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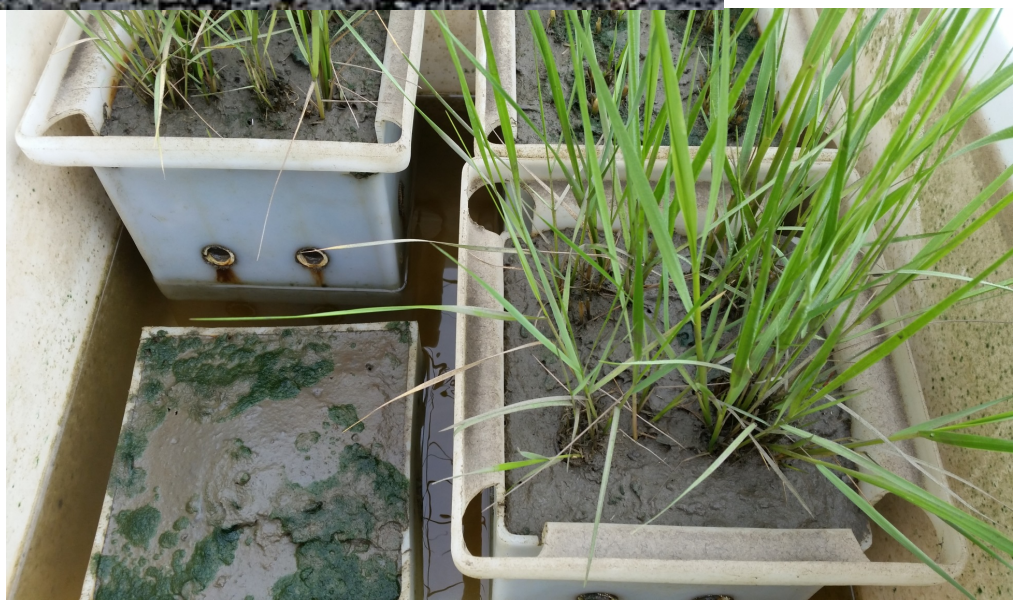
Efficacy and Ecotoxicological Effects of Shoreline Cleaners in Salt Marsh Ecosystems



NOAA Technical Memorandum NOS NCCOS #232

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Efficacy and Ecotoxicological Effects of Shoreline Cleaners in Salt Marsh Ecosystems

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ABSTRACT

Salt marsh ecosystems are sensitive habitats that may be susceptible to oil and oil spill mitigation chemicals during clean up. This research project assessed the toxicity of three shoreline cleaners (SLC) in laboratory and mesocosm exposures, determined petroleum hydrocarbon distributions in water and sediment, and evaluated efficacy of each SLC in oil (Louisiana Sweet Crude; LSC) removal from artificial substrates. The three SLC selected were PES-51, CytoSol, and Accell Clean SWA. In the mesocosm experiment, the greatest animal mortality (fish, snails, clams, amphipods and polychaetes) occurred in the Oil+Accell Clean treatment. Clam growth was reduced in the Oil+Accell Clean and CytoSol treatments. There was an increase in bacterial densities and a decrease in dissolved oxygen content in the Oil+Accell Clean treatment. Water column hydrocarbon concentrations were greatest in the Oil+Accell and Oil+CytoSol treatments after 7 d but decreased rapidly in all treatments after 30 d. In laboratory testing, grass shrimp were most sensitive to Accell Clean, followed by PES-51, then CytoSol. Effects on larval growth and development were observed. Accell Clean prepared as a chemically enhanced water accommodated fraction (CEWAF) with LSC oil was observed to act as a dispersant, mixing oil into solution and yielding greater concentrations of soluble hydrocarbons than PES-51 prepared as a CEWAF. Results of the oil-removal efficiency study with shoreline cleaners indicate that PES-51 and CytoSol were more effective at removing oil from the artificial substrates than Accell Clean. This new information on SLC product toxicity and chemical interactions with oil will allow managers to make more informed oil spill mitigation decisions.

INTRODUCTION

Oil spill response technology employs many tactics including the use of booms, skimmers, *in situ* burning, dispersants, and chemical cleaners. Chemical cleaners are applied in specific situations for removing oil from substrates such as shorelines, seawalls, or vegetation. There are 56 surface washing products (shoreline cleaners) approved for oil spill remediation as described in the U.S. EPA National Contingency Plan Product Schedule (U.S. EPA, 2017). As of 2017, the only product approved by Environment Canada as a surface washing agent is Corexit 9580 from Nalco (Fingas, 2013). Decisions as to where and when individual products will be utilized depend on understanding the efficacy, environmental fate, and environmental effects of these compounds.

Mechanism of Action

The USEPA categorized the chemical agents used to clean oiled shorelines into three categories: non-surfactant based solvents, chemical dispersants, and shoreline-cleaning agents (surfactant-based formulations specifically designed to release stranded oil from shoreline substrates) (Clayton, 1993). Shoreline-cleaning agents may act by separating the oil from the substrate, by dispersing the oil in the water used during the cleaning process, and/or by promoting biodegradation. Once the stranded oil is released by the shoreline cleaner, the goal may be to mechanically recover the removed oil. Non-surfactant based solvent cleaners function by lowering the viscosity of the stranded oil, thus allowing it to be rinsed off with a pressured-water application. The amount of water pressure needed will depend on oil composition and degree of weathering, amount of

adhesion to the substrate, and chemical composition of the cleaner (Clayton, 1993). A benefit of applying shoreline cleaners may be a reduction in the volume and temperature of wash-water required to remove the oil (Fiocco et al., 1991).

Surface washing agents that contain surfactants have a higher hydrophilic-lipophilic balance (HLB) than those in dispersants. Some surface-washing agents may result in dispersed oil, particularly under conditions of high wave energy (Fingas, 2013). The products can also be grouped into two basic types, 1) lift and disperse, and 2) lift and float (Michel and Rutherford, 2013). Lift and disperse products act to disperse, emulsify, or encapsulate the oil. The oil is not recoverable, so effluent must be contained, recovered, and properly treated. The lift and float products are specially formulated cleaners that dissolve or lift the oil without dispersing it. The oil forms surface slicks that can be recovered (Michel and Rutherford, 2013). PES-51 is listed as a "lift and float" surface washing agent (NOAA's Oil Spill Response Surface Washing Agents). Lift and float products are recommended for use on shorelines to allow oil recovery but should not be used in high energy environments where the oil cannot be recovered.

Shoreline cleaning agents work best with heavy crude oil, or light and medium crude oils that have weathered over time as constituents of the oil volatilize. The types of substrates best suited for the use of shoreline cleaning agents include man-made structures, rip-rap, boulders, cobble, bedrock, etc., that can be cleaned without trapping removed oil in inaccessible spaces. Guidelines for the use of shoreline cleaners include identification of certain habitats where they should not be applied (e.g. near living corals) (Michel and Benggio, 1995).

Product Background Information

Three shoreline cleaners were selected for testing: PES-51, CytoSol, and Accell Clean SWA. The products were selected to represent different chemical constituents, and were based on availability from the manufacturers. While Corexit 9580 was prioritized for study, it was not obtainable from the manufacturer.

PES-51

PES-51 is listed as a miscellaneous oil spill control agent and is manufactured by Practical Environmental Solutions (formerly known as Petroleum Environmental Services), San Antonio, TX. It is characterized as a biodegradable-surface-washing-agent. PES-51 chemical characteristics include some volatility, flammability at 124° F, and insolubility in water. PES-51 is used for shoreline and surface treatment, tank cleaning and equipment decontamination. It is used full strength and can be applied by hand sprayer. The manufacturer recommendations are to spray until saturation is attained, soak for 3-5 min, then rinse and recover with adsorbents. Water temperature and salinity are not reported to affect product performance. The manufacturer reports that the product/oil mixture has a density less than one, allowing it to float until it can be absorbed, skimmed, or vacuumed, and that a temporary protein film remains after treatment on the water surface that prevents the mobilized oil from re-depositing.

PES-51 consists primarily of d-Limonene (90-97% by weight). The water solubility of d-Limonene is 13.8 mg/L at 25°C (U.S. EPA, 2005). Limonene is a chemical with a lemon-like odor produced naturally by citrus plants and some coniferous trees. According to the manufacturer, PES-51 is also composed of bacterial fermentation by-products that, in combination with the carrier solvent, d-limonene, form a “unique

biological mixture" that surrounds hydrocarbon molecules and lifts them from surfaces. The product/oil mixture is stable and water-insoluble (Hoff et al., 1994).

The effectiveness of PES-51 to remove Bunker C oil was determined to be 42% at 22°C and 30% at 5°C (Guenette et al., 1998, data reproduced in Fingas, 2013). When tested with Orimulsion (a bitumen-based fuel), PES-51 effectiveness was 32% at 22°C and 23% at 5°C (Guenette et al., 1998, data reproduced in Fingas, 2013).

PES-51 was also tested during the 1994 Morris J. Berman oil spill in Puerto Rico, and while it increased the amount of oil removed compared to water spraying alone, it was noted that PES-51 required a repeat application. No dispersion of the oil was observed, and the released oil was recoverable (Michel and Benggio, 1995).

Two field test demonstrations of PES-51 were conducted; in Prince William Sound, Alaska on oil remaining from the Exxon Valdez oil spill, and in Tampa Bay, Florida following the Bouchard 155 oil spill. The Tampa Bay demonstration showed that PES-51 did remove oil from concrete and boulders, but not significantly more so than using hot-water washing (Hoff et al., 1994).

The PES-51 application in Sleepy Bay, Prince William Sound, AK (July 1997) was to a gravel beach with subsurface oil. The oil was weathered (8 years old), emulsified crude oil. The product was injected with air knives, followed by a water flush to release oil and recovery by skimmer/sorbent. Treatment of 9,490 square meters was completed over a 33-d period, producing a total of 20,007 pounds of oiled sorbent materials (Brodersen et al., 1998). PES-51 was considered effective in removing subsurface oil (PES-51 appeared to work very well at cleaning oil off rocky areas and out of the substrate) (Michel, 2015); however, laboratory tests conducted by Environment

Canada determined that it does not meet minimum qualifications for effectiveness as a surface-washing agent (Walker et al., 2003). The effectiveness reported in freshwater was 23% and in saltwater was 21% (Walker et al., 2003).

CytoSol

CytoSol™ is a surface washing agent derived from vegetable oil and animal fat methyl esters. It does not contain volatile hydrocarbons or petroleum constituents. It is manufactured by CytoCulture International, Inc., Point Richmond, CA. CytoSol has a reported water solubility of 14 ppm in freshwater and solubility ranges from 7 ppm to 230 ppm at 18 °C in seawater (Rial et al., 2010). Physical properties include a flash point of 360 °F, a specific gravity of 0.89 at 60° F, and a neutral pH. The methyl ester biosolvent is characterized by the manufacturer as "an excellent carbon/energy source for hydrocarbon-degrading bacteria", thus expediting the degradation of both the oil and the applied product (von Wedel et al., 2015). Rial et al. (2010) examined the chemical composition of CytoSol using GC-MS, and identified methyl esters of five fatty acids (hexadecanoic (palmitic) acid, octadecanoic (estearic) acid, 9-(z)-octadecanoic (oleic) acid, 9,12-(zz)-octadecadienoic (linoleic) acid, and 8,11-(zz)-octadecadienoic acid).

CytoSol may be used on weathered petroleum, heavily oiled shorelines that do not respond well to conventional treatments or that are considered too sensitive for mechanical/pressure wash strategies, coarse sand beaches, marsh areas and vegetated wetlands, concrete bulkheads, rip rap, piers, pilings, gravel or cobble shorelines, fisheries, hatcheries, mussel beds, river banks, and other sensitive or high impact sites.

Manufacturer recommendations are to apply the product full strength at an application ratio between 0.5:1 and 1:1 CytoSol to oil. It may be applied by hand sprayer

and should be applied as the tide is receding to maximize contact time. The product should be allowed to soak for at least one hour before rinse and recovery.

Use of CytoSol to clean light crude oil from rocky substrates after the Prestige oil spill was deemed successful, with an estimated efficacy of approximately 80% (Rial et al, 2010). When CytoSol was applied to remove crude oil from streambank vegetation at the Toro Creek Spill, CA (July 1997), the product was found to increase oil release over water application alone (Michel, 2015). Testing at high mixing energies noted that PES-51 and CytoSol dispersed the oil to a large degree and that to avoid dispersion low energy flushing must be used (Clayton, et al., 1995).

Accell Clean SWA

Accell Clean SWA (Accell Clean) is a surface washing agent (SWA) listed by the USEPA for use on oil-contaminated shorelines, mangroves, or seagrasses. Accell Clean is listed as soluble in freshwater and seawater. The recommended application method is a full strength product sprayed at 1 gallon per 100 square feet, followed by a 15-30 min soak period, then rinse and collect surface residue with skimmers/absorbent pads. According to the manufacturer (Advanced BioCatalytics, Irvine, CA), Accell Clean is not considered to disperse or solubilize oil into the water column (http://www.abiocat.com/accell-clean-swa.php). The product is a combination of surfactants and non-enzymatic proteins from baker's yeast that is designed to enhance natural biodegradation of petroleum contamination. The protein-surfactant complexes are meant to stimulate bacterial oil consumption without increasing bacterial biomass.

Accell Clean was used to clean oil off cobble substrate during the Refugio Oil Spill. The rocks were sprayed with 20% and 40% SWA, soaked 5 min and wiped using

shop rags. Limited success was noted with 20%, better with 40% SWA, and best with brush scrub instead of the rags (Faurot-Daniels, 2015).

Environmental toxicity data

There is potential for detrimental environmental effects resulting from shoreline cleaner application, including toxicity of the product and re-mobilized oil and possible movement of oil down the shoreline or into sub-surface habitats (Fingas, 2013). Toxicity does not necessarily correlate with effectiveness. A summary of available LC_{50} and EC_{50} values for Accell Clean, PES-51, and CytoSol are presented in Table 1.

Table 1. Comparison of available acute toxicity values (ppm) and 95% confidence interval (where provided) for selected shoreline cleaner products.

Test Species	Test Endpoint	PES-51	CytoSol	Accell Clean SWA
Mummichog, *Fundulus heteroclitus*	96 h LC_{50}	1425 (Hoff et al.,1994)		
Fathead minnow, *Pimpephales promelas*	96 h LC_{50}	810 (Hoff et al., 1994)		
Brine shrimp, *Artemia salinas*	48 h LC_{50}	840 (Hoff et al., 1994)		
Pacific oyster, *Crassostrea gigas*	48h EC_{50}	18.7 (Hoff et al., 1994)		
Rainbow trout, *Onchorhynchus mykiss*	96 h LC_{50}	98 (Hoff et al., 1994)		
Silversides minnow, *Menidia beryllina*	96h LC_{50} 96h LC_{50} 96h LC_{50} 7d LC_{50}	137 (Walker et al., 2003) 100 (Hoff et al., 1994) 21.7 (16.8-28) (Edwards et al., 2003) 20.3 (19.2-21.5) (Edwards et al., 2003)	578-738 (Walker et al., 1999)	24.12 (USEPA, 2011)
Mysid, *Americamysis bahia*	48h LC_{50} 96h LC_{50} 7d LC_{50}	54 (Walker et al., 2003) 20.0 (17.6-23.0) (Edwards et al., 2003) 15.4 (13.5-17.5) (Edwards et al., 2003)	121 (Walker et al., 1999)	59.46 (USEPA, 2011)
Purple sea urchin, *Paracentrotus lividus*	48h EC_{50} Embryo-larval		11.5 (10.7–12.4) (Rial et al., 2010)	
Mediterranean Mussel, *Mytilus galloprovincialis*	48h EC_{50} Embryo-larval		8.0 (7.7–8.3) (Rial et al., 2010)	
Blue Mussel, *Mytilus edulis*	48h EC_{50}	9.6 (Hoff et al., 1994)	8.0 (7.7–8.3) (Rial et al., 2010)	

PES-51

PES-51 contains d-Limonene, which is registered for use in pesticide products, and has been used as an ingredient in food products, soaps, and perfumes (USEPA, 2005). Toxicity of d-Limonene has been characterized by the US EPA as slightly toxic to freshwater fish and invertebrates (USEPA, 2005). Hoff et al. (1994) cited some evidence that aquatic degradation products of limonene may closely resemble the pesticide toxaphene and its breakdown products. Toxicity values available in the literature for PES-51 include a 48-h LC_{50} value of 54 ppm for *Americamysis bahia* and a 96-h LC_{50} value of 137 ppm for *Menidia beryllina* (USEPA, 1995). Additional 96-h LC_{50} values reported for PES-51 with fish include 1425 ppm (*Fundulus heteroclitus*) (Hoff et al., 1994) and 810 ppm (*Pimpephales promelas*) (Hoff et al., 1994). Bivalves such as the blue mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas*) were relatively more sensitive, with 48-h LC_{50} values of 9.6 ppm and 18.7 ppm PES-51, respectively (Hoff et al., 1994). Laboratory testing with *A. bahia* found significant effects on survival and growth after 7 d at 13 mg/L PES-51, and effects on fecundity at 21.6 mg/L (Edwards et al., 2003). The same study found significant effects on *M. beryllina* survival at 28 mg/L PES-51 (Edwards et al., 2003).

CytoSol

In a July 1997 application of CytoSol to remove crude oil from streambank vegetation at the Toro Creek Spill, CA, no increase in plant mortality was noted (Michel, 2015). Rial et al. (2010) examined the acute toxicity of CytoSol using 48h embryo-larval tests of the purple sea urchin, *Paracentrotus lividus*, and the mussel, *Mytilus galloprovincialis*. The toxicity values (EC50) determined were 11.5 ppm for the sea

urchin and 8.0 ppm for the mussel. These are lower than the toxicity values determined for mysids (121 ppm) and the fish *M. beryllina* (578-738 ppm) (Walker et al., 1999).

Rial et al. (2010) also tested *P. lividus* in a water-accommodated fraction (WAF) of CytoSol with Libyan light crude oil, and in runoff from CytoSol-treated rocky substrate with residues of the *Prestige* oil spill (NW Spain). The EC50 determined for the WAF was 23.1 ppm. The runoff water was determined to contain 49.7% CytoSol and 50.3% hydrocarbons. The CytoSol concentration in the aqueous runoff was 1.64 g/L, or 44% recovery. Exposure of the runoff water to the sea urchin resulted in an EC50 129 ppm. The mussel was more sensitive, with an EC50 of 64.3 ppm.

Accell Clean SWA

Environmental toxicity data were not available for Accell Clean SWA, except for the *A. bahia* and *M. beryllina* laboratory-derived LC_{50} values noted in Table 1 (U.S. EPA, 2011). The MSDS states that the product is not acutely toxic to algae.

Study Objectives

The overall goal of this research was to provide additional environmental toxicology and chemistry data for these chemical formulations to NOAA's OR&R. This project evaluated the efficacy and possible ecotoxicity of three shoreline cleaner products (Accell Clean, PES-51, and CytoSol) using a salt marsh mesocosm test system and laboratory exposures. The first objective was to compare the biological effects and chemical interactions with oil of the three shoreline cleaners when introduced into a simulated salt marsh ecosystem. The second objective was to establish acute toxicity thresholds for the three products alone and in conjunction with oil in adult and larval life stages of the grass shrimp, *Palaemonetes pugio*, and to examine sublethal effects in adult

shrimp and developmental effects in larval shrimp. The third objective was to assess the oil removal efficiency of the three products using artificial substrates in laboratory trials.

METHODS

Objective 1: Mesocosm testing

Each mesocosm system 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 20 systems used in this study were enclosed in a greenhouse, which incorporated natural light and temperature conditions (Figure 1), which during the time of testing were approximately 16 h light:8 h dark photoperiod and 25 °C mean temperature.

Figure 1. Individual mesocosm test systems enclosed in the greenhouse, NCCOS laboratory, Charleston SC.

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. The seawater was dispensed into the mesocosm tanks (443 L each) approximately 60 d prior to the exposure. A PVC pipe was installed in each tank to allow for water sample collection and water quality measurements to be taken without contact with the surface oil slick. Five tanks (one in each treatment; placed inside the PVC pipe) were monitored continuously with a YSI 5200A Continuous Aquaculture Monitor for water quality parameters (temperature, pH, dissolved oxygen, salinity). Pre-dose 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).

Sediments were also added to the mesocosms approximately 60 d 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 marsh grass (*Spartina alterniflora*) 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 mesocosm facility. The sediments were sieved through a course sieve (3mm) to remove larger benthic fauna and placed 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). Sediment trays were filled and placed randomly into each of the 20 mesocosm systems (3 trays with *Spartina* and one tray of

mud flat per system). Sediment trays were underwater at high tide and allowed to drain from the bottom at low tide to simulate tidal pumping and sediment drainage.

Ten days following the sediment collections, *S. alterniflora* 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 three *Spartina* sediment trays. *Spartina* was allowed to grow in the tank system 45 d before the addition of other species.

Fish (mummichogs, *Fundulus heteroclitus*) (4-6 cm in length) were collected from Cherry Point (N 32° 36' 04.29"; W 080° 11' 07.01"), Wadmalaw, SC. Adult grass shrimp, *Palaemonetes pugio* (2-3 cm in length) and adult mud snails, *Ilyanassa obsoleta*, (15-18 mm in length) were collected from Leadenwah Creek (N 32° 38' 51.00"; W 080° 13' 18.05") a tidal tributary of the North Edisto River, SC, USA. Clams, *Mercenaria mercenaria*, approximately 10-mm in diameter were acquired from Bay Shellfish, Co. (Terra Ceia Island, FL, USA). Juvenile amphipods, *Leptochierus plumulosus*, (≥500 and ≤710 µm in length) were obtained from Aquatic Biosystems Inc. (Fort Collins, CO, USA). Juvenile polychaetes, *Neanthes arenaceodentata*, (~2 weeks old, 10-15 mm in length) were obtained from Aquatic Toxic Support (Bremerton, WA, USA). Test species were acclimated to 20 ppt salinity and the same temperature and photoperiod conditions as in the greenhouse.

Grass shrimp (150) were added to each tank 27 d prior to dosing. Benthic species (clams, polychaetes, and amphipods) were added 5-7 d prior to dosing. Polychaetes (10 each) and amphipods (30 each) were added to plastic chambers filled with a sediment layer and covered with mesh, and placed on the bottom of the upper mesocosm tank.

Clams (10 each) were placed in cut plastic chambers with 100 mL (3 cm depth) of sieved sediment. Four clam chambers were then placed in a plexiglass box with mesh sides. Mud snails (30 per tank) were added 11 d prior to dosing. Six fish were added per tank 3 d prior to dosing. One tray of *Spartina* was cut 3 d prior to dosing to assess regrowth. The test duration was 30 d.

There were five treatments (Control, Oil, Oil+CytoSol, Oil+Accell, Oil+PES-51) with four replicate mesocosms per treatment. Ceramic tiles (12" x 12") were used to represent hard shoreline material such as concrete bulkhead/seawall. Five tiles were introduced into the bottom sump of each mesocosm system (Figure 2).

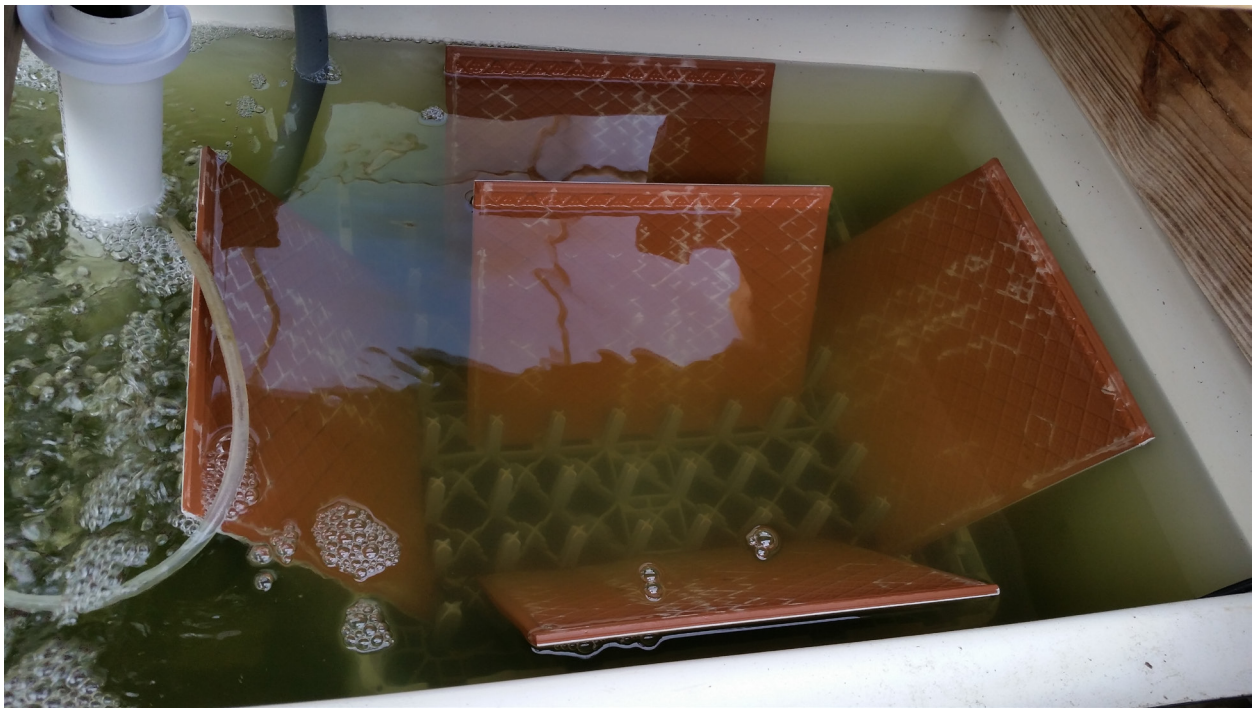

Figure 2. Ceramic tiles in place in the bottom sump of a mesocosm system.

LSC oil was added to the water surface of the bottom sump of each mesocosm system (except control) as a slick. To mimic a tidal re-oiling scenario, systems were dosed three times (0, 12, and 24 h). Each dose consisted of 74 mL, for a total of 222 mL. After the last dose, one tile was then removed from each system and weighed to assess oil mass (Figure 3).

Figure 3. Ceramic tiles after oil was applied to the treatments and one tile was removed to determine mass of oil on the tile.

Shoreline cleaners (Accell Clean, PES-51, and CytoSol) were applied to the tiles 24 h after the last oil dose, using a spray bottle of full strength product, with 8 sprays per tile side (approximately 192 mL total each mesocosm) (Figure 4).

Figure 4. Shoreline cleaner product being applied to the oiled tiles.

The shoreline cleaners were allowed to soak onto the oiled tiles for 30 min, after which the tiles were rinsed with 1 L seawater per tile side. The seawater was dispensed using a pressurized sprayer and the control and oil-only treatments received an equivalent application of seawater only.

Biological endpoints included fish, shrimp, snail, polychaete, amphipod, and clam survival, as well as clam growth, lipid peroxidation biomarker of enzyme activity (fish, snail, and clam), and impacts to salt marsh vegetation. Water samples were also collected from the upper tank of each mesocosms (using the PVC standpipe to avoid the slick) and analyzed for microbial endpoints including heterotrophic bacterial density and *Vibrio* bacteria (*V. vulnificus* and *V. parahaemolyticus*) densities. Additional water and

sediment samples were collected and preserved for microbial community composition analysis (specifically to assess groups of oil degrading bacteria) using nucleic acid sequencing methods.

Assessment of lipid peroxidation activity (LPX) was performed for surviving fish (liver tissue), clam and mud snails (tissue removed from shell) at the end of the 30 d mesocosm exposure. The LPX assay was performed according to the malondialdehyde method of Ringwood et al. (2003), adapted to microplate format. Tissues were homogenized on ice in 50 mM K_2PO_4 buffer (4:1 volume: sample weight). Homogenates were centrifuged at 13,000 g for ten min at 4°C, and 100 µL of each supernatant was transferred to a new microcentrifuge tube. Lipid peroxidation standards consisted of malondialdehyde (MDA) (3200 mM in K_2PO_4 buffer, final concentration of 12.5 – 1600 mM), and a blank of 100 µL K_2PO_4. A total of 1400 µL of 0.375% thiobarbituric acid (TBA) and 14 µL of 2% butylated hydroxytoluene (BHT) were added to 100 µL of each sample, standard, and blank. Samples and standards were then vortexed and heated at 92 °C for 15 min. Samples and standards were centrifuged at 13,000 *g* for five min at room temperature. Supernatant was transferred to a 96-well plate and absorbance was measured using a spectrophotometer at a wavelength of 532 nm.

Clam chambers were removed to assess survival, growth, and shell deformities at 7, 14, and 30 d post-dose. Clams were retrieved by sieving the water and sediment in the chambers 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 the sublethal assessments. Clams collected after 7 d and 14 d were

measured prior to tissue removal for biomarker assays. Clam shells were viewed under a dissection microscope and 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, Media Cybernetics, Rockville, MD). Shell volume (mm^3) was calculated as (major axis length)^2 x minor axis length. Mean values were calculated for each clam chamber. Clams collected after 30 d were weighed, dried 48-72 h at 68 °C, and weighed again to determine dry mass (mg). The batch dry masses were then divided by the number of clams per batch to obtain mean per clam dry mass estimates. A condition index was determined using the following ratio: dry mass (mg)/shell volume (mm^3).

Spartina growth was assessed using stem and shoot density and height measurements taken pre-dose, 14 d, and 30 d post dose. *Spartina* growth was also assessed using above ground biomass at the end of the exposure. The trays that were harvested pre-dosing were measured pre-dose, post-harvest, 14 d, and 30 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 meter stick to the nearest 1.0 cm. Above ground plant material was then separated, weighed, dried in an oven at 70 °C for 7 d, and reweighed to obtain above ground biomass.

Microbial assessments for water column densities of heterotrophic bacteria, *Vibrio vulnificus*, and *Vibrio parahaemolyticus* were conducted pre-dose, 24 h post-dose, 7 d, and 30 d post-dose using standard culture techniques. Heterotrophic marine bacteria

were selected for using Marine Broth 2216 and incubated at 25°C to mimic ambient mesocosm conditions. The agar plates were incubated for 24 h. *Vibrio* selective media (CHROMagar) plates were incubated for 24 h at 37°C. Colonies were identified by color; mauve colored colonies were presumptively *Vibrio parahaemolyticus* and turquoise colored colonies were presumptively *Vibrio vulnificus*. Colony forming units (cfu)/100 mL) were determined for each sample.

Water quality parameters (temperature, salinity, pH, and dissolved oxygen) were taken twice daily at approximately 9:00 a.m. and 3:00 p.m. using hand held instruments. In addition, each mesocosm treatment had one tank containing a multi-parameter probe for continuous water quality measurements.

Water and sediment samples for chemical analysis of total extractable hydrocarbons (TEH) and polycyclic aromatic hydrocarbons (PAHs) were collected 12 h post-oiling, and 24 h, 7 d, 14 d, and 30 d post-cleaner application. Samples were composited across replicates at 12 h, 14 d, and 30 d, but replicate mesocosm samples were analyzed at 24 h and 7 d.

To quantify PAH and TEH, water samples were acidified with 18% hydrochloric acid to a pH of 2 and then transferred into solvent rinsed 1 L separatory funnels to undergo liquid/liquid extraction. QA/QC measures for each batch (n = 7-10) included a blank, TEH spike (10 mg) and PAH spike (400 ng). All samples were spiked with PAH and TEH internal standards and mixed thoroughly. There were 18 deuterated PAH internal standards (d8-naphthalene, d_{10}-1-methylnaphthalene, d_8-acenaphthylene, d_{10}-acenaphthene, d_{10}-fluorene, d_8-dibenzothiophene, d_{10}-phenanthrene, d_{10}-anthracene, d_{10}-fluoranthene, d_{10}-pyrene, d_{12}-benz[a]anthracene, d_{12}-chrysene, d_{12}-benzo[b]fluoranthene,

d_{12}-benzo[k]fluoranthene, d_{12}-benzo[e]pyrene, d_{12}-benzo[a]pyrene, d_{12}-perylene and d_{12}-benzo[g,h,i]perylene [Cambridge Isotope Laboratories, Inc. Tewksbury, MA]) and 2 TEH internal standards (d_{26}-dodecane and d_{42}-eicosane perylene [Cambridge Isotope Laboratories, Inc. Tewksbury, MA]).

Samples were solvent extracted three times with the following solvents, dichloromethane, 50:50 dichloromethane/hexane and hexane. After extraction, samples were passed through GF/F paper containing anhydrous sodium sulfate and concentrated in a water bath (40°C) under a stream of nitrogen (14 psi). Extracts were cleaned-up using silica Solid Phase Extraction (SPE) (3 mL/0.5 g [Phenomenex Torrence, CA]) and spiked with a recovery standard (d_{14}-p-terphenyl [Cambridge Isotope Laboratories, Inc. Tewksbury, MA]) prior to instrumental analysis on GC/MS.

Sediment samples (top 1-2 cm) were collected from the mesocosm upper tanks using solvent rinsed metal spatulas. Sediments were extracted for the assessment of TEH and PAHs in a manner similar to the methods detailed in Balthis et al. (2015) and Cooksey et al. (2014). Approximately 10 g wet sediment was extracted under pressure using Accelerated Solvent Extraction (ASE200) (Dionex Inc.) with dichloromethane:acetone (1:1 v/v). The extracts were reduced in volume to 2mL under nitrogen and passed through a Biobead column via Gel Permeation Chromatography (GPC) to remove interferences. Additional clean-up was achieved by using silica SPE. The final volume was exchanged under nitrogen to hexane and the extracts analyzed for both PAH and TEH.

All extracts (water and sediment) were run on an Agilent 6890/5793N GC/MS with split/splitless injector containing a DB17ms 60m x 0.25 mm x 0.25µm analytical

column. The mass spectrometer was operated in SIM (selected ion monitoring) mode. Samples were injected twice, once for PAH analysis and once for TEH analysis. The instrument was calibrated with calibration standards ranging from 0.1-5000 ng/mL (PAHs) and 0.25-20 mg/mL (TEH). The TEH calibration curve was made by diluting Louisiana Sweet Crude. Continuing calibration verification standards were run every 10-15 samples to ensure the validity of the calibration curve. All analytes had a coefficient of determination (r^2) greater than or equal to 0.995. Data analysis was performed using MSD Chemstation software. Total PAH is reported for 50 parent and alkylated PAHs (Appendix 1).

Objective 2: Laboratory testing with grass shrimp

Adult grass shrimp (2-3 cm in length) were collected from Leadenwah Creek (N 32° 38' 51.00"; W 080° 13' 18.05") a tidal tributary of the North Edisto River, SC, USA. The shrimp were acclimated 7-14 d in 76-L tanks with 20 ppt saltwater and were fed Tetramin® fish flakes. Adult grass shrimp were tested in 4-L glass jars containing 2 L of test solution and 10 adult shrimp per jar. After preliminary range finding assays, the nominal shoreline cleaner concentrations tested were 12.3, 37, 111, 333, and 1000 mg/L, plus a seawater control. There were three replicate jars per treatment. The jars were aerated and kept at 25 °C and a 16 h light:8 h dark photoperiod. Every 24 h, water quality (temperature, dissolved oxygen, salinity and pH) was measured and the test solutions were renewed. Adult shrimp were not fed during the test. At the end of the 96 h exposure, mortality was determined and surviving shrimp were collected and stored frozen (-80 °C) for lipid peroxidation and glutathione bioassays.

Lipid peroxidation activity was determined using whole shrimp and the method described for the mesocosm experiment. Glutathione was assessed using the 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB)-glutathione reductase recycling protocol described in Ringwood et al. (2003). Shrimp were homogenized cold in 5% sulfosalicyclic acid (SSA) and centrifuged at 4°C for 5 min at 13,000 *g*. A 975 µL aliquot of a mixture of deionized water, 5,5'-dithiobis(2-nitrobenzoic) acid (DTNB), and β-nicotinamide adenine dinucleotide phosphate (NADPH) buffer was added to 25 µL sample supernatant. Glutathione standards were dissolved in SSA and 25 µL of each concentration (200, 100, 50, 25, 12.5, 6.25 µM) were combined with the previously described mixture. The blank consisted of SSA. Glutathione reductase (15 µL) was added to the samples and standards and absorbances were read in a spectrophotometer at 405 nm for 90 s with 15 s intervals. Data were expressed as nM of glutathione formed per gram of wet weight.

P. pugio larvae were obtained by placing ovigerous adult shrimp in brooding containers within 10-L aquaria. The brooding containers were designed to allow the embryos to hatch and the larvae to escape through the mesh. The larvae were fed 3-4 drops of newly hatched brine shrimp (*Artemia salina*) and tested at 24-48 h old. Larvae were exposed in 600 mL glass beakers with 400 mL of test solution and ten larvae per beaker. The same shoreline cleaner product concentrations were tested as per the adult exposures. There were three replicate beakers per treatment. The beakers were covered with aluminum foil, aerated, and kept at 25°C and a 16 h light:8 h dark photoperiod. Every 24 h, water quality (temperature, dissolved oxygen, salinity and pH) was measured

and the test solutions were renewed. Larvae were fed brine shrimp daily (1 mL per beaker) during the test. Larval mortality was determined at the end of the 96 h exposure.

The testing with the individual shoreline cleaners was repeated using Chemically Enhanced Water Accommodated Fractions (CEWAFs) of the Accell Clean and PES-51 products in mixture with Louisiana Sweet Crude (LSC) oil. CytoSol was not included in the CEWAF testing given the lack of solubility and toxicity noted in the product-alone testing. Preparation of the CEWAFs followed methods similar to Hemmer et al. (2011). A clean glass aspirator bottle was placed on a stir plate and the bottom outlet was closed with Tygon tubing and a glass stopper. A Teflon stir bar was placed in the bottom of the aspirator bottle. Seawater (19L, 20 ppt, see description above) was added to the aspirator bottle and the stirring was initiated to achieve minimal vortex. Next, LSC oil (25 g/L) was added to the center of the vortex. Oil was added using a graduated cylinder and the initial weight and weight after dispensing were recorded to determine the actual amount added by mass difference. The shoreline cleaner was then added to the center of the vortex using a glass pipette at a ratio of 1:10 product:oil, and again the delivery mass was calculated by difference in weight. The aspirator bottle was then sealed with a stopper, the mixing speed was increased to achieve a vortex 25% of the solution height, and the solution was stirred for 18 h. After letting the solution sit for 6 h, the stopper was removed, the bottom outlet was opened, and the CEWAF was dispensed into a collection container, without disturbing the slick layer.

The 100% CEWAF was then diluted with 20 ppt seawater to achieve additional treatments (50%, 16.7%, 5.6%, 1.85%, 0.62%, 0.21%). Similar test methods were used as for the product-alone testing, except that CEWAF testing was conducted using static

exposures, whereas the shoreline cleaners alone were tested using static renewal exposures. Water samples were collected from each 100% CEWAF and the dilutions and analyzed for TEH and PAH.

An additional study to examine larval grass shrimp development post-exposure was conducted. The method was similar to the larval aqueous static renewal 96-h bioassay described above for each SLC. Nominal SLC concentrations were selected based on the results of the definitive 96-h test (Accell Clean: 4.1, 12.3, and 37 ppm; PES-51: 12.3, 37, and 111 ppm; Accell Clean-CEWAF: 0.21%, 0.62%, 1.85%, and 5.6%; PES-51-CEWAF: 0.62%, 1.85%, 5.56%, 16.7%, 50%, and 100%). There were three replicate beakers per treatment with ten larvae per beaker, along with at least three replicate 6-well plates per treatment with one larvae per well. Before each daily water change, molts and dead larvae were counted and removed from the wells. The three beakers per treatment were terminated after 96 h and surviving larvae were frozen at -80 °C for ecdysteroid analysis.

Also after 96-h, larvae from the 6-well plates were moved to new clean plates containing clean seawater and post-exposure larval development was assessed. Each day, molts were counted and removed and larval developmental status was assessed. On Monday, Wednesday, and Friday water quality (temperature, salinity, pH, and dissolved oxygen) was assessed, the well plates were renewed with clean 20 ppt seawater, and the larvae were fed 50 µL of Artemia. The test was terminated when larvae in all concentrations reached post-larval status. P. pugio larvae were characterized as swimming upside down and backward and containing pairs of chromatophores (Key et al.

1998). Post-larval status was characterized as swimming right-side up and forward after the final larval molt and loss of the chromatophore pairs (Broad, 1957). Surviving larvae that reached post-larval status were oven dried for 48 h at 60 °C to determine dry weight (McKenney, 1986; Key and Fulton, 1993).

A modified ecdysteroid ELISA protocol was used to assess larval shrimp ecdysteroid activity after 96-h exposure (Cayman Chemical, 2009; Gelman et al., 2002; Tuberty and McKenney, 2005). Larval shrimp, 7-10 individuals depending on availability, were weighed, homogenized for two minutes on ice in 50 µL/shrimp of 80% methanol, and centrifuged at 14,000 g for 5 min at 4 °C to remove precipitated proteins and debris. The supernatant was transferred to new tubes and placed on ice. Fifty µL/shrimp of 80% methanol was added to the precipitates, homogenized for 1 minute, and centrifuged again at 14,000 g for 5 min at 4 °C. The second supernatant was added to the corresponding first supernatant on ice. The methanol was evaporated in a TurboVap under nitrogen. The sample was reconstituted by adding 50 µL/shrimp of EIA buffer to each sample tube and vortexed. One hundred µL of EIA buffer was added to the non-specific binding (NSB) wells and 50 µL to the maximum binding (B0) wells in a Cayman Chemical 96-well plate. Fifty µL of standards (32, 16, 8, 4, 1, 0.2, 0.1, 0.02 Fmol/ µL) and samples were added to the appropriate wells. Fifty µL of tracer was added to all wells except the blank (Blk) and total activity (TA) well and 50 µL of antiserum was added to all wells except Blk, TA, and NSB. The plate was covered with plastic film and incubated overnight (18 h) at 4 °C. Contents were discarded and wells were washed with wash buffer five times. A 200 µL aliquot of Ellman's reagent (DTNB) was added to each well and 5 µL of tracer was added to the TA well. The plate was developed in the

dark for 90 min. Absorbances were read in a spectrophotometer at a wavelength of 418 nm. Protein concentration (ng/g wet tissue weight) in each sample was calculated from a standard curve.

Objective 3: Oil-removal efficiency study with shoreline cleaners

A controlled laboratory study was performed in order to understand the efficiency of three shoreline cleaners. Pre-weighed ceramic tiles (4" x 4") were coated with Louisiana Sweet Crude oil on the glazed (smooth) side of the tile. The tiles were also weighed after oil application so that a mass of oil could be calculated. Oil was applied using a 4" foam roller. Three tiles were coated with oil for each treatment. The treatments included four rinsing treatments: Seawater (SW), PES-51 (PES), Accell Clean (ACC), and CytoSol (CYT). Oiled tiles were placed in a foil pan and weathered in a greenhouse for 10 d under ambient light and temperature conditions.

After 10 d, tiles were washed according to the manufacturer's label application instructions. Cleaners were applied using hand sprayers that were pre-calibrated to dispense approximately 3 mL per spray. PES was applied until saturation (three sprays of PES to each tile (~9 mL)) with a soak time of five min. For the ACC treatment, the recommended application is 1 gallon of cleaner per 100 sq. ft., which roughly equated to two sprays per tile (~6 mL). The soak time was 30 min. CYT application was 1:1 (cleaner mass: oil mass) with a soak time of 60 min; about 0.48 g of CYT was applied to each tile. For the SW treatment, in lieu of a SLC product, seawater was applied to the tile using a hand sprayer (three sprays, ~9 mL) and allowed to sit for 2 min. After soaking, tiles were

rinsed with a calibrated pressurized garden sprayer containing seawater for 30 sec. Volumes of rinse water ranged from 440-625 mL

For each tile from the SW, PES, ACC and CYT treatments, the seawater rinse was collected in 1 L pre-acidified, solvent rinsed amber bottles, and the washed tiles were placed into foil covered aluminum pans for transport to the lab. Water samples were extracted via liquid/liquid extraction as detailed in the mesocosm section.

Residual oil remaining on the tile was extracted using 100 mL of dichloromethane followed by 100 mL of hexane. Extracts were concentrated to a known volume, typically 10 mL Thereafter, 1 mL of the extract was cleaned-up with silica SPE before instrumental analysis. Deuterated PAH and alkane internal standards were added just prior to silica SPE.

All extracts (tile and water) were run on an Agilent 6890/5793N GC/MS as detailed in the mesocosm section. The instrument was calibrated with calibration standards ranging from 0.1-5000 ng/mL (PAHs) and 0.25-20 mg/mL (TEH). The TEH calibration curve was made by diluting weathered Louisiana Sweet Crude. The oil was weathered in the same manner as described earlier in this section. Continuing calibration verification standards were run every 10-15 samples to ensure the validity of the calibration curve. All analytes had a coefficient of determination (r^2) greater than or equal to 0.995. Data analysis was performed using MSD Chemstation software.

Statistical Analysis

Median lethal concentrations (96 h LC_{50} values) with 95% confidence intervals (CIs) were determined for the grass shrimp laboratory exposures based on nominal values using SAS Probit Analysis (PROC PROBIT, SAS V.9.1.3, Cary, NC, USA). Significant

differences ($p < 0.05$) between LC_{50}s of the different chemicals and life stages were determined using the LC_{50} ratio test (Wheeler 2006). Statistical differences among treatments were determined using analysis of variance (ANOVA). Where ANOVA revealed a significant difference among treatments ($p<0.05$), Dunnett's procedure for multiple comparisons was used to determine which treatments differed significantly from the control. The *Spartina* measurements from the mesocosm exposure were analyzed using repeated measures ANOVA with subsampling (two trays per tank), followed by Dunnett's test for each time point.

RESULTS AND DISCUSSION

Objective 1: Mesocosm testing

Fish, shrimp, and snail survival in the mesocosms was assessed after 30 d (Figure 5). Overall grass shrimp survival was poor, most likely due to predation by *Fundulus heteroclitus*. These fish were substituted in the experiment for *Cyprinodon variegatus* due to a disease outbreak at the aquaculture supplier. Unfortunately, *F. heteroclitus* is an efficient grass shrimp predator. As a result, treatment-related mortality or sublethal biomarkers could not be assessed on the shrimp. Mean fish survival was also relatively low in the controls, and we did observe some fish had jumped out of the mesocosm tanks. However, compared to the control and other treatments, the Oil+Accell treatment had significantly lower fish survival (0%; ANOVA, Dunnett's $p = 0.0037$). Mean mud snail survival was 74-85% in all treatments except the Oil+Accell treatment, which had 0% snail survival (ANOVA, Dunnett's $p < 0.0001$).

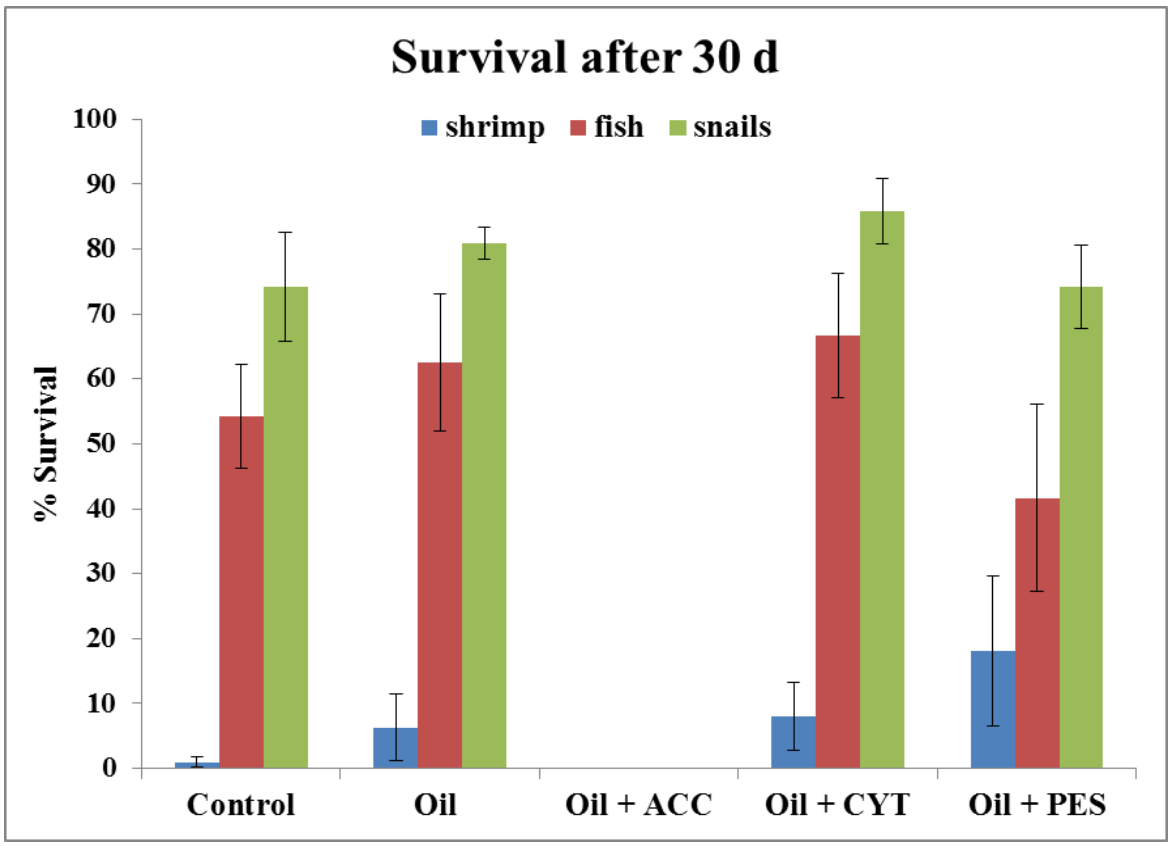

Figure 5. Survival of fish, shrimp, and snails after 30 d in the mesocosm treatments.

Clam survival was assessed after 7 d, 14 d, and 30 d (Figure 6). Mean clam survival was 90-100% at all time points in all treatments except for the Oil+Accell treatment, which had significantly lower survival after 30 d (ANOVA, Dunnett's $p < 0.0013$). Clam survival in the Oil + Accell treatment declined over time, with 62.5% mean survival after 7 d, 52.5% after 14 d, and 37.5% after 30 d.

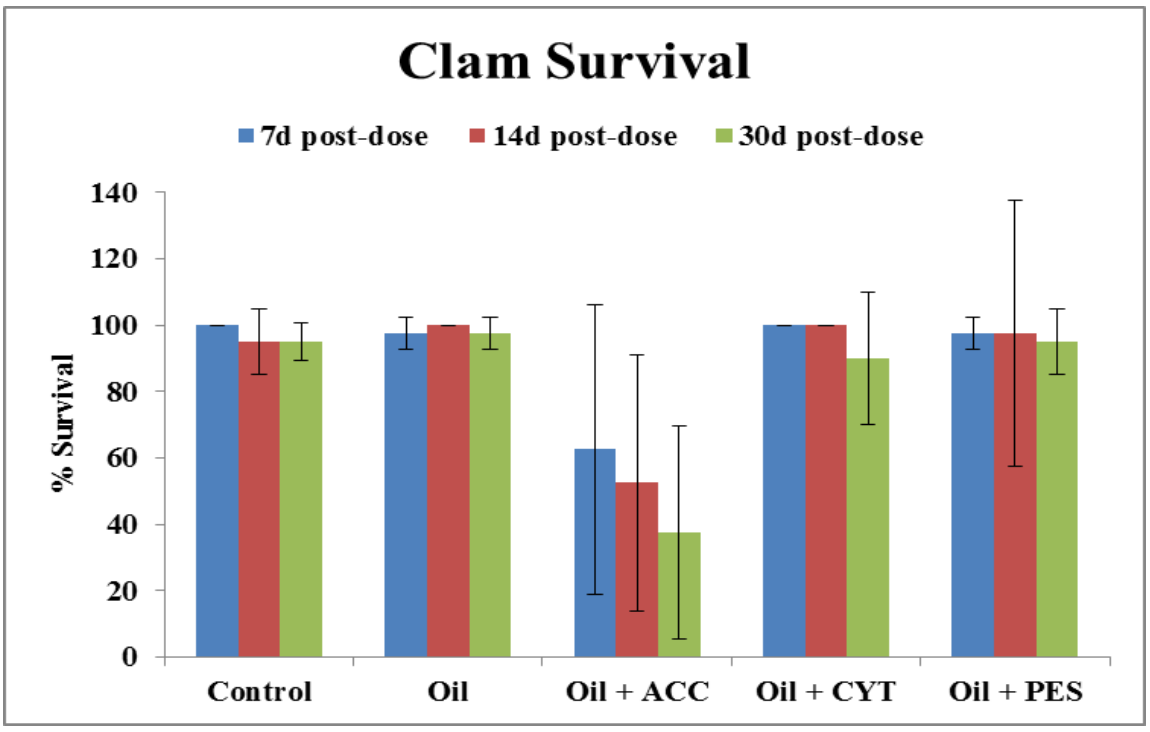

Figure 6. Survival of juvenile clams after 30 d in the mesocosm treatments.

There was a significant treatment effect on mean juvenile clam dry weight measured 30 d post-dose (ANOVA p = 0.0021), with both the Accell Clean and CytoSol treatments yielding significantly less clam mass than the control (Dunnett's test p = 0.0097 and p = 0.0044, respectively) (Figure 7). Shell size expressed as mean shell major axis length 30 d post-dose was also significantly different among treatments (ANOVA with nested sampling p = 0.0023), with the CytoSol treatment having significantly smaller shells than the control (Dunnett's test p = 0.0029) (Figure 8).

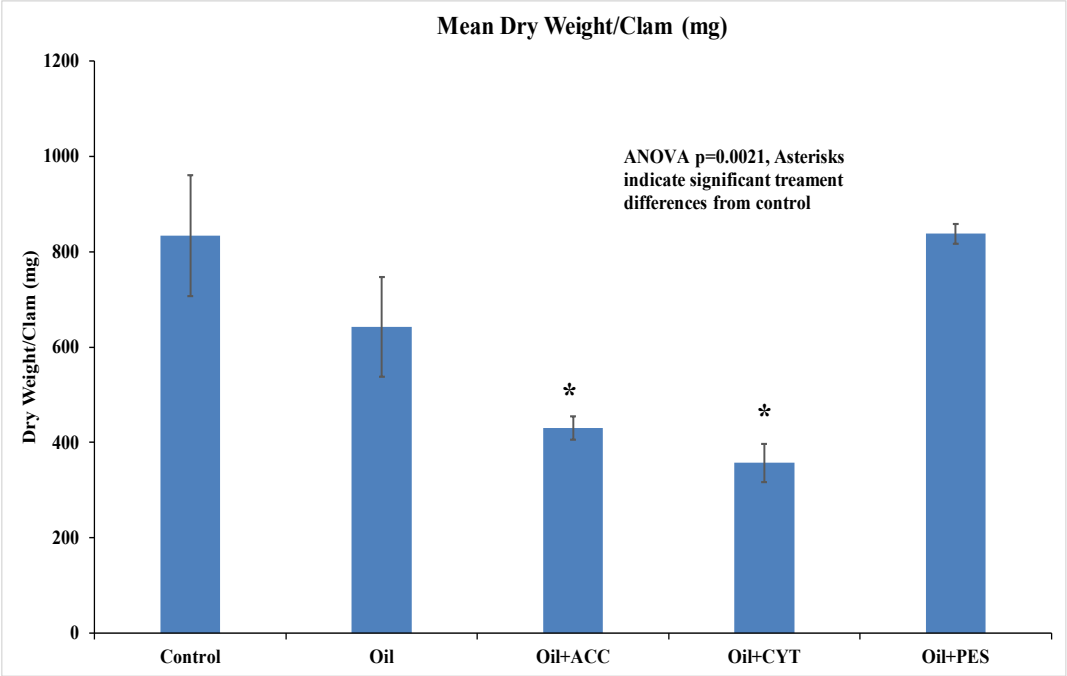

Figure 7. Juvenile clam dry weight after 30 d in the mesocosm treatments.

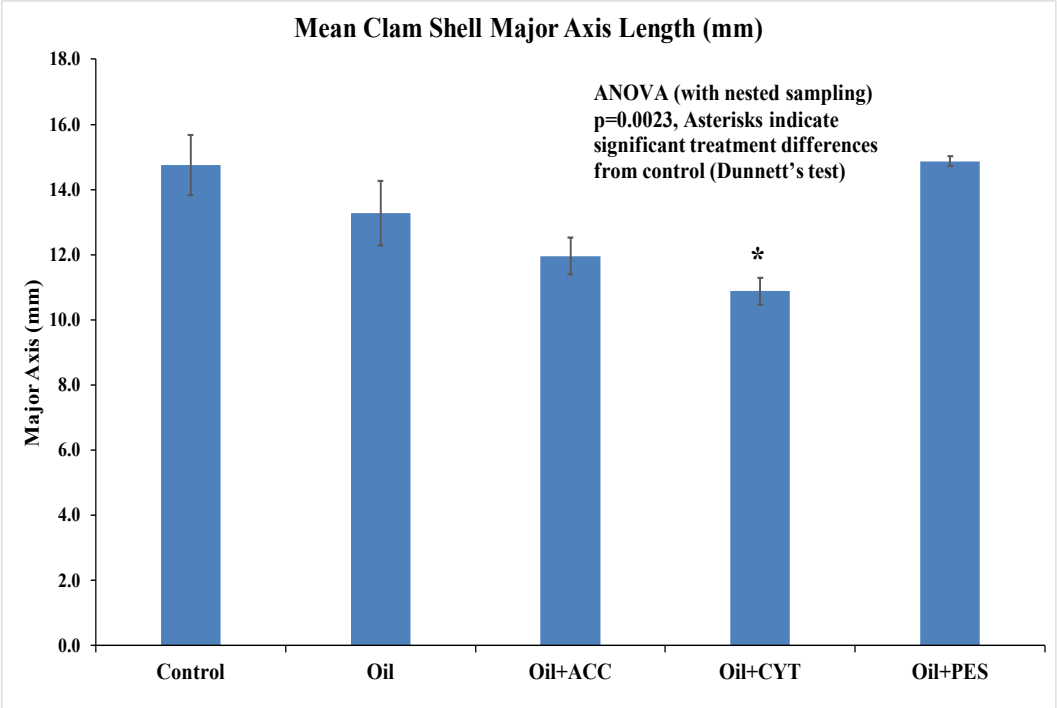

Figure 8. Juvenile clam shell major axis length after 30 d in the mesocosm treatments.

Mean juvenile clam condition index measured 30 d post-dose was not significantly different among treatments (ANOVA p = 0.5753). The results suggest that condition index was not the most sensitive measure of clam health. The effects on shell length and weight were in general agreement, however, with trends in decreased growth observed in the Oil+Accell and Oil+Cytosol treatments.

Polychaete and amphipod survival was assessed after 7 d and 14 d. Mean polychaete survival after 7 d and after 14 d was similar in each treatment, 65-67.5% in the control, 52.5-55% in the Oil+PES-51 treatment, 42.5% in the oil alone treatment, 22.5% in the Oil+CytoSol, and 0% in the Oil+Accell treatment (Figure 9). There was a significant effect on polychaete survival after 14 d in the Oil+CytoSol and Oil+Accell treatments (ANOVA, Dunnett's p values = 0.0038 and < 0.0001, respectively).

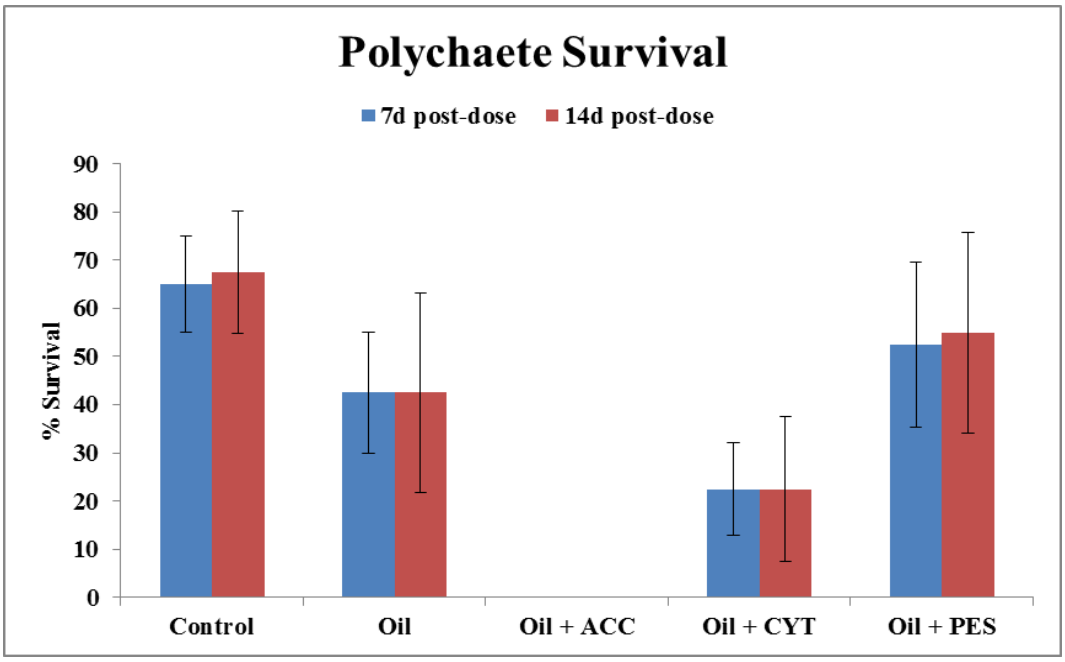

Figure 9. Survival of polychaetes after 7d and 14d in the mesocosm treatments.

Mean amphipod survival declined from 7 d to 14 d in each treatment (Figure 10). Greatest amphipod survival occurred in the control (77% after 7 d, 50% after 14 d), followed by the Oil+PES-51 treatment (60% after 7 d, 39% after 14 d), the Oil treatment (41% after 7 d, 30% after 14 d), the Oil+CytoSol treatment (32% after 7 d, <1% after 14 d), and the Oil+Accell treatment (0% survival after 7 d and 14 d). Amphipod survivial after 14 d was significantly lower in the Oil+CytoSol and Oil+Accell treatments than the control (ANOVA p = 0.0053, Dunnett's p values = 0.0051 and 0.0045, respectively).

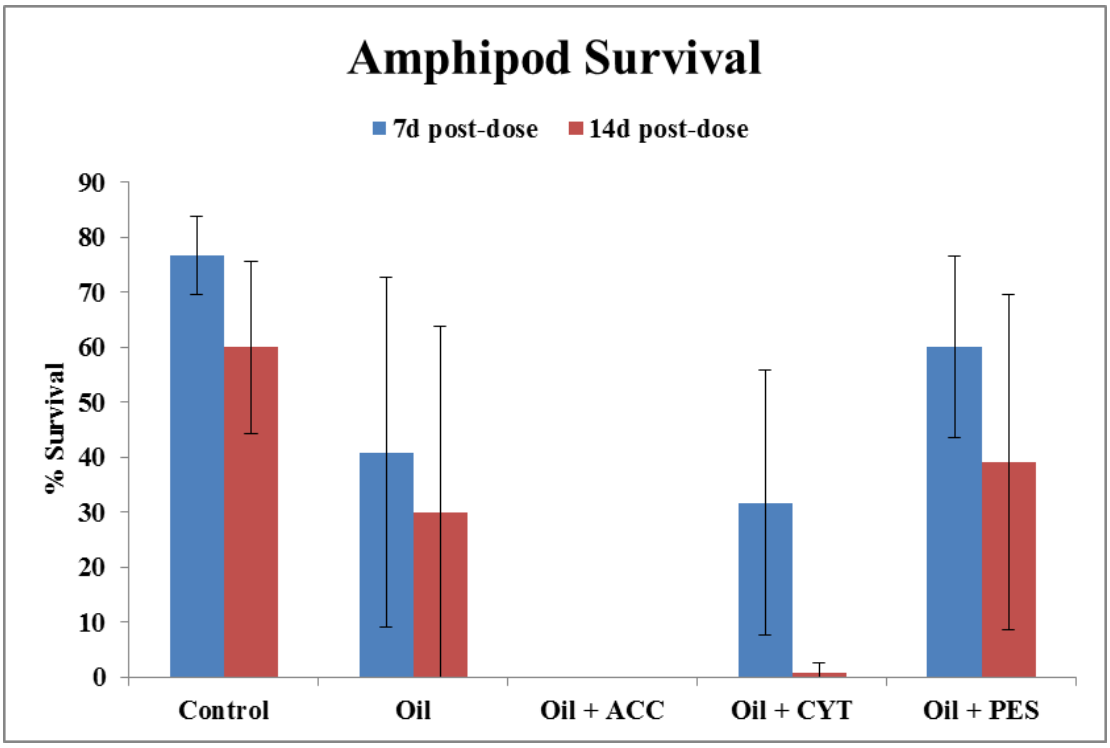

Figure 10. Survival of amphipods after 7d and 14d in the mesocosm treatments.

There was no significant difference in fish, clam and snail lipid peroxidation activity among the shoreline cleaner mesocosm treaments (Table 2). Analysis of variance p values were 0.5987 for fish, 0.4993 for clams, and 0.2345 for snails. There were not enough surviving fish or snails to analyze in the Oil+Accell treatment. Lipid peroxidation

is a measure of oxidative damange to cellular membranes. This has been a sensitive biomarker in previous short term exposures (e.g., DeLorenzo et al., 2014), but it is likely that the response was not detectable after the chronic 30 d mesocosm exposure.

Table 2. Lipid peroxidation activity in surviving fish (*C. variegatus* livers), clams (*M. mercenaria*), and snails (*I. obsoleta*) in each shoreline cleaner treatment at the end of the 30 d mesocosm exposure. Values are mean ± standard error.

	Fish	Clams	Snails
Control	52.54 (±14.49)	176.33 (±26.62)	81.08 (±14.20)
Oil	38.13 (±4.94)	174.72 (±19.40)	91.52 (±12.45)
Oil+CytoSol	41.76 (±5.15)	172.55 (±13.49)	101.44 (±12.19)
Oil+PES-51	46.24 (±6.97)	135.70 (±11.35)	68.41 (±6.26)
Oil+Accell	none surviving	146.01 (±14.30)	none surviving

Bacterial densities were assessed pre-dose, 24 h, 7 d, and 30 d post-dose. While the Oil+Accell treatment had the greatest animal mortality, it had the highest bacterial densities (cfu/mL) (Figure 11). Pre-dose mean heterotrophic bacteria densities ranged from 3175-18625 across all treatments. There was at least a 100-fold increase in heterotrophic bacterial density in the Oil+Accell treatment compared to all other treatments at 24 h post-dose and densities remained approximately four times higher after 7 d. After 30 d, all treatments had relatively similar heterotrophic bacteria densities, ranging from 2975-4350 cfu/mL It is unknown whether the increase in bacterial densities in the Oil+ Accell treatment was due the shoreline cleaner serving as a carbon source; or a result of the animal decomposition occurring in that treatment.

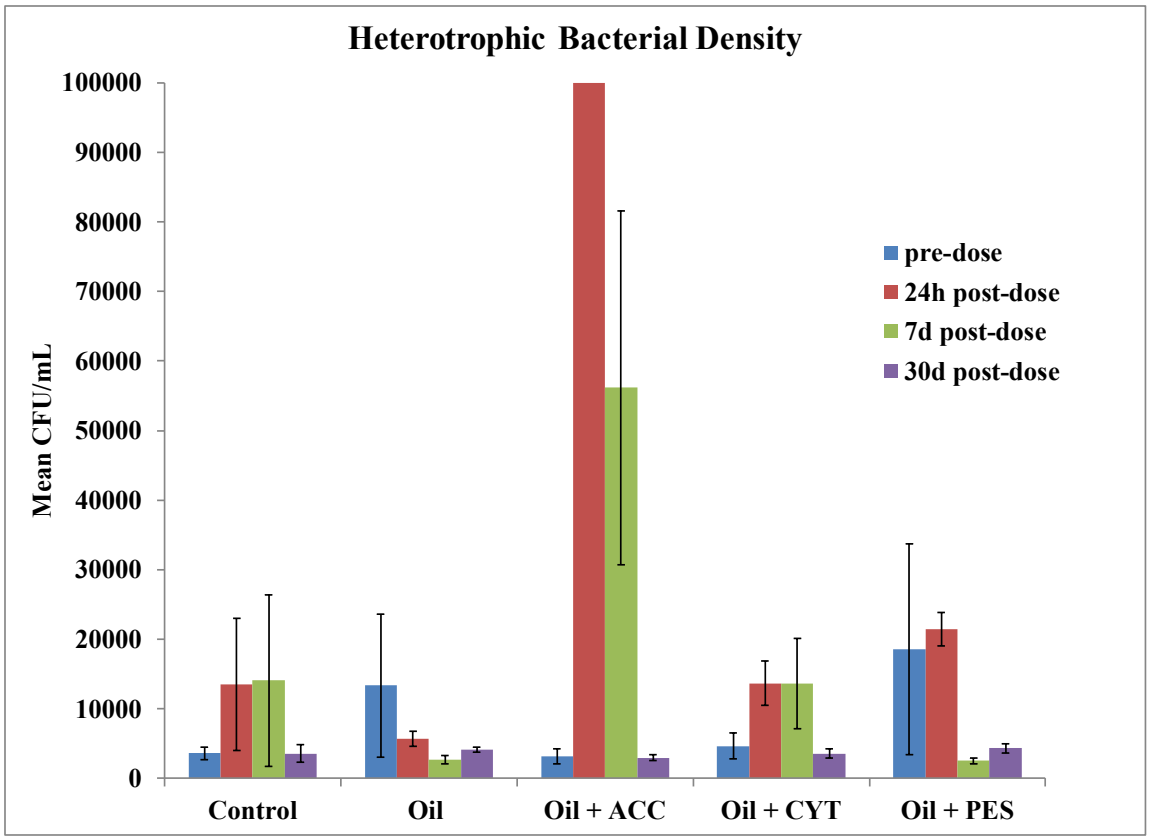

Figure 11. Heterotrophic bacterial densities measured at each timepoint in the mesocosm treatments.

Similar to the heterotrophic bacteria, *Vibrio* bacteria densities were also elevated in the Oil+Accell treatment after 24 h and 7 d. Pre-dose values for *V. vulnificus* ranged from 5-20 cfu/mL across treatments, compared to 24 h post-dose values of 25 cfu/mL (Control), 10 cfu/mL (oil alone), 15 cfu/mL (Oil+PES-51), 135 cfu/mL (Oil+CytoSol), and >10000 cfu/mL (Oil+Accell) (Figure 12). *V. parahaemolyticus* densities were higher than *V. vulnificus*, and ranged from 220-770 cfu/mL across treatments pre-dose. Mean *V. parahaemolyticus* densities 24 h post-dose were 390 cfu/mL (Control), 475 cfu/mL (oil alone), 955 cfu/mL (Oil+PES-51), 670 cfu/mL (Oil+CytoSol), and >10000 cfu/mL (Oil+Accell) (Figure 12).

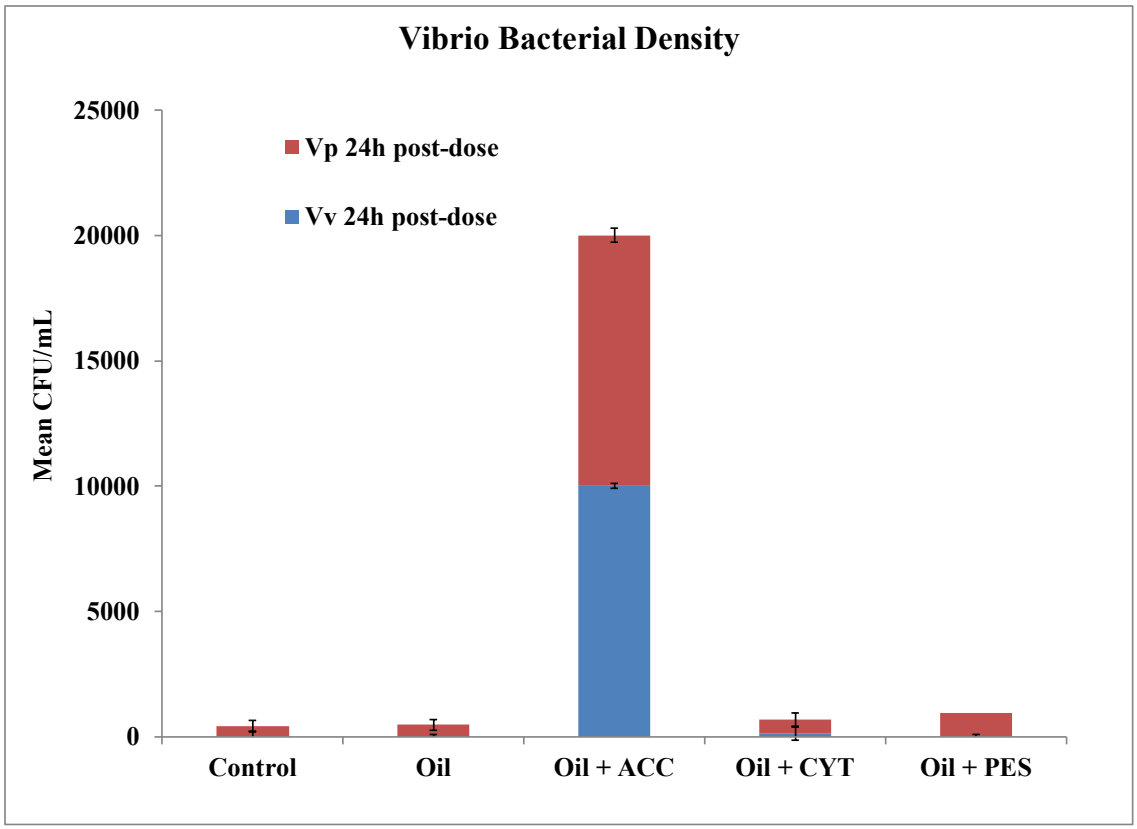

Figure 12. Densities of *Vibrio parahaemolyticus* (Vp), and *Vibrio vulnificus* (Vv) measured 24h post-dose in the mesocosm treatments.

After 7 d, *Vibrio* densities were elevated in the Oil+CytoSol treatment compared to controls, and remained at highest densities in the Oil+Accell treatment (Figure 13). After 30 d, the Oil+PES-51 treatment had elevated *Vibrio* densities compared to controls, while the Oil+Accell treatment had the lowest *Vibrio* densities (Figure 13). Water and sediment samples were collected at multiple time points for microbial community composition analysis. DNA extractions were performed and the samples are pending submission for sequence analysis.

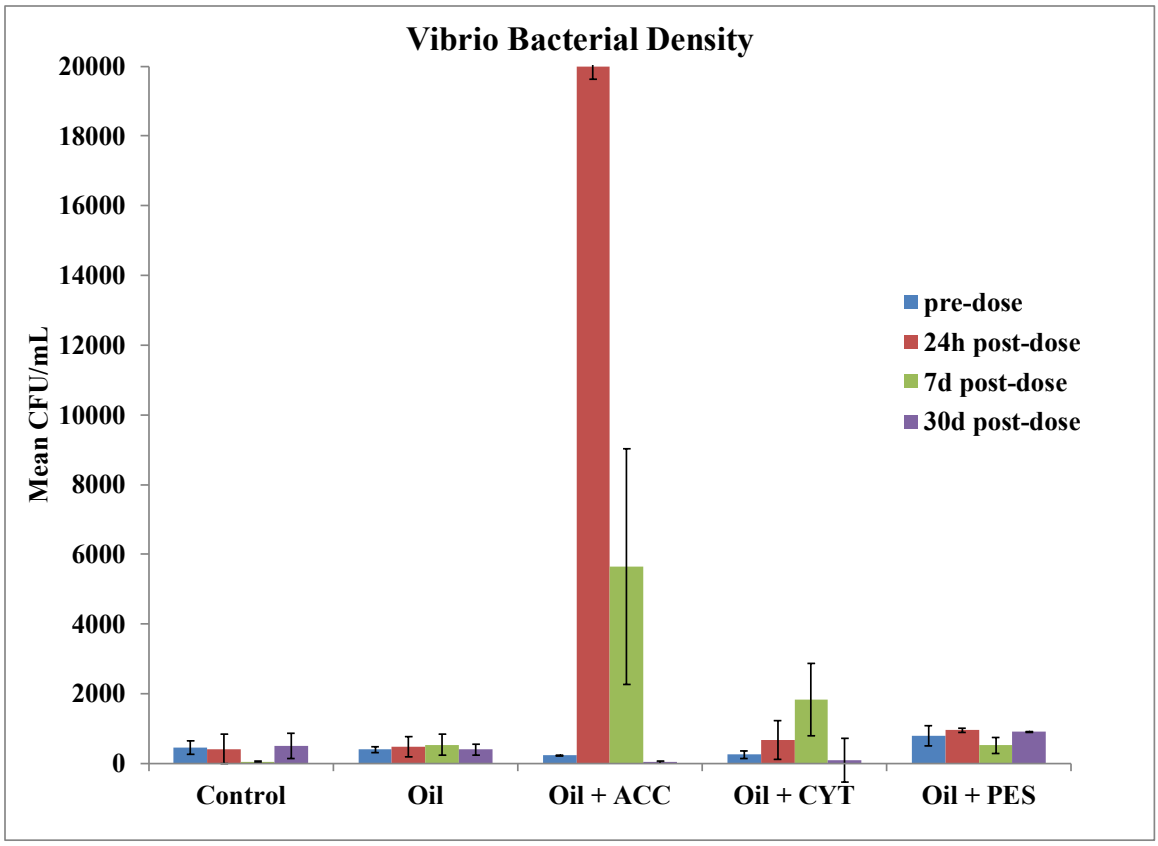

Figure 13. Total *Vibrio* bacterial densities (sum of *V. parahaemolyticus* and *V. vulnificus*) measured at each time-point in the mesocosm treatments.

In nearly all cases, *S. alterniflora* grew over time as expected throughout the study in the controls. Oil and oil plus shoreline cleaners did not significantly affect *Spartina* stem growth (Figure 14), or *Spartina* shoot growth (Figure 15), although the oil alone exposures did have the lowest stem and shoot densities. There was also no significant effect of treatment on *Spartina* stem or shoot re-growth in the trays that were harvested immediately prior to being dosed with oil or oil plus shoreline cleaners (Figures 16 and 17, respectively).

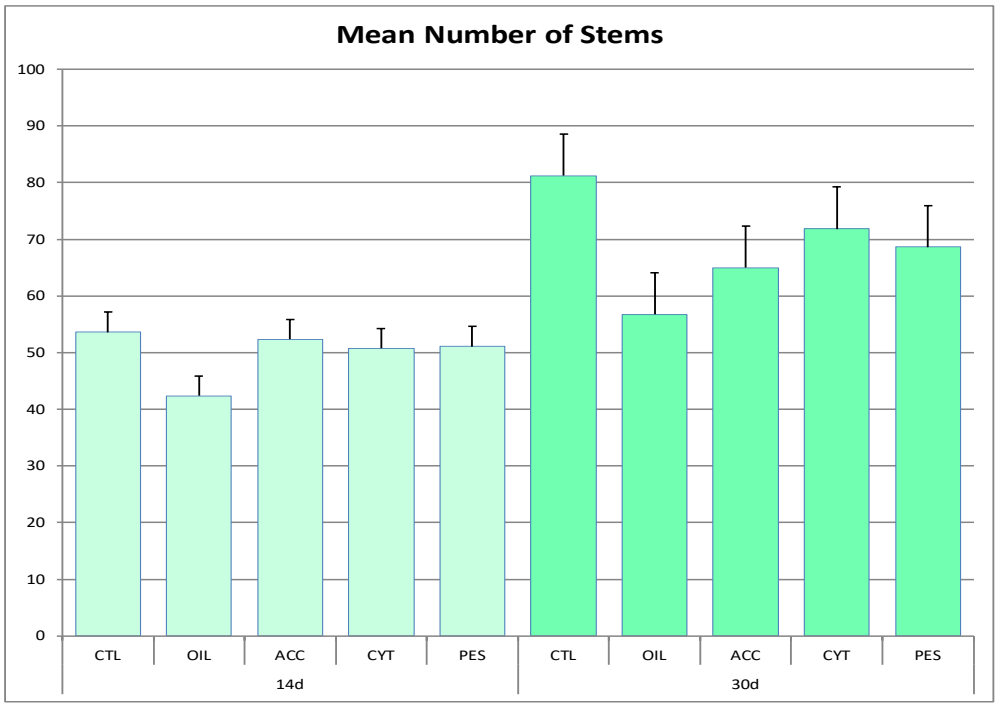

Figure 14. Mean number of *Spartina* stems measured in each mesocosm treatment. Repeated Measures ANOVA, no significant effect of treatment (p=0.1861).

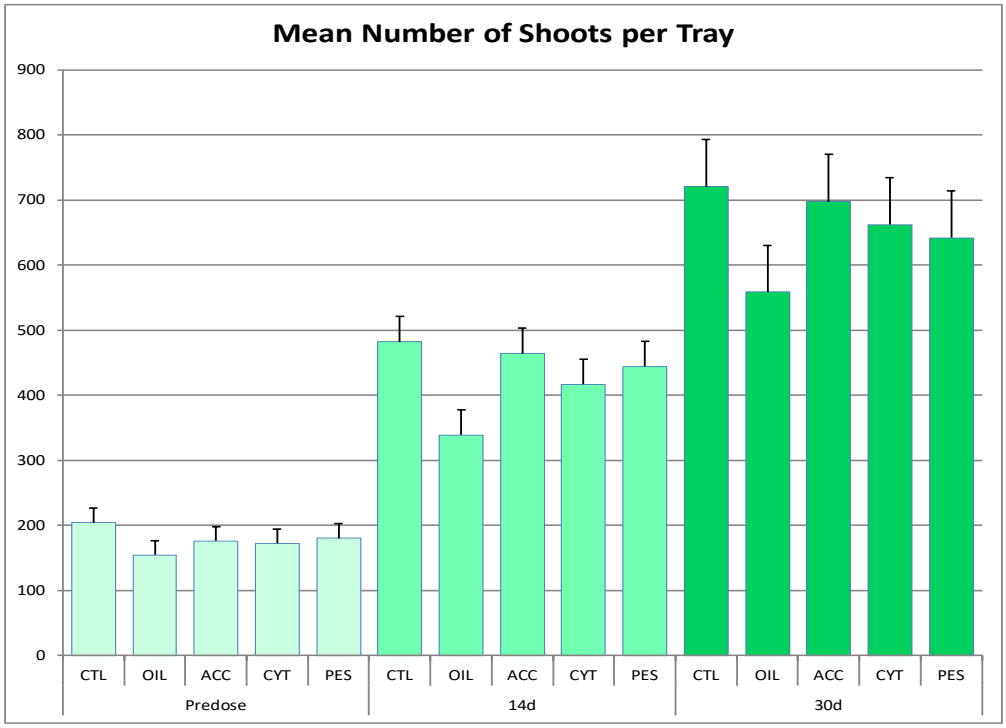

Figure 15. Mean number of *Spartina* shoots measured per tray in each mesocosm treatment. Repeated Measures ANOVA, no significant effect of treatment (p=0.3644).

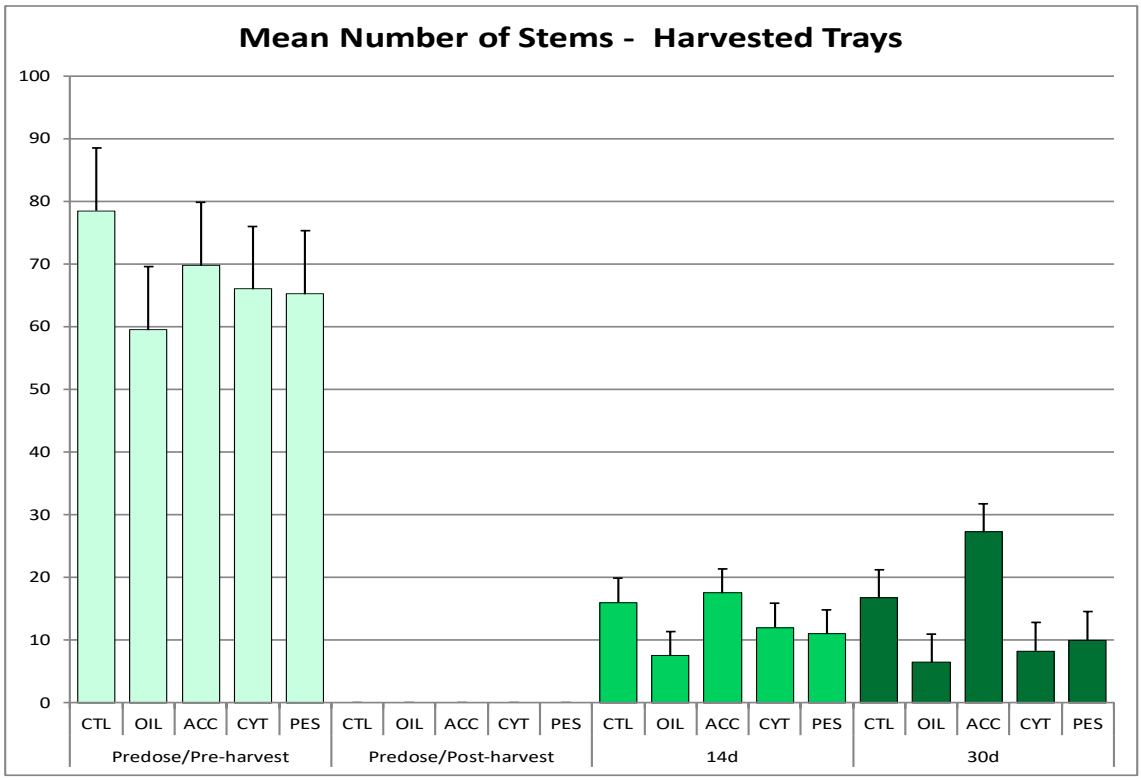

Figure 16. Mean number of *Spartina* stems in the harvested trays of each mesocosm treatment. Repeated Measures ANOVA, no significant effect of treatment (p=0.3616).

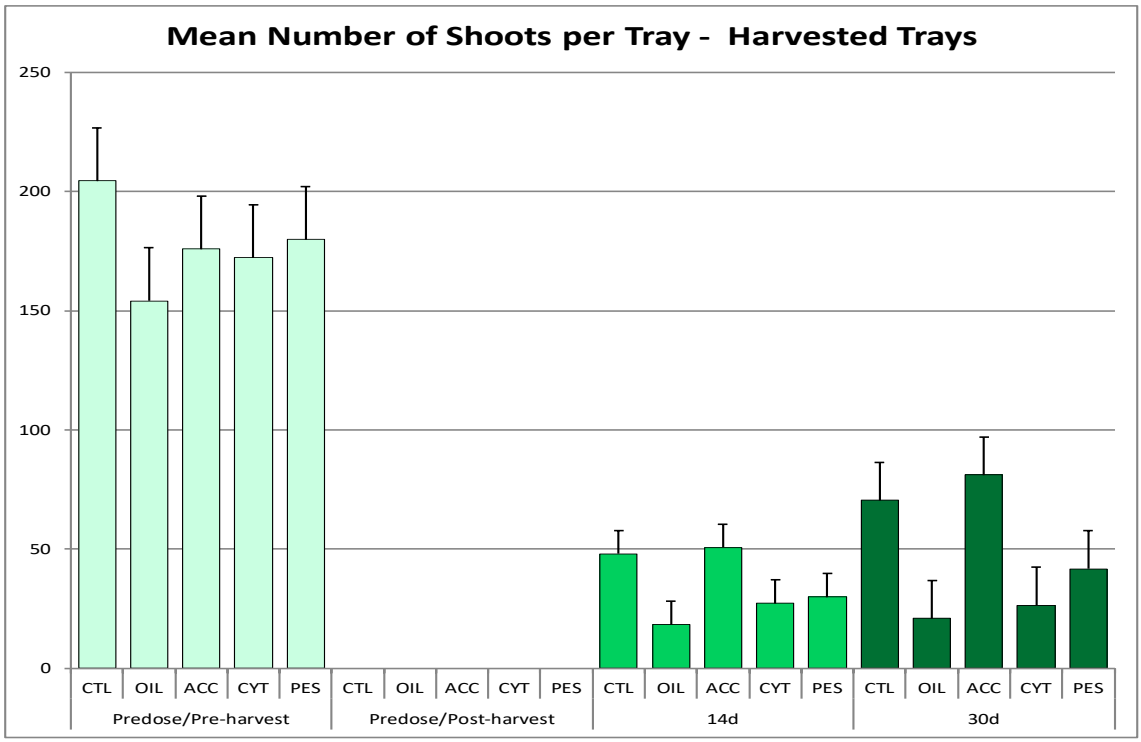

Figure 17. Mean number of *Spartina* shoots in the harvested trays of each mesocosm treatment. Repeated Measures ANOVA, no significant effect of treatment (p=0.0784).

There was no significant difference in *Spartina* shoot height before the mesocosms were dosed (Figure 18), and oil and oil plus shoreline cleaners did not significantly affect *Spartina* shoot height 14 d or 30 d post-dose (Figures 19 and 20, respectively).

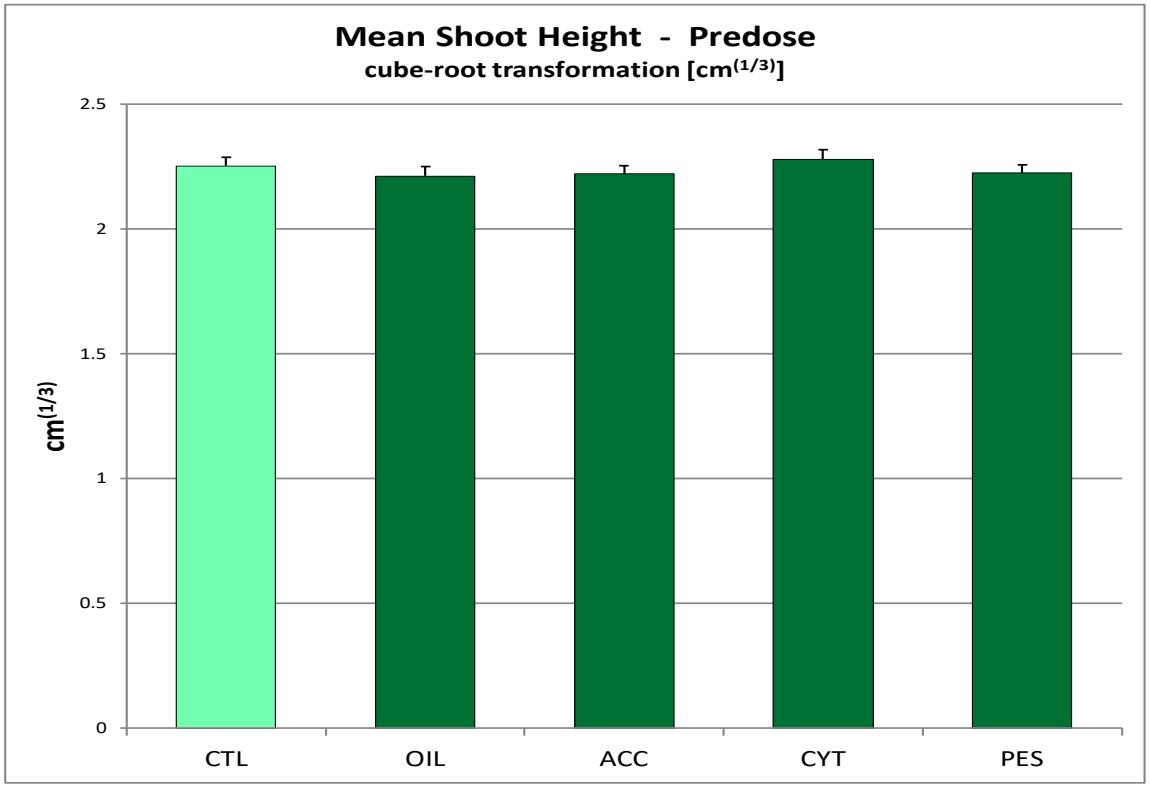

Figure 18. Mean *Spartina* shoot height measured in each mesocosm treatment pre-dose. One-way ANOVA (with nested sampling) p=0.6453.

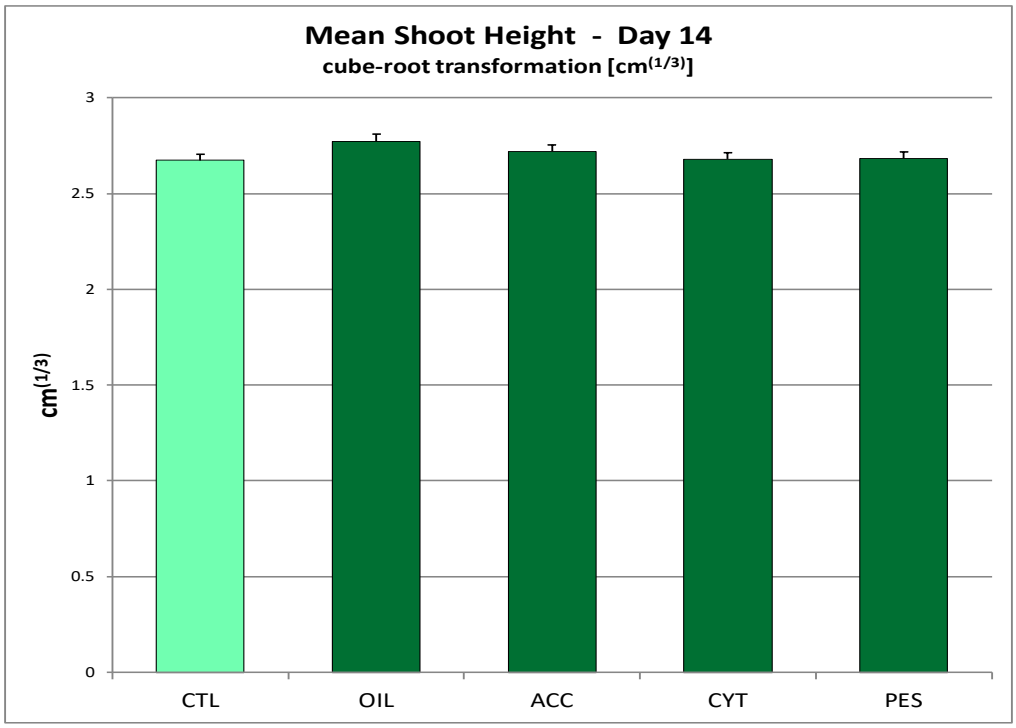

Figure 19. Mean *Spartina* shoot height measured in each mesocosm treatment 14 d post-dose. One-way ANOVA (with nested sampling) p=0.2789.

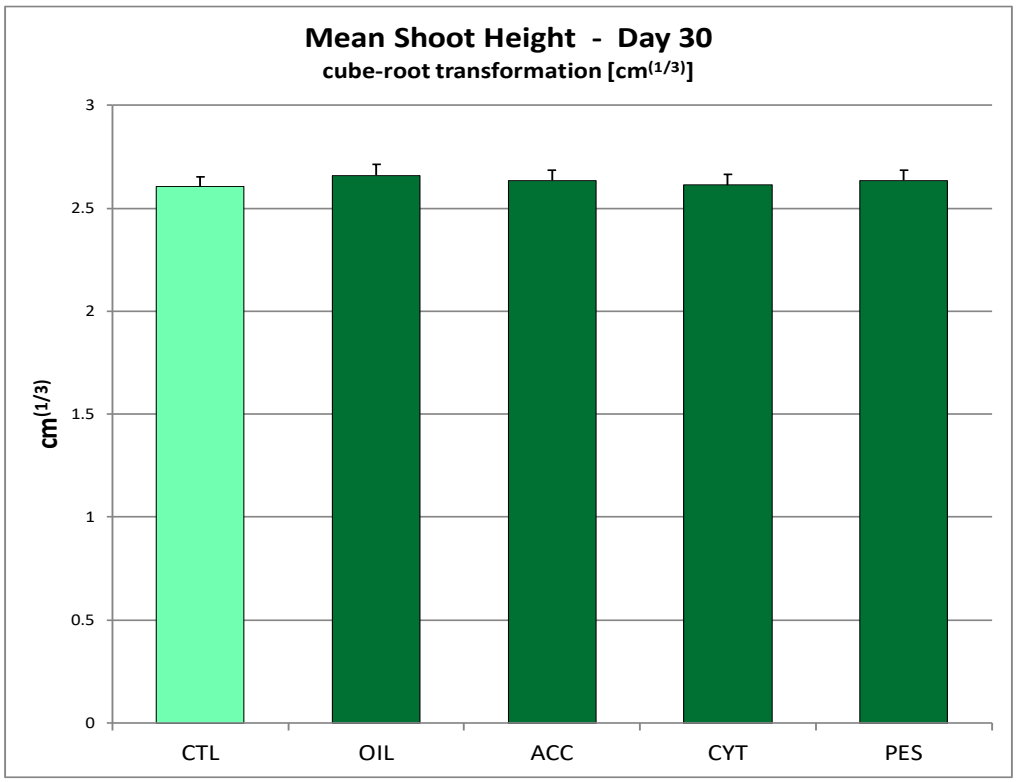

Figure 20. Mean *Spartina* shoot height measured in each mesocosm treatment 30 d post-dose. One-way ANOVA (with nested sampling) p=0.9501.

In the trays that were harvested immediately prior to being dosed with oil or oil plus shoreline cleaners, there was an effect on *Spartina* shoot height of the grass that grew after dosing in the oil-alone treatment (not significant after 14 d (Figure 21), but significant after 30 d (Figure 22)).

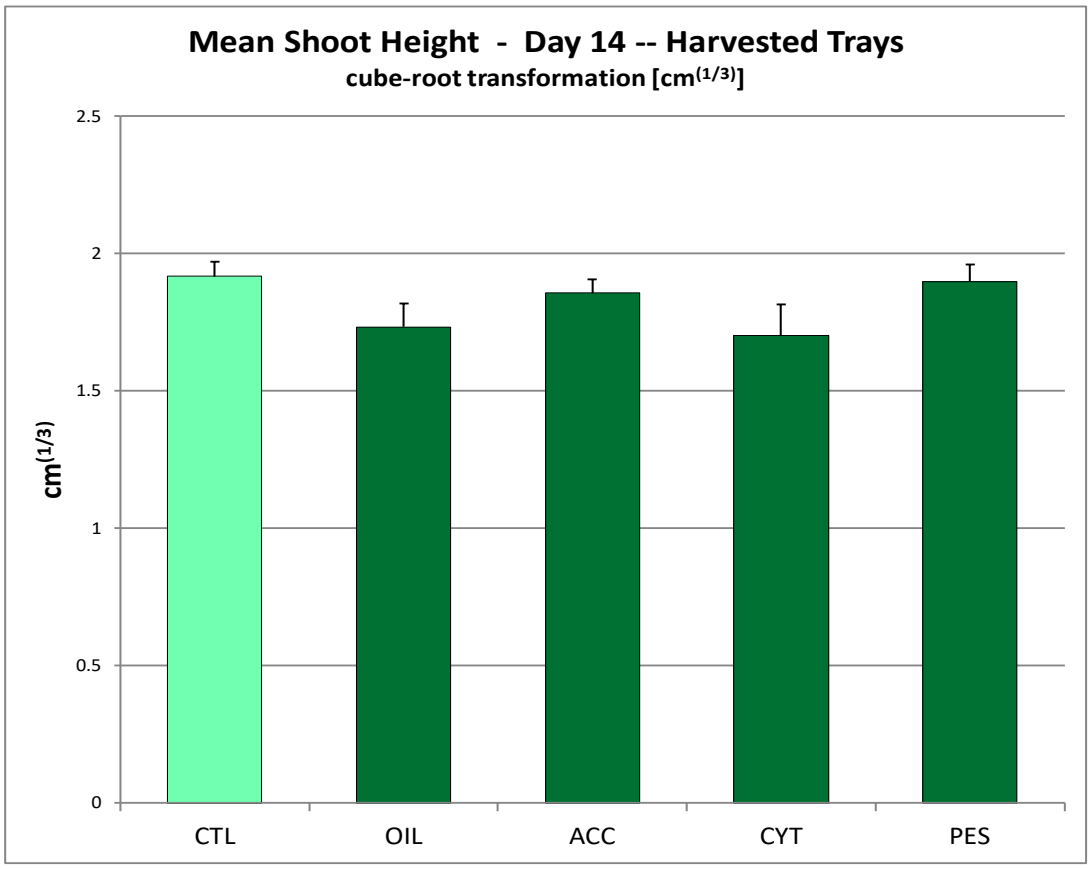

Figure 21. Mean *Spartina* shoot height measured in the harvested trays of each mesocosm treatment 14 d post-dose. One-way ANOVA (with nested sampling) p=0.2768.

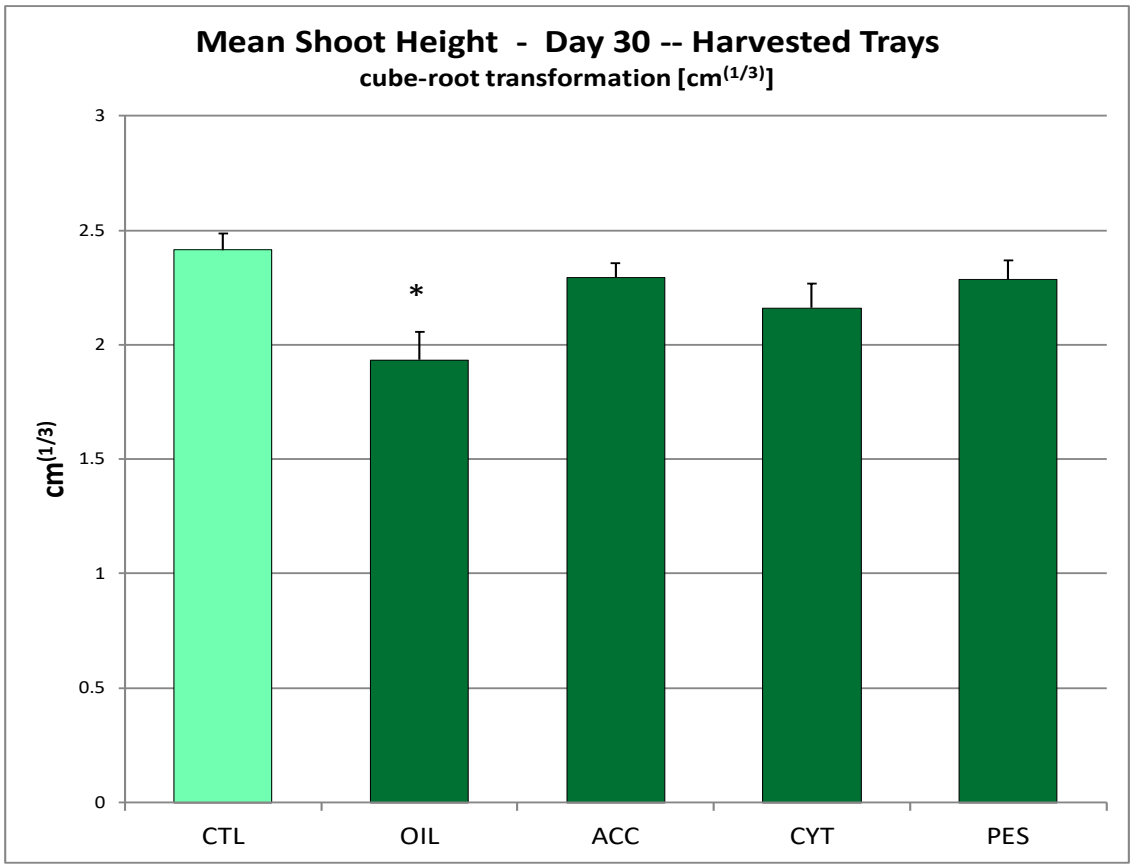

Figure 22. Mean *Spartina* shoot height measured in the harvested trays of each mesocosm treatment 30 d post-dose. One-way ANOVA (with nested sampling) p=0.0425, Dunnett's test oil treatment significantly different from control (p=0.0143).

There was also a signficant difference in *Spartina* above-ground biomass (AGB). Trays were harvested prior to dosing (Figure 23), allowed to regrow for 30 d and then havested again at the end of the experiment (Figure 24), and the analysis showed that the oil and oil+CytoSol treatments had significantly lower dry weight biomass levels than the control. However, when above ground biomass was determined from the trays that were not harvested prior to the start of the experiment, there was not a significant difference among treatments (Figure 25). Measures of *Spartina* photosynthetic activity would possibly have added to the interpretation of treatment effects.

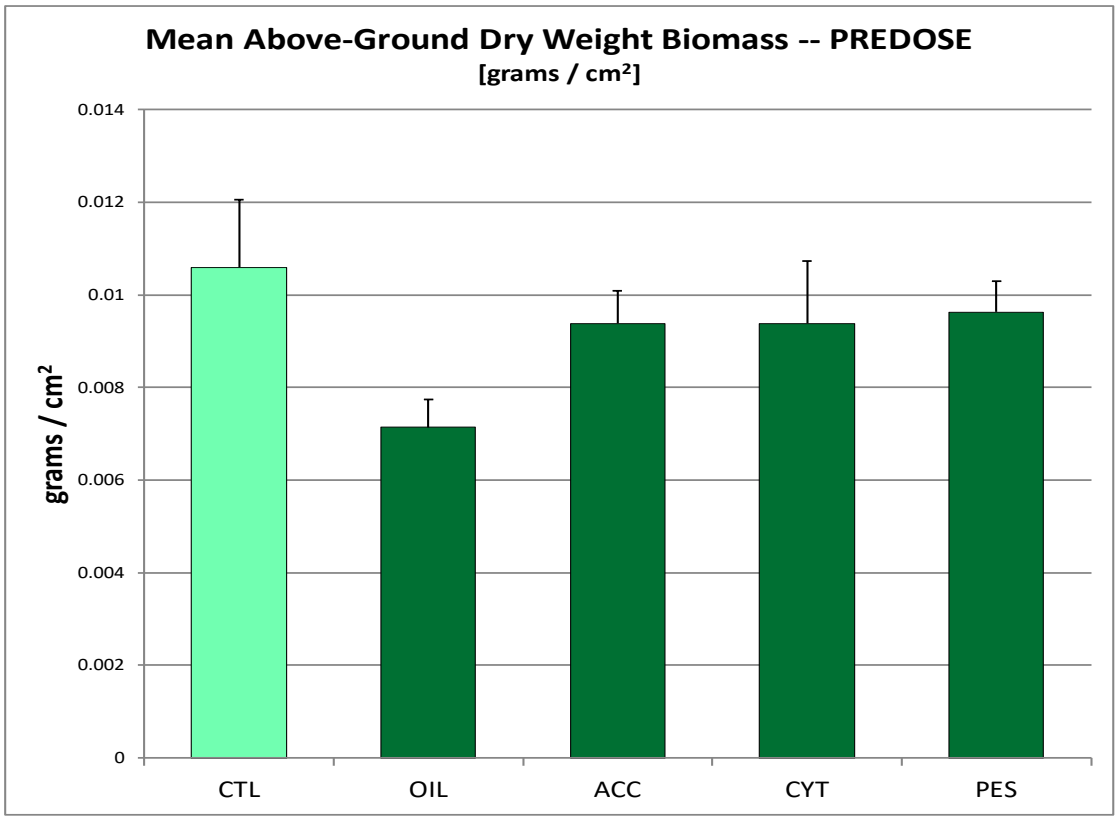

Figure 23. Mean *Spartina* above ground biomass measured in each mesocosm treatment pre-dose. One Way ANOVA p=0.2434.

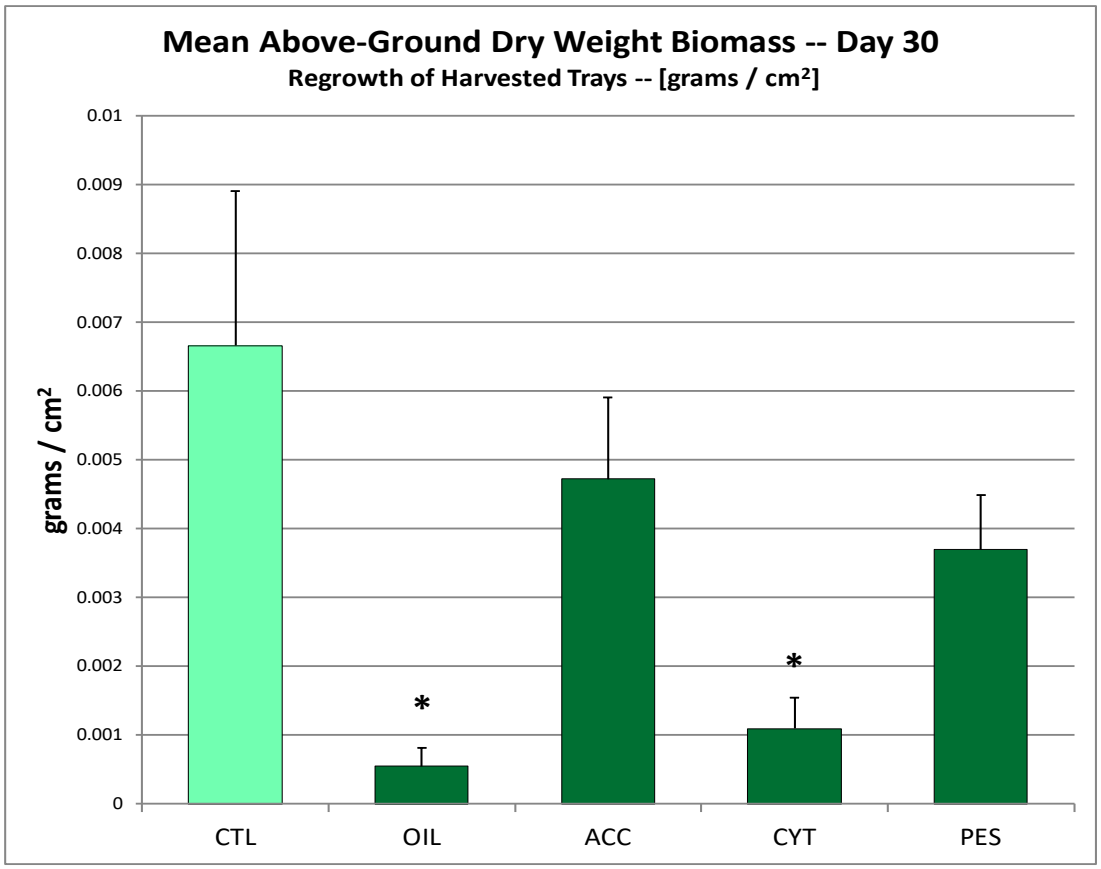

Figure 24. Mean *Spartina* above ground biomass measured in each mesocosm treatment 30 d post-dose. One way ANOVA p-value = 0.0146, Dunnett's test CYT (p=0.0179) and Oil (p=0.0095) treatments significantly different from Control.

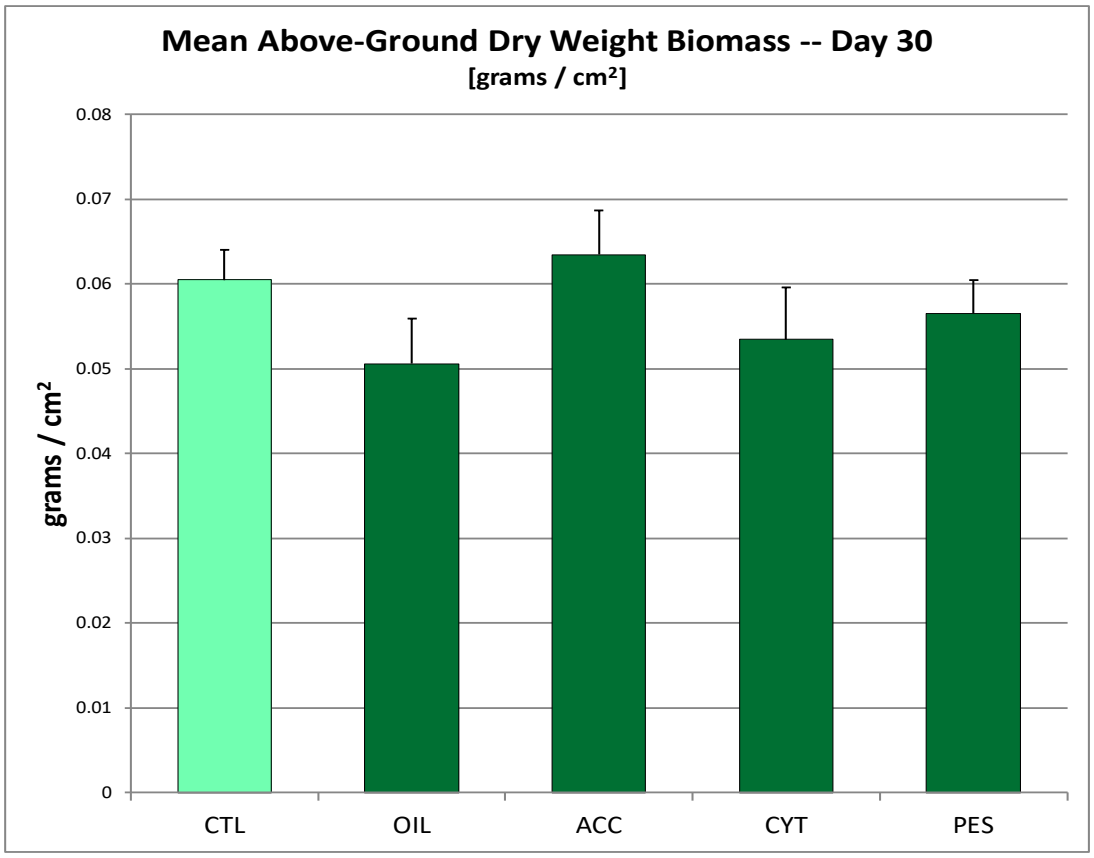

Figure 25. Mean *Spartina* above ground biomass (dry weight) measured in each mesocosm treatment 30 d post-dose. One way ANOVA (with nested sampling) p-value= 0.7004.

Dissolved oxygen (DO) significantly decreased in the Oil+Accell treatment (Figure 26). Decreases in dissolved oxygen in the Accell treatments were also noted in the laboratory studies; however, the aeration supplied to the test chambers kept the dissolved oxygen concentration within acceptable levels across all treatments. There was also some decrease in DO concentration in the Oil+CytoSol treatment. DO content in the mesocosms was not affected in the oil alone treatment. DO concentrations the Oil+Accell treatment decreased to hypoxic levels (< 2 mg/L) after the third dose was applied, and

hypoxic conditions lasted for approximately 4 d post-dose. Summary statistics for water quality parameters measured in the mesocosm treatments are presented in Appendix 2.

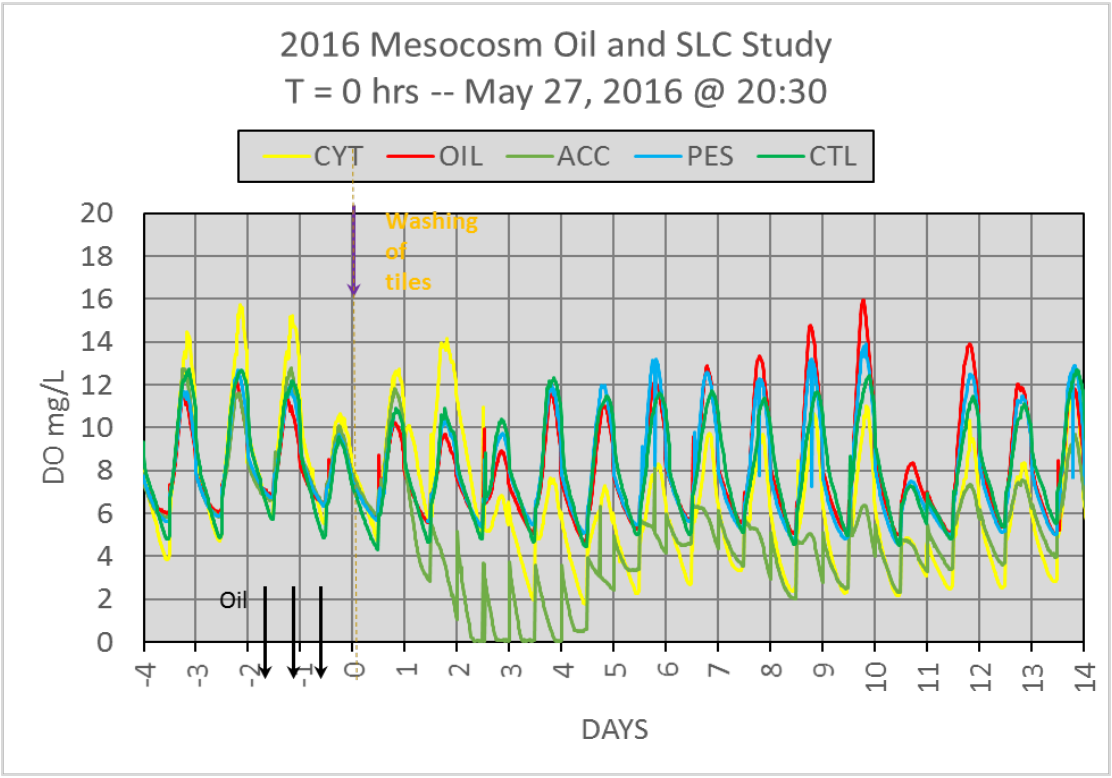

Figure 26. Water column dissolved oxygen concentration measured in the mesocosm treatments.

Mesocosm Chemistry Results

Treatment data were not distributed normally, thus a non-parametric Kruskal-Wallis test (non-parametric one-way ANOVA) followed by Wilcoxon Each Pair test was used to compare treatments at each of the time points with replicate samples (24 h and 7 d (168 h) post-cleaner application for water and 7 d post-cleaner application for sediments).

Water samples were collected from the upper chamber at high tide 12 h post-oiling, and 24 h, 7 d and 14 d post-cleaner application. Due to the intensive sampling

schedule, composite samples were collected at the 12 h and 7 d time points; thus there are no error bars for those data in Figures 27-30. Maximum average TEH concentrations generally were observed 24 h post-cleaner application (Table 3; Figure 27), while maximum PAH values were generally observed at 12 h post-oiling (Table 3; Figure 28).

Table 3. Water TEH (mg/L) and PAH50 (µg/L) concentrations measured during the mesocosm exposure (average; standard deviation where applicable).

		CTL	OIL	ACC	CYT	PES
12-h	TEH	0	1.77	1.74	2.92	1.38
36-h	TEH	0	1.64 (0.72)	6.07 (1.98)	5.07 (5.58)	1.43 (0.41)
7-d	TEH	0	0.55 (0.58)	2.21 (2.02)	3.49 (1.23)	0.19 (0.21)
14-d	TEH	0	0.47	0	0.61	0
30-d	TEH	0	0.31	0	0.86	0.39
12-h	PAH50	0.584	96.4	106	112	97.2
36-h	PAH50	0.286 (0.236)	68.1 (14.1)	157 (34.0)	107 (80.3)	67.3 (11.3)
7-d	PAH50	0.017 (0.013)	8.93 (4.31)	33.3 (19.1)	41.3 (11.1)	5.87 (1.03)
14-d	PAH50	0	6.80	4.31	11.3	4.37
30-d	PAH50	0	3.21	1.57	7.77	4.29

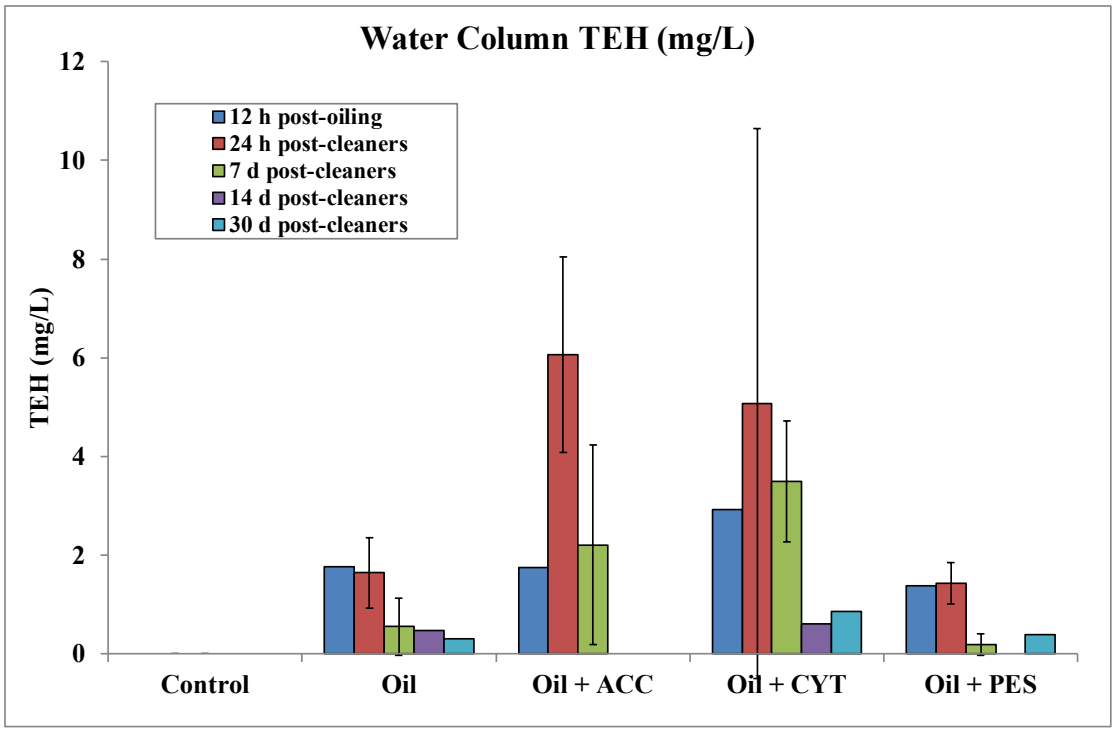

Figure 27. Water column Total Extractable Hydrocarbon (TEH) concentration (mg/L) measured at each time-point in the mesocosm treatments.

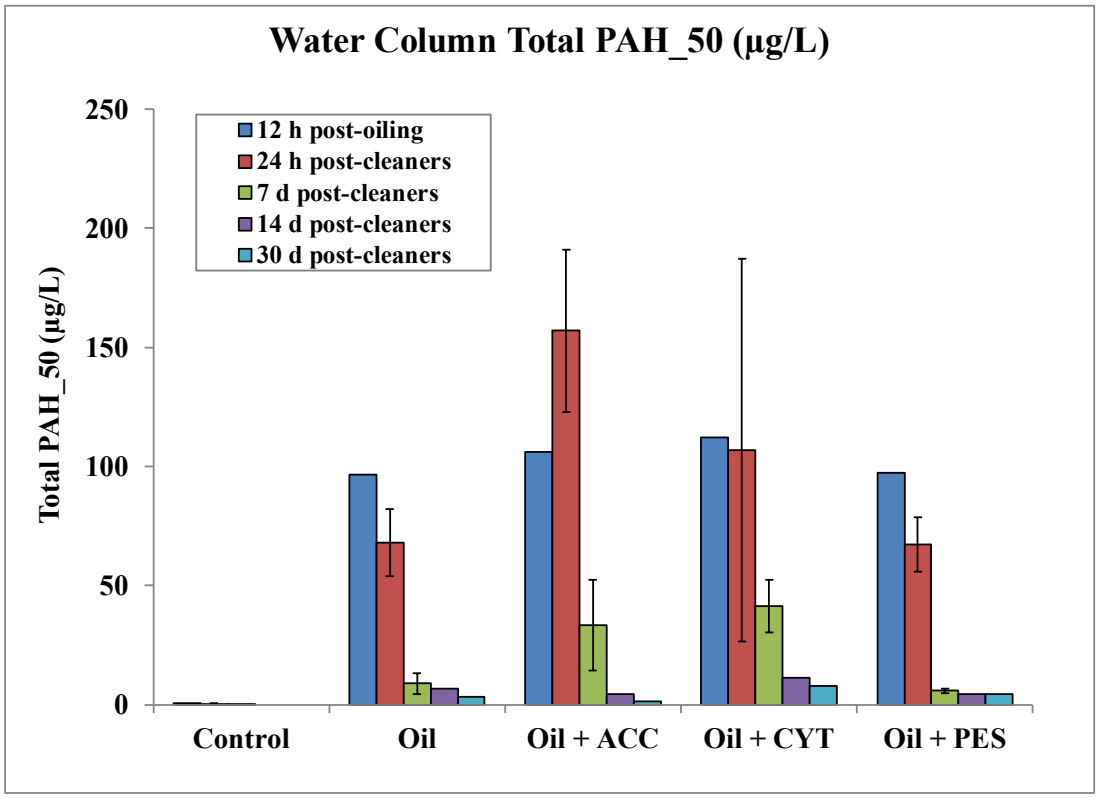

Figure 28. Water column Total PAH concentration (µg/L) measured at each time-point in the mesocosm treatments.

At 24 h and 7 d post-cleaner application, the Kruskal-Wallis analysis for TEH indicated significant differences among the treatment means (Chi^2 = 0.0057 and 0.0053 respectively). For Total PAHs, the Kruskal-Wallis analysis indicated significant differences among the treatment means at both the 24 h post-cleaner application (Chi^2 = 0.0103) and 7 d post-cleaner application (Chi^2 = 0.002) time points. Significant differences between treatments were identified and these differences are listed in Table 4.

Table 4. Pairwise Comparison results for treatment comparisons of water concentrations at A.) 36 h and B.) 7 d.
A '++' indicates a pairwise difference for both TEH and PAH50; '+' indicates a difference for TEH only and a '*' indicates a difference for PAH50 only.

A.) 36 h

	CTL	OIL	ACC	CYT	PES
CTL		++	++	++	++
OIL			++		
ACC					++
CYT					
PES					

B.) 7 d

	CTL	OIL	ACC	CYT	PES
CTL		++	++	++	++
OIL			++	*	+
ACC					*
CYT					*
PES					

Water column TEH values were less than the detection limit (approximately 0.25 mg/L) in the control at all timepoints. Mean measured TEH values 24 h post-cleaner addition were 1.6, 6, 5, and 1.4 mg/L in the Oil alone, Oil+Accell, Oil+CytoSol, and

Oil+PES-51 treatments, respectively (Figure 27). After 7 d, the TEH values declined to 0.6, 2, 3, and 0.2 mg/L in the Oil, Oil+Accell, Oil+CytoSol, and Oil+PES-51 treatments, respectively. After 14 d, the mean TEH concentrations in the oiled mesocosms were 0.5 mg/L (Oil), 0 mg/L (Oil+Accell), 0.6 mg/L (Oil+CytoSol), and 0 mg/L (Oil+PES-51) (Figure 27).

Mean measured Total PAH values 12 h post-oiling were 0.58 µg/L in the control, 96 µg/L in the Oil treatment, 106 µg/L in the Oil+Accell treatment, 112 µg/L in the Oil+CytoSol treatment, and 97 µg/L in the Oil+PES-51 treatment (Figure 28). Total PAH values 24 h post-cleaner addition were 0.29 µg/L in the control, 68 µg/L in the Oil treatment, 157 µg/L in the Oil+Accell treatment, 107 µg/L in the Oil+CytoSol treatment, and 67 µg/L in the Oil+PES-51 treatment. After 7 d, the Total PAH values had declined in each treatment, to 0.02 µg/L in the control, 9 µg/L in the Oil treatment, 33 µg/L in the Oil+Accell treatment, 41 µg/L in the Oil+CytoSol treatment, and 6 µg/L in the Oil+PES-51 treatment. After 14 d, the measured concentrations had decreased further to <MDL, 7, 4, 11, and 4 µg/L in the Control, Oil, Oil+Accell, Oil+CytoSol, and Oil+PES-51 treatments, respectively (Figure 28).

Mean measured sediment TEH values were low (< 0.3 mg/g dry weight) in all treatments throughout the 30 d experiment (Figure 29). TEH oncentrations were relatively similar across treatments, except that TEH was not detected in the Oil+PES-51 treatment until the 30 d time point (Figure 29). Sediment Total PAH concentrations generally declined in the mesocosm treatments over time, with the exception of a spike in Total PAH levels after 14 d in the Oil+Accell treatment (Figure 30).

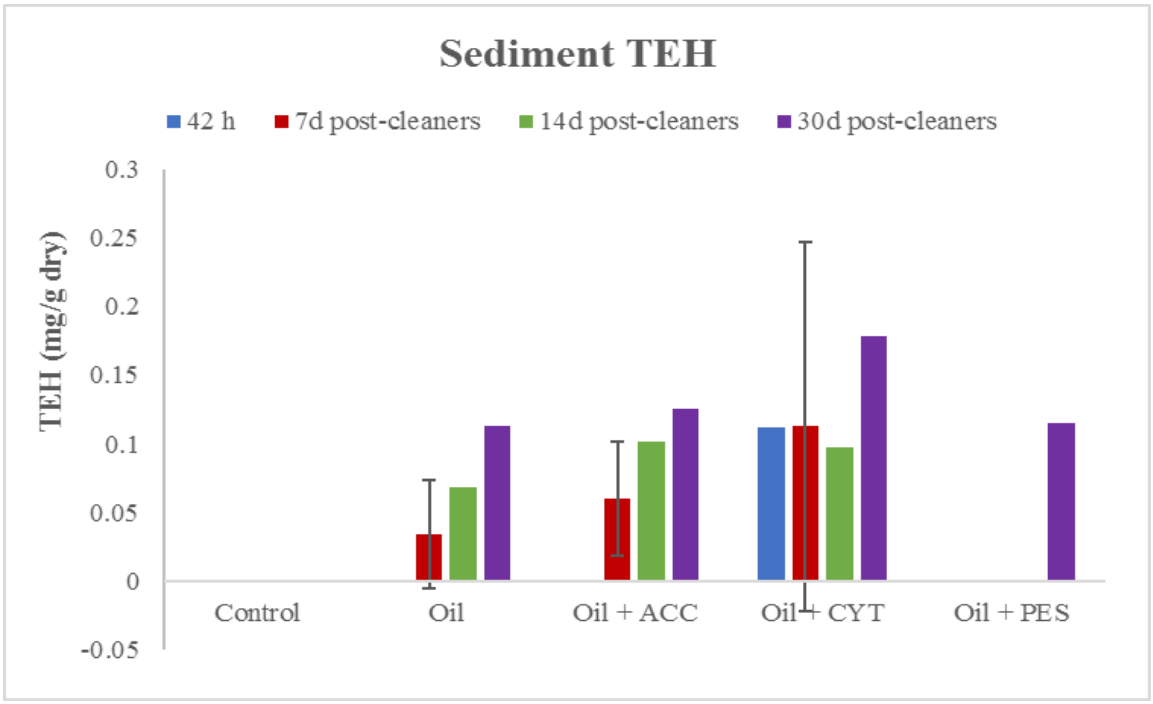

Figure 29. Sediment Total Extractable Hydrocarbon (TEH) concentration (mg/g dry weight) measured at each time-point in the mesocosm treatments.

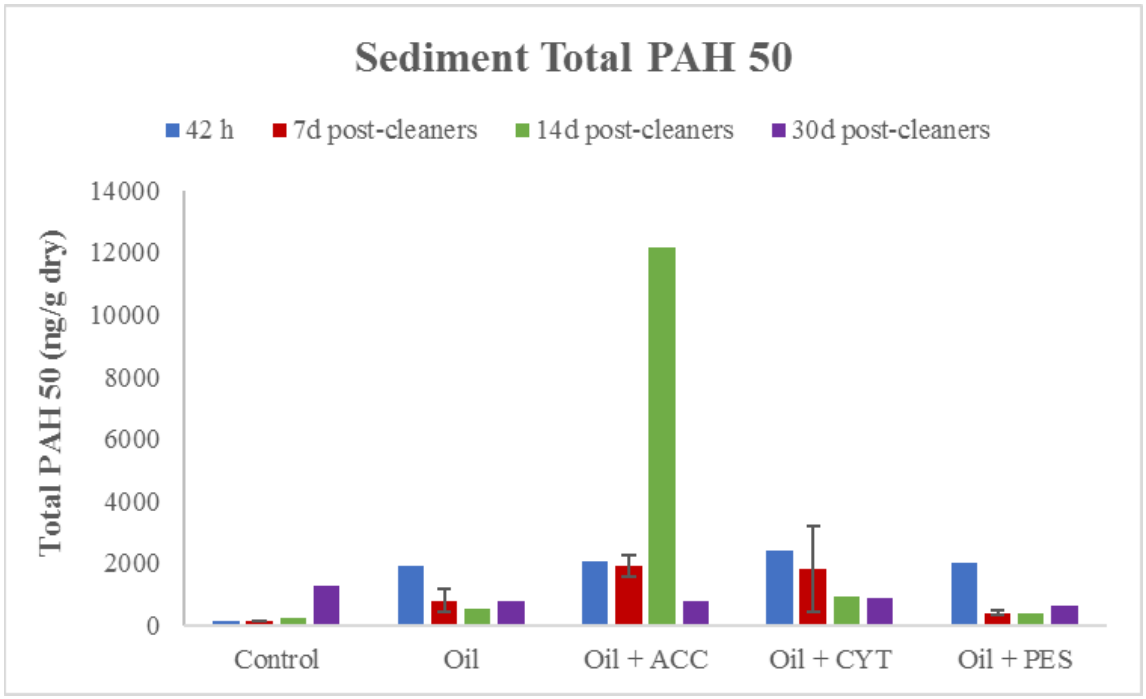

Figure 30. Sediment Total PAH concentration (ng/g dry weight) measured at each time-point in the mesocosm treatments.

Mesocosm PAH profiles in both water and sediment samples were plotted (Figures 31-35) to assess differences in the distribution of PAHs across treatments. Composite water samples were obtained from each treatment 12 h after the last dose of oil occurred. The average PAH profile across all treatments (Figure 31) indicated that lighter PAHs (naphthalene and C1-C4 napthalenes) were the most abundant PAHs. This is expected as LSC oil contains more light-mid weight PAHs rather than high molecular weight PAHs.

Following SLC application (Figure 32), lighter PAHs were still abundant in the water column. PAH profiles between treatments were generally similar; however, there was an enhancement of C2-C4 phenanthrenes/anthracenes in the water column with the Oil+CytoSol treatment. With regards to the sediment PAH profiles 24 h after SLC application, there was no consistent pattern observed in PAHs between treatments. This may be due to not having sufficient time for PAHs to have been distributed to the sediment.

Seven days after SLC application the PAH patterns were generally consistent between treatments in both water and sediment (Figure 33). There was a decrease in abundance of the lighter PAHs and an enhancement in the middle weight PAHs (C1-C3 fluorenes, C1-C4 phenanthrenes/anthracenes). Lighter weight PAHs, for example naphthalene and its alkylated constituents, are more susceptible to volatilization processes, which would explain a decrease in their abundances.

At 14 d (Figure 34), there was further reduction of lighter PAHs with a subsequent increase in mid to heavy weight PAHs in the water column. Patterns remained consistent between treatments in the water column. In 14 d sediment samples, the profiles

were also dominated by mid-heavy weight PAHs. The most obvious difference in pattern occurred with the Oil+Accell treatment. The Oil+Accell PAH profile had much higher proportions of fluoranthene, pyrene and chrysene/triphenylene at 14 d when compared to the other treatments. This treatment also had the highest sediment PAH concentration measured throughout the experiment (Figure 34). LSC is characterized as having high proportions of light PAHs, whereas fluoranthene, pyrene, chrysene/triphenylene, and other heavy molecular weight PAHs are very minor constituents of this oil. This observed pattern for Oil+Accell may be a result of Accell Clean product interactions with oil that contributed particular PAHs to the sediment.

At 30 d (Figure 35), PAH profiles in the water were similar to those at 7 d. Most notably, it was observed that the Oil+CytoSol treatment had higher proportions of C1 and C3 fluorenes. Sediment patterns were also consistent between treatments. The increased proportions of heavy molecular weight PAHs observed with Oil+Accell at 14 d were not detected at 30 d.

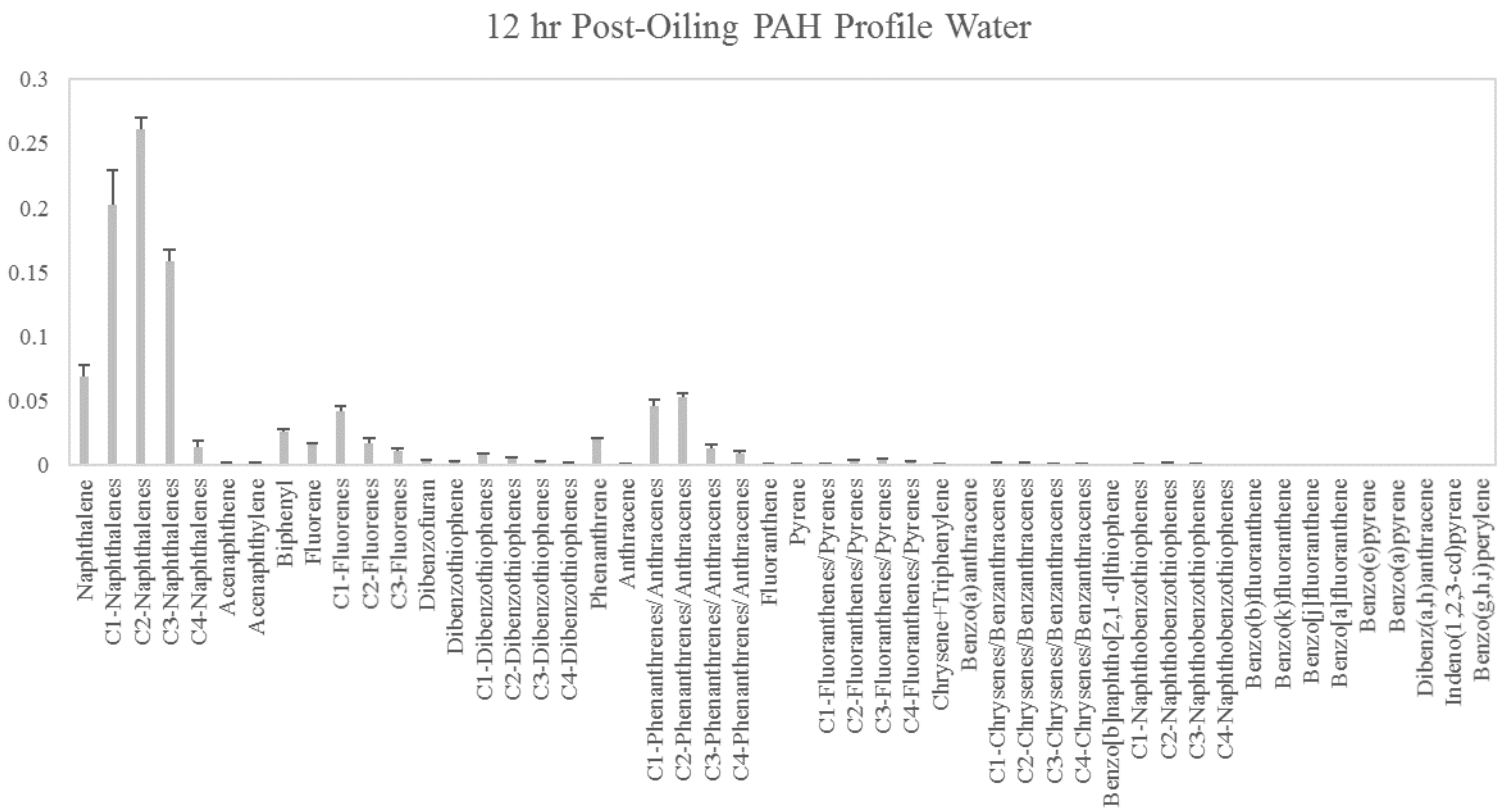

Figure 31. Average (with standard deviation bars) PAH profile in composite water samples from all treatments (OIL, ACC, CYT and PES).

Composites were taken 12 h after the last dose of oil but prior to the application of shoreline cleaners. PAH proportions shown here and in subsequent figures were obtained by dividing individual PAH concentrations by TPAH 50 concentrations.

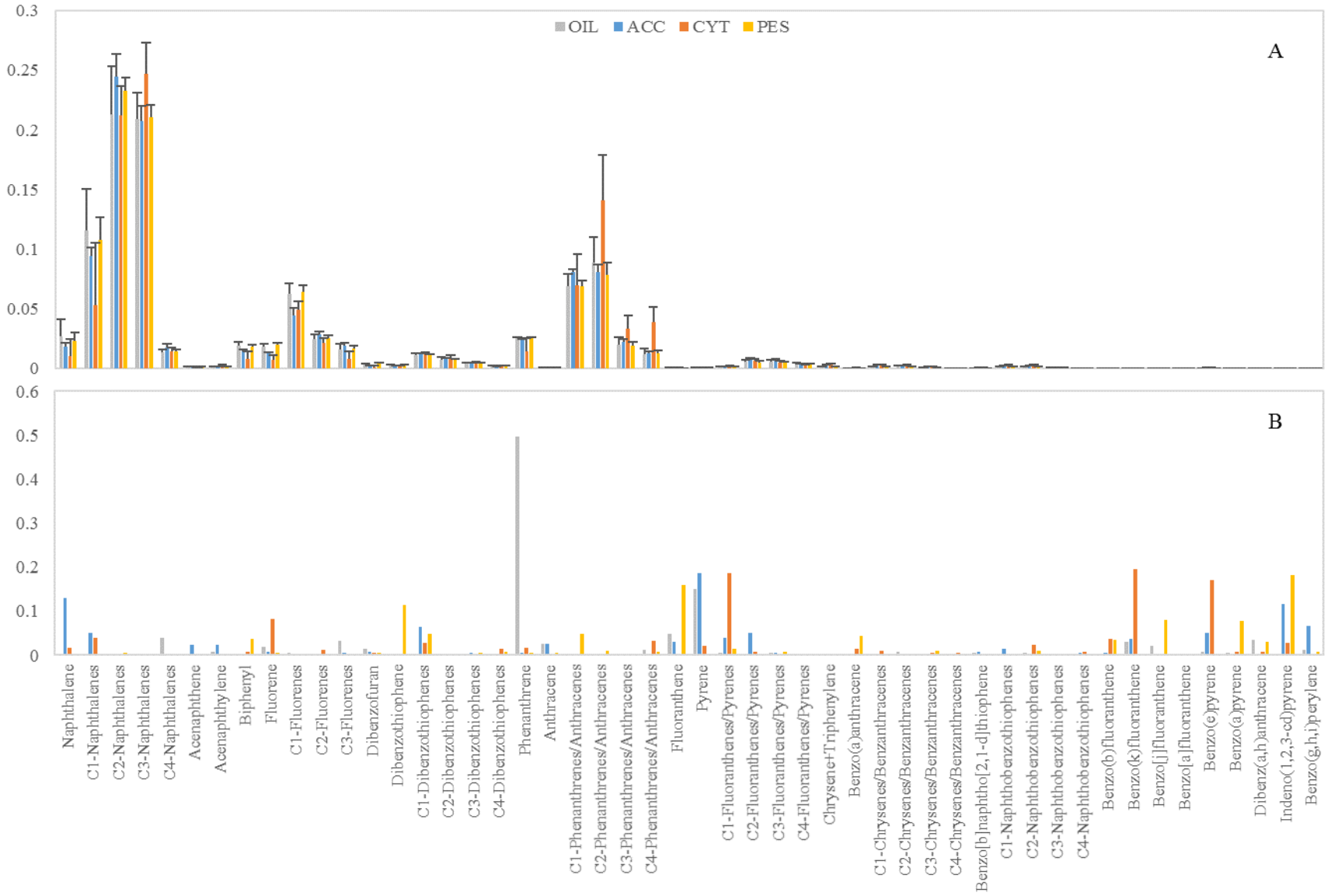

Figure 32. PAH profiles at 24 h post shoreline cleaner application in both water (A) and sediment (B).

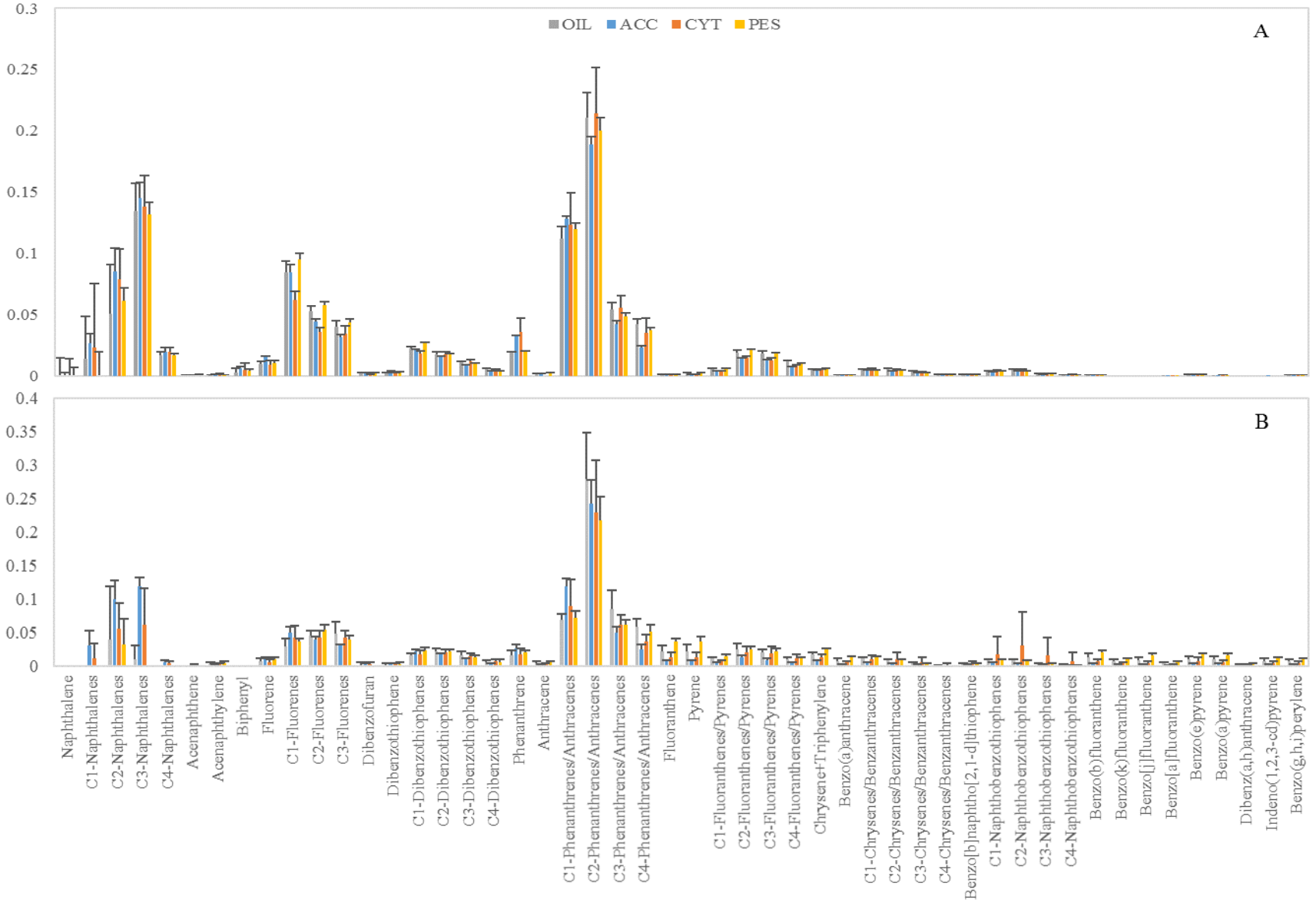

Figure 33. PAH profiles at 7 d post shoreline cleaner application in both water (A) and sediment (B).

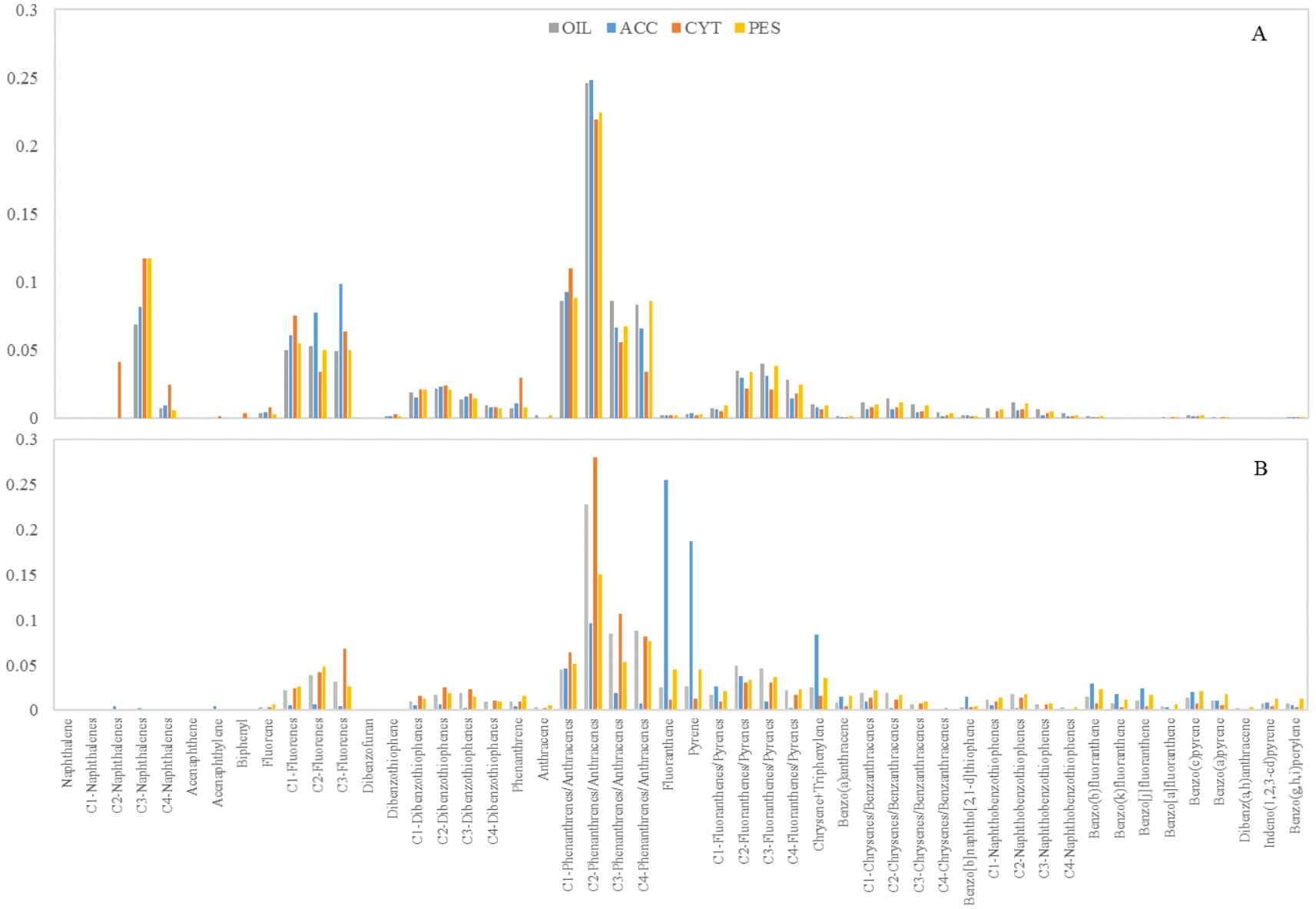

Figure 34. PAH profiles at 14 d post shoreline cleaner application in both water (A) and sediment (B).

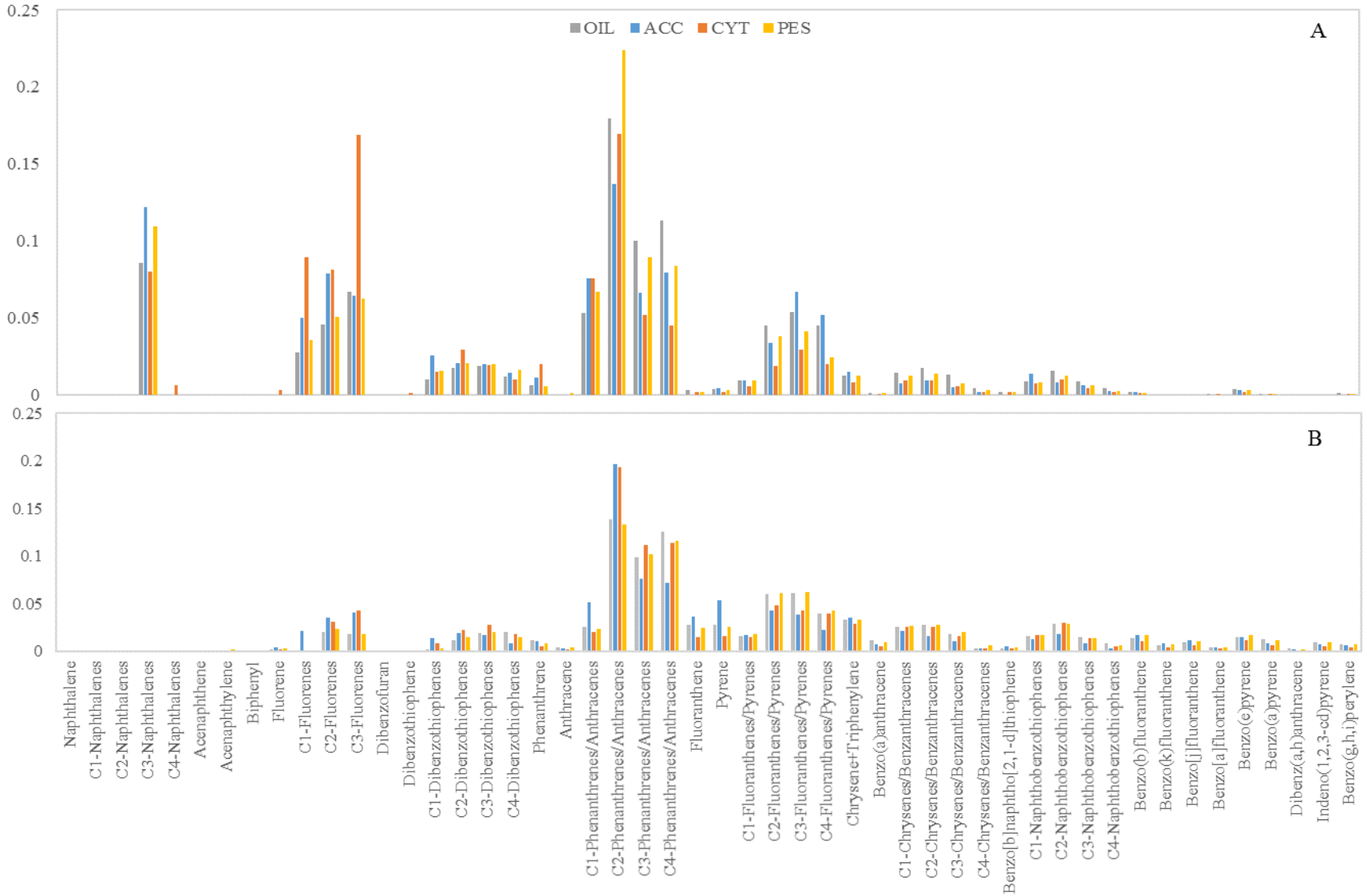

Figure 35. PAH profiles at 30 d post shoreline cleaner application in both water (A) and sediment (B).

Objective 2: Laboratory testing with grass shrimp

The shoreline cleaners Accell Clean, PES-51, and CytoSol were tested individually and in shoreline cleaner-CEWAFs with the grass shrimp, *Palaemonetes pugio.* Mortality was determined for each SLC product (Figure 36) and a median lethal toxicity value (LC_{50}) was determined after 96 h exposure (Table 5).

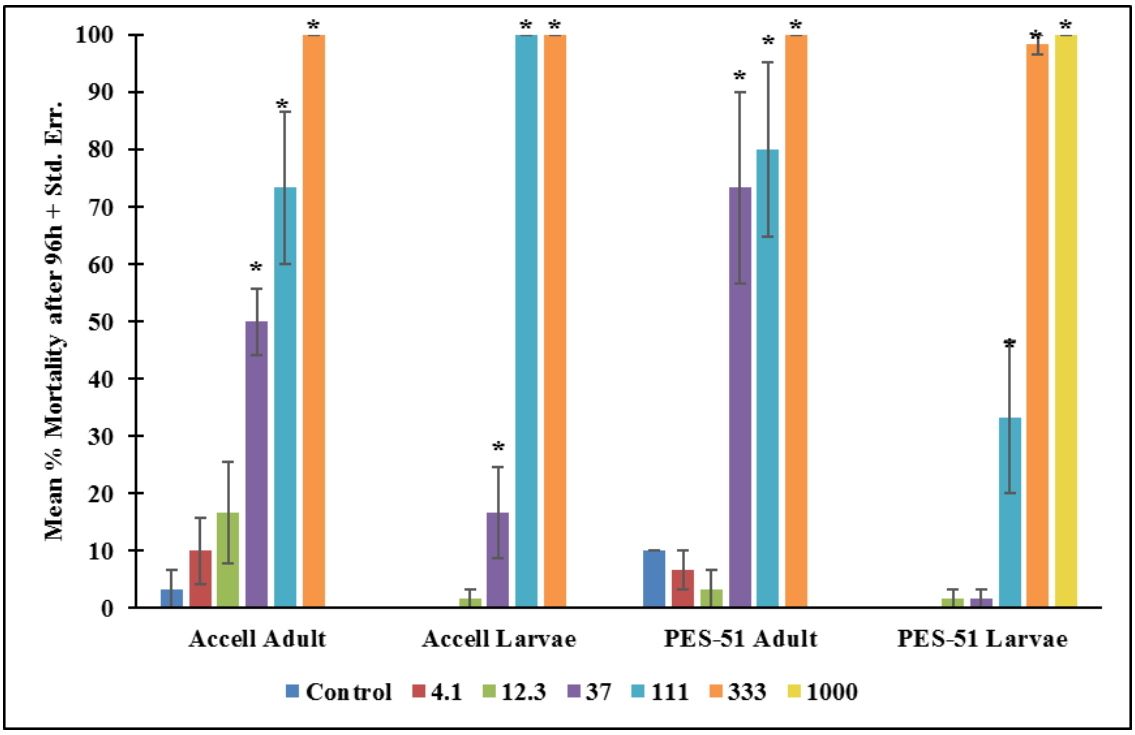

Figure 36. Adult and larval grass shrimp mortality after 96 h laboratory exposure to shoreline cleaners (ppm) only.
Larval shrimp were exposed to PES-51 from 12.3 - 1000 ppm. Asterisks (*) indicate significant differences from the control (ANOVA $p<0.0001$, Dunnett's test).

In the mesocosm experiment, grass shrimp mortality due to fish predation did not allow assessment of their sensitivity to shoreline cleaners. Toxicity due to cleaners alone was similar for Accell Clean and PES-51, and was much lower for Cytosol.

Table 5. Shoreline cleaner product laboratory testing with adult and larval grass shrimp. Toxicity values are 96 h LC_{50} values and 95% confidence intervals for shoreline cleaner products in seawater.
Asterisks (*) indicate a significant difference between Accell Clean and PES-51 LC_{50} values and crosses (+) indicate a significant difference between adult and larval shrimp LC_{50} values (Wheeler ratio test $p<0.05$).

ppm (nominal)	CytoSol	Accell Clean	PES-51
Adult	>10,000	44.18 (30.39-60.52)	38.75 (17.99-65.34)
Larvae	>10,000	48.64 (41.62-80.62)	155.42 (127.43-200.28) *+

CytoSol was not toxic at concentrations up to 10,000 ppm, and did not appear soluble in seawater at that concentration. CytoSol was not subsequently tested as CEWAF. A 96 h LC_{50} value of 44.18 ppm (95% confidence interval (CI): 30.39 - 60.52) was determined for Accell Clean for adult shrimp, and 48.64 ppm (95% CI: 41.62 - 80.62) for larval shrimp. The 96 h LC_{50} value determined for PES-51 was 38.75 ppm (95% CI: 17.99 - 64.43) for adult shrimp and 155.42 ppm (95% CI: 127.43 - 200.28) for larval shrimp. Adult grass shrimp mortality was 73.33% for 37 ppm PES-51 compared to 1.67% mortality for larval shrimp (Figure 36). Larval grass shrimp were significantly more tolerant to PES-51 than Accell Clean ($p < 0.0001$). Larval mortality was 33.33% at 111 ppm PES-51 compared to 100% mortality for larvae exposed to 111 ppm Accell Clean. A Wheeler LC_{50} ratio test determined that there was no significant difference between adult LC_{50} values for Accell Clean and PES-51 ($p = 0.1311$).The LC_{50} values determined for adult grass shrimp are similar to those determined for mysid (see Table 1 for toxicity values available in the literature).

When the Accell Clean and PES-51 cleaners were prepared as CEWAFs with LSC oil, there were significant differences in grass shrimp toxicity; with the Accell Clean-CEWAF having significantly greater toxicity than the PES-51-CEWAF ($p < 0.0001$) (Figure 37). LC_{50} values could not be determined for PES-51-CEWAF since less than 50% mortality occurred in the full-strength CEWAF (Table 6).

Table 6. Shoreline cleaner product laboratory testing with adult and larval grass shrimp in chemically enhanced water accommodated fractions (CEWAF) with Louisiana Sweet Crude oil (1:10 shoreline cleaner to oil).
Toxicity values, 96 h LC50 and 95% confidence interval, are based on nominal percent CEWAF concentration. Asterisks (*) indicate a significant difference between Accell Clean and PES-51 LC50 values and crosses (+) indicate a significant difference between adult and larval shrimp LC50 values (Wheeler ratio test $p<0.05$).

% CEWAF	Accell Clean-CEWAF	PES 51-CEWAF
Adult	20.22% (16.23-28.18) *	>100%
Larvae	12.00% (9.39-15.09) *+	>100%

Since the adult grass shrimp toxicity was similar for Accell Clean and PES-51 when tested as individual products, it is likely that the difference in product toxicity seen with the CEWAFs is a result of differences in how these two shoreline cleaners interact with oil. Toxicity values for Accell Clean-CEWAF were 20.22% (95% CI: 16.23 - 28.18) for adult shrimp and 12.00% (95% CI: 9.35 - 15.09) for larval shrimp, with the larvae being significantly more sensitive to the Accell Clean-CEWAF than the adults based on the Wheeler LC_{50} ratio test ($p <0.0001$).

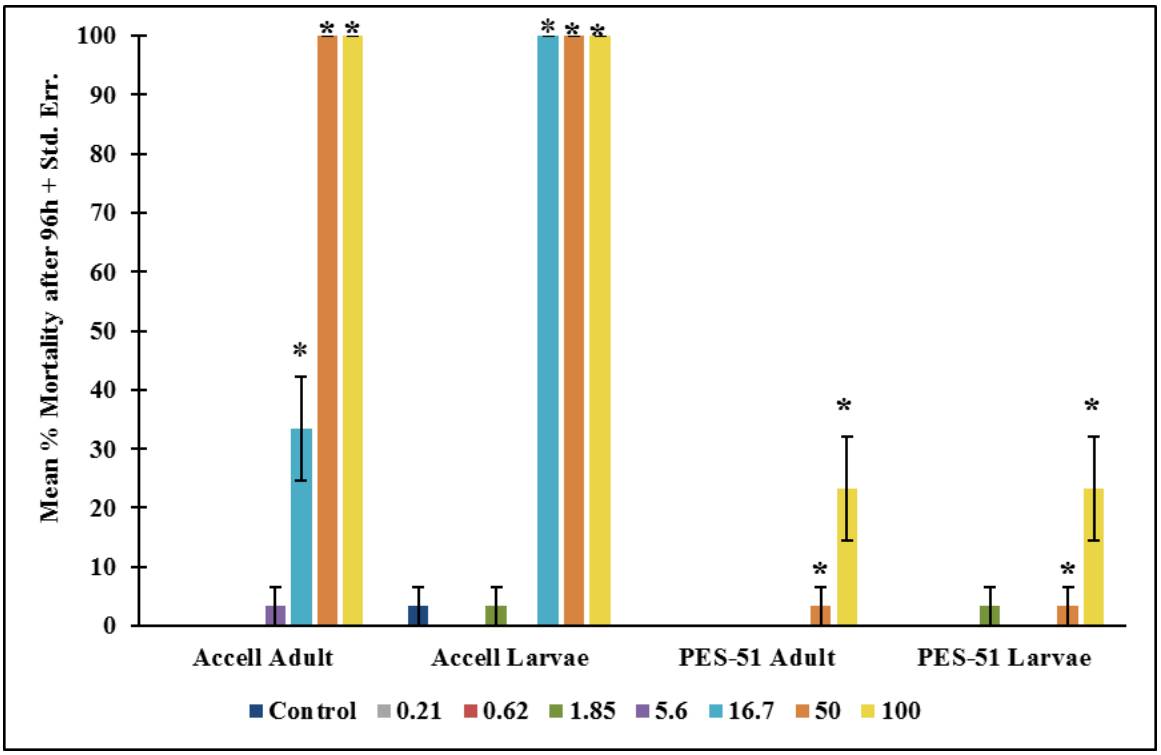

Figure 37. Adult and larval grass shrimp mortality after 96 h laboratory exposure to shoreline cleaner-CEWAF (% CEWAF).
Asterisks (*) indicate significant differences from the control, Dunnett's test (ANOVA: Accell adult $p<0.0001$; Accell larvae $p<0.0001$; PES-51 adult $p=0.0029$; PES-51 larvae $p=0.0056$).

The adult shrimp mortality at 16.7% Accell Clean-CEWAF was approximately 33% compared to 100% for larval grass shrimp at the same concentration (Figure 37). Larval grass shrimp are developing at a faster rate than adult grass shrimp, therefore larval shrimp likely have a higher metabolic rate and chemical uptake (larger surface area to volume ratio) than adult shrimp, as well as a less developed chemical metabolism pathway (DeLorenzo et al., 2006; DeLorenzo et al., 2012). This could lead to an increase in the uptake of contaminants and could make the grass shrimp larvae more sensitive to the Accell Clean-CEWAF, as well as the Accell Clean alone.

Total extractable hydrocarbons and Total PAHs were quantified in all PES-51 and Accell Clean CEWAF treatments (Table 7).

Table 7. Measured TEH and total PAH concentrations for the Accell Clean-CEWAF and PES-51-CEWAF treatments from the grass shrimp laboratory testing.

Accell Clean-CEWAF (% CEWAF)		
Treatment	**TEH (mg/L)**	**Total PAH (µg/L)**
0	0.00	0.00
0.21	0.00	2.56
0.62	0.34	7.14
1.85	0.36	18.11
5.6	0.81	56.19
16.7	1.53	100.66
50	16.51	412.21
100	72.34	951.08
PES-51-CEWAF (% CEWAF)		
Treatment	**TEH (mg/L)**	**Total PAH (µg/L)**
0	0.00	ND
0.62	0.00	5.17
1.85	0.00	10.79
5.6	0.00	14.78
16.7	0.57	37.14
50	3.24	93.30
100	7.60	528.50

Reported TEH concentrations for the PES-51-CEWAF test decreased from 7.6 mg/L (100x) to less than 0.25 mg/L (the detection limit) for 5.56x, 1.85x, 0.62x and the control treatments. Total PAH concentrations in the PES-51-CEWAF followed the same pattern: 528.50 µg/L (100x), 93.30 µg/L (50x), 37.14 µg/L (16.67x), 14.78 µg/L (5.56x), 10.79 µg/L (1.85x), 5.17 µg/L (0.62x), and less than detection for the control. Concentrations from the Accell Clean-CEWAF were higher than those reported in the PES-51-CEWAF. Reported TEH concentrations were 72.34 mg/L (100x), 16.51 mg/L (50x), 1.53 mg/L (16.67x), 0.81 mg/L (5.56x), 0.36 mg/L (1.85x), 0.34 mg/L (0.62x) and less than detection (<0.25 mg/L) for both 0.2x and the control. Total PAH concentrations were 951 µg/L (100x), 412 µg/L (50x), 101 µg/L (16.67x), 56.2 µg/L (5.56x), 18.1 µg/L (1.85x), 7.14 µg/L (0.62x), 2.56 µg/L (0.2x) and less than detection for the control.

Using the measured TEH concentrations, the toxicity values for Accell Clean-CEWAF were 1.86 mg/L (95% CI: 1.51 - 3.86) for adult shrimp and 1.14 mg/L (95% CI: 1.01 - 1.28) for larval shrimp, with adults being significantly more tolerant than larvae ($p = 0.0476$). The LC_{50} values for PES-51 were >7.60 mg/L (Table 7). Using the measured PAH concentrations, the toxicity values for Accell Clean-CEWAF were 113.99 µg/L (95% CI: 98.98-247.97) for adult shrimp and 80.61 µg/L (95% CI: 33.13-106.76) for larval shrimp, with adults being significantly more tolerant than larvae ($p = 0.0015$). The LC_{50} values for PES-51 were >528.50 µg/L (Table 8).

Table 8. Summary of 96h LC_{50} values (and corresponding 95% confidence intervals) for adult and larval grass shrimp, *Palaemonetes pugio*, calculated using measured TEH (mg/L) and total PAH (µg/L) concentrations in the CEWAF.

Asterisks (*) indicate a significant difference between Accell Clean and PES-51 CEWAF LC_{50} values and crosses ($^{+}$) indicate a significant difference between adult and larval shrimp LC_{50} values (Wheeler ratio test $p<0.05$).

Life Stage	LC_{50} TEH (mg/L) (95% CI)	
	Accell Clean-CEWAF	**PES-51-CEWAF**
Adult	1.86 (1.51-3.86)	>7.6
Larvae	1.14 (1.01-1.28)	>7.6

Life Stage	LC_{50} Total PAH (µg /L) (95% CI)	
	Accell Clean-CEWAF	**PES-51-CEWAF**
Adult	113.99 (98.98-247.97)	>528.50
Larvae	80.61 (33.13-106.76)	>528.50

To prepare the 100% CEWAFs, 2500 mg/L SLC was added in solution, which was more than twice as much as the highest concentration (1000 ppm) used for SLC alone exposure. This application rate was adapted from directions provided on product labels. Assuming 100% of the SLC product went into the CEWAF solution, the Accell Clean-CEWAF LC_{50} value for adult shrimp of 20.22% would be approximately equivalent to 506 mg/L Accell Clean. This is approximately 12 times higher than the LC_{50} value for adult shrimp with Accell Clean alone of 44.18 ppm, demonstrating that much of the shoreline cleaner added was not bioavailable in the CEWAF solution. The 100% PES-51-CEWAF, which conceivably could have contained as much as 2500 mg/L PES-51, resulted in < 30% mortality; which is 64 times greater than the

PES-51 alone LC_{50} of 38.75 mg/L. Possible reasons why the SLCs added were not bioavailable in the CEWAF solutions include: 1) degradation/loss of SLC during the 24 h preparation of the CEWAF and subsequent static exposure, 2) binding of SLC product constituents with LSC oil constituents, 3) chemical transformations/differential uptake of the SLC when prepared as CEWAF with LSC oil. Based on their different compositions and chemical properties, different reasons for the results of each SLC-CEWAF could apply; e.g. PES-51 as a lift-and-float product could be lost from solution as the CEWAF was prepared, whereas Accell Clean as a detergent containing proteins may bind or transform due to interactions with the oil. Given that chemical analyses to quantify the SLC concentrations in solution were not performed due to the proprietary nature of the products, these questions remain unanswered.

Lipid peroxidation activity in adult grass shrimp was significantly affected by Accell Clean exposure, increasing from 91.26 nmol/g wet weight (control) to 449.22 nmol/g wet weight at 111 ppm (ANOVA, $p = 0.0017$) (Table 9). With the exception of 37 ppm, MDA concentrations increased as Accell Clean concentrations increased (Table 8). No significant relationships between PES-51 concentration and MDA levels were determined (ANOVA, $p = 0.0633$). Lipid peroxidation activity in adult shrimp was not significantly affected by either the Accell Clean or PES-51 CEWAFs (ANOVA, $p > 0.05$) (Table 10).

Table 9. Lipid peroxidation activity based on malondialdehyde tetraethylacetal (MDA) levels and glutathione levels for adult grass shrimp after 96h Accell Clean and PES-51 exposure. Asterisks (*) indicate significant difference from the control based on ANOVA followed by a Dunnett's test.

Treatment	**MDA (nmol/g wet weight) (mean ± SE)**	**Glutathione (nmol/g wet weight) (mean ± SE)**
Accell Clean (ppm)		
0	91.26 (11.21)	257.34 (29.09)
4.1	215.46 (81.35)	292.32 (27.60)
12.3	240.40 (25.16)	313.94 (30.14)
37	69.04 (12.97)	632.68 (76.86)*
111	449.22 (123.85)*	602.44 (257.56)*
PES-51 (ppm)		
0	323.36 (75.85)	333.12 (37.79)
4.1	296.82 (79.25)	357.00 (26.41)
12.3	574.99 (184.50)	337.78 (30.23)
37	84.10 (15.22)	283.38 (82.97)
111	118.80 (22.99)	322.44 (0.00)

Table 10. Lipid peroxidation activity based on malondialdehyde tetraethylacetal (MDA) levels and glutathione levels for adult grass shrimp after 96h shoreline cleaner-CEWAF exposure. Asterisks (*) indicate significant difference from the control based on ANOVA followed by a Dunnett's test.

Treatment	**MDA (nmol/g wet weight) (mean ± SE)**	**Glutathione (nmol/g wet weight) (mean ± SE)**
Accell Clean-CEWAF (% CEWAF)		
0	60.64 (10.17)	208.05 (17.03)
0.21	88.53 (16.50)	239.94 (17.10)
0.62	52.10 (8.77)	245.11 (25.16)
1.85	46.35 (9.51)	234.81 (13.28)
5.6	83.39 (13.30)	250.81 (19.60)
16.7	102.72 (40.02)	353.20 (33.36)*
PES-51-CEWAF (% CEWAF)		
0	59.26 (14.01)	205.75 (21.31)
0.62	46.76 (6.23)	196.44 (12.53)
1.85	54.24 (13.90)	210.33 (21.71)
5.6	50.84 (7.97)	240.33 (11.09)
16.7	33.08 (6.30)	229.79 (16.36)
50	33.71 (6.09)	227.65 (22.33)
100	35.19 (5.53)	312.87 (25.18)*

Glutathione levels in adult shrimp were significantly higher in the 37 ppm and 111 ppm Accell Clean treatments compared to the control (ANOVA, $p = 0.0004$) (Table 9). There was no significant relationship between PES-51 concentrations and glutathione levels (ANOVA, $p = 0.8366$) (Table 9). Both the Accell Clean-CEWAF (16.7%) and PES-51-CEWAF (100%) significantly increased glutathione activity in adult grass shrimp compared to control levels (ANOVA, Accell Clean-CEWAF $p = 0.0011$; PES-51-CEWAF $p = 0.0027$) (Table 10). All other concentrations of both CEWAFs were relatively similar to the controls.

In this study, lipid peroxidation activity was significantly affected in adult shrimp exposed to Accell Clean, with increased MDA levels at 111 ppm compared to the control. MDA levels also tended to increase in the Accell Clean-CEWAF, but the trend was not significant. An effect on lipid peroxidation was not observed with PES-51 alone or in mixture with oil as a CEWAF. An alternative antioxidant response (e.g. superoxide 30 dismutase and catalase) may have been triggered with exposure to PES-51 (Fisher et. al., 2003). Glutathione (GSH) is a ubiquitous tripeptide and is one of the most important non-protein thiols in biological systems (Hoguet and Key 2007; Kosower and Kosower 1978; Mason and Jenkins 1996; Ringwood et al. 2003). Glutathione levels are commonly used to characterize the antioxidant status of an organism (Hoguet and Key, 2007). When exposed to the shoreline cleaners alone, glutathione levels were not significantly different from the control for grass shrimp exposed to PES-51. When exposed to Accell Clean, adult shrimp glutathione levels significantly increased at 37 and 111 ppm. A similar trend of increased glutathione levels compared to the control was seen when shrimp were exposed to the shoreline cleaners mixed with oil as a CEWAF. The oil and shoreline cleaner mixture may be metabolized differently than the shoreline cleaner alone. The Accell Clean-CEWAF and PES-51-CEWAF may be metabolized by the cytochrome P450

system, resulting in increases of glutathione transferases. An increase in glutathione levels has been measured as a result of adult grass shrimp exposure to the insecticide permethrin (DeLorenzo et al., 2006).

Larval shrimp ecdysteroid molting hormone levels were significantly higher in the 37 ppm Accell Clean treatment than the control (ANOVA $p = 0.0105$), whereas PES-51 had no significant effect on ecdysteroid (ANOVA, $p = 0.2772$) (Table 11).

Ecdysteroid levels increased from 9.89×10^4 ng 20-HE/g wet weight in the 0.62% CEWAF treatment to 2.03×10^5 ng 20-HE/g wet weight in the 5.6% Accell Clean-CEWAF (Table 11). A William's test for monotonic trend determined the lowest observable effect concentration for increasing ecdysteroid level the Accell Clean-CEWAF was at 5.6% ($p = 0.0426$) (Table 11).

Ecdysteroid levels were lower than the control for all concentrations of PES-51-CEWAF (Table 11). However, the control had a large standard error and no significant relationship was observed between ecdysteroid level and PES-51-CEWAF concentration (ANOVA, $p = 0.4098$). The decrease in ecdysteroid concentrations during exposure may indicate that PES-51 is an endocrine disruptor in grass shrimp and could over the long-term possibly disturb molting and development of the organism (Lafontaine et al., 2016).

Table 11. Ecdysteroid activity for larval grass shrimp after 96h exposure to Accell Clean and PES-51 individually and prepared with LSC oil as CEWAFs. Asterisks (*) indicate significant differences from the control (ANOVA p=0.0075, Dunnett's test).

Treatment	**Ecdysteroid (ng 20-HE/g wet weight) (mean ± SE)**	
ppm	**Accell Clean (ppm)**	**PES-51 (ppm)**
0	$5.59x10^{3}$ ($6.66x10^{3}$)	$1.64x10^{5}$ ($1.89x10^{4}$)
4.1	$8.00x10^{4}$ ($1.40x10^{4}$)	not tested
12.3	$6.22x10^{4}$ ($1.04x10^{4}$)	$1.62x10^{5}$ ($4.36x10^{4}$)
37	$1.34x10^{5}$ ($1.83x10^{5}$)*	$1.88x10^{5}$ ($1.89x10^{4}$)
111	no surviving larvae	$9.52x10^{4}$ ($9.57x10^{3}$)
Treatment	**Ecdysteroid (ng 20-HE/g wet weight) (mean ± SE)**	
(% CEWAF)	**Accell Clean-CEWAF**	**PES-51-CEWAF**
0	$1.49x10^{5}$ ($1.92x10^{4}$)	$1.74x10^{5}$ ($8.36x10^{4}$)
0.21	$9.98x10^{4}$ ($1.50x10^{4}$)	not tested
0.62	$9.89x10^{4}$ ($8.59x10^{3}$)	$2.38x10^{4}$ ($6.94x10^{3}$)
1.85	$1.79x10^{5}$ ($3.07x10^{4}$)	$7.37x10^{4}$ ($2.96x10^{4}$)
5.6	$2.03x10^{5}$ ($1.96x10^{4}$)	$3.91x10^{4}$ ($5.74x10^{3}$)
16.7	no surviving larvae	$8.09x10^{4}$ (0.00)
50	no surviving larvae	$9.11x10^{4}$ (0.00)
100	no surviving larvae	$5.25x10^{4}$ (0.00)

The number of molts until post-larvae was significantly lower in the 12.3 ppm Accell Clean treatment (ANOVA p = 0.0179), but was not significantly different at the next higher treatment (Table 12). The mean number of molts increased in the PES-51 treatments (from eight molts at 12.3 ppm to nine molts at 111 ppm) but there was no significant difference from the control (8.36 molts) (ANOVA, p = 0.4148) (Table 12). The mean number of days to reach post-larval stage was significantly lower in the 12.3 ppm Accell Clean treatment (ANOVA p = 0.0464), but was not significantly different at the next higher treatment (37 ppm) (Table 12). Larvae exposed to 12.3 ppm Accell Clean may have inadvertently been fed more Artemia than larvae exposed to other concentrations resulting in faster growth and increased dry weight.

PES-51 had no significant effect on mean number of days to reach post-larval stage (p = 0.0807) (Table 12). The mean dry weight of post-larval grass shrimp was not significantly different in any of the Accell Clean or PES-51 treatments (ANOVA, Accell Clean: p = 0.1056; PES-51: p = 0.2801) (Table 12).

Table 12. Grass shrimp development at the end of the larval stage after 96h exposure to Accell Clean and PES-51. Asterisks (*) indicate significant difference from the control based on ANOVA followed by a Dunnett's test.

Treatment	**Dry Weight (µg) (mean ± SE)**	**Days to Postlarvae (mean ± SE)**	**Number of Molts (mean ± SE)**
Accell Clean (ppm)			
0	707.73 (23.03)	16.40 (0.53)	7.07 (0.25)
4.1	776.60 (35.37)	15.40 (0.37)	6 .40 (0.22)
12.3	807.40 (38.45)	14.90 (0.31)*	5.80 (0.25)*
37	751.22 (21.59)	16.17 (0.25)	6.50 (0.26)
PES-51 (ppm)			
0	893.14 (27.86)	18.79 (0.59)	8.36 (0.31)
12.3	820.00 (34.64)	18.78 (0.39)	8.00 (0.31)
37	811.79 (30.82)	19.93 (0.60)	8.50 (0.23)
111	836.67 (31.94)	21.67 (0.33)	9.00 (0.00)
333	920.00 (0.00)	22.00 (0.00)	ND

Accell Clean-CEWAF exposure significantly increased mean dry weight of post-larval grass shrimp (ANOVA $p = 0.0037$) (Table 13), the mean number of molts until post-larval stage (ANOVA $p = 0.0022$) (Table 13), and the mean number of days to reach post-larval stage (ANOVA $p < 0.0001$) (Table 13) in the 5.6% treatment compared to the control. PES-51-CEWAF exposure significantly increased the mean number of molts until post-larval stage (ANOVA $p = 0.0033$) (Table 13) and the mean number of days to reach post-larval stage (ANOVA $p < 0.0001$) in the 100% treatment compared to the control (Table 13).

Table 13. Grass shrimp development at the end of the larval stage after 96h exposure to shoreline cleaner-CEWAFs. Asterisks (*) indicate significant difference from the control based on ANOVA followed by a Dunnett's test.

Treatment	**Dry Weight (µg) (mean ± SE)**	**Days to Postlarvae (mean ± SE)**	**Number of Molts (mean ± SE)**
Accell Clean-CEWAF (% CEWAF)			
0	745.35 (22.80)	16.59 (0.44)	6.35 (0.27)
0.21	800.00 (28.73)	17.56 (0.30)	6.83 (0.20)
0.62	718.33 (28.05)	16.50 (0.41)	6.61 (0.24)
1.85	735.00 (26.17)	16.71 (0.25)	6.94 (0.18)
5.6	858.20 (29.26)*	18.87 (0.34)*	7.67 (0.19)*
PES-51-CEWAF (% CEWAF)			
0	730.17 (21.86)	22.56 (0.52)	5.89 (0.23)
0.62	730.18 (22.94)	24.12 (0.66)	6.18 (0.20)
1.85	745.71 (18.77)	22.38 (0.39)	5.56 (0.22)
5.6	727.41 (15.33)	23.47 (0.37)	5.88 (0.17)
16.7	761.20 (17.93)	23.87 (0.54)	5.93 (0.28)
50	728.94 (16.76)	24.11 (0.27)	6 .33(0.14)
100	784.50 (33.51)	27.83 (0.95)*	7.17 (0.31)*

In the CEWAF larval life cycle tests, larvae exposed to the 5.6% Accell Clean-CEWAF had a significantly higher mean dry weight, number of days to postlarval status, and number of molts compared to the control. Similarly, larvae exposed to the 100% PES-51-CEWAF had significantly higher duration of development and number of molts compared to the control as

well as the highest mean dry weight. This suggests that larvae exposed to the shoreline cleaners mixed with oil may result in a longer duration of development, which results in more molts over a longer period of time and higher dry weights. This is of concern because a longer larval life stage may lead to increased predation on grass shrimp (McKenny and Hamaker, 1984). Molting is a vulnerable time during grass shrimp development. Any increase in the number of molts may lead to increased stress for the organism on top of predation pressures (Key, 2003).

Objective 3: Oil-removal efficiency study with shoreline cleaners

Historically, cleaners were evaluated for effectiveness using two protocols: 1) the inclined trough test and 2) the swirling coupon test (Clayton et al., 1995). Typically, these protocols are performed with consistent SLC application rates but use non-environmentally relevant substrates (i.e. a stainless steel trough or a plastic card or "coupon") in order to compare the effective oil removal from a substrate among different cleaning agents (Clayton et al., 1995). Recently, Koran et al (2009) reported on a more current protocol developed by the US EPA that standardizes oil application and substrate (sand and gravel), but even this test is engineered for cleaner comparisons for regulatory approval using non-diluted and standardized LSC application rates that are not detailed on the product's label. In this study, product application rates as described on the product label were followed in order to evaluate the effectiveness of the three cleaners selected for testing in our environmental simulation / mesocosm.

Instrumental analysis indicated that the shoreline cleaner products were interfering with the TEH signal, therefore, tiles lacking oil were washed using the same protocol as detailed above in order to calculate a background TEH concentration for each shoreline cleaner. The TEH signature for each cleaner was quantified in both the water rinse and the tile extract (Table 14).

These values were averaged and used to correct TEH values measured in the oil + shoreline cleaner portion of the study (Table 15).

Table 14. Contribution of TEH from each shoreline cleaner used in the efficiency study.

Shoreline Cleaner	Rep	TEH from tile (mg)	TEH in water rinse (mg)
PES-51	1	0.97	72.36
PES-51	2	1.03	37.92
PES-51	3	0.37	24.6
Accell Clean	1	0.32	66.75
Accell Clean	2	0.05	47.55
Accell Clean	3	0.27	53.25
CytoSol	1	2.97	69.57
CytoSol	2	4.03	56.88
CytoSol	3	3.79	53.49

Table 15. TEH and PAH 50 values for the tile and water fractions for each treatment as determined in the oil-removal efficiency study. TEH values have been corrected according to SLC TEH contribution in Table 16.

Treatment	Tile		Water	
	TEH (mg)	Total PAH (µg)	TEH (mg)	Total PAH (µg)
CTL 1	146	79.2	n/a	n/a
CTL 2	137	92.6	n/a	n/a
CTL 3	164	132	n/a	n/a
OIL 1	150	134	11.1	4.8
OIL 2	141	139	13.8	6.3
OIL 3	183	198	20.9	14.9
PES 1	14.1	20.7	133	133
PES 2	18.3	20.2	124	91.5
PES 3	11.2	28.1	162	152
ACC 1	174	254	108	79.9
ACC 2	134	87.0	66.9	45.1
ACC 3	166	107	44.7	47.4
CYT 1	63.7	53.6	109	152
CYT 2	53.9	36.7	91.4	131
CYT 3	36.3	42.5	162	214

A mass balance (Table 16) based on corrected TEH was calculated and the range based on nominal expected oil (as expressed by TEH) ranged from 56-134%.

Table 16. Mass balance for the amount of oil recovered from the tile and water fractions compared to the total amount of oil on the tile after ten d of weathering as determined in the oil-removal efficiency study.

Treatment	% Oil remaining on tile	% Oil in Water	% Oil accounted for
CTL 1	82 %	--	82 %
CTL 2	86 %	--	86 %
CTL 3	91 %	--	91 %
OIL 1	71 %	5 %	76 %
OIL 2	90 %	9 %	99 %
OIL 3	89 %	10 %	99 %
PES 1	5 %	47 %	51 %
PES 2	9 %	63 %	72 %
PES 3	5 %	68 %	73 %
ACC 1	83 %	51 %	134 %
ACC 2	67 %	33 %	100 %
ACC 3	83 %	22 %	105 %
CYT 1	28 %	47 %	75 %
CYT 2	28 %	48 %	76 %
CYT 3	16 %	70 %	86 %

Generally, the proportion of TEH found in the cleaner treatments was greater in the water rinse relative to rinse from the seawater only treatment (Figure 38.) The same can be reported for Total PAH (Figure 39).

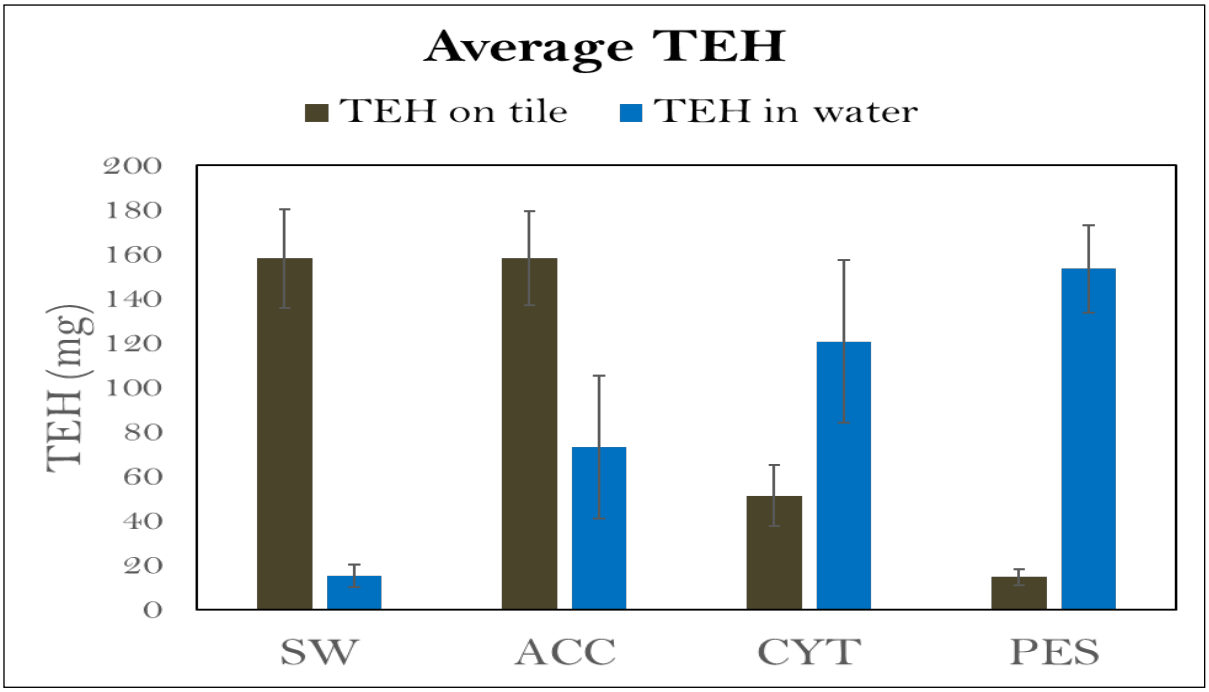

Figure 38. Average TEH in rinse water after SLC application and washing of oiled tiles. The rinsate included all oil related products in the water, floating oil was not separated from the water rinse.

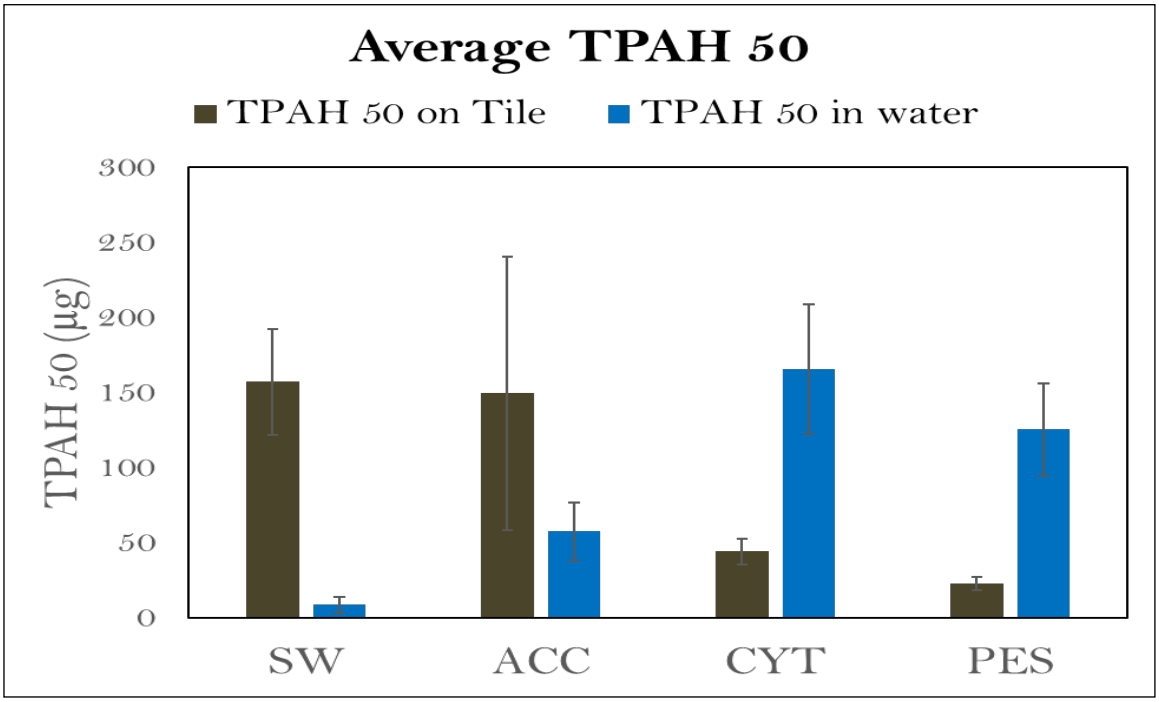

Figure 39. Average Total PAH50 in rinse water after SLC application and washing of oiled tiles. The rinsate included all oil related products in the water, floating oil was not separated from the water rinse.

Factors that may drive the high variability of data associated with this mass balance include the difficulties in tracking oil loss during weathering process and oil seepage into porous edges of the tile. Over reporting of oil may be attributed to contribution of hydrocarbons from the SLCs. The average percentages of oil remaining on the tiles were 83% (SW), 78% (ACC), 24% (CYT) and 6% (PES).

Statistical analysis of TEH and Total PAH data using Tukey-Kramer Pairwise comparisons generally showed significant differences between SW and ACC, and PES and CYT treatments for TEH and TPAH 50; significant differences were not observed between SW and ACC, nor were they observed between PES and CYT (Table 17). Results of this study indicate that PES and CYT were more efficient at removing oil from this substrate than ACC.

Table 17. Total PAH and TEH statistical analysis for tile and water samples.

A One-Way ANOVA revealed significant differences between treatments for both tile (a) and water (b) samples (TEH tile $p<0.0001$; Total PAH tile $p=0.0091$; TEH water $p=0.0066$; Total PAH water $p=0.0039$). Tukey-Kramer pairwise comparisons for tile and water data were run to discern where those differences were located. "++" indicates pairwise differences for both TEH and Total PAH, "+" indicates a pairwise differences for TEH only and "*" indicates a pairwise difference for Total PAH.

a.)

Tukey-Kramer Pairwise Comparison-Tile				
	SW	ACC	CYT	PES
SW			++	++
ACC			+	++
CYT				
PES				

b.)

Tukey-Kramer Pairwise Comparison-Water				
	SW	ACC	CYT	PES
SW			++	++
ACC			*	
CYT				
PES				

PAH profiles for both oil remaining on the tile and oil in the rinsate were plotted for each treatment (SW, ACC, CYT and PES; Figures 40-43) to determine if and how PAHs were preferentially removed from the tile during the efficiency study. For both the SW and ACC treatments (Figures 40 and 41), PAH patterns from both the tile and rinsate were similar to each other meaning that the treatment did not selectively remove certain PAHs from the tile. Conversely, in the CYT and PES treatments (Figures 42 and 43), it was observed that there were

some pattern differences between what was left on the tile and what was in the rinsate. For the CYT treatment, there were higher proportions of C1 and C2 phenanthrenes/anthracenes in the rinsate when compared to what remained on the tile. The oil residue remaining on the tile for CYT had higher proportions of C3-fluorenes, C1-C3-dibenzothiophenes and phenanthrene when compared to the rinsate. In the PES treatment, there were also higher proportions of C1 and C2-phenanthrenes in the rinsate when compared to the oil residue that remained on the tile. There were higher proportions of C4-phenenathrenes and alkylated fluoranthenes that remained on the tile for PES. Higher proportions of certain PAHs in the rinsate mean that the treatment was more effective in removing those PAHs from the tile while higher proportions on the tile can be interpreted as the treatment not being as effective as removing those PAHs. It also should be noted that treatments where pattern differences were observed between the tile and rinsate (CYT and PES) were also treatments that were more effective in removing oil from the tile.

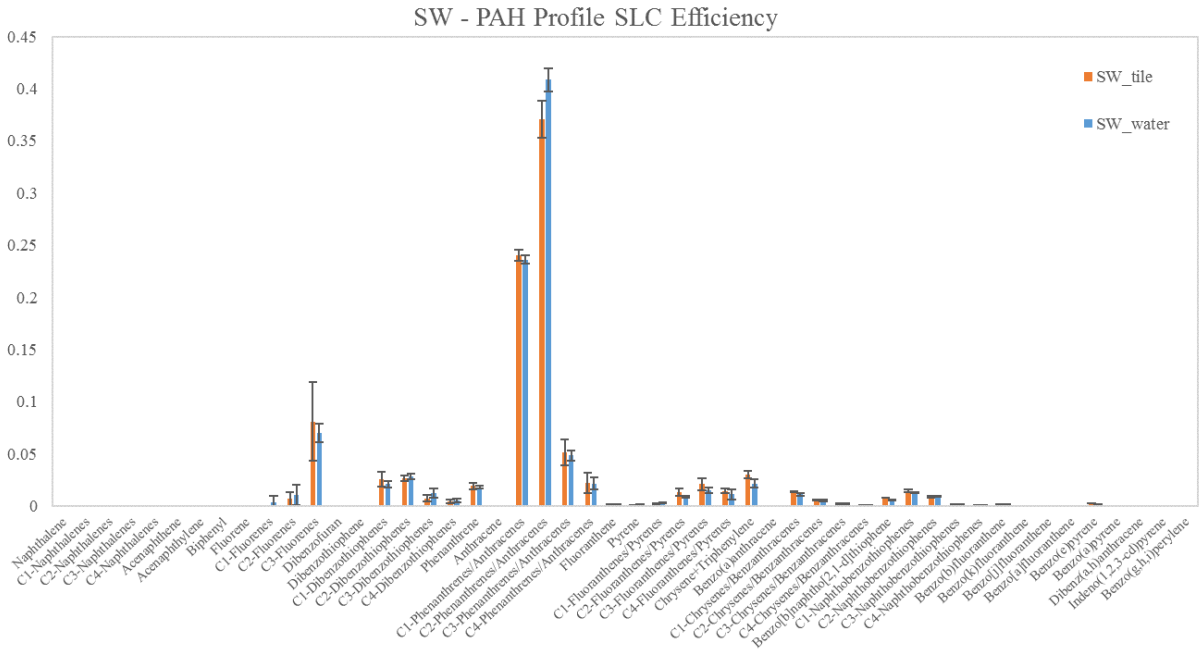

Figure 40. PAH profiles for oil remaining on the tile (orange) after SLC application/rinsing and oil in the rinsate (blue) for the seawater only protocol.

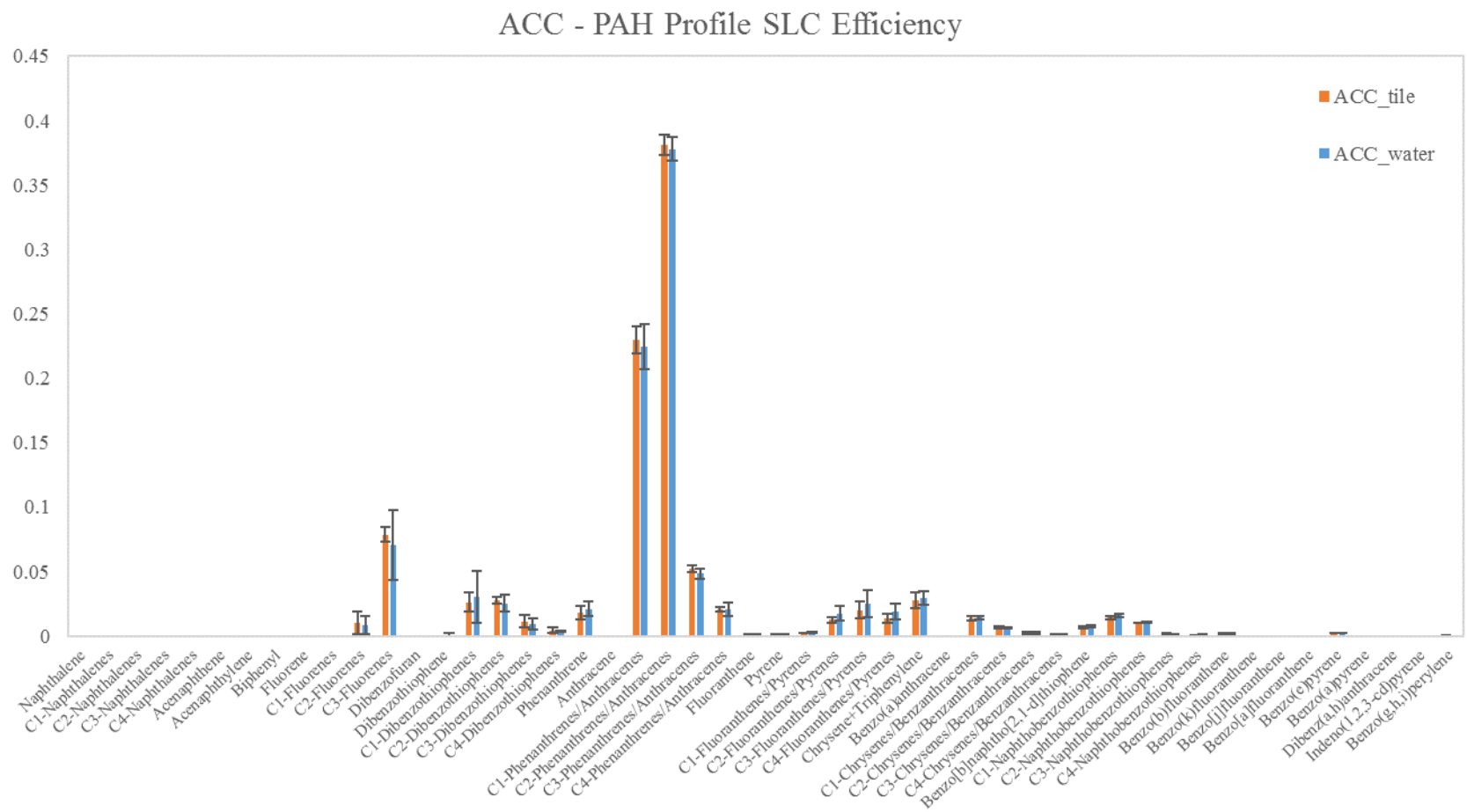

Figure 41. PAH profiles for oil remaining on the tile (orange) after SLC application/rinsing and oil in the rinsate (blue) for Accell.

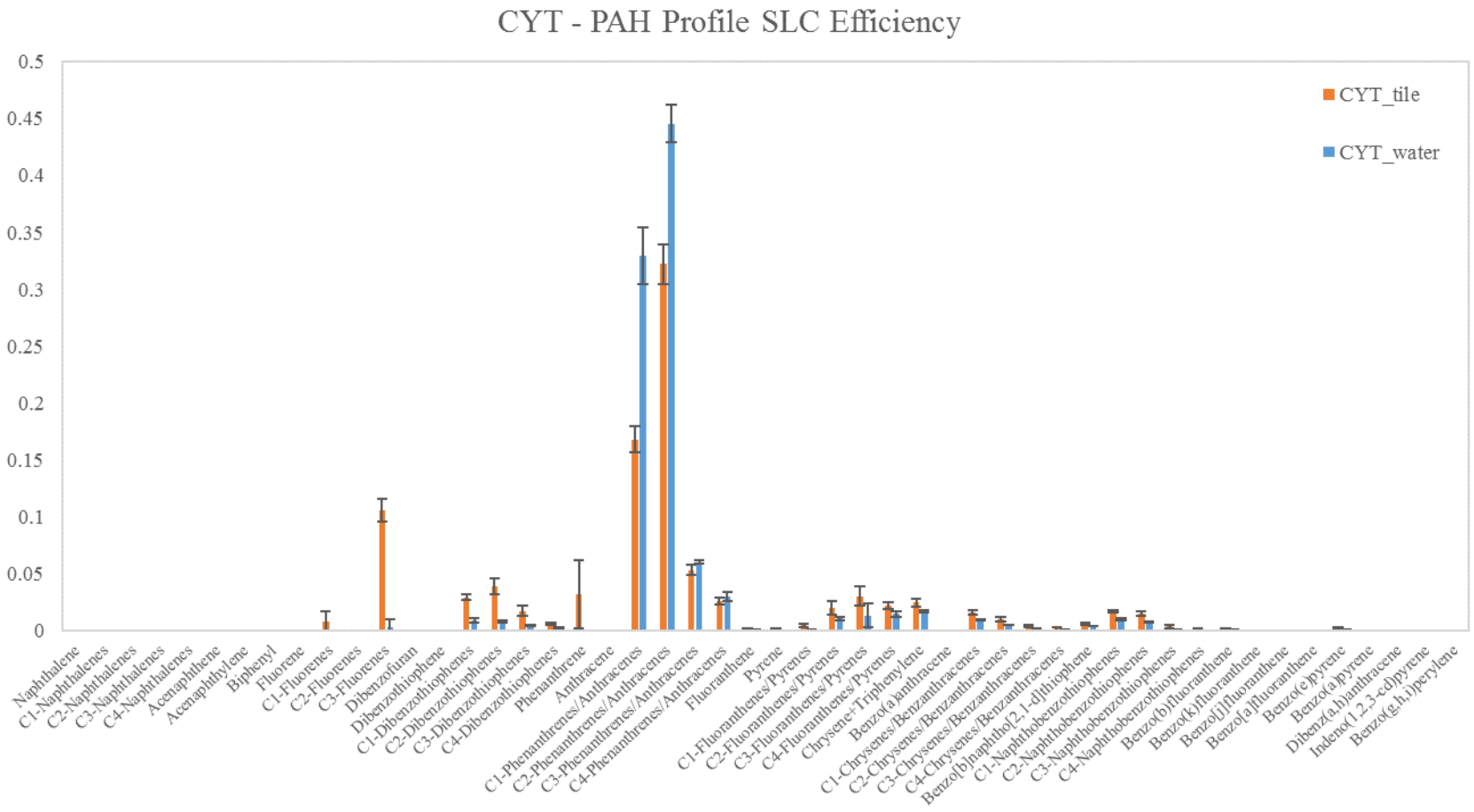

Figure 42. PAH profiles for oil remaining on the tile (orange) after SLC application/rinsing and oil in the rinsate (blue) for CytoSol.

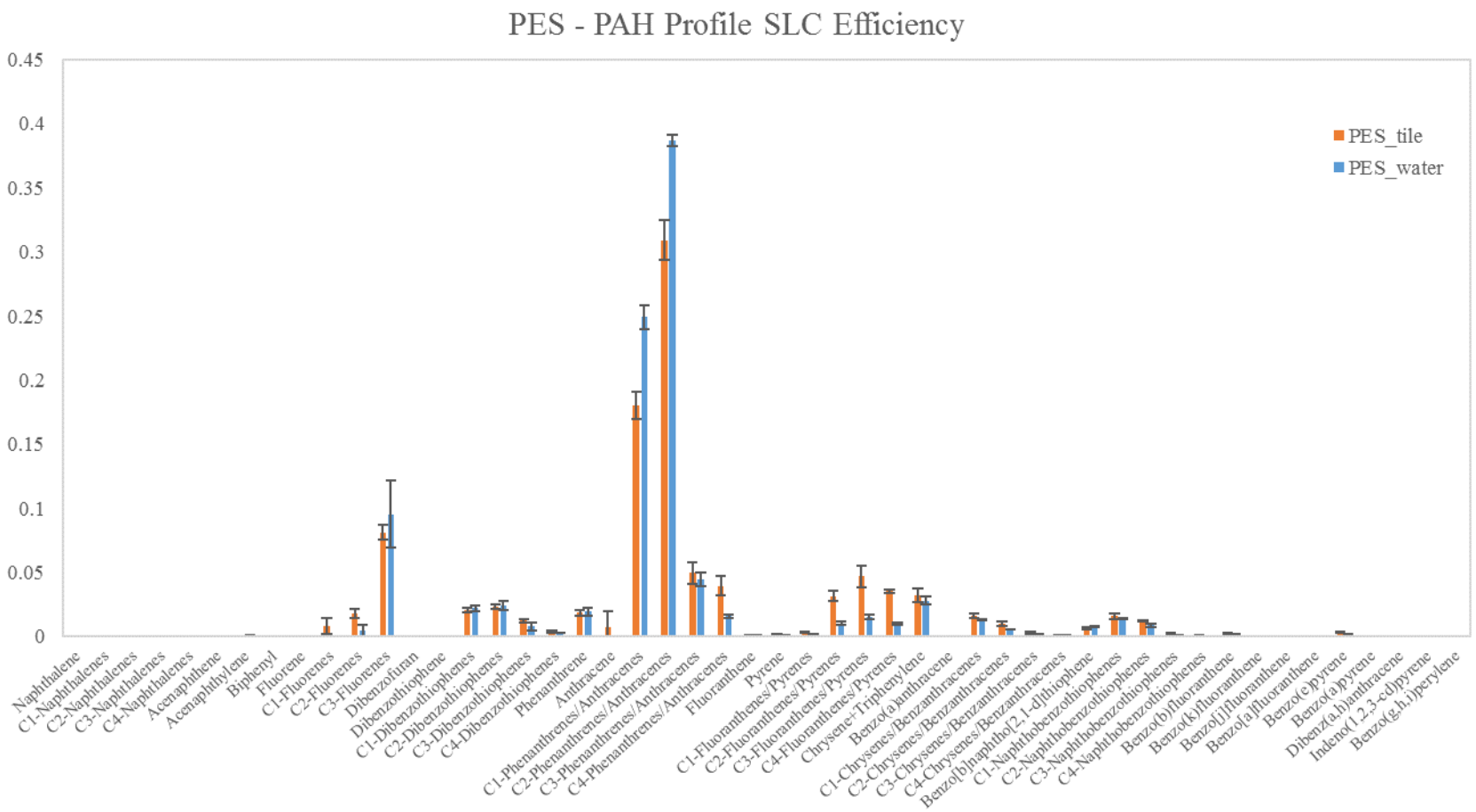

Figure 43. PAH profiles for oil remaining on the tile (orange) after SLC application/rinsing and oil in the rinsate (blue) for PES-51.

CONCLUSIONS

Shoreline cleaners can be valuable tools for oil spill mitigation, and understanding the potential toxic effects on coastal species is key to their appropriate use. The mesocosm study demonstrated that aquatic toxicity will depend on the product employed and the species present. Accell Clean resulted in the greatest mortality for mud snails in the mesocosm systems, followed by PES-51, then CytoSol. Polychaetes and amphipods were also most sensitive to Accell Clean, but the next most toxic compound was CytoSol, followed by PES-51. Clam survival was only affected by Accell Clean exposure. Fish mortality was also greatest in the Accell Clean treatment, followed by PES-51, then CytoSol, but given that some fish had jumped out of the mesocosm tanks, we cannot definitively conclude treatment differences. Most of these impacts are hypothesized to be related to the greater bioavailability of hydrocarbons in the water column in the Accell Clean treatment. Additional effects of Accell Clean in the mesocosm exposures included increased bacterial densities and decreased dissolved oxygen, which may be related to the chemical exposure itself, or the ecosystem interactions of animal mortality, bacterial decomposition, and resulting biological oxygen demand. The oil-alone treatment resulted in low toxicity to the aquatic species tested, however, it should be noted that the exposures did not incorporate ultraviolet light, which would be expected to have increased toxicity.

The results of this study also generated new toxicity thresholds for three shoreline cleaners in a common estuarine crustacean species, the grass shrimp. The data indicate that CytoSol is relatively insoluble in seawater and was not toxic to grass shrimp. PES-51 and Accell Clean were similar in toxicity when the products were tested in seawater, and both Accell Clean and PES-51 would be categorized as slightly toxic on the EPA scale. PES-51 would be

categorized as practically nontoxic to larval shrimp. A significant difference in toxicity was observed, however, when the products were tested in mixture with LSC oil (CEWAFs). Accell Clean was significantly more toxic to grass shrimp than PES-51 when the products were mixed with oil. The PES-51 product did not mix the oil into the water column, and did not result in sufficient mortality to obtain a threshold value for grass shrimp. The Accell Clean product was observed to act more as a dispersant and mixed the oil into the CEWAF solution, yielding greater concentrations of soluble hydrocarbons. Sublethal effects on larval shrimp development were observed. Accell Clean and Accell Clean-CEWAF treatments had increased lipid peroxidation activity and glutathione levels, indicating disruption to cellular homeostasis and cellular membrane damage. In addition, Accell Clean and Accell Clean-CEWAF treatments had increased ecdysteroid levels, increased number of days to post larvae, and increased number of molts, indicating effects on larval shrimp development.

Results of the oil-removal efficiency study with shoreline cleaners indicate that PES-51 and CytoSol were more effective at removing oil from the substrates tested than Accell Clean. Differences in oil-removal efficiency are likely due to differences in chemical composition among the products tested. Hydrocarbon binding and removal is probably driven by solvent properties within the products. The proprietary nature of the product formulations prevents further description of the chemical interactions of shoreline cleaner products and oil.

This research project addresses the NOAA priority of understanding ecosystem responses to chemical stressors. Working with OR&R, we will provide ecosystem assessments of oil spill mitigation products for use in spill response. The information generated on shoreline cleaner product toxicity to sensitive estuarine species and product efficacy in oil removal will allow managers to make more informed decisions regarding the future use of shoreline cleaners.

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Appendix 1. List of individual and alkylated PAHs that are included in Total PAH.

Individual and Alkylated PAHs in Total PAH	
napthalene	C1-Naphthalenes
biphenyl	C2-Naphthalenes
acenapthene	C3-Naphthalenes
acenapthylene	C4-Naphthalenes
fluorene	C1-Fluorenes
dibenzofuran	C2-Fluorenes
dibenzothiophene	C3-Fluorenes
phenanthrene	C1-Dibenzothiophenes
anthracene	C2-Dibenzothiophenes
fluoranthene	C3-Dibenzothiophenes
pyrene	C4-Dibenzothiophenes
benz(a)anthracene	C1-Phenanthrenes/Anthracenes
benzo(b)naphtho(2,1-d)thiophene	C2-Phenanthrenes/Anthracenes
chrysene + triphenylene	C3-Phenanthrenes/Anthracenes
benzo(a)fluoranthene	C4-Phenanthrenes/Anthracenes
benzo(b)fluoranthene	C1-Fluoranthenes/Pyrenes
benzo(j)fluoranthene	C2-Fluoranthenes/Pyrenes
benzo(k)fluoranthene	C3-Fluoranthenes/Pyrenes
benzo(a)pyrene	C4-Fluoranthenes/Pyrenes
benzo(e)pyrene	C1-Chrysene/Benzanthracene
dibenzo(a,h)anthracene	C2-Chrysene/Benzanthracene
indeno(1,2,3-c,d)pyrene	C3-Chrysene/Benzanthracene
benzo(g,h,i)perylene	C4-Chrysene/Benzanthracene
	C1-Naphthobenzothiophenes
	C2-Naphthobenzothiophenes
	C3-Naphthobenzothiophenes
	C4-Naphthobenzothiophenes

Appendix 2. Water quality parameters measured in the mesocosm treatments.

WQ_parm	trt	mean	std.err.	max	min	n
Summary statistics through 96 h						
Cond	ACC	33197.35	50.834	33744.31	19710.3	382
Cond	CTL	33335.53	9.539	33779	33052	383
Cond	CYT	33234.81	50.772	33558.01	19691.18	382
Cond	OIL	33311.12	5.328	33514.05	33134.73	381
Cond	PES	33084.37	8.232	33441.84	32817.14	322
DO	ACC	3.65	0.172	11.81	0.01	382
DO	CTL	7.74	0.111	12.34	4.29	383
DO	CYT	7.65	0.151	14.16	1.97	382
DO	OIL	7.48	0.081	11.55	5.05	381
DO	PES	7.78	0.099	12.04	5.39	322
Sal	ACC	20.80	0.025	21.1	11.7	382
Sal	CTL	20.87	0.006	21.1	20.66	383
Sal	CYT	20.80	0.034	21	11.7	382
Sal	OIL	20.86	0.003	21	20.7	381
Sal	PES	20.71	0.005	20.9	20.5	322
Sat	ACC	48.85	2.333	164	0	382
Sat	CTL	104.60	1.647	180.2	55.9	383
Sat	CYT	103.51	2.086	193	26	382
Sat	OIL	101.10	1.221	168	67	381
Sat	PES	105.26	1.506	176	71	322
Temp	ACC	24.06	0.081	29.5	22.5	382
Temp	CTL	24.20	0.076	29.05	22.58	383
Temp	CYT	24.40	0.078	29.8	22.7	382
Temp	OIL	24.21	0.072	28.8	22.6	381
Temp	PES	24.31	0.090	29.5	22.7	322
pH	ACC	7.57	0.017	8.29	7.19	382
pH	CTL	8.04	0.009	8.46	7.69	383
pH	CYT	8.01	0.011	8.5	7.47	382
pH	OIL	7.95	0.008	8.32	7.65	381
pH	PES	8.03	0.009	8.43	7.78	322

WQ_parm	trt	mean	std.err.	max	min	n
Summary statistics through 14 d						
Cond	ACC	33322.72	19.266	34119.7	19710.3	1339
Cond	CTL	33692.30	9.398	34535	33052	1342
Cond	CYT	33309.03	21.468	51514.95	19691.18	1332
Cond	OIL	33433.09	5.126	33971.3	33097.23	1339
Cond	PES	33359.88	6.655	34007.84	32817.14	1276
DO	ACC	4.33	0.063	11.81	0.01	1339
DO	CTL	7.99	0.061	12.7	4.29	1342
DO	CYT	6.10	0.075	14.16	1.78	1332
DO	OIL	8.05	0.068	15.94	4.67	1339
DO	PES	7.99	0.068	13.9	4.62	1276
Sal	ACC	20.86	0.011	21.4	11.7	1339
Sal	CTL	21.10	0.006	21.67	20.66	1342
Sal	CYT	20.84	0.027	52.8	11.7	1332
Sal	OIL	20.93	0.004	21.3	20.6	1339
Sal	PES	20.87	0.004	21.2	20.5	1276
Sat	ACC	58.89	0.879	164	0	1339
Sat	CTL	109.74	0.930	191.7	55.9	1342
Sat	CYT	84.48	1.079	193	24	1332
Sat	OIL	110.27	1.021	233	62	1339
Sat	PES	110.46	1.043	207	63	1276
Temp	ACC	24.63	0.058	30.8	19.5	1339
Temp	CTL	24.91	0.055	30.89	20.17	1342
Temp	CYT	25.47	0.060	31.5	20.6	1332
Temp	OIL	24.79	0.053	30.3	20.1	1339
Temp	PES	25.32	0.060	31	20.8	1276
pH	ACC	7.66	0.007	8.3	7.19	1339
pH	CTL	8.08	0.005	8.46	7.68	1342
pH	CYT	7.94	0.007	8.61	7.47	1332
pH	OIL	8.04	0.006	8.6	7.58	1339
pH	PES	8.13	0.006	8.64	7.71	1276

WQ_parm	trt	mean	std.err.	max	min	n
Summary statistics through 28 d						
Cond	ACC	33212.77	19.354	75467.55	19710.3	2674
Cond	CTL	33597.30	6.666	34535	31702	2685
Cond	CYT	33214.54	19.479	74199.55	19691.18	2669
Cond	OIL	33368.67	5.099	33971.3	32497.5	2677
Cond	PES	33243.39	6.503	34007.84	31998.2	2571
DO	ACC	8.40	0.123	34.62	0.01	2674
DO	CTL	7.86	0.045	13.1	3.01	2685
DO	CYT	8.02	0.098	26.8	1.78	2669
DO	OIL	8.87	0.070	21.33	4.3	2677
DO	PES	10.03	0.100	29.85	4.59	2571
Sal	ACC	20.74	0.014	52.1	11.7	2674
Sal	CTL	21.00	0.005	21.67	19.67	2685
Sal	CYT	20.73	0.018	52.8	11.7	2669
Sal	OIL	20.84	0.004	21.3	20.2	2677
Sal	PES	20.75	0.005	21.2	19.8	2571
Sat	ACC	121.38	1.897	500	0	2674
Sat	CTL	111.86	0.716	205.7	43.4	2685
Sat	CYT	116.65	1.535	421	24	2669
Sat	OIL	126.47	1.118	332	59	2677
Sat	PES	145.11	1.600	464	63	2571
Temp	ACC	26.67	0.060	34.8	19.5	2674
Temp	CTL	26.97	0.058	34.34	20.17	2685
Temp	CYT	27.44	0.058	34.9	20.6	2669
Temp	OIL	26.75	0.056	34.1	20.1	2677
Temp	PES	27.31	0.060	34.7	20.8	2571
pH	ACC	8.00	0.008	8.91	7.19	2674
pH	CTL	8.14	0.004	8.6	7.68	2685
pH	CYT	8.11	0.006	8.91	7.47	2669
pH	OIL	8.07	0.005	8.66	7.55	2677
pH	PES	8.23	0.006	9.01	7.68	2571

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