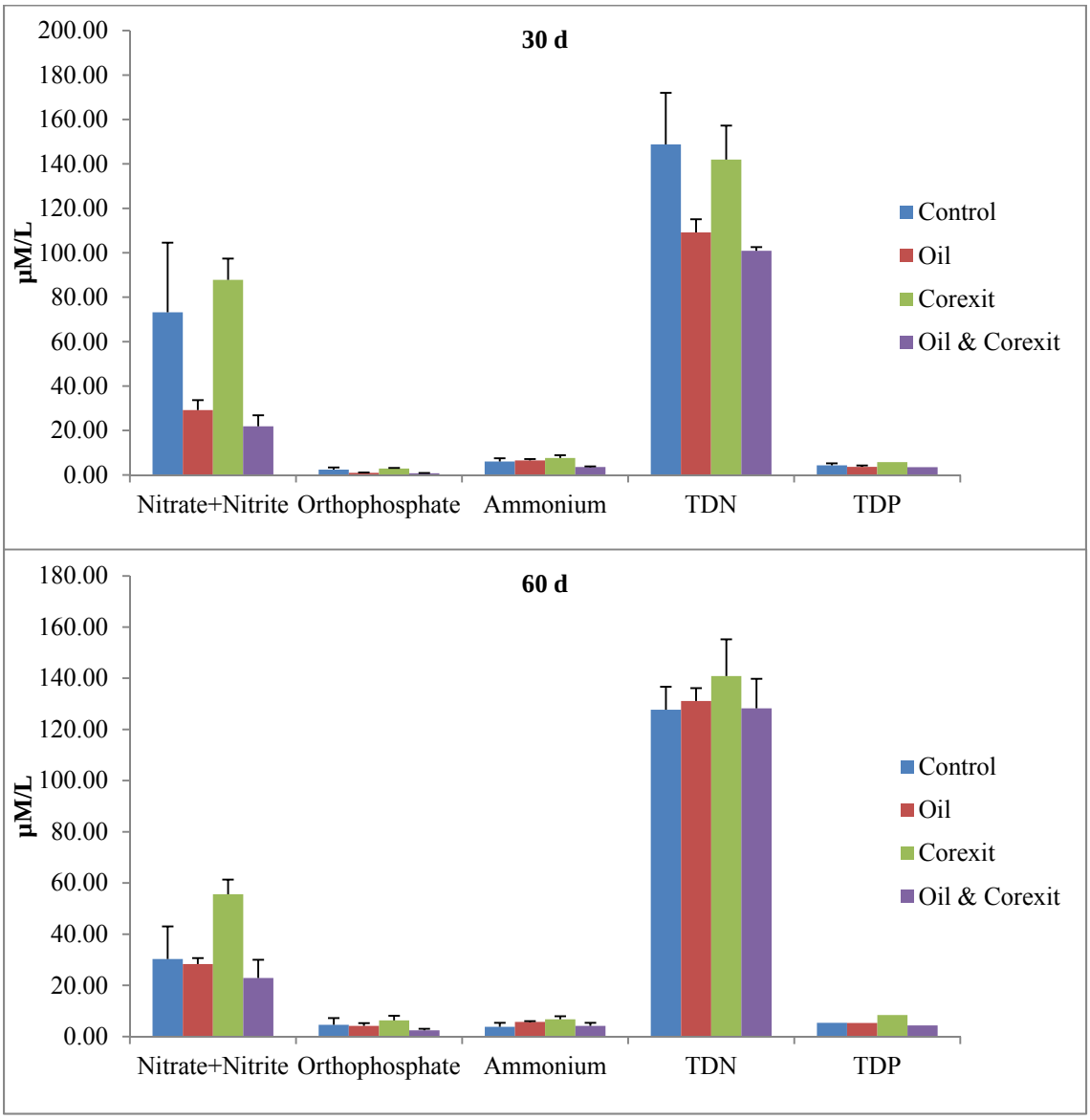
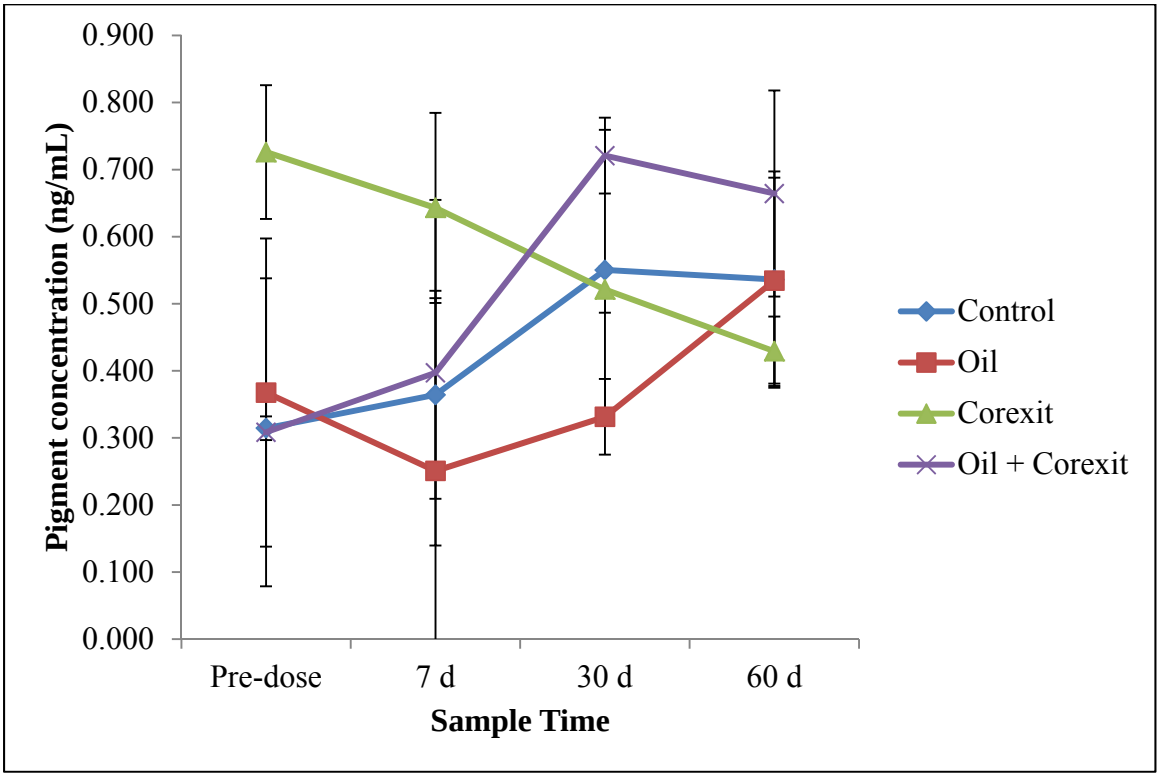


Figure 15. Continued.



HPLC was used to target 19 phytopigments with known taxonomic importance for phytoplankton identification. The results illustrated in Figure 16 were variable. There was a slight reduction in phytoplankton pigments within the first week of exposure to the oil treatment, which then recovered to control conditions between 1 and 2 months. However, this was not statistically significant (one way ANOVA, $p > 0.05$). There was a decrease in phytoplankton in the Corexit treatments over time, but an increase occurred in the other treatments including controls. One possible explanation is that since diatoms utilize nitrate, the diatoms could not survive this treatment and died off, leaving a surplus of nitrate relative to the other treatments where the diatoms fared better (see Figure 15).

Figure 16. Concentrations of diatoms and dinoflagellates-A sampled at four time periods during the experiment. Error bars represent the standard error of the means.



Chemical Assessments

TEM in water

Mean TEM (total extractable matter) concentrations in pre-dose water samples ranged from 1.6 mg/L in the Corexit treatment to 2.1 mg/L in the oil+Corexit treatment (Figure 17). Pre-dose TEM levels were not significantly different for any of the treatments (one way ANOVA, $p=0.7382$). At 12 h, TEM levels ranged from 2.1 mg/L in the oil treatment to 4.6 mg/L in the Corexit treatment (Figure 17). TEM levels in both the Corexit and oil+Corexit treatments were significantly ($p<0.05$) higher than the control at 12 h. At 24 h, TEM concentrations ranged from 2.0 mg/L in the control to 3.1 mg/L in the oil+Corexit treatment (Figure 17). Again, TEM concentrations in the Corexit and oil+Corexit treatments were significantly ($p<0.05$) higher than the control. The 48 h TEM concentrations ranged from 1.6 mg/L in the controls to 2.3 mg/L in the oil+Corexit treatment (Figure 17). None of the treatments were significantly different from the control ($p=0.2685$). At 7 d, the TEM concentrations ranged from 1.9 mg/L in the control to 2.9 mg/L in the oil+Corexit treatment (Figure 17). Again, none of the treatments were significantly different from the control ($p=0.3471$). After 14 d, TEM concentrations ranged from 1.8 mg/L in the control to 2.2 mg/L in the oil+Corexit treatment (Figure 17) with no significant differences among treatments ($p=0.5987$).

TEH in sediments

Mean TEH (total extractable hydrocarbons) concentrations in sediments at 7 d ranged from 116.7 mg/Kg in the control to 273 mg/Kg in the oil treatment (Figure 18). All three treatments had higher levels of TEH compared to controls, though none of these differences were statistically significant (one way ANOVA, $p=0.1181$). At 30 d, TEH concentrations ranged from 163.3 mg/Kg in the control to 340.0 mg/Kg in the oil treatment. TEH concentrations were not significantly different from the controls in any of the treatments ($p=0.3176$), although slightly elevated levels persisted in the oil and oil+Corexit treatments. Finally at 60 d, TEH concentrations ranged from 96.7 mg/Kg in the control to 323.3 mg/Kg in the oil+Corexit treatment. Once again, none of the

treatment concentrations were significantly different from the controls (p=0.0747), although slightly elevated levels persisted in the oil and oil+Corexit treatments.

Figure 17. TEM concentrations in water measured at five time points. Bars represent the mean of three samples. Error bars represent the standard error of the means. *Significantly different from corresponding control.

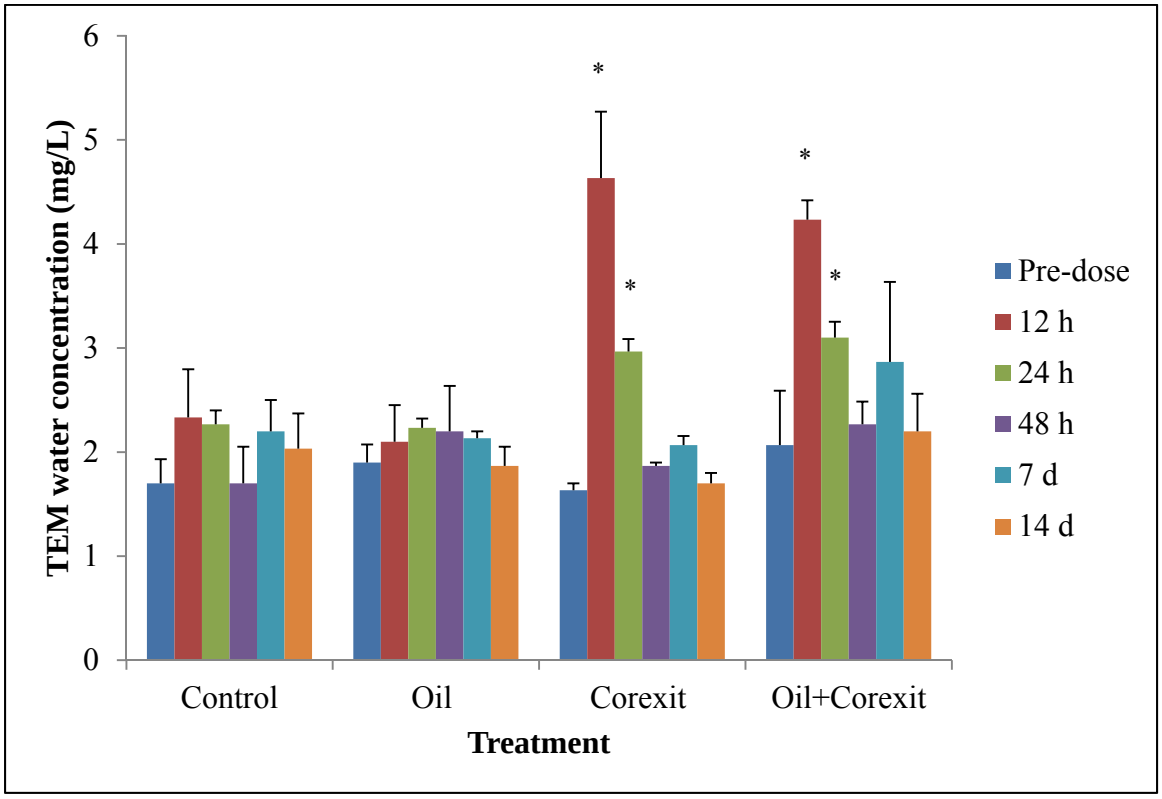
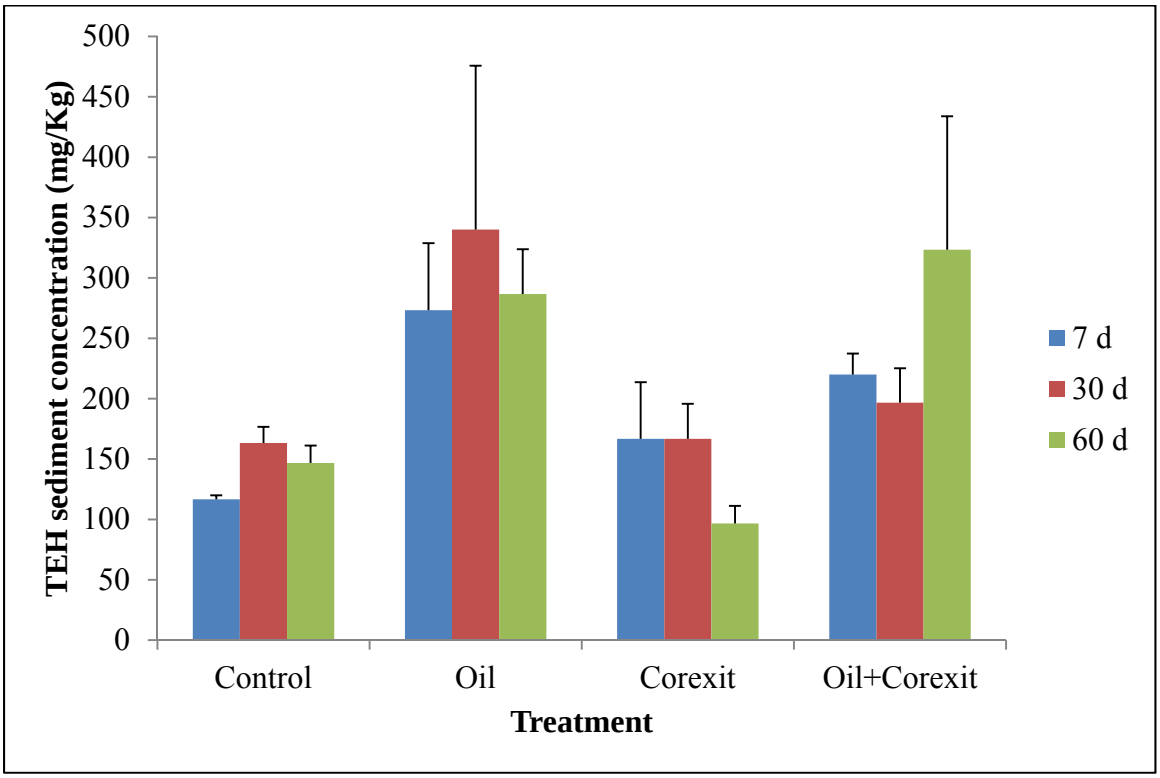


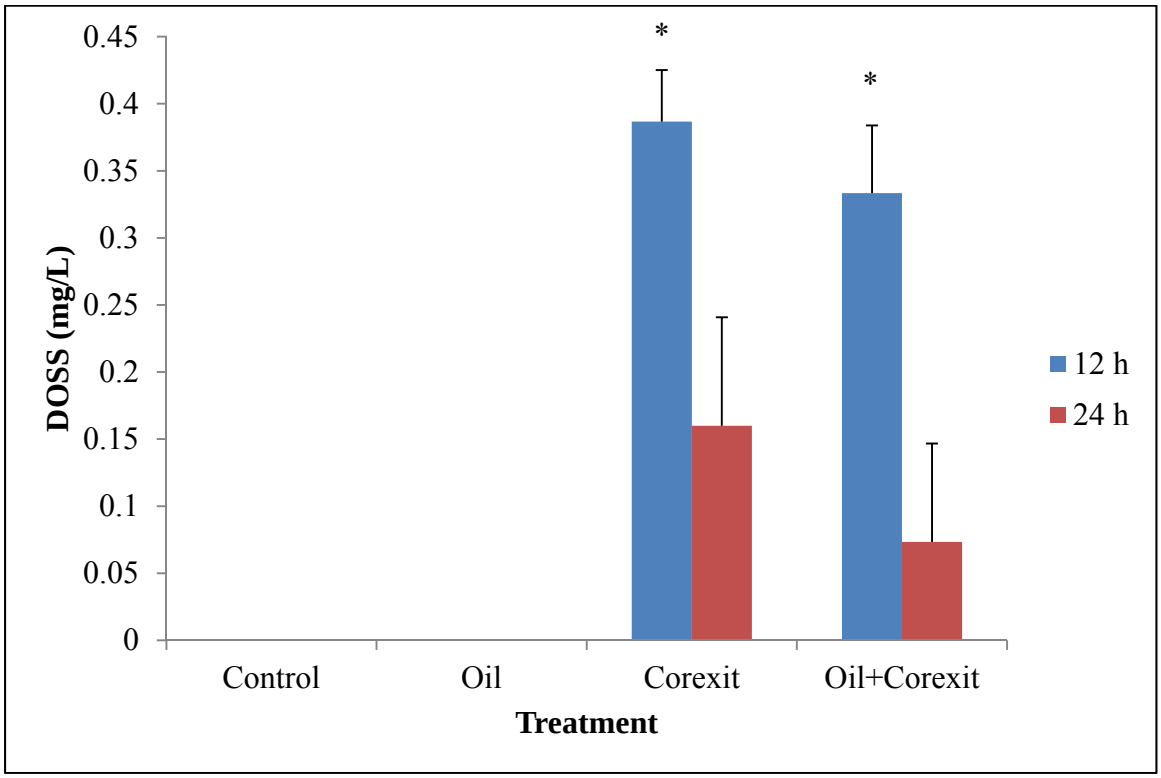
Figure 18. TEH concentrations measured in sediments at three time points. Bars represent the mean of three samples. Error bars represent the standard error of the means.



DOSS in water and tissue

DOSS was not detected in any of the pre-dose treatments. At 12h, mean DOSS concentrations ranged from below detection limits in the control and oil treatments to 0.39 mg/L in the Corexit treatment (Figure 19). The Corexit concentrations in the Corexit and oil+Corexit treatments were significantly higher (one way ANOVA, $p<0.05$) than the control. DOSS concentrations at 24 h ranged from <DL in the control and oil treatments to 0.16 mg/L in the Corexit treatment (Figure 19). Corexit concentrations were not significantly different in any of the treatments at 24 h ($p=0.2011$). Corexit concentrations were below detection limits in all treatments at 48 h, 7 d, and 14 d. DOSS was not detected in either fish or grass shrimp tissues at 60 d.

Figure 19. DOSS concentration in water measured at two time points. Bars represent the mean of three samples. Error bars represent the standard error of the means. *Significantly different from corresponding control.



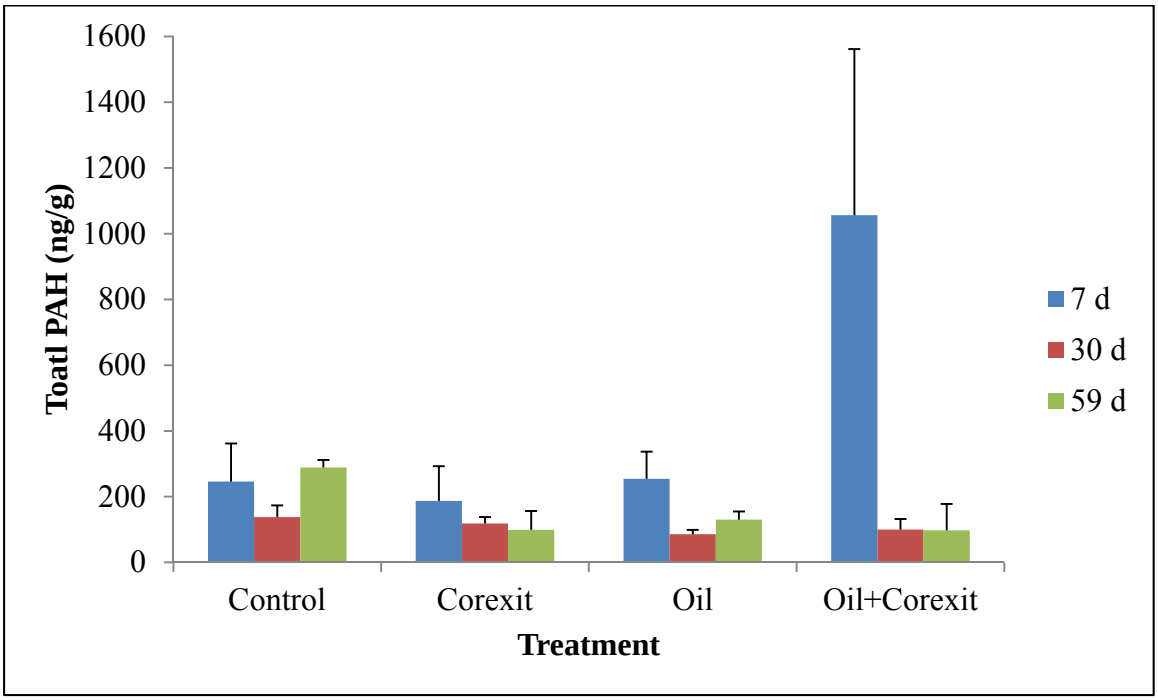
Total PAHs in sediment

Total PAHs were measured in sediment at three time points (Table 13 and Figure 20.) A background measurement at the sediment collection site was also conducted. PAH concentrations were variable throughout the tanks and throughout the experiment duration, however, there was a trend toward higher concentrations in the oil and oil+Corexit treatments at 7 d. PAH concentrations dropped in the 30 d samples and remained comparatively similar in the 59 d samples.

Table 13. Total sediment PAHs measured in each treatment tank at three time points.

Tank #	Treatment	Sample Time	Total PAH (ng/g)
		Baseline Pre-dose	71.729
G1	Oil+Corexit	7 d	1976.269
H1	Oil+Corexit		235.580
K3	Oil+Corexit		957.848
G3	Oil		264.135
H2	Oil		392.069
L3	Oil		106.405
H3	Corexit		396.830
K1	Corexit		65.738
L1	Corexit		99.238
G2	Control		49.746
L2	Control		449.973
K2	Control		238.710
G1	Oil+Corexit	30 d	143.456
H1	Oil+Corexit		190.979
K3	Oil+Corexit		79.914
G3	Oil		142.101
H2	Oil		96.742
L3	Oil		115.824
H3	Corexit		59.949
K1	Corexit		72.937
L1	Corexit		124.663
G2	Control		63.551
L2	Control		170.923
K2	Control		65.372
G1	Oil+Corexit	59 d	269.237
H1	Oil+Corexit		436.731
K3	Oil+Corexit		160.862
G3	Oil		50.149
H2	Oil		113.597
L3	Oil		132.834
H3	Corexit		240.683
K1	Corexit		100.889
L1	Corexit		48.357
G2	Control		139.785
L2	Control		90.779
K2	Control		62.198

Figure 20. Average total sediment PAHs measured in each treatment at three time points for Phase I.



Water Quality

Water quality was consistent across all treatments for the duration of the experiment as shown in Table 14.

Table 14. Mean water quality values as measured in the mesocosm tanks for each treatment for the experimental duration (May 2011 to July 2011).

Parameter	Control			Corexit			Oil			Oil+Corexit		
	Mean	**SE**	**n**	**Mean**	**SE**	**n**	**Mean**	**SE**	**n**	**Mean**	**SE**	**n**
Conductivity [mS/cm]	32.41	0.143	136	33.05	0.132	134	32.67	0.129	134	33.33	0.133	135
Specific Conductivity [mS/cm]	31.53	0.026	136	31.76	0.020	134	31.48	0.033	134	31.82	0.0199	135
Salinity [‰]	19.59	0.016	136	19.73	0.014	134	19.54	0.024	134	19.76	0.014	135
pH	8.25	0.015	136	8.17	0.013	134	8.24	0.013	134	8.30	0.016	135
Dissolved Oxygen [mg/L]	7.91	0.190	136	7.35	0.148	134	7.93	0.184	134	8.23	0.188	135
Dissolved Oxygen [%Sat]	109.88	2.655	136	103.18	2.061	134	111.25	2.644	134	116.69	2.795	135
Temperature [°C]	26.44	0.216	136	27.13	0.215	134	27.00	0.214	134	27.48	0.214	135

MATERIALS AND METHODS PHASE II

In Phase II of the mesocosm testing, the same 12 mesocosm setups were reused. The purpose of Phase II was to simulate a saltmarsh being re-oiled over a period of time due to incoming tides. The dosing strategy for Phase II consisted of repeated applications of oil and Corexit into the same treatment mesocosms used in Phase I. Benthos, fish, shrimp and clams were all newly added for Phase II testing. The *Spartina* sediment trays remained from Phase I. During the interval from the end of Phase I (7 July 2011) to the beginning of Phase II (11 Oct 2011), water quality continued to be measured and salinity adjusted as needed. The mesocosm tank setup for Phase II remained as illustrated in Figure 4.

Mesocosm Setup

Benthos, fish, shrimp and clams were all obtained from the same sources as in Phase I. Benthos was collected and added to the system approximately 1 month prior to the start of the experiment (20 Sept 2011). *F. heteroclitus* were collected and added to the lower tanks (25 fish per tank) one week prior to dosing (4 Oct 2011). This was the same as with Phase I. The fish were fed daily during acclimation. Grass shrimp were collected and added to upper tanks (200 shrimp per tank) one week prior as with Phase I. An additional 10 caged shrimp per tank were added to the upper tanks with the cage placed under the *Spartina* trays. The caged shrimp were not fed during the 7 d exposure. The caged shrimp were collected at set time points for future microarray analysis. An additional 10 caged fish per tank were also added to the upper tanks and were fed during the 7 d exposure. The caged shrimp were collected at set time points for future microarray analysis.

Caged clams, *M. mercenaria*, (150 clams per cup; 4 cups per tank; obtained from the Phase I supplier) were added to the upper tanks one week prior to dosing (4 Oct 2011). A new design was implemented for this phase due to the missing status of some clams from the open trays in Phase I. Polypropylene jars (473 mL) were used as test chambers. Jars were cut with a remaining height of 50 mm. Approximately 100 g of sieved and homogenized sediment were added to each jar. Four test jars were then placed

in a holding chamber. Chambers were constructed from polypropylene with a dimension of 215 mm x 205 mm x 105 mm. Four sections were constructed from that measuring 95 mm x100 mm. The lid measured 215 mm x 215 mm. The sides of the chamber were covered with 400 µm nylon mesh screen. The test chambers were placed into mesocosm tanks on 22 September to allow for the holding period. *Spartina* sediment trays remained in the tanks from Phase I testing and were used for Phase II testing. The *Spartina* in one tray of each tank was cut to sediment level before Phase II dosing in order to assess regrowth.

Dosing procedure

At high tide (8:00 a.m.), oil and oil+Corexit tanks were dosed with 40 mL oil and dosing was repeated at 12 h, 24 h, 36 h, and 48 h (40 mL x 6 tanks x 5 doses = 1200 mL total). This represented a maximum concentration of 2356.66 mg/L oil after five doses. Corexit was applied at a 1:20 ratio (2 mL x 6 tanks x 5 doses = 60 mL total) for a maximum concentration of 128.7 mg/L after five doses (Table 15).

Table 15. Dosing scheme for mesocosm test Phase II which consisted of three treatments and a control. Dosing of oil and Corexit occurred at 12h, 24h, 36h, and 48h.

TREATMENT (# replicates)	Amount of Oil added (per dose)	Amount of Corexit added (per dose)	Oil* Conc. after one dose	Final Oil* Conc. after 5 doses[1]	Corexit* Conc. after one dose	Final Corexit* Conc. after 5 doses[2]
Control (3)	–	–		–		–
Oil (3)	40 mL	–	78.55 mg/L	2356.66 mg/L		
Corexit (3)	–	2 mL		–	4.3 mg/L	128.7 mg/L
Oil+Corexit (3)	40 mL	2 mL	78.55 mg/L	2356.66 mg/L	4.3 mg/L	128.7mg/L

* Assuming a total system volume of 443 L and that all of the dosed material goes completely into solution.
1. The density of the oil is 0.87 g/mL.
2. The density of the Corexit is 0.95 g/mL.

Sample Collection

On 10 Oct 2011, the following samples were taken before the addition of any oil or dispersant (see also Table 16):

1. Clear cut marsh grass in one tray in each tank to measure re-growth.
2. One benthic tray and one clam cup from each tank.
3. Sediment for Microtox and PAH analysis as in Phase I.
4. Water samples for chlorophyll, primary productivity, and bacterial productivity analysis (250 mL each) as in Phase I.
5. Water samples for chemistry analysis (500 mL for TEM, 15 mL for DOSS, 15 mL for PAH).
6. Fish and shrimp for future enzyme activity/microarray analysis.

After dosing, the collection schedule was as follows (see also Table 16):

7 d. One benthic tray and clam cup from each tank. Water and sediment collected as for pre-dose. Caged fish and shrimp collected for enzyme activity/microarray analysis and shrimp for DOSS analysis.

30 d (last day of test). One benthic tray and clam cup from each tank. Sediments and water for chemistry analysis as for Day 7. Fish and shrimp for survival analysis, enzyme activity/microarray analysis and shrimp for DOSS analysis.

Table 16. Sample collection times for mesocosm Phase I. "X" denotes matrix was collected.

Sample Time	**Matrix Collected**					
	Water	**Sediment**	**Benthic Tray**	***F. heteroclitus***	***P. pugio***	***M. mercenaria***
Pre-dose	X	X	X	X	X	X
7d	X	X	X	X	X	
60d	X	X	X	X	X	X

M. mercenaria Assessment

Clams were assessed at pre-dose, 7 d and 30 d. At the termination of the experiment, survival, mass, and size were assessed. Clams were determined to be dead if they exhibited gaping shells, lack of response to stimuli, and/or shell closure for more than 5 min. Dead clams were excluded from sublethal endpoint analysis. Clams were then weighed, dried overnight at 68°C, and weighed again to determine dry masses (mg) as for Phase I.

S. alterniflora Assessment

At the end of Phase II, the growth of the clear-cut sections of marsh grass was measured as in Phase I.

Benthic Infauna Assessment

During Phase II benthic infauna samples were collected just prior to dosing, 1 week after dosing, and 1 month after dosing. Benthic samples were handled as for Phase I. Samples for sediment characterization and TOC analysis were collected by scraping the top 2-3 cm of sediment from the intertidal saltmarsh trays at the end of Phase II. Sediment characterization during Phase II focused only on silt-clay content.

Microtox Solid Phase Assessment

Sediment samples were collected and analyzed as in Phase I.

F. heteroclitus EROD Activity Assessment

Mud minnows were collected and samples were processed for EROD activity as in Phase I.

Chlorophyll and Productivity Assessment

Water samples were collected and analyzed for chlorophyll concentration, bacterial productivity, phytoplankton productivity, phytoplankton pigment analysis, and nutrient analyses as in Phase I.

Chemical Assessment

Water and sediment samples were collected and analyzed for DOSS, TEM, TEH and PAHs as in Phase I. During Phase II, only pre-dose, 48 h and 7 d post-dose water samples were collected as described for Phase I except that 1 L water samples were collected for TEM analysis. Pre-dose, 7 d, and 30 d sediment samples were collected for TEH analysis. Tissue samples (grass shrimp) were collected at 30 d for DOSS analysis.

RESULTS PHASE II

M. mercenaria Assessment

Survival in clams after 30 d ranged from an average of 74.4% in the controls to 3.3% in the Corexit treatments (Table 17). Survival in the oil, Corexit and oil+Corexit treatments was significantly lower than control ($p<0.0004$). There were no missing clams. Average shell area decreased from pre-dose measurements to 30-d measurements in control, oil and Corexit treatments but increased in the oil+Corexit treatments (Table 18). After one-way ANOVA analyses, there were no significant differences for shell area ($p=0.76$) and major/minor axis lengths ($p=0.85$/$p=0.64$) at 7 d nor 30 d (shell area $p=0.091$; major/minor axis lengths ($p=0.078$/$p=0.15$).

Table 17. *M. mercenaria* survival after 30 days. *Significantly lower than control.

Tank #	Treatment	# Clams Alive	# Clams Dead	# Clams Missing	% Survival	Mean % Survival	SE
G2	Control	44	16	0	73.33	74.44	0.0056
L2	Control	45	15	0	75.00		
K2	Control	45	15	0	75.00		
G3	Oil	26	34	0	43.33	33.89*	0.0862
L3	Oil	25	35	0	41.67		
H2	Oil	10	50	0	16.67		
H3	Corexit	2	58	0	3.33	3.33*	0.0192
K1	Corexit	0	60	0	0.00		
L1	Corexit	4	56	0	6.67		
G1	Oil+Corexit	15	45	0	25.00	42.78*	0.0934
H1	Oil+Corexit	28	32	0	46.67		
K3	Oil+Corexit	34	26	0	56.67		

Table 18. *M. mercenaria* shell area, major axis length and minor axis length at three time points. SE is standard error of the mean. NC is not calculated due to 100% mortality.

	Pre-Dose			7 d			30 d		
Treatment and Tank #	**Shell Area (mm^2)**	**Major Axis (mm)**	**Minor Axis (mm)**	**Shell Area (mm^2)**	**Major Axis (mm)**	**Minor Axis (mm)**	**Shell Area (mm^2)**	**Major Axis (mm)**	**Minor Axis (mm)**
Control									
G2	1.583	1.496	1.335	1.483	1.446	1.297	1.427	1.426	1.267
K2	1.496	1.444	1.295	1.436	1.429	1.276	1.366	1.389	1.248
L2	1.572	1.498	1.324	1.566	1.491	1.325	1.384	1.397	1.254
Mean	1.550	1.479	1.318	1.495	1.456	1.299	1.393	1.404	1.256
SE	0.027	0.018	0.012	0.038	0.018	0.014	0.018	0.011	0.006
Oil									
G3	1.348	1.393	1.232	1.618	1.503	1.351	1.394	1.411	1.257
L3	1.551	1.484	1.326	1.603	1.508	1.347	1.399	1.410	1.264
H2	1.315	1.376	1.211	1.432	1.434	1.274	1.317	1.399	1.204
Mean	1.405	1.418	1.257	1.551	1.482	1.324	1.370	1.407	1.242
SE	0.074	0.034	0.035	0.060	0.024	0.025	0.027	0.004	0.019
Corexit									
H3	1.436	1.419	1.280	1.490	1.504	1.269	NC	NC	NC
K1	1.564	1.486	1.331	1.420	1.408	1.283	NC	NC	NC
L1	1.409	1.414	1.263	1.691	1.537	1.383	1.348	1.368	1.259
Mean	1.470	1.439	1.291	1.534	1.483	1.312	-	-	-
SE	0.048	0.023	0.021	0.081	0.039	0.036	-	-	-
Oil + Corexit									
G1	1.436	1.419	1.280	1.467	1.442	1.289	1.454	1.437	1.278
H1	1.417	1.409	1.274	1.504	1.461	1.308	1.652	1.532	1.363
K3	1.599	1.508	1.343	1.571	1.489	1.335	1.461	1.446	1.284
Mean	1.484	1.445	1.299	1.514	1.464	1.311	1.523	1.472	1.308
SE	0.058	0.032	0.022	0.031	0.014	0.013	0.065	0.030	0.027

Average dry masses decreased from pre-dose to 30 d in the controls and Corexit treatments but increased in the oil and oil+Corexit treatments (Table 19). After one-way ANOVA analyses, there was no significant difference for dry mass at 7 d ($p=0.61$) nor 30 d ($p=0.34$). Condition indices generally remained constant from pre-dose to 30 d and there was no significant difference at 7 d ($p=0.45$) nor 30 d ($p=0.66$; Table 19).

Table 19. *M. mercenaria* dry mass and condition index (CI) at three time points.

	Pre-Dose		7 d		30 d	
Treatment and Tank #	**Dry Mass (mg)**	**CI**	**Dry Mass (mg)**	**CI**	**Dry Mass (mg)**	**CI**
Control						
G2	0.833	0.279	0.855	0.315	0.673	0.261
K2	0.780	0.305	0.655	0.251	0.614	0.255
L2	0.823	0.263	0.671	0.228	0.653	0.267
Mean	0.812	0.282	0.727	0.265	0.646	0.261
SE	0.016	0.012	0.064	0.026	0.017	0.003
Oil						
G3	0.607	0.254	0.764	0.251	0.581	0.243
L3	0.445	0.152	0.750	0.245	0.615	0.211
H2	0.613	0.267	0.675	0.258	0.853	0.372
Mean	0.555	0.225	0.730	0.251	0.683	0.275
SE	0.055	0.036	0.028	0.004	0.086	0.049
Corexit						
H3	0.643	0.250	0.300	0.105	NC	NC
K1	0.841	0.286	0.636	0.250	NC	NC
L1	0.724	0.287	0.812	0.248	0.478	0.203
Mean	0.736	0.274	0.583	0.201	-	-
SE	0.057	0.012	0.150	0.048	-	-
Oil + Corexit						
G1	0.508	0.197	0.625	0.233	0.636	0.241
H1	0.671	0.265	0.673	0.241	0.808	0.253
K3	0.804	0.263	0.771	0.260	0.682	0.254
Mean	0.661	0.242	0.689	0.245	0.708	0.249
SE	0.085	0.022	0.043	0.008	0.052	0.004

S. alterniflora Assessment

At the end of Phase II, *Spartina* regrowth showed a significant reduction in shoot height after 30 d in the oil only treatments as compared to controls (Figure 21). Correspondingly, there was a significant reduction in biomass in the oil treatments after 30 d compared to controls (Figure 22). The average number of *Spartina* shoots regrown

was significantly lower in both the oil and oil+Corexit treatments compared to controls (Figure 23). There was no difference in the number of stems regrown (data not shown).

Figure 21. Average height of *Spartina* shoot regrowth after 30 d in Phase II. Error bars represent the standard error of the means. * Significantly different from control.

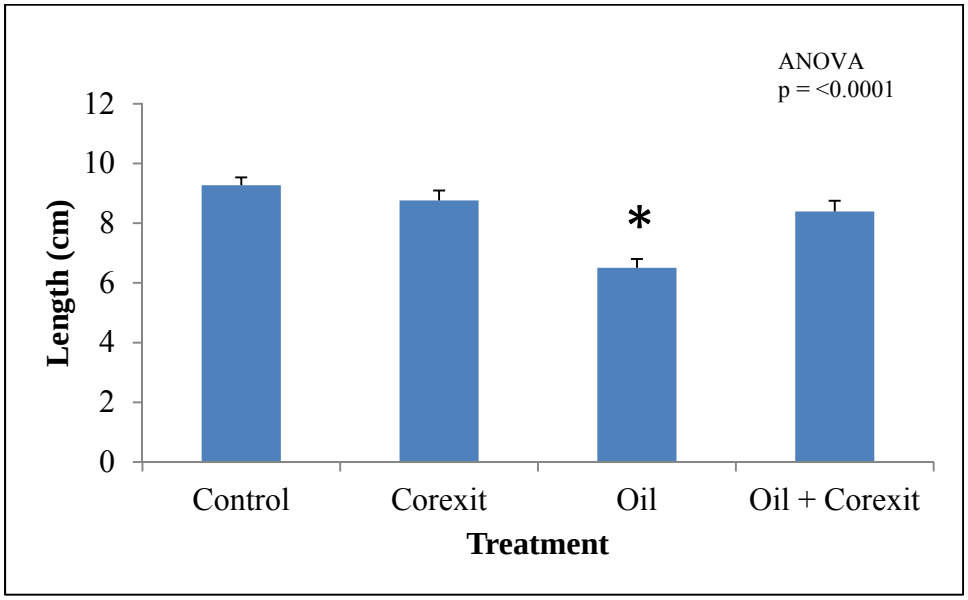


Figure 22. Dry mass of Spartina regrowth after 30 d in Phase II. Error bars represent the standard error of the means. * Significantly different from control.

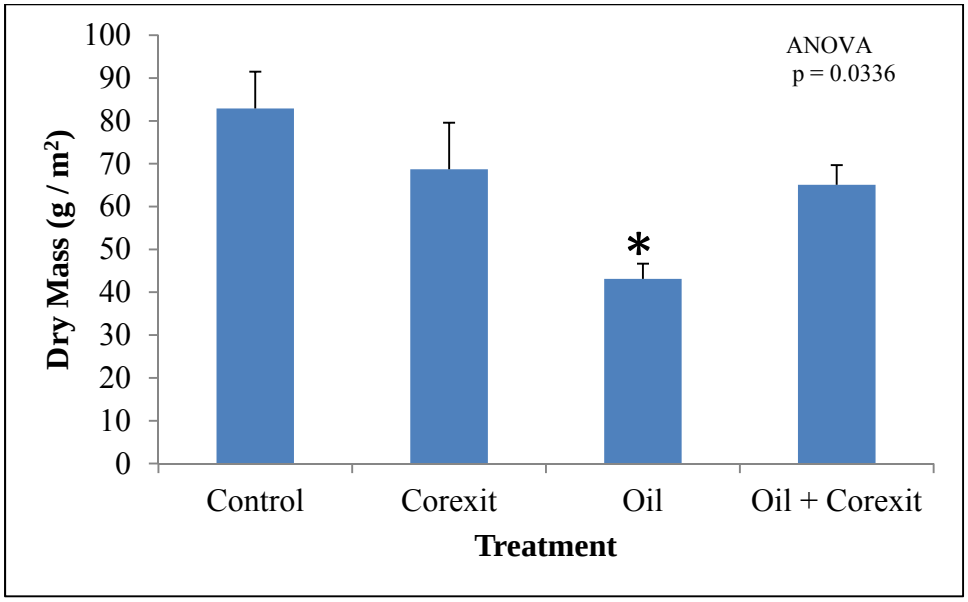
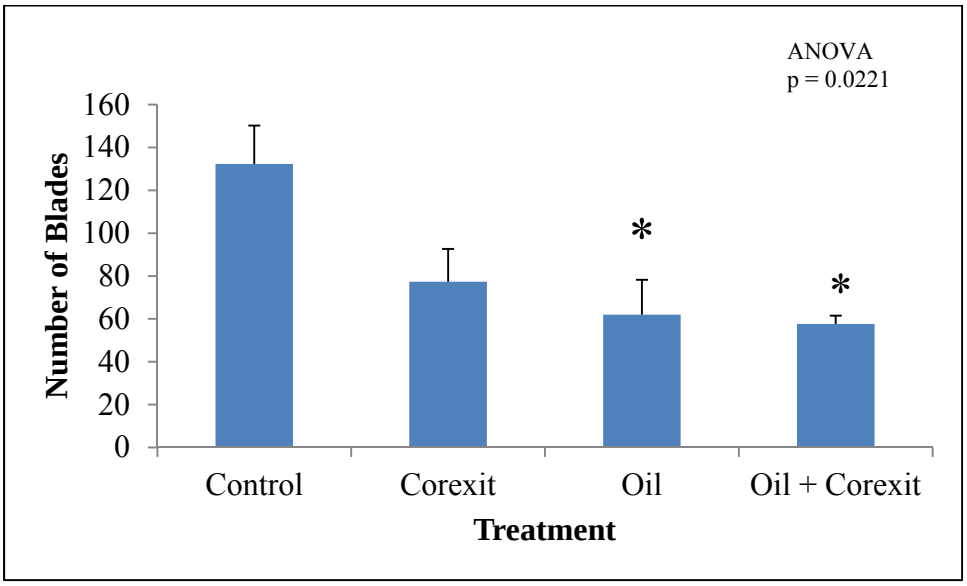


Figure 23. *Spartina* regrowth in terms of number of blades after 30 d in Phase II. Error bars represent the standard error of the means. * Significantly different from control.



Benthic Infauna Assessment

Benthic infauna community characteristics (density, number of taxa, diversity, and B-IBI) were similar across all treatments during Phase II (Table 20 and Figure 24). While no significant effects related to treatment were detected by two-way ANOVA, there were slight reductions in number of taxa and densities in the oil and Corexit treatments compared to controls at 7 d (Table 21). Also, these slight reductions persisted to one month, especially with respect to number of taxa.

Separate one-way ANOVAs among treatments at each time point were completed to further investigate the possibility of a treatment effect (Table 22). During the one month post-dose sampling event, density was found to be significantly different between the oil+Corexit treatment and the Corexit only treatment. Both *Capitella capitata* and *Streblospio benedicti* increased in abundance in the oil+Corexit treatment. Separate one-way ANOVAs among treatments were completed for percent silt-clay. No significant differences were detected for silt-clay (Table 23 and Figure 25).

Table 20. Benthic community characteristics by phase, treatment and time. [a] Following Van Dolah et al. (1999)

Time	Treatment	Number of Taxa	Diversity (H')	Density (m^{-2})	B-IBI[a]
Pre-dose	Control	8	1.99	2108	2.2
	Corexit	7	1.97	1158	1.8
	Oil	6	1.73	1325	2.0
	Oil+Corexit	8	1.70	2717	2.0
One week post-dose	Control	8	1.98	2992	2.3
	Corexit	5	1.63	625	1.7
	Oil	6	1.83	1008	1.8
	Oil+Corexit	8	2.20	1217	2.2
One month post-dose	Control	10	2.45	1933	2.5
	Corexit	7	2.18	842	2.2
	Oil	7	1.81	1675	2.2
	Oil+Corexit	8	2.09	4050	2.5

Table 21. Results of two-way ANOVA of benthic infaunal data.

Model	*df*	*F*	*Pr > F*	Effect	*df*	*F*	*Pr > F*
H' = treatment + time + (treatment x time)	11	0.80	0.6408	Treatment	3	0.89	0.3154
				Time	2	1.21	0.3154
				Treatment x time	6	0.61	0.7172
# Taxa = treatment + time + (treatment x time)	11	1.00	0.4760	Treatment	3	2.32	0.1011
				Time	2	1.02	0.3742
				Treatment x time	6	0.33	0.9149
Density= treatment + time + (treatment x time)	11	1.42	0.2282	Treatment	3	2.90	0.0560
				Time	2	0.61	0.5523
				Treatment x time	6	0.95	0.4801
B-IBI= treatment + time + (treatment x time)	11	0.77	0.6692	Treatment	3	1.40	0.2661
				Time	2	1.68	0.2068
				Treatment x time	6	0.14	0.9893

Table 22. Results of separate one-way ANOVAs by time of sampling of benthic infaunal data.

Effect	Time	*df*	*F*	*Pr > F*	Significant Differences
H'	Pre-dose	3	0.18	0.9059	
	One week post-dose	3	1.00	0.4414	
	One month post-dose	3	2.85	0.1052	
# Taxa	Pre-dose	3	0.26	0.8519	
	One week post-dose	3	1.27	0.3493	
	One month post-dose	3	3.61	0.0650	
Density	Pre-dose	3	0.87	0.4964	
	One week post-dose	3	0.91	0.4721	
	One month post-dose	3	**5.07**	**0.0295**	Oil + Corexit vs. Corexit only
B-IBI	Pre-dose	3	0.24	0.8644	
	One week post-dose	3	0.61	0.6294	
	One month post-dose	3	1.07	0.4158	

Table 23. Results of separate one-way ANOVAs of sediment characteristics data.

Effect	Time	*df*	*F*	*Pr > F*
Percent Silt-Clay	One month post-dose	3	2.65	0.1200

Figure 24. Benthic infauna community characteristics for three sampling periods during Phase II.

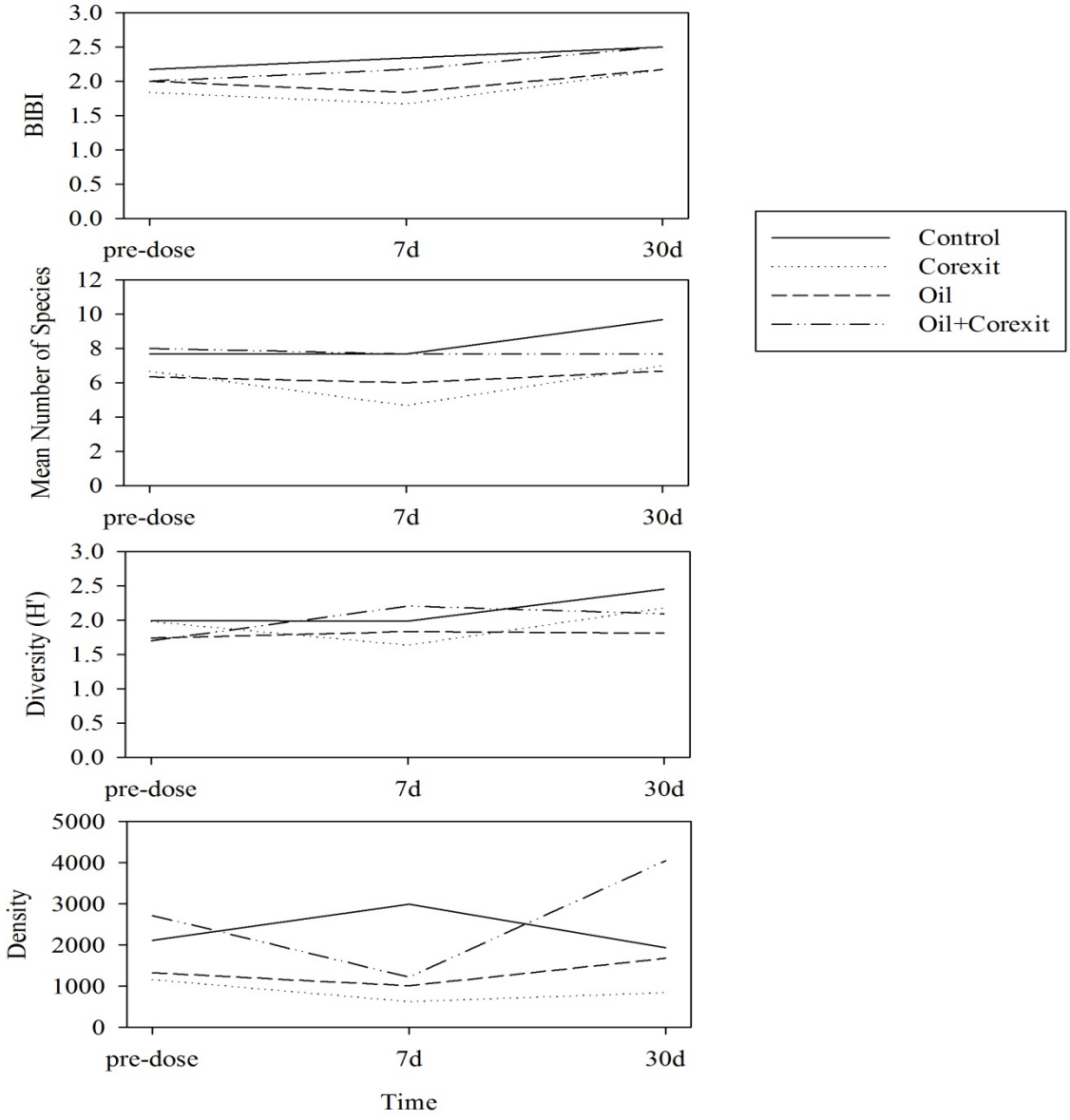
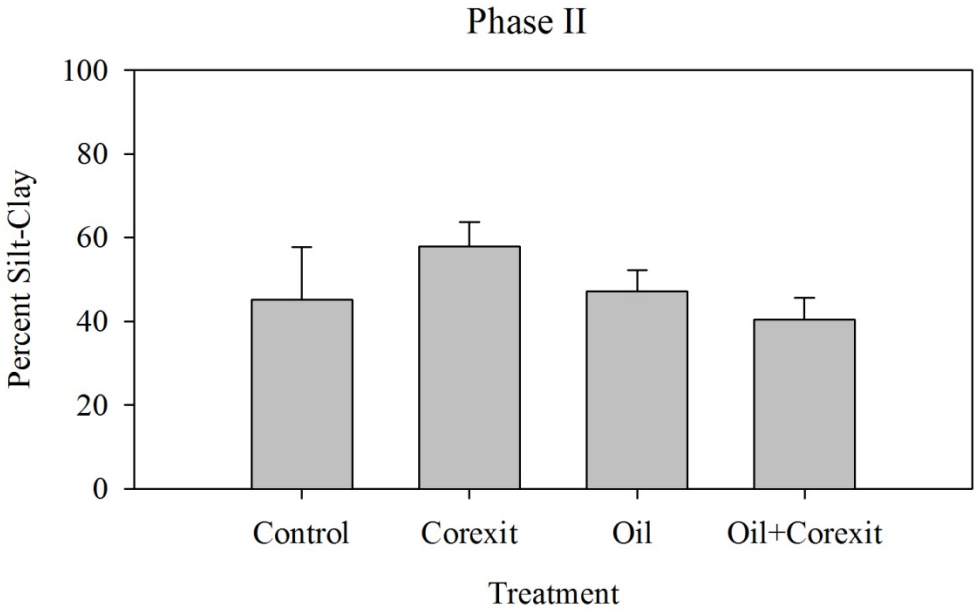


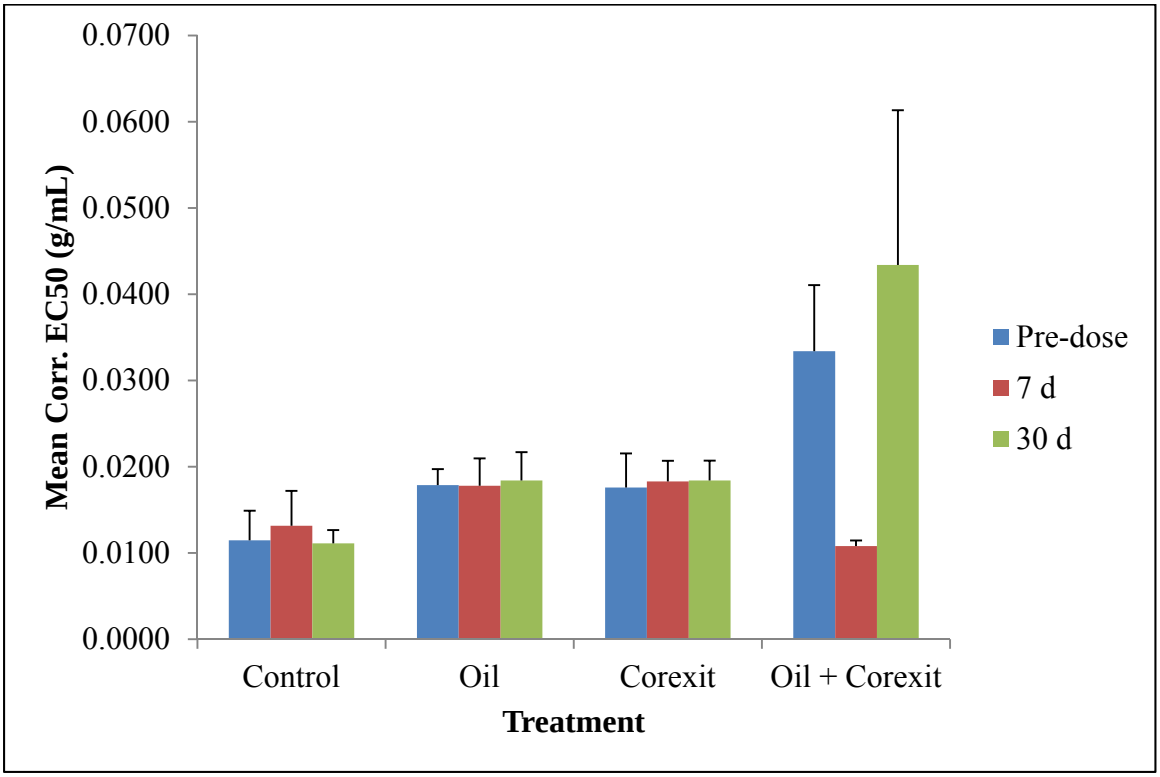
Figure 25. Benthic sediment characteristics for each treatment during Phase II. Error bars represent the standard error of the means.



Microtox Solid Phase Assessment

The results of the Microtox assays showed a general increase in EC50s in oil and Corexit treatments compared to controls but this was not significant (Figure 26). The EC50s for the oil+Corexit treatments were highly variable.

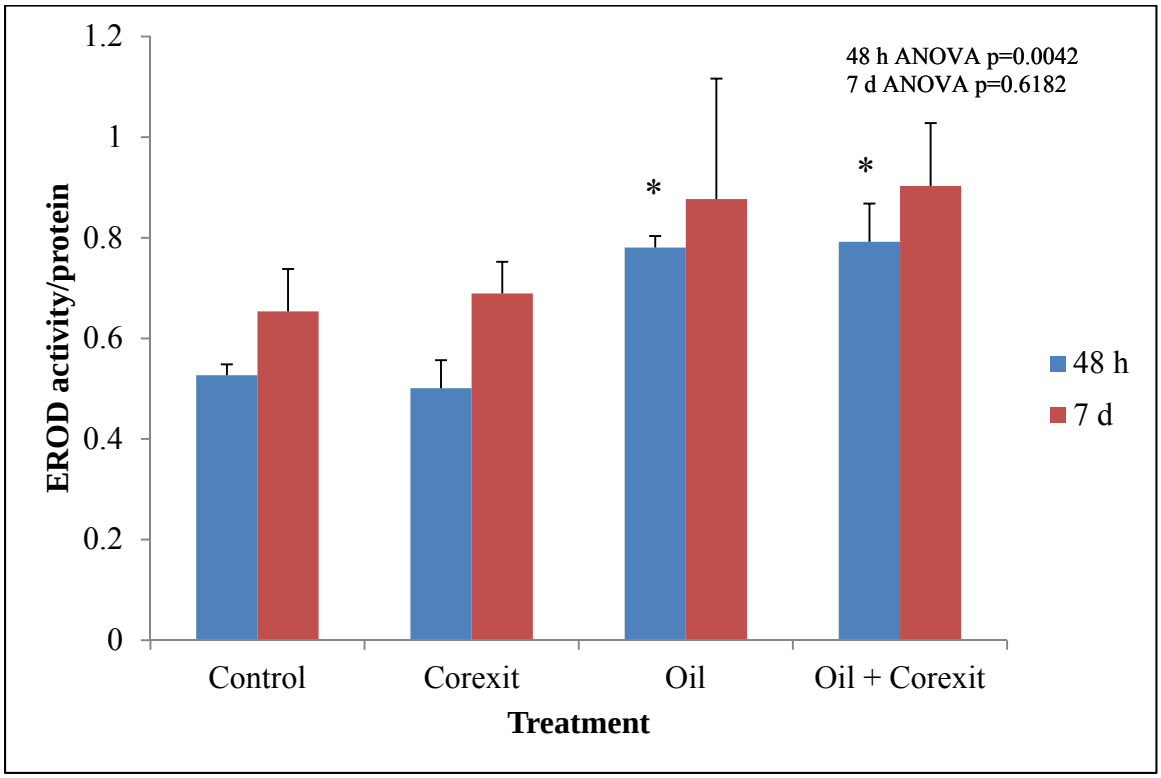
Figure 26. Microtox sediment assays expressed as EC50s assessed at three time points. Error bars represent the standard error of the means.



F. heteroclitus EROD Activity Assessment

EROD activity for mud minnows exposed to the four treatments for 7 d is shown in Figure 27. After 48 h, EROD activity was significantly induced in the oil only and oil+Corexit treatments compared to controls. EROD activity was not different in any of the treatments after 7d.

Figure 27. Phase II *F. heteroclitus* liver EROD activity measured at two time points. Error bars represent the standard error of the means. * Significantly different from corresponding control.



Chlorophyll and Productivity Assessment

Chlorophyll concentrations in Phase II significantly decreased after 7 d exposure in the oil+Corexit treatments compared to controls (Figure 28). There was increase in the Corexit treatments but this was not significant. There were no significant effects on bacterial productivity (Figure 29) or primary productivity (Figure 30); however, the oil+Corexit treatments tended to have higher productivity at the 7 day measurement.

Figure 28. Phase II chlorophyll concentrations measured at two time points. Error bars represent the standard error of the means. * Significantly different from corresponding control.

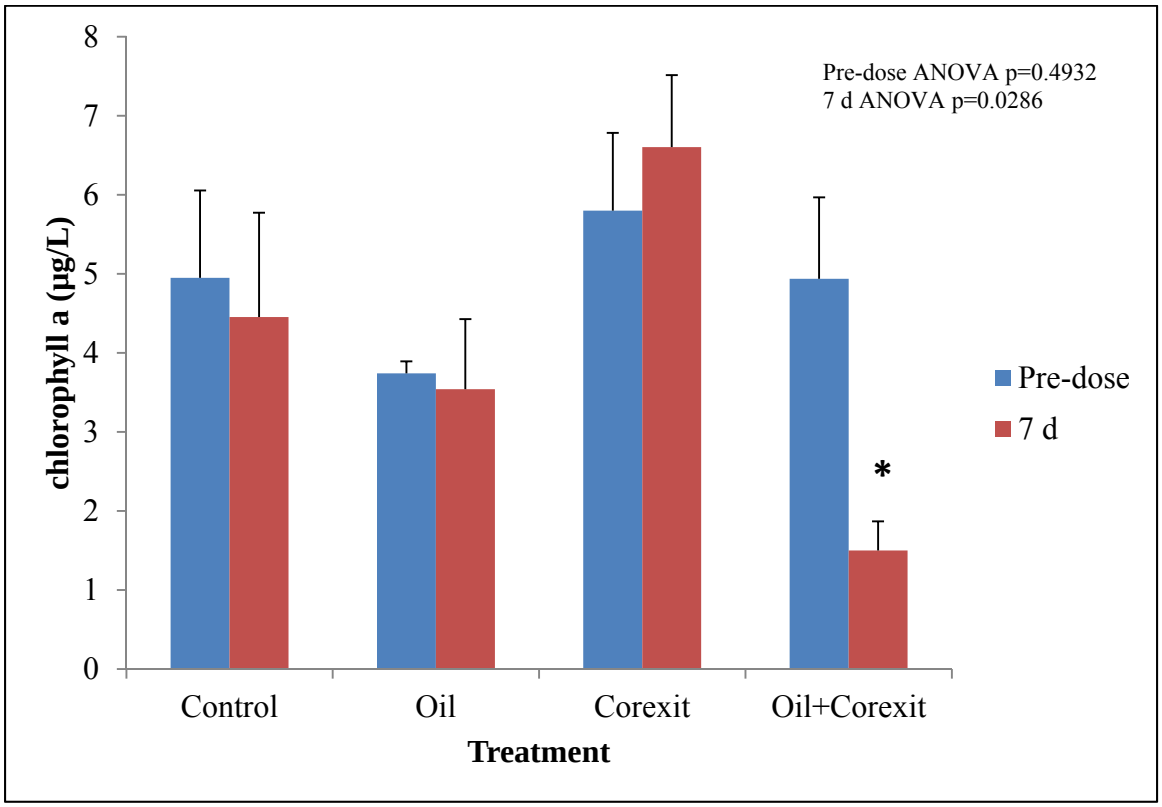


Figure 29. Phase II bacterial productivity measured at two time points. Error bars represent the standard error of the means.

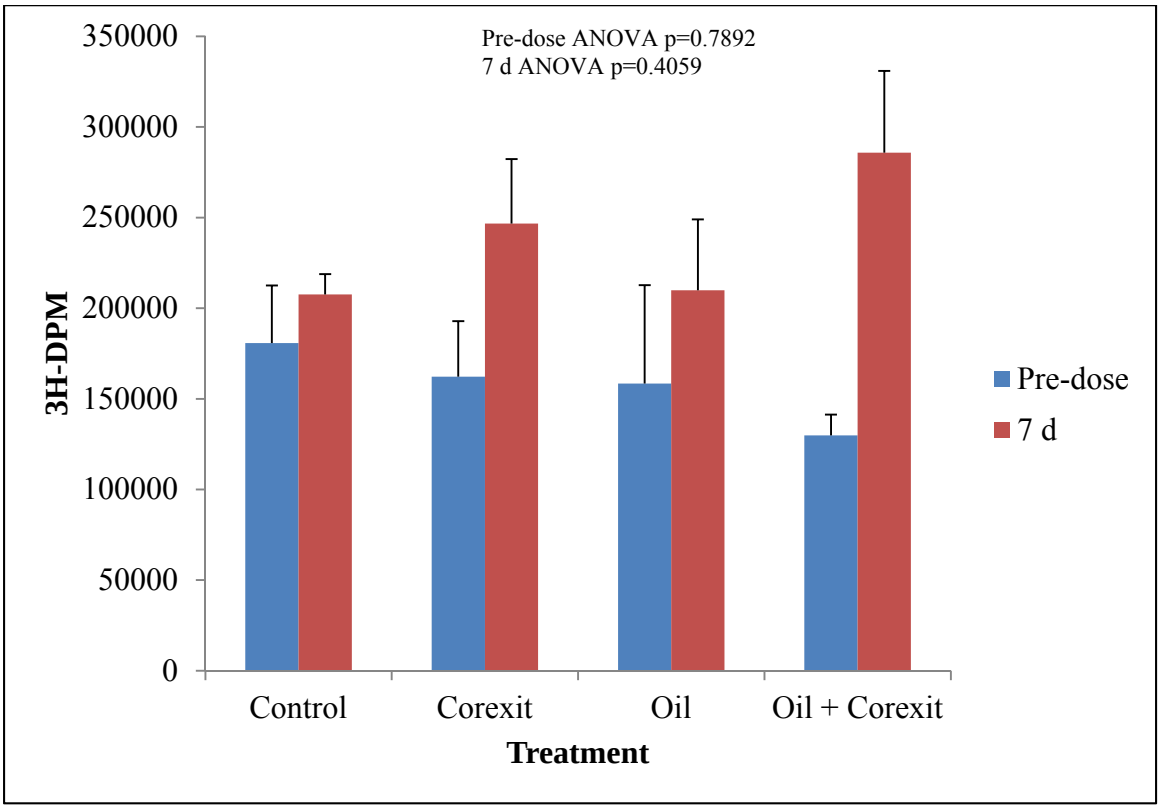
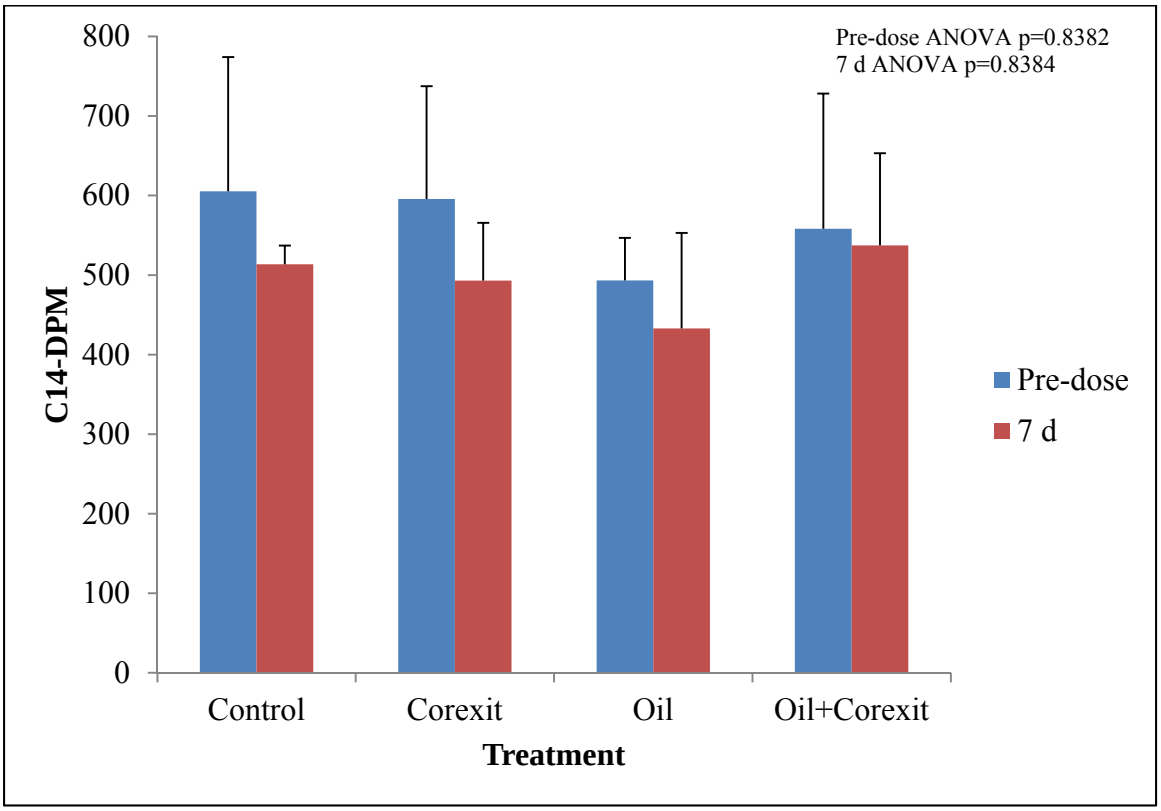


Figure 30. Phase II primary productivity measured at two time points. Error bars represent the standard error of the means.



Chemical Assessment

Mean TEM water concentrations in pre-dose samples ranged from 0.20 mg/L in the controls to 0.43 mg/L in the oil+Corexit treatment (Figure 31). Both the oil and the oil+Corexit treatments were significantly (one-way ANOVA for all analyses, $p<0.05$) higher than the controls, though concentrations in all tanks were considered to be at low background levels. At 48 h after dosing, TEM ranged from 0.25 mg/L in the controls to 8.3 mg/L in the oil+Corexit treatment (Figure 31). TEM concentrations were significantly ($p<0.05$) higher than the control in the Corexit and oil+Corexit treatments. Finally at 7 d, TEM concentrations ranged from 0.9 mg/L in the controls to 2.0 mg/L in Corexit treatment (Figure 31). TEM concentrations in the oil, Corexit, and oil+Corexit treatments were all significantly ($p<0.05$) higher than in the control.

Mean TEH sediment concentrations in pre-dose samples ranged from 91.0 mg/Kg in the Corexit treatment to 180.0 mg/Kg in the oil+Corexit treatment (Figure 32). None of the TEH concentrations in any of the pre-dose treatments were significantly different from the controls ($p=0.1181$). TEH concentrations at 7 d after dosing ranged from 108.0 mg/Kg in the control to 1,267.7 in the oil+Corexit treatment. At 30 d, TEH concentrations ranged from 90.3 mg/Kg in the Corexit treatment to 2550.0 mg/Kg in the oil/Corexit treatment. TEH concentrations in the oil and oil+Corexit treatments were higher compared to controls at both 7 d and 30 d, though such differences were statistically significant ($p<0.05$) only in the oil+Corexit treatment at 7 d.

DOSS concentrations measured in the water after 48 h were significantly elevated ($p<0.05$) compared to controls in the Corexit and oil+Corexit treatments (Figure 33). DOSS levels in grass shrimp tissue were below the detection limit.

Total sediment PAHs measured were elevated after 7 d in the oil and oil+Corexit treatments compared to the other treatments and compared to pre-dose levels (Table 24 and Figure 34), however only the oil treatment was significantly different from control ($p=0.0022$).

Figure 31. TEM concentrations measured in water at pre-dose, 48 h and 7 d. Error bars represent the standard error of the means. *Significantly different from corresponding control.

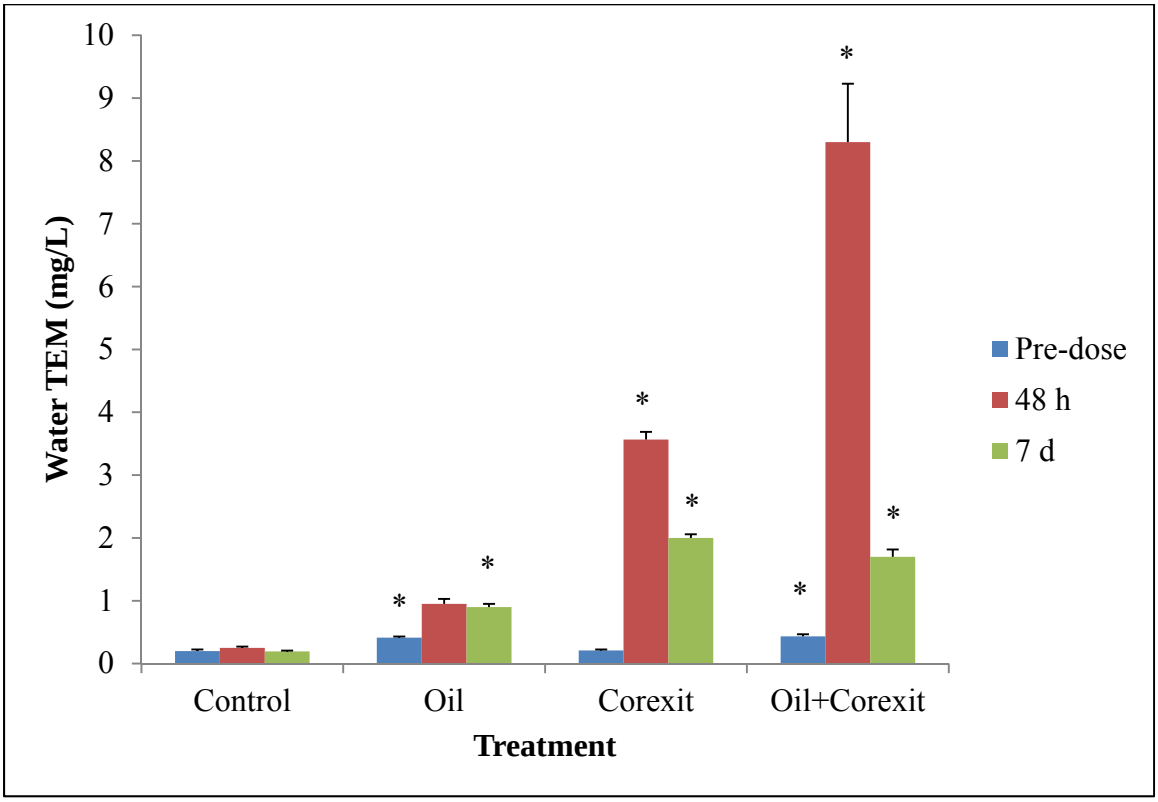


Figure 32. TEH concentrations measured in sediment at pre-dose7 d and 30 d. Error bars represent the standard error of the means. *Significantly different from corresponding control.

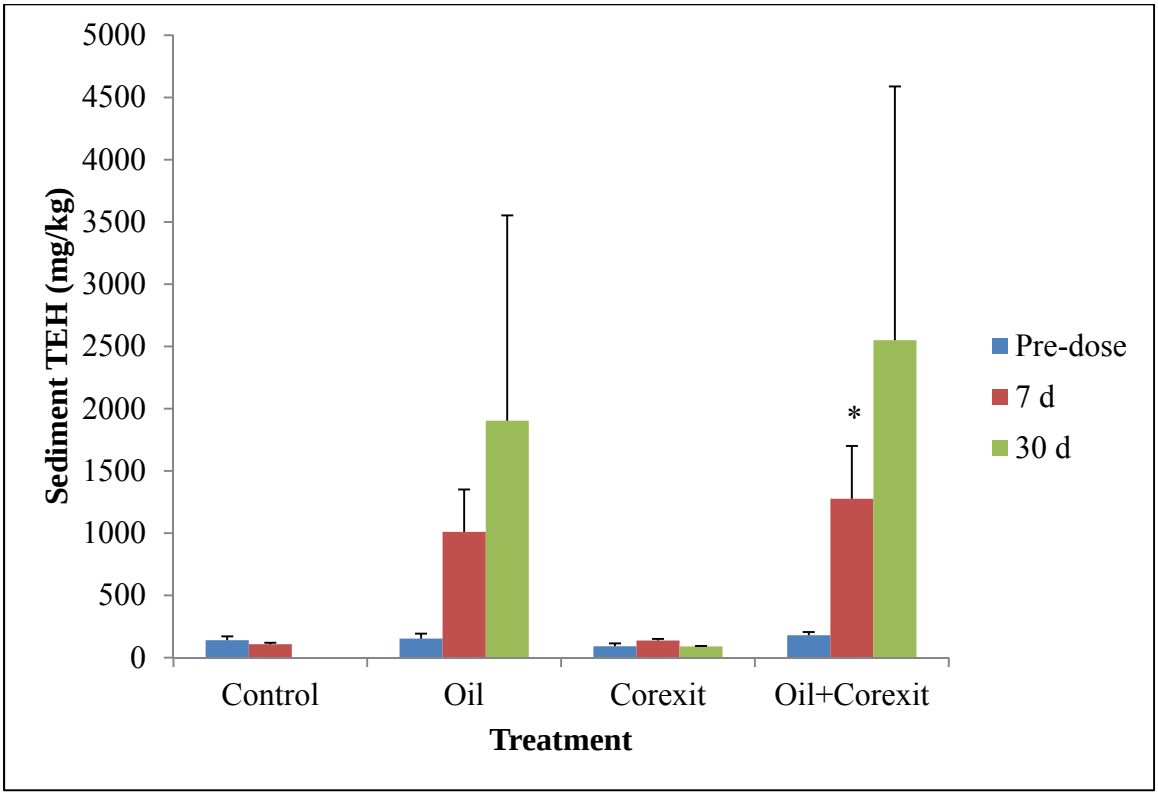


Figure 33. DOSS concentrations in water and grass shrimp tissue measured at four time points. Error bars represent the standard error of the means. *Significantly different from corresponding control.

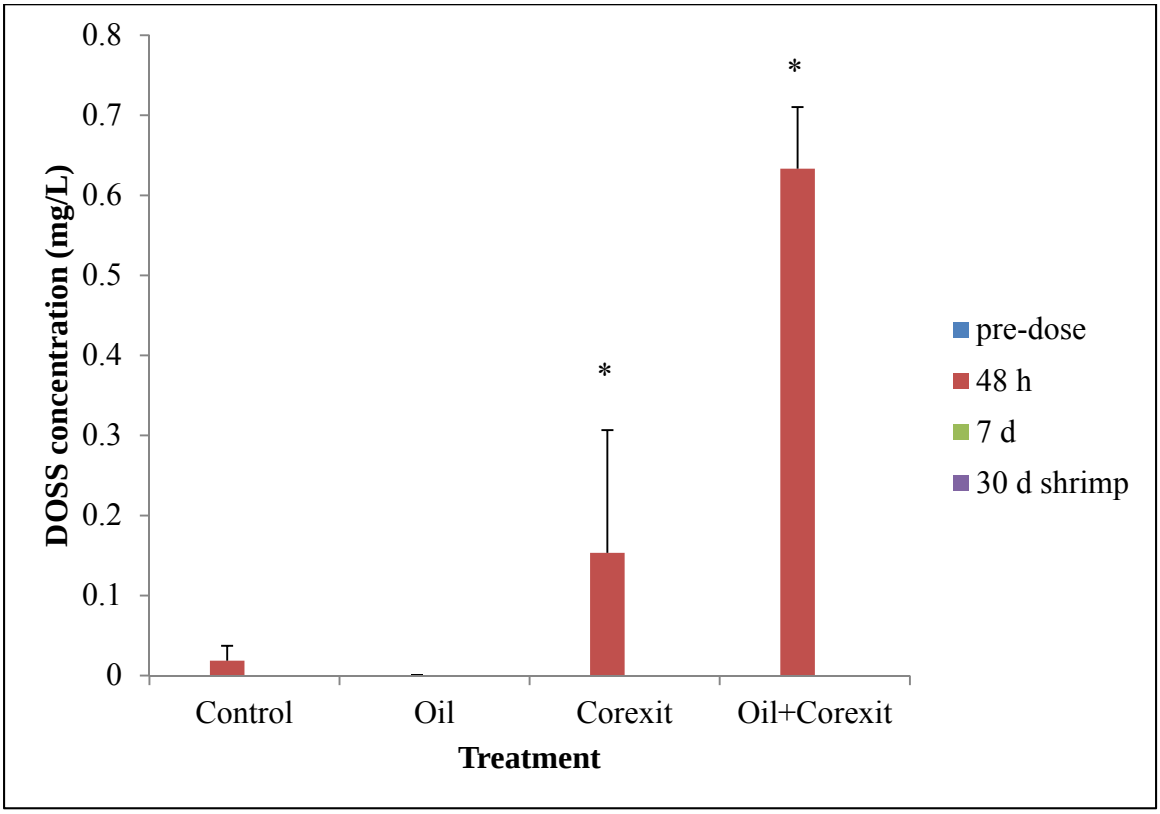
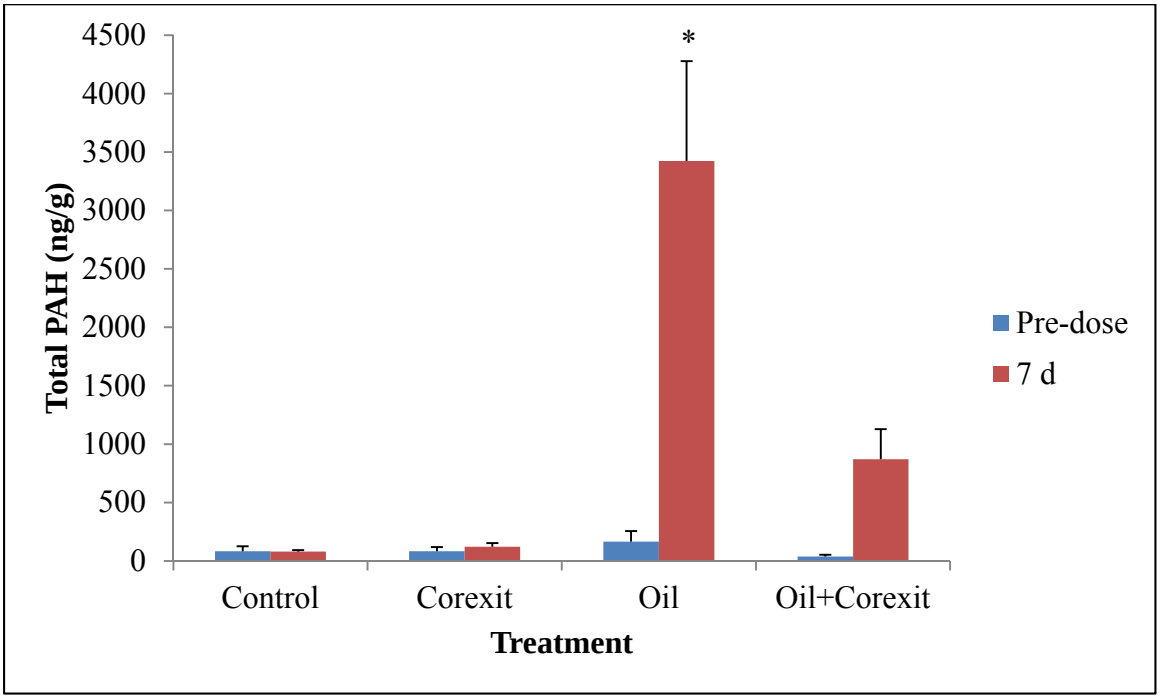


Table 24. Total sediment PAHs measured in each treatment tank at two time points for Phase II.

Tank #	Treatment	Sample Time	Total PAH (ng/g)
G1	Oil+Corexit	Pre-dose	12.376
H1	Oil+Corexit		33.301
K3	Oil+Corexit		67.143
G3	Oil		95.896
H2	Oil		52.578
L3	Oil		345.984
H3	Corexit		154.275
K1	Corexit		51.468
L1	Corexit		43.728
G2	Control		163.777
K2	Control		22.836
L2	Control		64.682
G1	Oil+Corexit	7 d	492.117
H1	Oil+Corexit		1361.728
K3	Oil+Corexit		756.790
G3	Oil		4850.078
H2	Oil		3524.984
L3	Oil		1894.125
H3	Corexit		61.984
K1	Corexit		134.757
L1	Corexit		169.132
G2	Control		56.915
K2	Control		81.762
L2	Control		100.394

Figure 34. Average total sediment PAHs measured in each treatment at two time points for Phase II. Error bars represent the standard error of the means. *Significantly different from corresponding control.



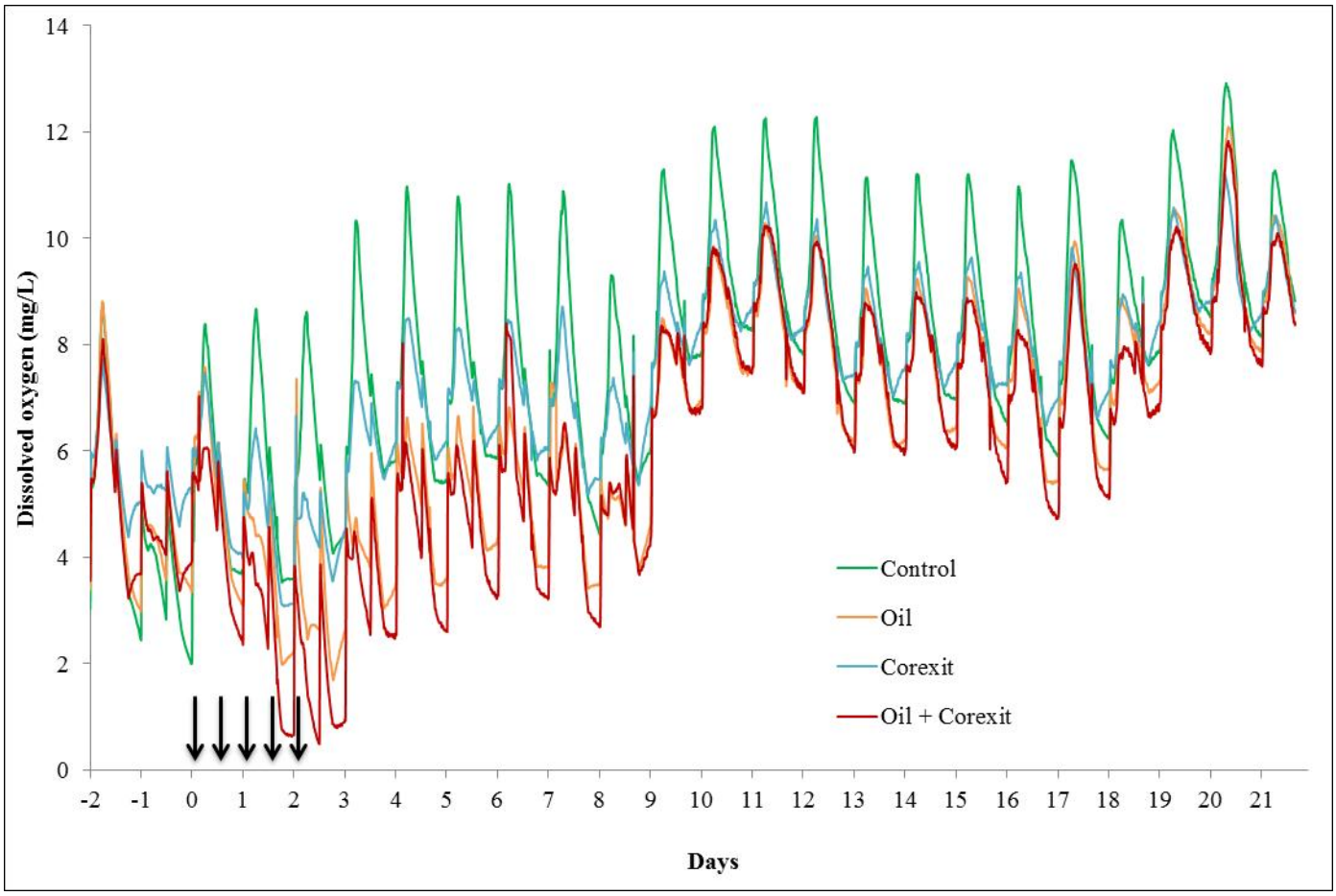
Water Quality

Water quality across all treatments for the duration of the experiment is shown in Table 25. There was a decreasing trend in dissolved oxygen concentration from control to Corexit treatments to oil treatments to oil+Corexit treatments. This is seen graphically in Figure 35 which also shows that dissolved oxygen levels remained suppressed compared to control even after the experimental period ended. Hypoxia (dissolved oxygen <2.0 mg/L) occurred in oil and oil+Corexit treatments during the first 72 hours of exposure (Figure 35). Dissolved oxygen levels recovered to near control values after 20 days.

Table 25. Mean water quality values as measured in the mesocosm tanks for each treatment for Phase II up until 14 days after the end of the experiment (October 2011). The lower dissolved oxygen levels are highlighted.

Parameter	Control			Corexit			Oil			Oil+Corexit		
	mean	SE	n	mean	SE	n	mean	SE	n	mean	SE	n
Conductivity [mS/cm]	27.28	0.2637	68	26.97	0.4778	70	27.30	0.2791	70	27.22	0.2680	69
Specific Conductivity [mS/cm]	32.26	0.0653	68	31.50	0.4623	70	31.76	0.0817	70	31.62	0.0704	69
Salinity [‰]	20.17	0.0437	68	19.68	0.2894	70	19.83	0.0552	70	19.74	0.0475	69
pH	8.02	0.0101	68	8.06	0.0143	70	8.03	0.0167	70	8.02	0.0172	69
Dissolved Oxygen [mg/L]	8.28	0.1313	68	8.02	0.1480	70	7.07	0.2056	70	6.99	0.2139	69
Dissolved Oxygen [% Sat]	95.78	0.8071	68	93.44	1.0852	70	82.26	1.8787	70	81.34	1.9791	69
Temperature [°C]	16.93	0.4397	68	17.53	0.4389	70	17.64	0.4433	70	17.72	0.4383	69

Figure 35. Mean dissolved oxygen levels for all treatments in Phase II up until 14 days after the end of the experiment. Arrows show times of hypoxic conditions.



SUMMARY

The purpose of this project was to assess acute and chronic impacts on various biological constituents of a simulated (mesocosm) saltmarsh community. The project was conducted in two phases: Phase I focused on simulating the impacts of a single incoming crude oil slick and Phase II simulated multiple re-oiling events with incoming tides. Treatments in Phase I and Phase II included crude oil only, the dispersant Corexit only and a crude oil+Corexit mixture. Results from Phase I showed that oil (TEM) and Corexit (DOSS) concentrations were elevated at 12 h to 24 h in the oil only and oil+Corexit treatments, but measured concentrations declined rapidly thereafter. Oil (TEH) concentrations in sediments were variable, but were slightly elevated in the oil only and oil+Corexit treatments at 30 and 60 d. No treatment-related effects were observed on the survival of crustaceans, molluscs, or fish; and there were no treatment-related effects on the growth of marsh grass or the benthic community.

Phase II results showed waterborne oil concentrations were significantly elevated in both the oil only and oil+Corexit treatments at 7 d. Oil concentrations in sediments were elevated in oil only and oil+Corexit treatments at 7 and 30 d. Clam survival was reduced in all oil and Corexit treatments. Growth of marsh grass was reduced in the oil only and oil+Corexit treatments at 30 d. There was a trend toward decreased benthic community diversity in the oil only and Corexit only treatment after 7 d, but these were not significant. No significant effects on the survival of fish or grass shrimp were observed; however, EROD activity was induced in the fish in both the oil only and oil+Corexit treatments at 7 d. Reductions in dissolved oxygen were observed throughout most of the exposure (~ 20 d) in the oil only and oil+Corexit treatments, and were most extreme in the oil+Corexit treatment.

In Phase I, both oil and Corexit were rapidly removed from the water column and there was little accumulation in sediments. Few significant effects were observed in biota suggesting that oil and Corexit concentrations were below thresholds necessary to elicit effects. The repeated oiling scenario in Phase II resulted in more measurable impacts to water quality and the biota. These results taken together suggest that the mesocosm approach is useful for evaluating oil and dispersant impacts to coastal ecosystems under different exposure scenarios.

Acknowledgements and Disclaimer

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