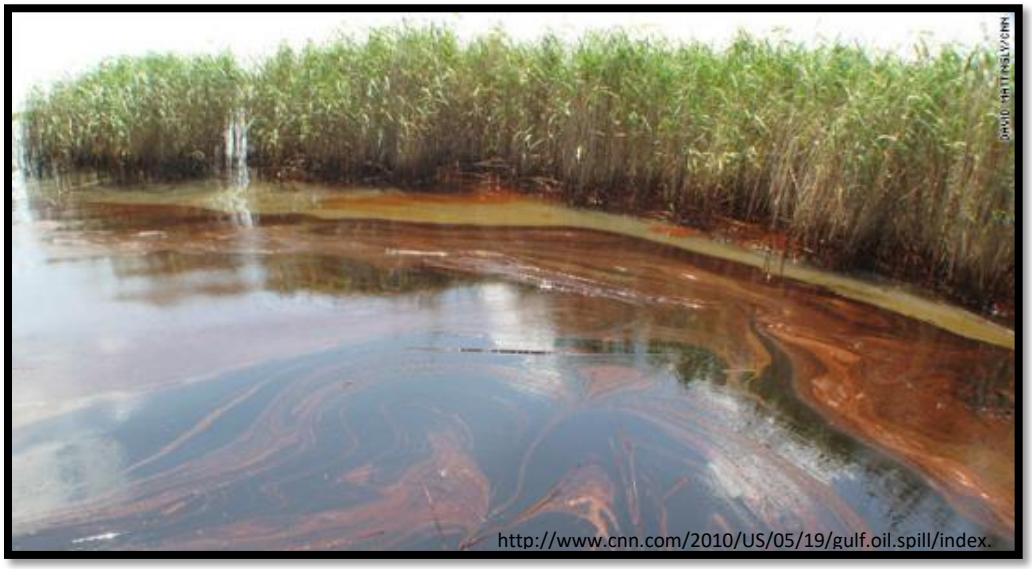
Fate and Effects of Chemical Contaminants Program Review



Volume 2: Ecotoxicology

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Volumes of the Fate and Effects of Chemical Contaminants Program (F&ECCP) Review

Volume 1: Introduction and F&ECCP Overview

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Fate and Effects of Chemical Contaminants Program Review

Volume 2: Ecotoxicology Branch

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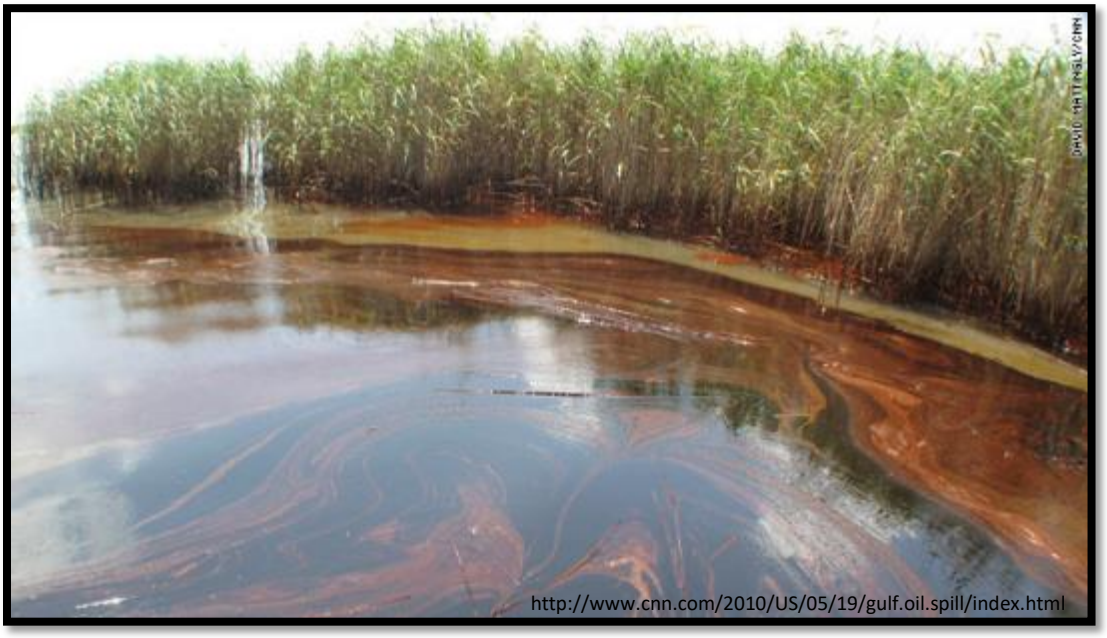

Title: Ecotoxicology in Coastal Ecosystems: An Overview of the Ecotoxicology Program

Authors: Marie DeLorenzo[1], Pete Key[1], Ed Wirth[1], Paul Pennington[1], Katy Chung[2], Emily Pisarski[2], James Daugomah[1], Blaine West[2], Allisan Beck[2], Brian Shaddrix[2], Lou Ann Reed[1], Joe Wade[1]

Affiliations: [1]NOAA, NOS, NCCOS, Charleston, SC; [2]CSS Inc.

Abstract:

The NCCOS Ecotoxicology Branch assesses the fate and effects of chemical contaminants in coastal ecosystems. Located in the NCCOS Charleston Laboratory, this team conducts environmental chemistry and toxicological testing focused on understanding the environmental distribution, fate and effects of chemical contaminants in coastal ecosystems. This multi-disciplinary approach includes standardized acute and chronic aqueous and sediment toxicity assays where chemical effects on survival, growth, reproduction, behavior, and cellular and molecular biomarkers are observed. Thresholds of effect are established and used to compare across chemicals and species. Additional factors influencing toxicity are also considered. Multi-stressor assessments include climate variables such as temperature, salinity, pH, dissolved oxygen, and ultraviolet light. The interactive effect of contaminants are also assessed as chemical mixtures. A tiered-approach is utilized in assessing chemical toxicity. We begin with very controlled laboratory exposures with a single chemical and species. Once a clear dose-response relationship has been established, additional parameters such as the addition of sediment are added. To evaluate how well laboratory-derived toxicity values predict effects in the natural environment, mesocosm testing is then employed. This ecosystem-level testing incorporates more environmental features such as ambient lighting and temperature, tidal flux, and community interactions such as nutrient cycling, predation, and competition. The Ecotoxicology Program also utilizes field testing, for example deploying caged animals to assess toxicological response to site specific chemical contamination. The Ecotoxicology Program has organic and inorganic chemistry laboratories to address a variety of research questions related to concentration-response, uptake and depuration, chemical fate and transformation, and seasonal and spatial distribution in water, sediment, and organism tissues. By measuring the chemical concentrations using these effects-based tests and determining the distribution and concentration of these chemicals in the environment, we are able to evaluate the potential hazard these chemicals pose in the environment.

Introduction

Ecotoxicology is a multidisciplinary science (Figure 1) incorporating chemistry, biology, physiology, ecology, statistics and modeling. Aquatic organisms are unique in that 1) they are generally inescapably immersed in the water column throughout their lives, and 2) they often serve as reservoirs for chemical pollutants. The fundamental principle of toxicology is that toxicity is a function of concentration of the chemical and duration of the exposure. Thus, it is

necessary to establish thresholds of effect and then characterize the potential for exposure in order to predict chemical impacts in the environment.

The Ecotoxicology Branch conducts research to evaluate and predict the effects of chemical contaminants and other environmental stressors on coastal ecosystems. Estuarine ecosystems include salt marshes, barrier islands, maritime forests, tidal creeks, and rivers. Important ecosystem services provided by estuaries include water filtration, habitat, flood and erosion control, nutrient cycling, primary productivity, commercial and recreational fisheries, and tourism. These systems are vulnerable to many anthropogenic stressors, including land development, agriculture, urban and resort runoff, and point and nonpoint source inputs. Estuarine environments can serve as sinks for many chemical contaminants bound to particulate matter as they move through urban and agricultural watersheds into rivers and are deposited in coastal areas where sedimentation rates are high. Headwater streams, such as tidal creeks in the coastal zone, are most susceptible to chemical runoff. These areas also serve as critical habitats supporting nursery grounds for estuarine fish and invertebrate species.

Contaminants: classes and sources

Chemical classes can be broadly separated into organic and inorganic contaminants. Inorganic chemicals include metals and metalloids, and radionuclides. Organic chemicals include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins, and pesticides (insecticides, herbicides, fungicides, etc.). These organic chemicals are generally referred to as POPs (persistent organic pollutants) as they are known to be persistent in the environment, bioaccumulate in organisms, and are toxic (PBT). Many times these PBT chemicals are found in industrial and sewage outfalls and in non-point source or surface run-off.

Contaminants of emerging concern (CECs) describe a wide range of chemicals that are broadly defined as any synthetic or naturally occurring chemical that is not commonly monitored in the environment but has the potential to enter the environment and cause known or suspected adverse ecological and/or human health effects. In some cases, release of emerging contaminants to the environment has likely occurred for a long time, but may not have been recognized until new detection methods were developed. In other cases, synthesis of new chemicals or changes in use and disposal of existing chemicals can create new sources of emerging contaminants. CECs include contemporary pesticides, pharmaceuticals and personal care products (PPCPs), surfactants and foams used to control fires (i.e., PFAS), polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFCs), microplastics, and nanomaterials.

Chemical contaminants can enter the coastal aquatic environment through both point and nonpoint sources. Nonpoint sources include surface runoff from agriculture, golf courses, lawns, and roads and other impervious surfaces, as well as atmospheric deposition. Point sources include municipal wastewater treatment plants, industrial discharges, and hazardous waste disposal sites. Chemicals also enter waterways through groundwater contamination, dredging, and spills. The fate of a chemical is controlled by the physical and chemical properties of the chemical (e.g. molecular structure, water solubility, vapor pressure, etc.), the physical and chemical properties of the environment (e.g. temperature, salinity, pH, depth, flow, total suspended solids, sediment

carbon content and particle size), and the sources and rates of input into the environment. Often multiple contaminant classes co-occur in runoff and can be measured in estuarine systems. The fine silt-clays of salt-marsh tidal creek sediments have a high surface area to volume ratio and high organic carbon content, making them particularly prone to adsorption of lipophilic, persistent organic contaminants.

Objectives

The NCCOS Ecotoxicology Branch has a long history of studying the effects of environmental pollution in estuaries. Our mission is to conduct research that evaluates and predicts the effects of chemical contaminants and other environmental stressors on coastal ecosystems. Our research ***priorities*** include:

- Determine bioeffects associated with environmental pollution
- Develop sublethal indicators of contaminant exposure and stress
- Develop sensitive analytical methods for identification and quantification of legacy and emerging environmental chemical pollutants
- Evaluate impacts of priority contaminants, contaminant mixtures, and multiple stressors
- Improve risk assessments for environmental and human health
- Characterize chemical transport and fate
- Support national and regional chemical contaminant assessments
- Provide science to support NOAA's mandate for spill response and restoration

Capabilities

We employ a wide range of analytical and laboratory capabilities that exist within our branch to successfully address contaminant research priorities. In addition to the expertise of our team, we collaborate with other researchers from across academia and federal organizations that bring new and targeted capabilities that support NCCOS goals.

Our in-house testing capabilities include extensive experience designing and performing acute and chronic aqueous toxicity testing with a variety of estuarine fish (mummichog, sheepshead minnow, red drum, seatrout) and invertebrates (e.g., shrimp, mysids, amphipods, molluscs, phytoplankton, microbial community, Microtox). In addition, this team is also experienced in sediment toxicity testing, using both field-collected and spiked sediments. Toxicity testing requires the ability to test under various exposure scenarios and, in addition to static-renewal assays, we have the capability and experience for conducting effluent testing, flow through testing, and life cycle testing (e.g. grass shrimp). We also conduct multi-stressor experiments to assess contaminant toxicity along with changes in environmental parameters such as temperature, salinity, UV light, etc. In order to support these laboratory toxicity assays, we have a set of environmental chambers at our facility that we use to control temperature and lighting for each exposure. Our mesocosm systems are a unique capability that allows for communities and populations to be tested at an ecosystem-level, allowing for more complex interactions among the various compartments of a natural estuarine system to occur during chemical exposure. Evaluating simulated-estuarine systems requires space that is larger than an environmental

chamber, and we have a unique, greenhouse based system of modular estuarine systems ('mesocosms') that allows for replicated, systems level testing.

In addition to the organismal level data we collect, we have numerous capabilities for sublethal toxicity assessment, including cellular and molecular biomarkers (e.g., acetylcholinesterase, lipid peroxidase, glutathione, p450, cholesterol, protein, lipids, and genetic markers), chemoreception, imposex, growth, reproduction, and behavior. We also have expertise in microbiology, and use these capabilities as they relate to chemical contaminant effects on antibiotic resistance, pathogens, community changes, etc. We have trucks, small boats, and field sampling gear at our NCCOS Charleston Facility that allow for collection of coastal samples.

Our experienced chemistry team has capabilities for the quantification of inorganic and organic contaminants in various matrices including water, sediment, and tissues. We conduct chemical uptake and depuration studies and quantify trophic transfer. We are also highly involved in long-term monitoring studies that describe the distribution of pollutants in coastal areas. Historically, these efforts were focused on legacy pollutants such as organochlorine pesticides (i.e. DDT), oil and oil related compounds such as polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs), but over the past decade or so, we have increased our ability to determine contaminants of emerging concern (CEC). By developing these analytical extraction and detection protocols, our CEC efforts have supported laboratory, mesocosm and field research with a diverse list of contaminant classes (i.e. polybrominated diphenyl ethers (PBDEs), antifoulants, musks, organic sunscreen products, pharmaceuticals and phthalates). The analytical instrumentation includes inductively coupled plasma mass spectrometry (ICP-MS), Direct Mercury Analysis, gas chromatography/mass spectrophotometry (GC/MS), and liquid chromatography/mass spectrophotometry (LC/MS).

Experimental procedures

Selection of test species

Our research focuses on the areas of greatest potential chemical exposure (i.e. edge-of-field effects). In the southeastern coastal zone, the headwaters of tidal creeks are typically the point of entry for both point and nonpoint pollution from urban, suburban, industrial, resort, and agricultural activities. Representative estuarine species that we have selected for study include the grass shrimp (*Palaemonetes pugio*), the sheepshead minnow (*Cyprinodon variegatus*), the mysid (*Americamysis bahia*), the mummichog (*Fundulus heteroclitus*), the mud snail (*Tritia obsoleta*), the hard clam (*Mercenaria mercenaria*), the Eastern oyster (*Crassostrea virginica*), the amphipod (*Leptocheirus plumulosus*), the polychaete (*Neanthes* spp.), the copepod (*Amphiascus tenuiremis*), and the green alga (*Dunaliella tertiolecta*). These species represent a diversity of trophic levels, habitats, and feeding types within the estuary. They also have qualities associated with model toxicity test species, such as being ecologically and/or economically important, widely distributed, abundant, easy to collect or culture, tolerant of handling stress, and sensitive to chemicals. Juvenile forms of recreationally important fish species are also tested, including red drum (*Sciaenops ocellatus*) and spotted seatrout (*Cynoscion nebulosus*). Additional species are

included when relevant to specific chemical or ecological impacts. Individuals used for toxicity testing must be collected from the same source, be uniform in size, age, and physiological condition, and devoid of visible parasites. Our methods also minimize collection/handling stress and provide gradual acclimation to test conditions.

Selection of test chemicals

The purpose of our research is to provide chemical fate and effects data that are relevant to current environmental issues and that can be used to improve management of chemicals in coastal ecosystems. The selection of test chemicals for study is determined by several factors. The first is an identified data gap regarding the environmental concentrations and/or toxicological effects of a given chemical or contaminant class. This research need often becomes apparent after an industrial chemical has been spilled in the coastal zone, or after a non-target effect is suspected for a pesticide already in use. Requests for this type of study often come from state and federal management agencies. The second way we identify chemicals for testing is to respond to trends in the manufacturing, use, and disposal of chemicals. For example, we responded to the need for marine monitoring and toxicity data for PPCPs (pharmaceuticals and personal care products). Similarly we are initiating testing to quantify the effects of PFAS (per-and polyfluoroalkyl substances) and the mandated fluorine free fire-fighting foams as a replacement for current PFAS containing foams. These studies are often prompted by proposal calls from other government agencies such as the Environmental Protection Agency (EPA) or Department of Defense (DOD). The third way we determine which chemicals to study are through long-term research collaborations with NOAA, NOS partners. For example, a significant portion of our research portfolio is invested in serving data to NOAA's Office of Response and Restoration. We have a long-standing collaboration with ORR to study the fate and effects of oil and chemicals used in oil spill mitigation, such as dispersants and shoreline cleaners.

Test methods

The Ecotoxicology program utilizes a tiered approach to assessing contaminant effects in coastal ecosystems. Laboratory experiments to determine dose-response relationships and develop toxicity thresholds are the first tier of study. Each chemical contaminant is tested under controlled conditions with independent (chemical concentration) and dependent (survival, growth, number of lesions, etc.) variables and often following standard methods that are recognized internationally by various regulating organizations (ASTM, ISO, OECD, EPA). The exposures are hypothesis driven, where the null hypothesis states that chemical concentration does not affect the endpoint measured, and the alternative hypothesis then follows that chemical concentration will affect the endpoint measured, in a dose-dependent manner. The primary goal of each toxicity test is to establish thresholds of response by dose and time. The statistically-derived toxicity values obtained include EC50/LC50 (median effective/lethal concentration at which 50% of the test population responds), NOEC and LOEC (no observable effects concentration and lowest observable effects concentration), and MATC (maximum allowable toxicant concentration). Toxicity testing usually begins with a range-finding assay using log-spaced chemical doses, followed by definitive tests with the criteria of five or more test concentrations, spaced closer together, and yielding a response range of <35% to >65% effect. This tier of study is conducted

using standardized methods of testing, which allow for direct statistical comparisons of toxicity across test chemicals and across test species (see ASTM, US EPA, and OECD methods cited in the References section).

While standard methods are utilized to the greatest extent possible, some variations are needed for individual test species. Where modifications are necessary, we consider the following experimental design criteria: selecting the minimum number of test animals per treatment: 10 for static, 20 for flow-through (max. recommended biomass = 0.5-0.8 g/L static), sufficient replication, avoiding pseudoreplication, avoiding cross-contamination, required types of controls: negative, carrier/solvent, positive (reference).The treatments must be randomized within the test chamber to avoid spatial differences in temperature and lighting. Chemical and physical data are monitored daily.

The next tier of study is mesocosm testing. The goal of aquatic toxicology is to predict the effects of contaminants in an ecosystem, and mesocosms enable testing of more ecosystem components under more environmentally realistic conditions than laboratory testing. The NCCOS Charleston laboratory has developed and maintained a simulated estuarine mesocosm facility since ~1998 Briefly, the mesocosms include large saltwater tanks enclosed in a greenhouse (Figure 2). Each tank system incorporates a controlled tidal flux. There are 24 individual units, which allows robust experimental design, such as testing of five treatments with four replicates each. Generally, the ecosystem components incorporated in these tests are saltmarsh sediment, vegetation, and multiple species representing multiple trophic levels. These systems provide an important level of environmental realism, while still allowing control of most variables and sufficient replication for statistical analysis.

Ultimately, we strive to relate our laboratory findings to the environment, thus the final tier of testing is field research. This research requires creative but robust experimental designs and can involve deploying caged animals at sites of various chemical contamination and examining effects on survival. It also involves monitoring water, sediment, and animal tissues for the distribution of chemicals and markers of exposure/effect. The Ecotoxicology Branch uses a long-term reference tidal creek on Wadmalaw Island, SC, Leadenwah Creek (N 32° 38′ 50.89″; W 080° 13′ 18.05″), a tidal tributary of the North Edisto River, SC, USA, as a reference site. The surrounding watershed is relatively undeveloped and the levels of contaminants measured in the water and sediment are considered low, or “background”. This location has been the source of toxicity test animals such as grass shrimp for over 20 years. It also serves as a site for continuous water quality monitoring. A datasonde is maintained at the site for collection of water temperature, salinity, dissolved oxygen, and pH.

Data Quality and Data Management

In addition to using published standard methods for toxicity testing (ASTM, OECD, U.S. EPA), the Ecotoxicology Branch has compiled a set of Standard Operating Procedures (SOPs) that are used to assure consistency in methodology and data quality. These SOPs describe a wide range of common laboratory procedures (e.g. sample collection/receipt, glassware washing, preparation of liver microsomal fractions). SOPs provide documentation of methodology, instructions for staff, and consideration of safety and environmental compliance. The Ecotoxicology SOPs are available

in a folder on the NCCOS O: drive. An example of the format and a list of available SOPs are included (Appendix 1).

Each SOP also touches on the importance of quality assurance/quality control (QA/QC). QA/QC methods can include many aspects. For example, specific assays have requirements that must be met in order for the test to be considered valid (e.g. a coefficient of variability <30%, or ≤10% mortality in the controls, or a coefficient of determination (R^2) value ≥ 95%). It also includes good laboratory practices such as use of reference toxicants, instrument calibration, and data entry validation. Analytical chemistry methods also have specific QA/QC guidelines such as use of spikes (reagent and matrix), blanks, and standard reference materials (SRMs).

Given the large volume of data that are generated within the Branch, it is critical that we have a defined data management plan that allows us to keep project data organized and accessible. A project folder for each project is created and stored on the shared NCCOS server. Data generated by the chemistry team is tracked from sample receipt to data delivery and archiving in a proprietary laboratory information management system (ChemLIMS). All laboratory and field notes and data sheets are electronically scanned. Handwritten values from datasheets and numerical data from instruments are entered/exported to Excel spreadsheets, including metadata. Chain-of-custody forms, statistical analysis program outputs, etc. are included in the folder. All data are thus archived and available should they be requested. The storage location promotes collaboration among Branch scientists, and safeguards against loss of data from individual researcher computers. A graphic of the Ecotoxicology Branch data flow plan is provided in Figure 3.

Collaborators/Partnerships

The Ecotoxicology Branch collaborates with other Branches within NCCOS, especially the Monitoring and Assessment Branch and the Key Species and Bioinformatics Branch. The overlapping interests in assessing chemical contaminant impacts to corals and marine mammals, and characterizing the distribution of chemicals in coastal ecosystems allows for a natural interaction of testing and analysis. The Ecotoxicology Branch also works closely with other NOS offices; particularly the Office of Response and Restoration (OR&R). We have collaborations with all three divisions in OR&R (Emergency Response Division, Assessment and Restoration Division, Marine Debris Division). There are also significant partnerships within the state of South Carolina (South Carolina Department of Natural Resources, College of Charleston, Clemson University, University of South Carolina), and with other federal agencies (National Institutes of Standards and Technology, US Army Engineer Research and Development Center (ERDC)). We also conduct research with the Southern California Coastal Water Research Project (Emerging Contaminants Program).

Budget and staffing

The Ecotoxicology Branch consists of seven full-time federal employees and five full-time CSS contract staff (Table 1). The Branch structure includes three programmatic areas: Chemistry, Toxicology, and Mesocosm/Field. Each program has an Ph.D. level FTE program lead who also serves as Principal Investigator (PI) on related research projects. Most projects within the Branch

involve cross-program coordination. We have two organic chemists (one with a B.S.and one with a M.S. degree) and one inorganic chemist (Ph.D. degree). We have one B.S. level laboratory biologist, two M.S. level laboratory biologists, and one M.S. level and one B.S. level field and mesocosm biologists.

The PIs (Drs. Marie DeLorenzo, Peter Key, Paul Pennington, and Ed Wirth) have over 70 years of acute and chronic toxicity testing and chemical analysis experience focused on the marine environment. Abbreviated curricula vitae for the PIs are provided (Appendix 2).

The Ecotoxicology Branch funding (contractor labor, supplies, and travel) for FY19 was approximately $468,832 from NCCOS base, $48,040 from other NOAA sources, and $34,588 from external reimbursables. This distribution will change for FY20, with a greater proportion coming from external reimbursables due to the Department of Defense Strategic Environmental Research and Development Program (SERDP) grant.

Results

Research products

A list of research projects conducted by the Ecotoxicology Branch (Oct. 2016-2020) are provided in Table 2. These studies covered a range of chemical contaminants and estuarine taxa.Within the scope of this program review, the Ecotoxicology Branch has published 15 peer-reviewed journal articles and 6 NOAA Technical Memorandums, and delivered 30 platform presentations and 16 poster presentations (Available in Inventory of Accomplishments). In addition, we have disseminated data at scientific workshops and training events.

The data generated by our Branch are of particular value to the environmental management of contaminants because 1) fewer chemical toxicity threshold values exist for estuarine and marine organisms, 2) we can deliver a holistic chemical assessment through the tiered-approach to testing described previously.

Many of the laboratory species we utilize are not part of pesticide registration testing or chemical manufacturing testing requirements. The estuarine species we use in our testing broaden the understanding of potential chemical impacts in the environment, and sometimes capture unique responses that would be otherwise missed. For example, our research determined that the phenylpyrozole insecticide, fipronil, was significantly more toxic to estuarine crustaceans than freshwater species (Key et al. 2003). The chemical toxicity thresholds generated by our Branch have been catalogued in a number of databases including the NOAA Chemical Aquatic Fate and Effects (CAFE) database and the U.S. EPA Ecotoxicology Database (ECOTOX).

Beyond laboratory testing, our ecosystem level testing has been very effective in characterizing chemical fate and effects. Estuarine mesocosm data are typically lacking in risk assessment, and the use of mesocosms in marine and estuarine toxicology is somewhat limited. The NCCOS Charleston mesocosms have been employed to study a wide array of chemical contaminants including, herbicides, insecticides, fungicides, PAHs, metals, PPCPs, nanomaterials, oil,

dispersants, shoreline cleaners, and bioremediation products. They have primarily been used to model the intertidal zone, but have also been used to represent subtidal environments and to assess effects of thin-layer dredge disposal of contaminated sediments. Mesocosm level testing provides valuable data linking laboratory results, where environmental interactions are limited to a very specific range, to complex factors that are found in a field setting. The primary results that are provided by mesocosm testing that go beyond laboratory results are that we can characterize both direct and indirect effects (mortality, growth, reproduction, predator/prey interactions) while characterizing chemical partitioning, transformation and degradation. For example, results from our mesocosm facility have included verifying published sediment quality guidelines (ERL/ERM), assessing proposed water quality guidelines and chronic toxicity of contemporary use pesticides, describing decreases in bivalve growth related to herbicide impacts to microalgal communities, and describing persistence and transformation of chemical parent compounds to degradation products. The systems have also been used for technology testing, such as to validate datasonde sensors and to evaluate the efficacy of passive and active water sampling devices. The estuarine mesocosms have the added advantage of promoting teamwork and research collaboration. These experiments typically include investigators from multiple programs and agencies, along with graduate student participation.

This program review will describe recent and current (Oct. 2016-2020) research within the Branch. This briefing book will include case studies to provide examples of our laboratory, mesocosm, and field research. Herein will be results of comparative assessments across species and chemicals. The study examples include oil spill research such as the effects of thin oil sheens and their interactions with abiotic stressors such as ultraviolet (UV) light, temperature, and salinity. A multigenerational study with grass shrimp, oil and UV light is discussed. The briefing book will also describe work with oil spill mitigation chemicals such as dispersants and shoreline cleaners. New research with PFOS and fluorine-free AFFF compounds is presented. Restoration research is described with an ongoing marsh grass replanting study. A project with field-deployed mesocosms for chemical spill response is included. An analytical chemistry study characterizing the transformation of floating oil is presented. An example of technology testing in the mesocosms is provided, and a representative regional monitoring study example is provided with the Southern California Bight 2018 Regional Monitoring Program.

Research transition

We have worked closely with the end users of our chemical contaminant data to assure the relevance and utility of the research conducted. The applied nature of this research has yielded data used by management agencies to make informed decisions about chemical impacts in the coastal zone. Examples of our data use include the integration of toxicity data into the Office of Response and Restoration spill response databases, U.S. Environmental Protection Agency Pesticide Advisory Committee discussions, consultations on chemical ecotoxicity provided to state and regional agencies including Charleston County Mosquito Control, SC Department of Health and Environmental Control, and Clemson Department of Pesticide Regulation. One example was invited participation in the 2017 Workshop on Innovation and Regulation in Agriculture to contribute our expertise in Ecotoxicology. The continued requests for chemical fate

and effects data from local, state, and federal entities demonstrate how ecotoxicology research results are important to protecting coastal resources.

Education and Outreach

The Ecotoxicology Branch places significant emphasis on the importance of Education and Outreach. This an important aspect of our work and a central part of NOAA's mission. It is our opportunity to mentor students and inform the public on current environmental issues. Through these efforts, we foster career interest in the coastal sciences. We actively participate in numerous NOAA student training programs including the Hollings Scholar Program, the Educational Partnership Program, and the Knauss Fellowship. There are also requests for STEM support from local schools and colleges. Ecotoxicology Branch staff participate in events ranging from K-12 career fairs and science fairs to mentoring of doctoral level students. The Ecotoxicology Branch has collectively mentored hundreds of undergraduate and graduate students from institutions across the U.S. An abbreviated list of students mentored for Oct. 2016-2020 is provided in Appendix 3. Additionally, there is a list of Educational and Outreach Activities (Oct. 2016-2020) (Appendix 4).

Discussion

Our research approach is guided by accepted principles of toxicology and the corresponding assumptions associated with the discipline (e.g., Hodgson, 2004; Klassen, 2018; Malins and Ostrander 2004; Nikinmaa 2014; Rand, 1995). For example, all chemicals have the potential to be toxic, but for a chemical to elicit an adverse effect it must 1) come in contact with a biological receptor, 2) be present at a high enough concentration, and 3) for a long enough period of time for that response to occur. We consider the physical-chemical properties of the contaminant as well as organism physiological function when assessing environmental risk. We do not overstate or understate the potential for environmental harm, but rather place all chemical effects data in context of likelihood of environmental exposure.

Prior to any dissemination of data, a rigorous evaluation of the data quality and statistical relevance is performed. Statistical analyses must be used in accordance with assumptions surrounding the data and experimental design. In some cases, a statistically significant result may require additional interpretation based on ecological knowledge. For example, an experimental outcome may show oil causes a statistically significant decrease in plant growth, but we must consider the magnitude of the effect – is a 2 mm change in shoot height ecologically significant in terms of overall biomass and productivity? In contrast, we may observe an experimental outcome that we know could have meaningful ecological consequences, such as reduced larval swimming activity, but the effect is not statistically significant. In this case, it may be necessary to repeat the experiment with greater replication to further characterize the chemical effect.

These distinctions play an important role in how toxicological data are used in risk management. It is critical to base all management decisions on the best available data and with an application of knowledge and experience specific to the resources in question. Our tiered approach to

ecotoxicology includes the essential components of risk assessment (Figure 4). Through laboratory and mesocosm toxicity testing, we establish effects levels that can then be compared to exposure models and monitoring data to predict the likelihood of environmental impact.

One of the keys to a successful research program is to understand the importance of iterative planning and decision-making. This involves a collaborative effort between researchers and managers known as co-production (Djenontin and Meadow, 2018). The chemical contaminants work we do is applied science, and we take a very customer-driven approach in determining what environmental stressors to study. An example of the co-production model is seen in our work with ORR (Figure 5). They determine what data gaps exist in their response and restoration knowledge. We provide the science to answer those research questions. OR&R can then apply the information in response and restoration. The effectiveness of those management actions can then be measured through monitoring and those data feed back into our overall assessment. This is also known as adaptive management. Because it is an iterative process, the initial study and environmental management application may lead to more research questions. Working together at every step of the research and incorporating multiple knowledge streams will help ensure that as data are collected and shared, questions can be addressed, changes in course can be proposed, etc. that will improve the overall quality of the research product. As summarized by Williams (2011), "The feedback between learning and decision making is a defining feature of adaptive management, with learning contributing to management by helping to inform decision making, and management contributing to learning through interventions that are useful for investigating resource processes and impacts."

Challenges and Future Directions

One of the challenges we have is maintaining scientific equipment, particularly analytical chemistry instrumentation. For NCCOS to maintain a leading role in environmental chemical analysis, it is critical for our scientists to have technology that allows for the measurement of these legacy and emerging contaminants at environmentally relevant concentrations in a time efficient manner. Instruments critical in assessing environmental concentrations have rapidly decreased the detection limits, increased direct analysis of samples (thus reducing sample extraction time and costs and increasing sample throughput) and increased automation (eg. in-line sample clean-up or processing). Our instrumentation (GC/MS, LC/MS and ICP/MS platforms) are all greater than 10 years old (with the exception of one GC/MS acquired in 2019 for routine legacy/PAH analysis). Instrumentation required for sample extraction and preparation is ~20 years old (i.e. microwave-assisted digestion supporting inorganic analysis and gel permeation chromatographs supporting organic sample preparation). For NCCOS to remain and become a more widely regarded leader in ecotoxicological research, acquiring and supporting the analytical platforms required is critical.

Another challenge for the Ecotoxicology Branch is staff funding. Of the FY20 branch budget, approximately $500,000 is allocated to contract staff labor. This situation stems from a historical shortage of FTE backfills and new hires. We are staffing NCCOS mission work with the uncertainty of soft funding. Perhaps most critically, because many of our contract staff have been

in the branch more than 10 years, we have a long-term investment in cultivating expertise, which can be lost with short term horizon project planning.

Future staffing plans should consider maintaining critical NCCOS core research capabilities, while planning for potential areas to add new capabilities. There are research tools and techniques that would be desirable for growth within the Branch. For example, we would benefit from gaining experience with histopathology, integrating our research with the social sciences and ecosystem modelling.

Acknowledgments

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Tables

Table 1. Ecotoxicology team members

Name	**Position within Branch**	**FTE or CSS Contractor**	**Education Level**
Marie DeLorenzo	Branch Chief	FTE	Ph.D.
Pete Key	Program Lead Toxicology	FTE	Ph.D.
Paul Pennington	Program Lead Mesocosms	FTE	Ph.D.
Ed Wirth	Program Lead Chemistry	FTE	Ph.D.
LouAnn Reed	Inorganic Chemist	FTE	Ph.D.
James Daugomah	Biologist	FTE	M.S.
Joe Wade	Biologist	FTE	B.S.
Allisan Beck	Physiologist	CSS	M.S.
Katy Chung	Toxicologist	CSS	M.S.
Emily Pisarski	Organic Chemist	CSS	M.S.
Brian Shaddrix	Organic Chemist	CSS	B.S.
Blaine West	Biologist	CSS	B.S.

Table 2. Ecotoxicology Branch Projects Oct 2016-2020

- Southern California Bight 2018 Regional Monitoring Program: Sediment Toxicity
- South Carolina Coastal and Estuarine Assessment Program 2017-2018
- South Carolina Coastal and Estuarine Assessment Program 2019-2020
- An Assessment of Contaminants of Emerging Concern in Chesapeake Bay, MD and Charleston Harbor, SC.
- Integrated Assessment of Ecosystem Condition and Stressor Impacts in Submerged Habitats of the Guana Tolomato Matanzas (GTM) National Estuarine Research Reserve (NERR)
- Multi-stressor effects of ultraviolet light, temperature, and salinity on Louisiana Sweet Crude oil toxicity in larval estuarine organisms
- Comparing the survival and growth implications of photo-enhanced thin oil sheens on newly hatched and one week old sheepshead minnows (*Cyprinodon variegatus*), spotted seatrout (*Cynoscion nebulosus*), and red drum (*Sciaenops ocellatus*)
- Ecotoxicity of Perfluorooctane Sulfonate and Fluorine-Free Fire Fighting Foams to Estuarine Organisms
- Comparative Toxicity of Two Chemical Dispersants and Dispersed Oil in Estuarine Organisms
- Effect of Louisiana Sweet Crude Oil on a Pacific Coral, *Pocillopora damicornis*, Aquatic Toxicology
- Lionfish (*Pterois volitans*) as biomonitoring species for oil pollution effects in coral reef ecosystems.
- An interlaboratory comparison exercise for the determination of microplastics in standard sample bottles.
- Assessment of Hydrocarbon Carryover Potential for Six Field Cleaning Protocols.
- Depth-dependent temperature variability in the Southern California bight with implications for the cold-water gorgonian octocoral *Adelogorgia phyllosclera*.
- Toxicity Comparison of the Shoreline Cleaners Accell Clean® and PES-51® in Two Life Stages of the Grass Shrimp, *Palaemonetes pugio*
- Efficacy and ecotoxicological effects of shoreline cleaners in salt marsh ecosystems.
- Urinary phthalate metabolites in common bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, FL
- Effects of Salinity on Oil Dispersant Toxicity in the Mud Snail, *Ilyannasa obsoleta.*
- Comparative Toxicity of Two Chemical Dispersants and Dispersed Oil in Estuarine Organisms.
- Toxicity of oil and dispersant on the deep water gorgonian octocoral *Swiftia exserta*, with implications for the effects of the Deepwater Horizon oil spill.
- Exposure of the grass shrimp, *Palaemonetes pugio*, to antimicrobial compounds affects associated *Vibrio* bacterial density and development of antibiotic resistance.
- Effects of Salinity on Oil Dispersant Toxicity in the Grass Shrimp, *Palaemonetes pugio*.
- Mercury bioaccumulation in offshore reef fishes from waters of the Southeastern USA.
- Comparison of Chemical Contaminant Measures Using CLAM, POCIS, and PED Samplers in Estuarine Mesocosms

- An assessment of the impact of crude oil and UV light exposure on fertilization and early development in the variegated sea urchin, *Lytechinus variegatus*.
- Chronic developmental and reproductive effects in estuarine species following acute larval exposures to thin oil sheens and ultraviolet light.
- Field-based mesocosms: in situ deployments for assessing impacts of chemical spills in coastal areas.
- An Assessment of NPS Runoff Pollution in Coastal Stormwater Ponds of SC and the Potential for Development of Antibiotic Resistant Microbes.
- The verification of a benthic injury dose-response model for polychlorinated biphenyls
- Analysis of Floating Oil Exposed to Ultraviolet Light Under Different Environmental Conditions
- Assessment of oil spill effects and restoration methods for smooth cordgrass in salt marsh ecosystems.

Figures

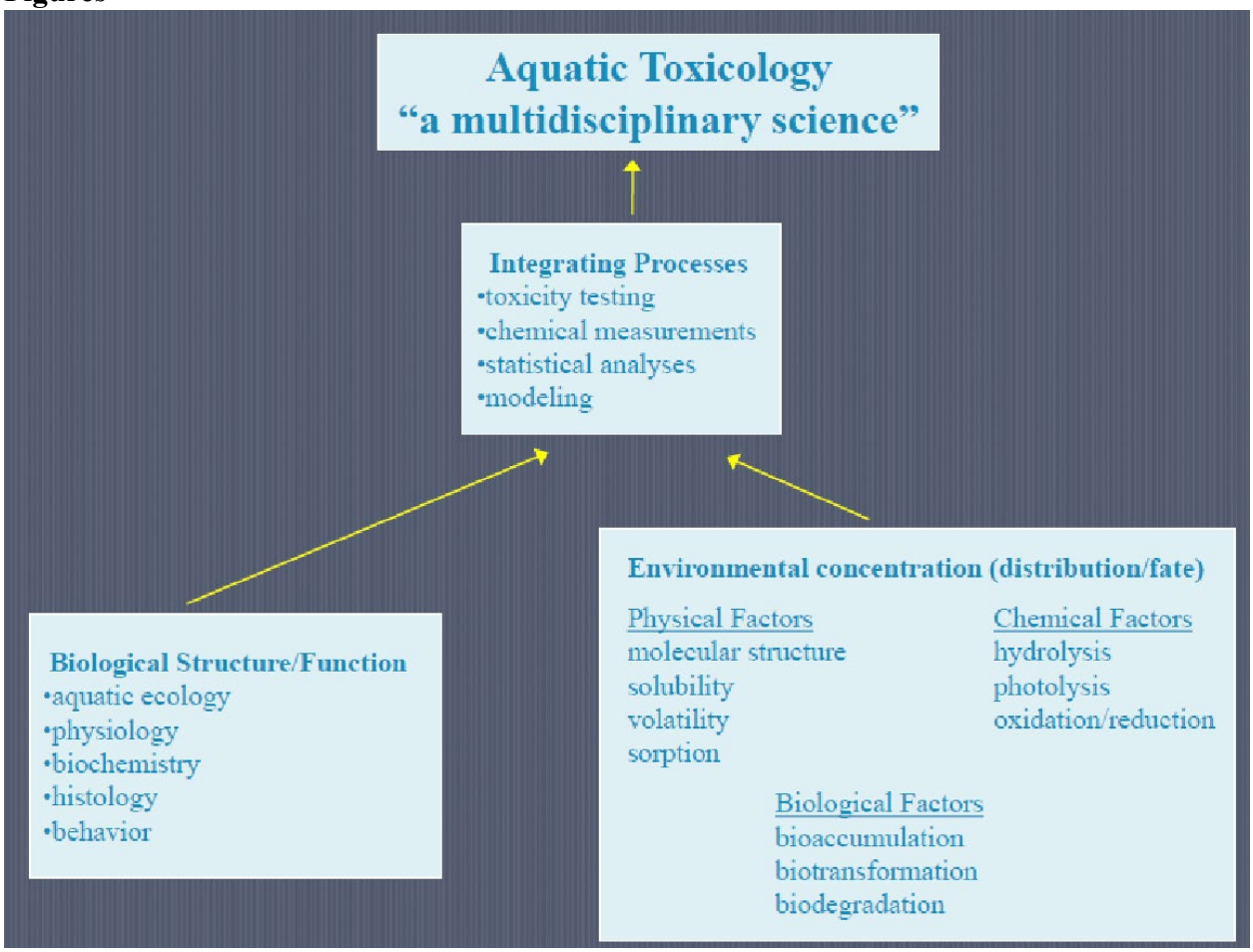

Figure 1. The interdisciplinary nature of aquatic toxicology, adapted from Rand, 1995.

Figure 2. Estuarine mesocosm facility at NCCOS Charleston.

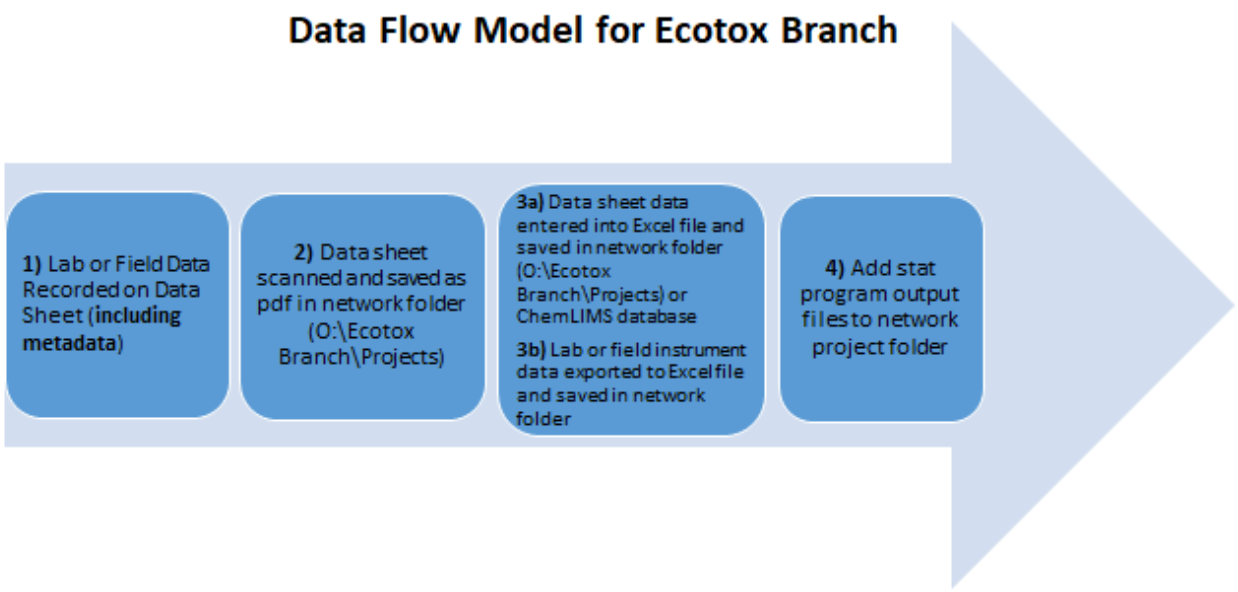

Figure 3. The model for how lab and field data are managed with the Ecotoxicology Branch.

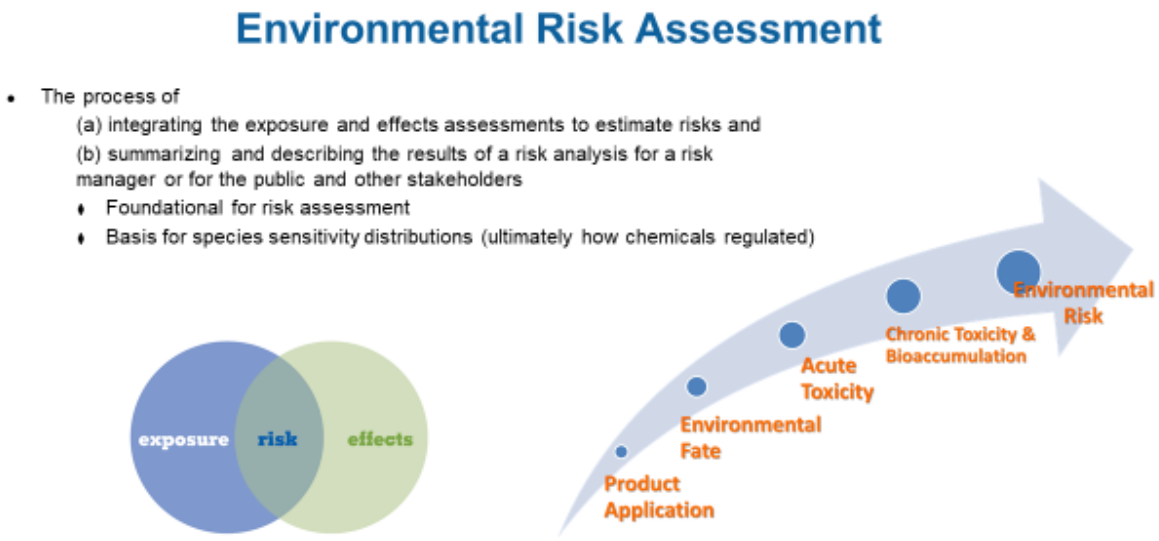

Figure 4. Environmental risk assessment components and process.

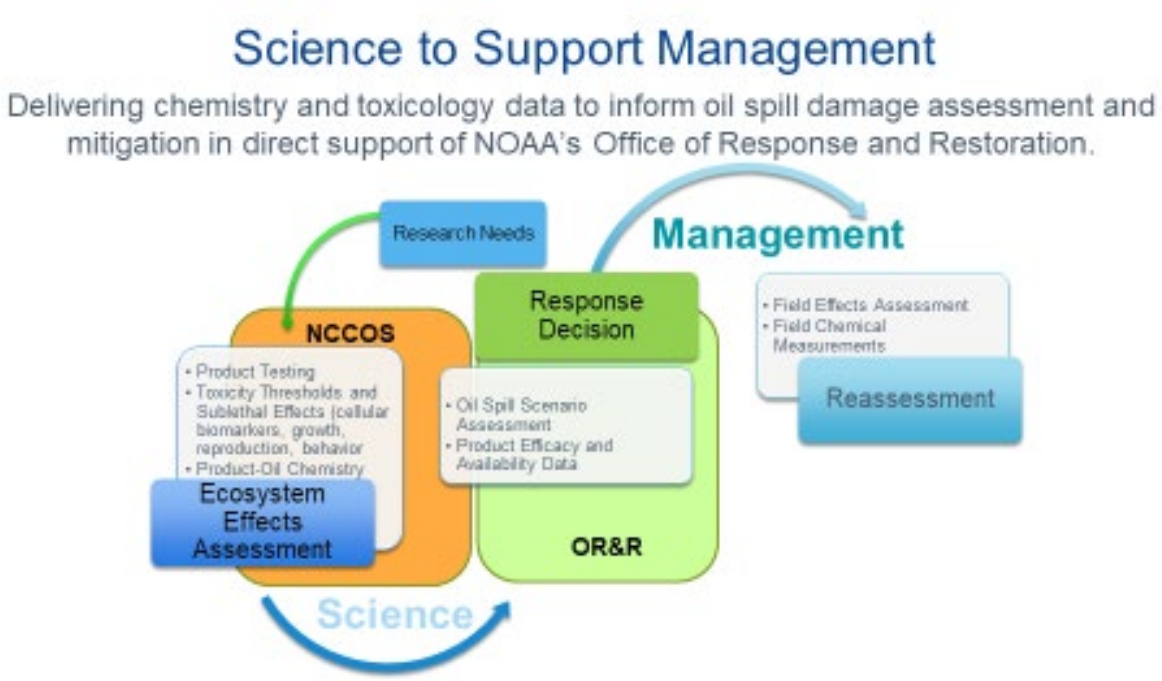

Figure 5. Example of the co-production model - how the Ecotoxicology Branch works with OR&R.

Appendix 1. Example SOP and List of Ecotoxicology Branch SOPs.

ECOTOX/SOP #001
Page 1 of 3
Created on 12/6/2000 8:42 AM
Revised on 8/26/2010

Title: **FORMAT FOR WRITING ECOTOX STANDARD OPERATING PROCEDURES**

Author:	Marie E. DeLorenzo	Date:_____
Program Manager:	Peter B. Key	Date:_____
Branch Chief:	Michael H. Fulton	Date:_____

1.0 OBJECTIVE

This section describes the purpose of the standard operating procedure (SOP).

2.0 HEALTH AND SAFETY

This section describes personal protective equipment, where the task should occur, and other safety considerations.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

This section describes the training and supervision required.

4.0 REQUIRED AND RECOMMENDED MATERIALS

This section lists the required supplies and equipment:

00000000000	0000000000000
00000000000	0000000000000

5.0 PROCEDURE

5.1 Main Heading 1

5.1.1 Subheading 1

- Bulleted steps should follow.

5.1.2 Subheading 2

- Bulleted steps should follow.

5.2 Main Heading 2

5.2.1 Subheading 1

- Bulleted steps should follow.

5.2.2 Subheading 2

- Bulleted steps should follow.

Add additional headings and steps as needed.

6.0 QUALITY CONTROL/QUALITY ASSURANCE

This section describes QA/QC requirements such as condition of equipment, instrument calibration, use of reference toxicants, record keeping, and data evaluation. For general guidance on good laboratory practices related to toxicity testing, see: USEPA, 1979, 1980; and DeWoskin, 1984.

7.0 REFERENCES

DeWoskin, R.S. 1984. Good laboratory practice regulations: a comparison. Research Triangle Institute, Research Triangle Park, North Carolina. 63 pp.

USEPA. 1979. Good laboratory practice standards for health effects. Part 772 - Standards for development of test data. Fed. Reg. 44:27362-27375, May 9, 1979.

USEPA. 1980. Physical, chemical, persistence, and ecological effects testing; good laboratory practice standards (proposed rule). 40 CFR 772, Fed. Reg. 45:77353-77365. November 21, 1980.

8.0 TABLES

This section includes tables such as recipes for stock solutions, example data sheets, etc.

9.0 EMS STATEMENT

This protocol of standard operating procedures (SOPs)/ guidelines (SOGs) fully incorporates the NOAA/NCCOS Environmental Management Systems (EMS) requirements and conforms to E.O.13423 and E.O.13514. The SOPs/SOGs include appropriate considerations regarding evaluating and minimizing an environmental footprint, and implementing energy and water conservation directives.

List of Ecotoxicology Branch Standard Operating Protocols and Guidelines

https://drive.google.com/drive/folders/1aNYdP63HCmaGjXzddsymNtnPdlTrmFNF

Toxicology and Physiology Program

Ecotox SOP Number	Title	Created Date	Revised Date	Author
001	Ecotox SOP Format			
002	Chronic Algal Toxicity Test	11/21/2000	09/06/2006	MED
003	Microscope Care and Use	11/21/2000	03/11/2006	MED
004	Bacterial Enumeration Using Acridine Orange Direct Counts	06/07/1999	03/11/2004	MED
005	Fluorometric Determination of Chlorophyll a	11/21/2000	03/11/2004	MED
006	Artificial Substrate (PFU) Deployment and Retrieval	12/06/2000	02/07/2005	MED
007	Microbial Sample Preservation	12/06/2000	03/11/2004	MED
008	Phototrophic Carbon Assimilation Assay	12/07/2000	03/11/2004	MED
009	Glassware Disposal Method	12/07/2000	03/11/2004	MED
010	Acid Bath Maintenance	02/18/2001	04/23/2004	KWC
011	Acute Clam (*Mercenaria mercenaria*) Toxicity Test	02/15/2001	04/23/2004	KWC
012	Chronic Clam (*Mercenaria mercenaria*) Toxicity Test	02/15/2001	04/23/2004	KWC
013	Nitrogen, Ammonia Test	02/21/2001	04/23/2004	KWC
014	Mutatox™ Toxicity Test	02/15/2001	04/23/2004	KWC
015	Microtox® Basic Test (Phenol Standard)	02/15/2001	04/23/2014	KWC
016	Microtox® Solvent Extract Test	02/15/2001	04/23/2014	KWC
017	Microtox® Solid-Phase Test (SPT)	02/15/2001	04/23/2014	KWC
018	Collection of Natural Seawater from Cherry Point	12/06/2000	02/07/2005	PLP
018a	Collection of Natural Seawater from Hollings Marine Lab (HML) [In Progress]			KWC
019	Water Grab Sample Collection	02/13/2001	02/07/2005	PBK
020	Methods for Large Volume Water Extraction (“Pepsi Cans”)	03/11/2004	06/30/2004	EFW
021	Sediment Collection for Analytical Chemistry Suite and Toxicity Testing	02/12/2001	02/07/2005	PBK

022	Procedure for Washing Labware for Pesticide Residue Quality (PRQ)	02/07/2001	03/11/2004	PBK
023	Boat Operating Procedures	05/12/2003	09/02/2010	JBW
024	SCECAP Push-net Sampling	09/02/2003	03/22/2004	JBW
025	*Artemia* Culturing	02/08/2001	03/11/2004	PBK
026	Grass Shrimp Culturing Techniques	12/14/2000	03/23/2004	PBK
027	Grass Shrimp Embryo (*Palaemonetes pugio*) Toxicity Test	12/11/2000	08/20/2009	KWC
028	Grass Shrimp Population Assessment and Monitoring	01/03/2001	02/04/2005	JBW
029	Push-netting - Monthly Samples	01/02/2001	02/07/2005	JBW
030	Hydrolab Minisonde Setup and Calibration	02/12/2001	03/11/2004	PBK
031	Water Chemistry: Polyclonal and Monoclonal Antibody Test Kits Procedures for Detection of Pesticides -- EnviroGard® (Strategic Diagnostics, Inc.)	11/13/2003	03/11/2004	PLP
032	Water Chemistry: Polyclonal and Monoclonal Antibody Test Kits Procedures for Detection of Pesticides – RaPID Assay® (Strategic Diagnostics, Inc.)	11/01/2003	12/01/2005	PLP
033	Ohaus Analytical Balance Model AP250D Standard Operating Procedures	02/16/2001	03/11/2004	PBK
034	Acetylcholinesterase Assay for Spectronic 601 Spectrometer	12/01/2003	03/11/2004	PBK
035	Protein Assay Protocol	02/13/2001	03/11/2004	PBK
036	Acute 96-hr Toxicity Test	02/14/2001	03/11/2004	PBK
037	Procedure for Creating a Pesticide Stock from Powdered Reagent	01/30/2001	03/11/2004	EFW
038	Amplex® Red Cholesterol Assay	10/27/2005	10/27/2005	JH
039	Lipid Extraction (Crustaceans)	01/30/2001	03/11/2004	EFW
040	Water Quality Instruments – YSI 55 and 85	01/29/2004	01/29/2004	KWC
041	Acetylcholinesterase Assay for Ultrospec 4300pro Spectrometer	11/25/2003	03/11/2004	PBK
042	Lowry Protein Assay Protocol for Ultrospec 4300 pro Spectrophotometer	11/25/2003	03/11/2004	PBK
043	New Chemical Receipt	03/31/2004	06/30/2004	EFW
044	Western Blot Standard Operating Procedures	05/13/2004	06/30/2004	JPE
045	*Leptocheirus plumulosus* Culture Protocol	07/08/2004	09/01/2010	KWC
046	28-d *Leptocheirus plumulosus* Sediment Bioasasay	01/14/2004	--	EFW

047	General Standard Operating Procedure for the Replicated Modular Estuarine Mesocosm: Materials Collection, Setup, Experimentation, and Breakdown	09/09/2004	09/13/2004	PLP
048	Water Quality/Parameter Data Collection with the YSI 556 MPS – Calibration, Maintenance, Usage, and Storage	02/16/2005	--	PLP
049	Glutathione Assay (GSH) Protocol for Ultrospec 4300 pro Spectrophotometer	10/13/2005	10/13/2005	JH
050	Lipid Peroxidation (LPX) Protocol for Ultrospec 4300 pro Spectrophotometer (using cuvettes)	10/13/2005	10/13/2005	JH
050a	Lipid Peroxidation (LPX) Protocol for Ultrospec 4300 pro Spectrophotometer (using 96-well plates)	05/14/2016	09/13/2016	KWC
050b	Lipid Peroxidation (LPX) Protocol for Ultrospec 4300 pro Spectrophotometer (using 96-well plates)			AB
051	Microcystin-LR Toxin Test	11/23/2005	12/09/2005	LSS
052	Clam Enumeration and Size	10/31/2006	11/02/2006	JH
053	10-d *Leptocheirus plumulosus* Sediment Bioasasay	09/01/2010	09/01/2010	KWC
054	Acetylcholinesterase Buffers and Reagents	01/23/2007	01/23/2007	JH
055	Water Quality/Parameter Data Collection with the YSI 6920 Data Sondes – Calibration, Maintenance, Usage, and Storage	03/08/2007	--	JBW
056	Mummichog (*Fundulus heteroclitus*) (Aqueous) Toxicity Test	07/06/2007	--	KWC
057	Grass Shrimp Adult (*Palaemonetes pugio*) (Aqueous and Sediment) Toxicity Test	07/06/2007	08/26/2010	KWC
058	mFC Agar Plates with 1% Rosolic Acid	04/20/2006	07/06/2007	LSS
059	Grass Shrimp Larval (*Palaemonetes pugio*) (Aqueous and Sediment) Toxicity Test	07/06/2007	--	KWC
060	*Americamysis bahia* Culturing Techniques	05/28/2009	--	KWC/ JJV
061	*Artemia* Culturing Techniques for Mysids	04/07/2009	--	KWC
062	*Americamysis bahia* Two-Generation Test	05/28/2009	--	KWC/ VM/ PBK
063	10-day Freshwater Amphipod (*Hyalella azteca*) Sediment Bioassay	04/11/2013	04/23/2014	KWC
064	mFC AGAR PLATES WITH 1% ROSOLIC ACID			
065	Spectrophotometric Method for Carbohydrate Analysis	07/30/2008	--	TDB
066	Spectrophotometric Method for Glycerol Analysis	07/29/2008	--	TDB
067	Mud Snail Adult (*Ilyanassa obsoleta*) (Aqueous and Sediment) Toxicity Test	01/29/2004	--	MCF

068	Mud Snail Culturing Techniques	01/29/2004	--	MCF
069	Mud Snail Larval (*Ilyanassa obsoleta*) (Aqueous and Sediment) Toxicity Test	01/29/2004	--	MCF
070	Nile Red Method for Lipid Analysis	07/28/2008	--	TDB
071	Plant Response and Toxicity Test Assays for estuarine marsh plants (*Spartina alterniflora* and other species) in laboratory and mesocosm studies	04/24/2014	--	PLP
072	UV Toxicity Test [In Progress]			KWC
073	Multistressor Toxicity Testing (salinity, temperature, pH) [In Progress]			KWC
074	Oil and Dispersant Toxicity Testing [In Progress]			KWC
075	Sheepshead minnow husbandry (collection, breeding) [In Progress]			KWC
077	Use of Power Tools and Dissecting Instruments [In Progress]			KWC
078	Field Deployment Cages (Construction and Deployment) [Draft]			JWD/ JBW/ PLP
079	Infaunal Processing (benthic ecology)			JD
080	Grain Size SOP (benthic ecology)			JD

Chemistry Program

Number	Title	Date	Updated	Auth or
CCR-001	CCR SOP Template	9/23/2009	6/9/2020	N/A
CCR-002	Organic Glassware Cleaning Protocol	9/23/2009	6/8/2020	ECP
CCR-003	Dry Weight Determination	9/23/2009	6/9/2020	BSS/ ECP
CCR-004	Dionex ASE 200 Extraction System	9/23/2009	6/26/2020	BSS/ ECP
CCR-005	ASE Extract Filtering Protocol	9/23/2009	6/26/2020	BSS/ ECP
CCR-006	TurboVap II Concentration Work Station	9/25/2009	4/27/2020	BSS/ ECP

CCR-007	Protocol for the Operation of the J2 Scientific Gel Permeation Chromatography System	9/23/2009	9/2/2010	BSS
CCR-008	SPE Clean-up Using Zymark Rapid Trace System (Silica)	9/23/2009		BSS
CCR-009	Cyanopropyl Column Cleanup for Sediment Extracts	6/10/2008	9/2/2010	GM
CCR-010	Alumina Column Cleanup for Organic Extracts	9/23/2009		DL
CCR-011	Cyanopropyl Column Cleanup for Sediment Extracts	6/10/2008	9/2/2010	GM
CCR-012	Lipid Analysis for Tissue Samples	9/23/2009	6/29/2020	ECP
CCR-013	Elution Protocol for Water Samples	6/10/2008	9/2/2010	BSS
CCR-014	Florisil PR Cleanup for Water Samples	6/10/2008	9/2/2010	BSS
CCR-015	South Florida Water Management District Elution Protocol for Water Extractions	6/10/2008	9/2/2010	DL
CCR-016	Extraction of Pyrethroids in Sediments	6/10/2008	9/2/2010	DL
CCR-017	Liquid-Liquid Extraction Of Bifenthrin From Seawater	6/10/2008	9/2/2010	DL
CCR-018	Solid Phase Extraction Of Antifouling Compounds From Seawater	6/10/2008	9/2/2010	YS
CCR-019	Solid Phase Extraction Of Estrogenic Compounds From Seawater And Wastewater	6/10/2008	9/2/2010	YS
CCR-020	Protocol for the Determination of POPs in Marine Mammal Serum and Plasma (draft)	4/1/2008	9/23/2009	GM
CCR-021	Protocol for the Determination of POPs in Marine Mammal Tissue	4/1/2008	9/23/2009	GM
CCR-022	Cleaning Of Labware For Metals Analysis	6/10/2008	9/29/2009	LAR
CCR-023	Acid Bath Maintenance	6/10/2008	9/29/2009	LAR
CCR-024	Drying And Grinding Sediments	6/10/2008	9/29/2009	LAR
CCR-025	Microwave Digestion Of Sediments Using Nitric Acid	6/10/2008	9/29/2009	LAR
CCR-026	Microwave Digestion Of Sediments Using Nitric And Hydrofluoric Acids	6/10/2008	9/29/2009	LAR
CCR-027	Microwave Digestion of Tissue Samples	9/29/2009	4/18/2019	LAR
CCR-028	Hotplate Digestion of Sediment Samples	6/10/2008	9/29/2009	LAR

CCR-029	Acid Volatile Sulfides And Simultaneously Extractable Metals	6/10/2008	9/29/2009	LAR
CCR-030	Inorganic Instrumental Analysis	6/10/2008	9/29/2009	LAR
CCR-031	Mercury Analysis	6/10/2008	9/29/2009	LAR
CCR-032	Sewage Treatment Plant Effluent Water Sample Collection for the Monitoring of Trace Levels of Pharmaceuticals	6/10/2008	9/29/2009	LAR
CCR-033	CHN Analyzer Procedures	6/27/2006		LSS
CCR-034	Environmental Water Sample Collection for the Monitoring of Trace Levels of Pharmaceuticals	9/30/2009		LAR
CCR-035	QUALITY ASSURANCE PROCEDURES and GUIDELINES	3/5/2004	11/6/2008	EFW
CCR-036	Determination of munitions compound residue by HPLC-MS/MS and HPLC-DAD [IN PROGRESS]	12/6/2000	9/2/2010	JMR
CCR-037	Prochloraz Solution (12 Mg/L) Preparation In 19 L Carboy For Diluter Test	5/7/2009	9/23/2009	YS
CCR-038	Solid Phase Extraction And LC-MS-MS Analysis Of Prochloraz In Seawater	3/5/2009	9/23/2009	YS
CCR-039	Sulfur Removal with Activated Copper Wool	12/13/2010		DL
CCR-040	Dilution of high concentration samples for analysis by HPLC	2/27/2013		JMR
CCR-041	Integration of sample chromatograms. [IN PROGRESS]	4/27/2020		ECP
CCR-042	Integration of procedural blanks for MDL calculation [DRAFT]	2/27/2013	4/27/2020	JMR
CCR-043	Analysis of Persistent Organic Pollutants by GC-MS [DRAFT]	4/29/2013		JMR
CCR-044	Combustion Cleaning of Sodium Sulfate for ASE Extraction [DRAFT]	4/30/2013		JMR
CCR-045	Sediment Extraction for Microtox and/or Reporter Gene Assay Extracts	4/30/2013		EFW
CCR-046	Cleaning of ASE Extraction Cells	5/2/2013		JMR
CCR-047	CCR Sample Receipt	4/30/2013		EFW

CCR-048	Sample Homogenization – Whole Tissue Composite [DRAFT]	10/29/13		JMR
CCR-049	Twister Extraction With Solvent Desorption	5/31/2011		DL
CCR-050	GC-MS/MS Basic Maintenance	1/23/2019		ECP
CCR-051	Extraction of PCBs from water samples [In Progress]			ECP
CCR-052	SPE Extraction for TEH and PAH in Seawater	1/1/2015	2/2/2017	ECP
CCR-053	Liquid-liquid extraction and cleanup of PAH-TEH from Seawater	9/23/2018		ECP
CCR-054	Acid Extraction for PPCPs in Sediments	1/11/2019		ECP
CCR-055	Acid Extraction for in Tissues	1/14/2019		ECP
CCR-056	PPCP Group 4 Base Extraction for Sediments	1/15/2019		ECP
CCR-057	PPCP Group 4 Base Extraction for Tissues	1/15/2019		ECP
CCR-058	Extraction of Munition Compounds from Marine Tissues via ASE	9/23/2018		ECP
CCR-059	Extraction of APs and APEOs from sediments	1/17/2019		ECP
CCR-060	Extraction of APs and APEOs from tissues	1/18/2019		ECP
CCR-061	Azure ChemLIMS Database	1/17/2019		ECP
CCR-062	DOSS Extraction from Seawater using QuEChERs	2/5/2015		ECP
CCR-063	Sample Collection, Filtration and Extraction Protocol for seawater samples from St. Croix for UV Filter Analysis	7/12/2017		ECP
CCR-064	SOP Hotblock Digestion of Dolphin Blood Samples	1/28/2010		LAR
CCR-065	SOP Mercury Analysis of Dolphin Skin Samples	1/28/2010		LAR
CCR-066	SOP Mercury Analysis of Dolphin Whole Blood Samples	1/28/2010		LAR
CCR-067	SOP Microwave Digestion of Dolphin Skin Samples	1/28/2010		LAR
CCR-068	Operation of A211 pH meter	1/15/2018		ECP
CCR-069	LC-MS/MS Troubleshooting	1/23/2019		ECP
CCR-070	Extraction of UV Sunscreens from Seawater [In Progress]	5/28/2020		ECP
CCR-071	LC-MS/MS Operation [In Progress]	12/5/2018		ECP

CCR-072	Extraction of Phthalate Metabolites from Dolphin Urine [In Progress]	8/7/2019		ECP
CCR-073	Extraction of Fipronils and Pyrethroids from Sediments	9/3/2019		ECP
CCR-074	Extraction of Fipronils and Pyrethroids from Silicone Bands [In Progress]			ECP
CCR-075	Preparation of Passive Samplers [In Progress]	4/3/2020		ECP
CCR-076	Extraction of Passive Samplers [In Progress]			ECP

Appendix 2. Abbreviated Curricula Vitae (CV) (3 pages max. per CV):

Marie Elizabeth DeLorenzo

219 Fort Johnson Road
Charleston, SC 29412
phone: (843) 460-9685
email: marie.delorenzo@noaa.gov

EDUCATION

1997	Ph.D. Environmental Toxicology, Clemson University, Clemson, SC
1994	M.S. Ecology, Penn State University, University Park, PA
1992	B.S. Environmental Resource Management, Minor in Marine Sciences, Penn State University, University Park, PA, *magna cum laude*

RESEARCH INTERESTS

Environmental toxicology, microbiology, physiological ecology, community ecology, food web dynamics, effects and mechanisms of pesticide toxicity, oil spill toxicity and response, bioaccumulation and trophic transfer, mesocosms, coastal resource management

PROFESSIONAL EXPERIENCE

2018-present *Supervisory Environmental Scientist, ZP-5*, U.S. Dept. Commerce, NOAA, National Ocean Service, National Centers for Coastal Ocean Science, Charleston, SC; Branch Chief, Ecotoxicology Branch

2006-2018 *Research Ecologist, ZP-4*, U.S. Dept. Commerce, NOAA, National Ocean Service, National Centers for Coastal Ocean Science, Charleston, SC; Program Lead, Environmental Physiology Program, Ecotoxicology Branch (2012-2018); 4-month detail as Acting Protected Areas and Resources Branch Chief, ZP-5 (2016); 4-month detail as Acting Monitoring and Assessment Branch Chief, ZP-5 (2018)

1998-2006 *Research Assistant Professor*, University of South Carolina School of Public Health, Environmental Health Sciences Department, affiliation with U.S. Dept. Commerce, NOAA, National Ocean Service, National Centers for Coastal Ocean Science, Charleston, SC; Ecotoxicology Branch

2014-pres. *Adjunct Graduate Faculty*, Florida A&M University, School of the Environment

2000-pres. *Adjunct Graduate Faculty*, College of Charleston, Charleston, SC, Masters Program in Environmental Studies

1999-pres. *Adjunct Graduate Faculty*, Medical University of South Carolina, Charleston, SC

1998-pres. *Adjunct Graduate Faculty*, Department of Biology, College of Charleston, Charleston, SC, Graduate Program in Marine Biology

PROFESSIONAL ORGANIZATIONS & COMMITTEES

NOAA representative to the National Water Quality Monitoring Council, an interagency and state forum for coordinating national efforts on water quality issues (Sept. 2016-present)

Board member of the Slocum-Lunz Foundation, a charitable, non-profit corporation whose purpose is the advancement of scientific knowledge and education through the support of students in marine biology and related natural sciences (April 2017-present)

College of Charleston, Marine Biology Program, Curriculum and Academic Planning Committee, 2000-2002; 2008-2010; 2014-2017; Marine Biology Graduate Council, 2004-2007; Nominations Committee, 1999-2000; Faculty Student Relations Committee, 1998-1999; Funding and Cooperative Research Committee, 2012-2014; Secretary, 2017-2019

Southeastern Estuarine Research Society member 1997-present, Program Chair 2002-2004, President Elect 2004-2006, President 2006-2008, Past President 2008-2010, Local meeting co-host 2013

Coastal Pesticide Advisory Committee, Charleston, SC, 1998-present, Chair, 2018-present

Society of Environmental Toxicology and Chemistry (SETAC) member, 1993-present; SETAC Pharmaceuticals Advisory Group member, 2005-2008; Awards Committee, 2016-present; Diversity and Inclusion Committee, 2018-present

Coastal and Estuarine Research Federation, governing board member 2006-2008

Carolina's Chapter of the Society of Environmental Toxicology and Chemistry (SETAC), member 1997-present, Board member, 2002-present, Student Chapter Relations Committee Chair, 2002-present, Program Chair 2003, Vice President 2004 and 2016; President 2005 and 2017; Past President 2006 and 2018

UNDERGRADUATE STUDENTS MENTORED = 31

GRADUATE STUDENTS MENTORED = 40

SELECTED JOURNAL PUBLICATIONS (out of 53)

Baxter, S.E., **DeLorenzo, M.E**., Key, P.B., Chung, K.W., Beckingham, B., Fulton, M.H. (2018) Toxicity Comparison of the Shoreline Cleaners Accell Clean® and PES-51® in Two Life Stages of the Grass Shrimp, *Palaemonetes pugio*. *Environ Sci Poll Res* 25(11):10926-10936. DOI: 10.1007/s11356-018-1370-2

DeLorenzo, M.E., Evans, B., Chung, K.W., Key, P.B., Fulton, M.H. 2017. Effects of Salinity on Oil Dispersant Toxicity in the Mud Snail, *Ilyannasa obsoleta. Environ Sci Poll Res* 24(26):21476-21483. DOI 10.1007/s11356-017-9784-9

DeLorenzo, M.E., Key, P.B., Chung, K.W., Pisarski, E., Shaddrix, B., Moore, J.G., Wirth, E.F., Pennington, P.L., Wade, J., Franco, M., Fulton, M.H. 2017. Comparative Toxicity of Two Chemical Dispersants and Dispersed Oil in Estuarine Organisms. *Arch Environ Contam Toxicol* 74(3): 414-430. DOI 10.1007/s00244-017-0430-9

Frometa, J., **DeLorenzo, M.E**., Pisarski, E.C., Etnoyer, P.J. 2017. Toxicity of oil and dispersant on the deep water gorgonian octocoral *Swiftia exserta*, with implications for the effects of the Deepwater Horizon oil spill. *Mar Poll Bull*. 122:91-99.

DeLorenzo, M.E., Eckmann, C.A., Chung, K.W., Key, P.B., Fulton, M.H. 2016. Effects of salinity on oil dispersant toxicity in the grass shrimp, *Palaemonetes pugio*. *Ecotoxicology and Environmental Safety*. 134:256–263.

Garcia, R.N., **DeLorenzo, M.E.,** Curran, M.C. (2014) Individual and mixture effects of two PPCPs, caffeine and sulfamethoxazole, on the daggerblade grass shrimp *Palaemonetes pugio* following ovigerous female exposure. *Environmental Toxicology and Chemistry*. 33(9):2120-2125.

Garcia, R.N., Chung, K.W., Key, P.B., Burnett, L., Coen, L., **DeLorenzo, M.E.** (2014) Interactive effects of mosquito control insecticide toxicity, hypoxia and elevated CO2 on larval and juvenile Eastern oysters and hard clams. *Arch. Environ. Cont. Toxicol*. 66:450–462.

DeLorenzo, M.E., Key, P.B., Chung, K.W., Sapozhnikova, Y., Fulton, M.H. (2013). Comparative toxicity of pyrethroid insecticides to two estuarine crustacean species, *Americamysis bahia* and *Palaemonetes pugio*. *Environmental Toxicology*.

DeLorenzo, M.E., Chung, K.W., Key, P.B., Fulton, M.H. (2012). Mixture toxicity of crude oil and Corexit® 9500 to estuarine organisms. *International Journal of Environmental Science and Engineering Research (IJESER)*. 3(3):161-169.

DeLorenzo, M.E. and Fulton, M.H. (2012) Comparative risk assessment of permethrin, chlorothalonil, and diuron to coastal aquatic species. *Marine Pollution Bulletin*. 64:1291-1299.

DeLorenzo, M.E., Danese, L.E., Baird, T.D. (2013) Influence of increasing temperature and salinity on herbicide toxicity in estuarine phytoplankton. *Environmental Toxicology.* 28(7):359-371.

Parent, L., **DeLorenzo, M.E.**, Fulton, M.H. (2011). Effects of the synthetic pyrethroid insecticide, permethrin, on two estuarine fish species *Journal of Environmental Science and Health,* Part B. 46:615–622.

Key, P.B., Chung, K.W., Hoguet, J., Sapozhnikova, Y., **DeLorenzo, M.** (2011).Toxicity of the mosquito control insecticide phenothrin to three life stages of the grass shrimp (*Palaemonetes pugio*). *Journal of Environmental Science and Health,* Part B. 46(5):426-431.

DeLorenzo, M.E., De Leon, R.G. (2010). Toxicity of the insecticide etofenprox to three life stages of the grass shrimp, *Palaemonetes pugio*. *Archives of Environmental Contamination and Toxicology*. 58(4): 985-990.

Baird, T.D., **M.E. DeLorenzo**. (2010). Descriptive and mechanistic toxicity of conazole fungicides using the model test alga *Dunaliella tertiolecta* (Chlorophyceae). *Environmental Toxicology*. 25(3):213-220.

DeLorenzo, M.E., Wallace, S.C., Danese, L.E., Baird, T.D. (2009). Temperature and salinity effects on the toxicity of common pesticides to the grass shrimp, *Palaemonetes pugio*. *Journal of Environmental Science and Health,* Part B. 44(5):455-460.

Finnegan, M.C., Pittman, S., **M.E. DeLorenzo**. (2008). Lethal and sublethal toxicity of the antifoulant compound Irgarol 1051 to the mud snail, *Ilyanassa obsoleta. Archives of Environmental Contamination and Toxicology*. 56(1):85-95.

NAME: Peter B. Key

PROFESSIONAL ADDRESS:
National Oceanic and Atmospheric Administration
National Ocean Service
National Centers for Coastal Ocean Science at Charleston
219 Ft. Johnson Road
Charleston, SC 29412

POSITION: Research Fishery Biologist

TELEPHONE: 843-460-9661

E-MAIL: Pete.Key@noaa.gov

EDUCATION:

University	Major	Degree	Date
Clemson University	Economic Biology	BS	1981
University of South Carolina (Environmental)	Public Health	MS	1985
University of South Carolina (Environmental)	Public Health	PhD	1995

AREAS OF RESEARCH EXPERTISE:
Oil and oil-dispersing chemicals effects on vertebrate and invertebrate aquatic animal.
Pesticide and pharmaceutical effects on vertebrate and invertebrate aquatic animal enzyme systems.
Toxicology of insecticides, pharmaceutics, metals and PAHs in crustaceans and fish.
Sediment toxicity tests utilizing benthic and pelagic aquatic animals.
Utilization of aquatic animal enzymes as biomarkers of exposure.
Detecting nonpoint source runoff effects on estuarine ecosystems.
Field sampling of water, sediments and aquatic animals.
Maintaining field instrumentation (datasondes, DO meters, pH meters, refractometers, etc.)

PROFESSIONAL EXPERIENCE:

Dates	Position	Employer
2002-Present Toxicology Program Lead Ecotoxicology Branch	Research Fishery Biologist	NOAA/National Ocean Service
1993 - 2002	Research Fishery Biologist	NOAA/National Ocean Service
1991-1993	Biological Lab Technician	NOAA/National Ocean Service
1988-1991	Graduate Research Technician	University of South Carolina

PROFESSIONAL AFFILIATIONS:

Delta Omega Society, Mu Chapter
Society of Environmental Toxicology and Chemistry
Southeastern Estuarine Research Society
Carolina Society of Environmental Toxicology and Chemistry, President 2015-2016
South Carolina Coastal Pesticide Advisory Council

Charleston County Adopt-A-Highway Laboratory Coordinator
NOAA Hollings Undergraduate Scholarship Program Review Panel

ACADEMIC AFFILIATIONS:

College of Charleston, Graduate Program in Marine Biology, Graduate Faculty. 1998 - present

RECENT PUBLICATIONS (out of 60):
Developmental and reproductive effects in grass shrimp (Palaemon pugio) following acute larval exposure to a thin oil sheen and ultraviolet light. P. Key, K. Chung, B. West, P. Pennington, M. DeLorenzo. 2020. Submitted to Aquatic Toxicology.

Toxicity Comparison of the Shoreline Cleaners Accell Clean and PES-51 in Two Life Stages of the Grass Shrimp, Palaemonetes pugio. S. Baxter, M. DeLorenzo, P. Key, K. Chung, B. Beckingham, M. Fulton. 2018.
Environmental Science and Pollution Research 25 (11):10926-10936.

Comparative Toxicity of Two Chemical Dispersants and Dispersed Oil in Estuarine Organisms. M. DeLorenzo, P. Key, K. Chung, E. Pisarski, B. Shaddrix, E.Wirth, P. Pennington, J. Wade, M. Franco, M. Fulton. 2018. Archives of Environmental Contamination and Toxicology. 74:414-430.

Effects of Salinity on Oil Dispersant Toxicity in the Eastern mud snail, Ilyanassa obsoleta. M. DeLorenzo, B. Evans, K. Chung, P. Key, M. Fulton. 2017. Environmental Science and Pollution Research. 24: 21476-21483.

Effects of Salinity on Oil Dispersant Toxicity in the Grass Shrimp, Palaemonetes pugio. M. DeLorenzo, C. Eckmann, K. Chung, P. Key, M. Fulton. 2016. Ecotoxicology and Environmental Safety. 134 (Part 1): 256-263.

Toxicity, uptake and enzymatic side effects of the monooxygenase inhibitor piperonyl butoxide to the grass shrimp (Palaemonetes pugio). E. Küster, J. Ragland, B. Shaddrix, K. Chung, P. Key, E. Wirth, M. Fulton, M. DeLorenzo. 2016. Archives of Environmental Contamination and Toxicology. (In Prep.)

Marine Debris Impacts on Coastal and Benthic Habitats. P. B. Key, S. McLaughlin. 2016. National Oceanic and Atmospheric Administration Marine Debris Program. Silver Spring, MD: National Oceanic and Atmospheric Administration Marine Debris Program. https://marinedebris.noaa.gov/reports/marine-debris-impacts-coastal-and-benthic-habitats

Assessment of crude oil and a dispersant in a simulated Spartina alterniflora salt marsh ecosystem. P. B. Key, K. W. Chung, C. L. Cooksey, M. E. DeLorenzo, M. H. Fulton, D. I. Greenfield, T. W. Greig, J. L. Hyland, J.L., B. C. Nelson, V. Patel, P. L. Pennington, E. J. Petersen, and E. F. Wirth. 2014. NOAA Technical Memorandum NOS NCCOS 186. 89 pp. National Ocean Service. National Centers for Coastal Ocean Science, Silver Spring, MD.

Interactive effects of mosquito control insecticide toxicity, hypoxia, and increased carbon dioxide on larval and juvenile Eastern oysters and hard clams. R. N. Garcia, K. W. Chung, P. B. Key, L. E. Burnett, L. D. Coen, M. E. DeLorenzo. 2014. Archives of Environmental Contamination and Toxicology. 66 (3): 450-462.

Relationship between land use classification and grass shrimp Palaemonetes spp. population metrics in coastal watersheds. J. W. Daugomah, P. B. Key, J. B. West, N. R. Shea, S. McDaniel, P. L. Pennington, M. H. Fulton. 2014. Environmental Monitoring and Assessment. 186 (6):3445-3453.

Insecticide toxicity in fish. M. Fulton, P. Key, M. DeLorenzo. In: Organic chemical toxicology of fishes. K. Tierney, A. Farrell, C. Brauner, eds. Academic Press: London. 2014; 309-368.

RECENT PRESENTATIONS AND POSTERS (out of 90):

Toxicity of common environmental contaminants on two estuarine species following multi-stressor impacts. P. Key, K. Chung, C. Collins, A. Beck, B. Shaddrix, M. DeLorenzo. Platform presentation by Key at 2020 Gulf of Mexico Oil Spill and Ecosystem Science Conference, Tampa, FL, 5 February 2020.

Assessment of oil spill effects and restoration methods for smooth cordgrass in salt marsh ecosystems. P. Key, P. Pennington, K. Chung, M. DeLorenzo. Poster presented by Key at 2019 CERF 25th Biennial Conference, Mobile, AL, 5 Nov 2019.

Comparison of chemical contaminant measures using CLAM, POCIS, and PED samplers in estuarine mesocosms. P. Pennington, D. Whitall, E. Wirth, P. Key, M. DeLorenzo. Platform presented by Pennington at 2019 Society of Environmental Toxicology and Chemistry, Toronto, Canada, Nov 2019.

Effects of oil spill mitigation products on the microbial community and water quality in estuarine mesocosm systems. P. Pennington, P. Key, E. Wirth, B. West, K. Chung, J. Wade, M. DeLorenzo. Platform presented by Pennington at PRIMO 20 Conference, Charleston, SC 20 May 2019.

Chronic developmental and reproductive effects in estuarine species following acute larval exposures to thin oil sheens and ultraviolet light. M.E. DeLorenzo, P.B. Key, K.W. Chung, E. Pisarski, P. Pennington, B. West, E. Wirth. Platform presentation by DeLorenzo at 2019 Gulf of Mexico Oil Spill and Ecosystem Science Conference, New Orleans, LA, 6 Feb 2019.

Developmental and reproductive effects in grass shrimp (Palaemonetes pugio) following acute larval exposures to thin oil sheens and ultraviolet light. P.B. Key, K.W. Chung, B. West, M.E. DeLorenzo. Platform presentation by Key at 2018 Society of Environmental Toxicology and Chemistry, Sacramento, Ca, 6 Nov 2018.

Multi-stressor effects of ultraviolet light, temperature, and salinity on oil toxicity in estuarine species. M.E. DeLorenzo, P.B. Key, K.W. Chung, P.L. Pennington, E. Pisarski, E. Wirth, M.H. Fulton. Platform presentation by DeLorenzo at 2018 Society of Environmental Toxicology and Chemistry meeting, Sacramento, Ca, 8 Nov 2018.

Chronic developmental and reproductive effects in estuarine species following acute larval exposures to thin oil sheens and ultraviolet light. M.E. DeLorenzo, P.B. Key, K.W. Chung, E. Pisarski, P. Pennington, B. West, E. Wirth. Platform presentation by DeLorenzo at 2019 Gulf of Mexico Oil Spill and Ecosystem Science Conference, New Orleans, LA, 6 Feb 2019.

Paul L. Pennington

CONTACT INFORMATION

NOAA, National Ocean Service
NCCOS Charleston and the Hollings Marine Laboratory
331 Fort Johnson Rd.
Charleston, SC 29412
843-460-9699
paul.pennington@noaa.gov

Professional and Academic Credentials

University of South Carolina, School of Public Health, Doctor of Philosophy, 2002
University of Charleston, South Carolina, Graduate Program in Marine Biology, Master of Science, 1996
College of Charleston, Department of Biology, Bachelor of Science, 1991

Additionally:

Coastal and Estuarine Research Federation, Member
Society of Environmental Toxicology and Chemistry, Member
Adjunct Faculty, Marine Biology Program, College of Charleston
Adjunct Faculty, Marine Environmental Studies Program, College of Charleston

Research Interests:

- Oil spill and oil mitigation research
- Salt marsh restoration methods following oil and chemical spills
- Monitoring of emerging contaminants in aquatic systems
- The effects of urban, suburban, agricultural, industrial, and resort runoff on aquatic organisms
- The usage of laboratory bioassays, microcosm and mesocosms to perform aquatic ecosystem health assessments
- Development and validation of new environmental technologies for water quality monitoring and assessment
- Non-point source runoff in aquatic systems
- Coastal land use and population growth
- Statistical analysis methods for toxicology, ecotoxicology, and environmental science

Professional Experience:

Dates	Position	Employer	Supervisor
4/20/2015 -- Present	Marine Biologist ZP-0401-4	NOAA National Ocean Service NCCOS Charleston	Dr. Marie DeLorenzo (843)460-9685
8/30/2014 – 4/17/2015	Scientist	JHT Incorporated / NOAA CCEHBR	Ms. Ann Skradski mollyb863@gmail.com

8/30/2010 – 8/29/2014	Marine Biologist ZP-0401-3/4-TERM	NOAA National Ocean Service CCEHBR	Dr. Michael Fulton fulton29492@yahoo.com
10/1/2003 – 8/29/2010	Senior Biologist	JHT Incorporated / NOAA CCEHBR	Mr. Wayne Alderman
5/1997 - 9/2003	Research Specialist II	University of South Carolina / NOAA CCEHBR	Dr. Geoffrey Scott (803)777-8964
9/1996 - 4/1997	Biological Science Laboratory Technician	Prof. and Technical Services, Inc. / NOAA CCEHBR	Dr. Geoffrey Scott (803)777-8964
10/1995 - 8/1996	Graduate Assistant	University of South Carolina / NOAA NMFS Charleston	Dr. Geoffrey Scott (803)777-8964
1/1993 - 9/1995	Biological Science Laboratory Technician GS-0404-4	NOAA NMFS Charleston Lab.	Dr. Geoffrey Scott (803)777-8964
6/1991 - 5/1995	Boatswain, senior crew	Southern Windjammer, Ltd.	Capt. Bob Marthai castlewizz@gmail.com

Publications:

Emily C. Pisarski, Edward F. Wirth, Paul L. Pennington, S. Ian Hartwell, Brian S. Shaddrix, David R. Whitall, Dennis A. Apeti, Greg Baker (In Review). Assessment of Hydrocarbon Carryover Potential for Six Field Cleaning Protocols. Target Journal is Environ. Monitor. Assess.

May, Lisa., Athena Burnett, Carl Miller, Emily Pisarski, Laura Webster, Zachary Moffitt, Paul Pennington, Ed Wirth, Gregory Baker, Robert Ricker, Cheryl Woodley. (2020) Effect of Louisiana Sweet Crude Oil on a Pacific Coral, Pocillopora damicornis. Aquatic Toxicology pre-proof available: https://doi.org/10.1016/j.aquatox.2020.105454

DeLorenzo, M., P. Key, K. Chung, E. Pisarski, B. Shaddrix, E. Wirth, P. Pennington, J. Wade, M. Franco and M. Fulton (2018). "Comparative toxicity of two chemical dispersants and dispersed oil in estuarine organisms." Archives of Environmental Contamination and Toxicology 74(3):1-17.

Downs, C. A., E. Kramarsky-Winter, R. Segal, J. Fauth, S. Knutson, O. Bronstein, F. R. Ciner, R. Jeger, Y. Lichtenfeld, C. M. Woodley, P. Pennington, K. Cadenas, A. Kushmaro and Y. Loya (2016). "Toxicopathological Effects of the Sunscreen UV Filter, Oxybenzone (Benzophenone-3), on Coral Planulae and Cultured Primary Cells and Its Environmental Contamination in Hawaii and the U.S. Virgin Islands." Archives of Environmental Contamination and Toxicology 70(2): 265-288.

Scott, G. I., D. E. Porter, R. S. Norman, C. H. Scott, M. I. Uyaguari-Diaz, K. A. Maruya, S. B. Weisberg, M. H. Fulton, E. F. Wirth, J. Moore, P. L. Pennington, D. Schlenk, G. P. Cobb and N. D. Denslow (2016). "Antibiotics as CECs: An Overview of the Hazards Posed by Antibiotics and Antibiotic Resistance." Frontiers in Marine Science 3(24).

Bratkovics, S., E. Wirth, Y. Sapozhnikova, P. Pennington and D. Sanger. 2015. Baseline monitoring of organic sunscreen compounds along South Carolina's coastal marine environment. Mar Pollut Bull 101: 370-377. doi:10.1016/j.marpolbul.2015.10.015..

Reed, L.A., W.E. McFee, P.L. Pennington, E.F. Wirth and M.H. Fulton. 2015. A survey of trace element distribution in tissues of the dwarf sperm whale (Kogia sima) stranded along the South Carolina coast from 1990-2011. Mar Pollut Bull 100: 501-506. doi:10.1016/j.marpolbul.2015.09.005.

Daugomah, J. W., P. B. Key, J. B. West, N. R. Shea, S. McDaniel, P. L. Pennington, and M. H. Fulton. 2014. Relationship between land use classification and grass shrimp Palaemonetes spp. population metrics in coastal watersheds. Environmental Monitoring and Assessment 186:3445-3453.

Downs, C. A., E. Kramarsky-Winter, J. Fauth, R. Segal, O. Bronstein, R. Jeger, Y. Lichtenfeld, C. Woodley, P. Pennington, A. Kushmaro, and Y. Loya. 2014. Toxicological effects of the sunscreen UV filter, benzophenone-2, on planulae and in vitro cells of the coral, *Stylophora pistillata*. Ecotoxicology 23:175-191.

Moore, J. G., A. Ruple, K. Ballenger-Bass, S. Bell, P. L. Pennington, and G. I. Scott. 2014. Snapshot of *Vibrio parahaemolyticus* densities in open and closed shellfish beds in Coastal South Carolina and Mississippi. Environmental Monitoring and Assessment:1-12.

Pennington, P. L., H. Harper-Laux, Y. Sapozhnikova, and M. H. Fulton. 2014. Environmental effects and fate of the insecticide bifenthrin in a salt-marsh mesocosm. Chemosphere 112:18-25

Wirth, E. F., P. L. Pennington, C. Cooksey, L. Schwacke, L. Balthis, J. Hyland, and M. H. Fulton. 2014. Distribution and sources of PCBs (Aroclor 1268) in the Sapelo Island National Estuarine Research Reserve. Environmental Monitoring and Assessment, DOI: 10.1007/s10661-014-4039-4

Burns, J. M., P. L. Pennington, P. N. Sisco, R. Frey, S. Kashiwada, M. H. Fulton, G. I. Scott, A. W. Decho, C. J. Murphy, T. J. Shaw, and J. L. Ferry. 2013. Surface charge controls the fate of au nanorods in saline estuaries. Environ Sci Technol 47:12844-12851.

Scott, G. I., M. H. Fulton, M. E. DeLorenzo, E. F. Wirth, P. B. Key, P. L. Pennington, D. M. Kennedy, D. Porter, G. T. Chandler, C. H. Scott, and J. L. Ferry. 2013. The Environmental Sensitivity Index and Oil and Hazardous Materials Impact Assessments: Linking Prespill Contingency Planning and Ecological Risk Assessment. Journal of Coastal Research:100-113.

Cleveland, Danielle, Stephen E. Long, Paul L. Pennington, Emily Cooper, Michael H. Fulton, Geoffrey I. Scott, Timothy Brewer, Jeff Davis, Elijah J. Petersen, Laura Wood. 2012. Pilot estuarine mesocosm study on the environmental fate of Silver nanomaterials leached from consumer products. Science of The Total Environment, Volumes 421–422 (2012) pages 267-272.

Fuquay, J. M., Muha, N., Pennington, P. L., & Ramsdell, J. S. (2012). Domoic acid induced status epilepticus promotes aggressive behavior in rats. Physiology & Behavior, 105(2), 315-320

Edward F. Wirth

CONTACT INFORMATION
NOAA, National Ocean Service
NCCOS Charleston and the Hollings Marine Laboratory
331 Fort Johnson Rd.
Charleston, SC 29412
843-460-9782
ed.wirth@noaa.gov

Professional and Academic Credentials
University of South Carolina, School of Public Health, Doctor of Philosophy, 1999
University of South Carolina, Marine Science; Master of Science, 1993
Lebanon Valley College, Biochemistry, Bachelor of Science, 1990

Additionally:
Society of Environmental Toxicology and Chemistry
Adjunct Faculty, Marine Biology Program, College of Charleston
Adjunct Faculty, Marine Environmental Studies Program, College of Charleston

Relevant Activities

Research interests include topics related to the toxicological effect (particularly sublethal effects) and quantification of chemical contaminants in the environment. Current environmental chemistry research efforts focus on evaluating analytical methods for the identification and quantification of new and emerging chemical contaminants in both aqueous, sediment, and tissue matrices in support of laboratory and field based research. Previous toxicological research projects included studies of pharmaceuticals, contemporary use pesticides and other anthropogenic contaminant effects on crustaceans, oysters and fish at both lethal and sublethal concentrations. In particular, research is focused on the fate and effects of emerging contaminants, including immunological, reproductive and behavioral changes within laboratory exposures as well as understanding the distribution of contaminants in the environment.

Previous research experience focused on the effects of contaminants on various benthic and epibenthic crustacean species, including evaluating the potential effects of endocrine disrupting chemicals. This research specifically examined the bioaccumulation of legacy pollutants in a harpacticoid copepod and required the development of methods for extracting PCBs in sub milligram mass of copepods. Subsequently, my research interests focused on the effects of sublethal pesticide exposure on the reproduction and physiology of the estuarine grass shrimp. Current use pesticides were found to affect the rate at which grass shrimp mated. This population effect was related to alterations in lipids chemistry and vitellogenin.

Selected Publications

Hart, L.B., B. Beckingham, R.S. Wells, M.A. Flagg, K. Wischusen, A. Moors, J.Kucklick, E. Pisarski, E. Wirth. 2018. Urinary Phthalate Metabolites in Common Bottlenose Dolphins (*Tursiops truncatus*) from Sarasota Bay, FL, USA. GeoHealth. 2(10):313-326. doi.org/10.1029/2018GH000146.

DeLorenzo, M.E., P.B. Key, K.W. Chung, E. Pisarski, B. Shaddrix, E.F. Wirth, P.L. Pennington, J. Wade, M. Franco, M.H. Fulton. 2017. Comparative Toxicity of Two Chemical Dispersants and Dispersed Oil in

Estuarine Organisms. Archives of Environmental Contamination and Toxicology. DOI 10.1007/s00244-017-0430-9.

Whitall, D., Ramos, A., Wehner, D., Fulton, M. Mason, A., Wirth, E., West, B., Pait, A., Pisarski, E., Shadrix, B., Reed, L. 2016. Contamination of the queen conch (*Strombus gigas*) in Vieques, Puerto Rico. *Regional Studies in Marine Science*. 5:80-86.

Balthis, W.L., C. Cooksey, M.H. Fulton, J.L. Hyland, G.H.M. Riekerk, R.F. VanDolah, E.F. Wirth. 2015. An integrated assessment of habitat quality of National Estuarine Research Reserves in the southeastern United States. *Integrated Environmental Assessment and Management*. 11(2):266-275.

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Appendix 3. List of students mentored by Ecotoxicology Branch Personnel, Oct. 2016-2020

UNDERGRADUATE STUDENTS MENTORED

1. Kaitlin Aaby, St. Mary's College of Maryland, St. Mary's City, MD, NOAA Hollings Scholar Program 2017
2. Deanna Hausman, University of Texas at Austin, Austin, TX, College of Charleston NSF REU Program 2017
3. Cheldina Jean, American University, Washington, D.C., College of Charleston NSF REU Program 2018
4. Max Zavell, University of Rhode Island, Kingston, RI, NOAA Hollings Scholar Program 2019
5. Carolina Rios, New York University, New York, NY, College of Charleston NSF REU Program 2019
6. Silvia Sdary, Lebanon Valley College, Annville, PA, College of Charleston NSF REU Program 2020 (upcoming)
7. Chloe Weyer, School of Public Health, Environmental Health Sciences, University of South Carolina, Columbia, SC
8. Philip Tanabe, NOAA Hollings Scholar , University of Miami, 2017
9. Joey Winston, NOAA Hollings Scholar, Louisiana State University, 2018
10. Kayla Laria, Miami University, Miami, FL, NOAA Hollings Scholar Program 2018
11. Cameron Collins, NOAA Hollings Scholar, University of South Carolina, 2019

GRADUATE STUDENTS MENTORED

Served as committee member:

1. Shannon Bley, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2020-present
2. Elizabeth Gugliotti, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2017-2018
3. Cassandra Horton, Ph.D. in Environmental Health Sciences, University of South Carolina, Columbia, SC, 2017-pres.
4. Edwina Mathis, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2017-present
5. Elizabeth Harris, M.S. in Marine Biology, Texas A&M University, Corpus Christi, TX, 2020-present
6. Rajaa Alyassein, Ph.D. in Environmental Health Sciences, University of South Carolina, Columbia, SC, 2017-2019
7. Catharine Parker, M.S. in Marine Environmental Studies, College of Charleston, Charleston, SC, 2017-2018
8. Kimberly Prince, Ph.D. in Environmental Engineering, University of Florida, Gainesville, FL, 2018-present
9. Fallon Parker, M.S. in Environmental Studies, College of Charleston, SC, Charleston, SC, 2016-18.
10. Jenna Klingsick, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2018-present
11. Brooke Blosser, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2019-present
12. Caroline Vill, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2018-present
13. Elizabeth Gugliotti, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2017-2018

14. Maria Zubizarretta, M.S. in Public Health, University of South Carolina, Columbia, SC, 2018-2019
15. Sarah Kell, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2017-present
16. Shelby Butz, Ph.D. in Public Health, Environmental Health Sciences, University of South Carolina, Columbia, SC, 2018-2019
17. Danielle Beers, College of Charleston, 2017-2020t
18. Rachel Leads, College of Charleston, 2016 - 2018
19. Sarah Baxter, College of Charleston, 2016 - 2017
20. Michelle Franco, College of Charleston, 2015 - 2017
21. Hannah Rutter, College of Charleston, 2015 - 2017

Served as major advisor:

1. Tiffany Baskerville, Ph.D. Florida A&M University, FL, 2013- 2017 – served as co-major advisor
2. Sarah Baxter, M.S. in Environmental Studies, College of Charleston, Charleston, SC, 2016-2017
3. Danielle Beers, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2017-2020
4. Jessica Ramirez, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2019-present
5. Breanna Hanson, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2019-present
6. Chloe VanderMolen, M.S. in Environmental Science, College of Charleston, Charleston, SC, 2019-present
7. Jenna Klingsick, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2019-present
8. Miranda Dzoibak, M.S. in Marine Environmental Studies, College of Charleston, Charleston, SC, 2019-present
9. Johnthan Stewart, M.S. in Marine Biology, College of Charleston, 2020-in progress
10. Hannah Rutter, M.S. in Marine Biology, College of Charleston, Charleston, SC, 2015-2017

Appendix 4. List of Education and Outreach Activities by Ecotoxicology Branch Personnel, Oct. 2016-2020

1. SeaPerch Charleston Challenge for Middle and High School Students, Katy Chung, Pete Key, Joe Wade, Paul Pennington, James Daugomah, Served on the organizing committee and assisted in the judging process for the underwater robotics competition 3/11/2020, 3/15/2019, 3/8/2018, 3/9/2017

2. NOAA Hollings Scholar Program Applications Reviewer, Katy Chung, Pete Key, March 2020, March 2019

3. Ashley Hall "Introduce a Girl to STEM" event, Sherri Fields, Chloe VanderMolen, and Marie DeLorenzo, Ashley Hall School, Charleston, SC, 2/20/20.

4. Sangaree Middle School Career Day, Ladson, SC, Joe Wade, 11/13/19

5. Interns from Congressman Joe Cunningham's D.C. office visited the NCCOS Charleston lab to learn about coastal pollution issues. Marie DeLorenzo, Blaine West, James Daugomah, 2 Aug 2019

6. Mentored University of South Carolina PhD student, Marie DeLorenzo, James Daugomah, Blaine West, Katy Chung, Pete Key, University of South Carolina student Rajaa Al-Yassein presented her dissertation research "The Effects of Climate Change on the Ecotoxicology of Contaminants of Emerging Concern on the Estuarine Grass Shrimp, Palaemonetes pugio". Her research was supported by the Ecotoxicology Branch through assistance with field collections and representation on her dissertation committee. 7/10/2019

7. Student Travel Award Reviewer, Society of Environmental Toxicology and Chemistry Annual Meeting, Marie DeLorenzo, Reviewed applications for the SETAC student travel awards. Recipients will receive funding to attend the annual meeting in Toronto, Canada in November7/15/2019

8. Mentored NOAA Hollings Scholar, Marie DeLorenzo, Katy Chung, University of Rhode Island Max Zavell studied the interactive effects of oil exposure and UV light, temperature and salinity on larval oyster survival May 2019-present

9. Leads MS student advisory committee, Ed Wirth, College of Charleston Graduate Program; Masters in Environmental Sciences, Participates on the thesis committee of MS student Miranda Dziobak; focuses on phthalte metabolites in the marine environment May 2019-present

10. Leads MS student advisory committee, Ed Wirth, College of Charleston Graduate Program in Marine Biology students, Leads thesis committee of MS student Jenna Klingsick; focuses on linking trace elements in coastal otter populations to land use May 2019-present

11. Leads MS student advisory committee, Paul Pennington, College of Charleston Graduate Program in Marine Biology students, May 2019-present

12. Leads thesis committee of MS student Jessica Ramirez; focuses on marsh replanting as tactic for oil spill marsh recovery

13. Mentored NOAA Hollings Scholar, Pete Key, Katy Chung, Cameron Collins, University of South Carolina, Cameron is studying the impacts of three contaminants on larval fish and shrimp. May - August 2019

14. Hosted 9th Grade Field Trip from Highpoint NCNCCOS Charleston, Wesleyan Christian Academy (83 Students and 8 Chaperones), Students chose two activities from the following focal groups: ecotoxicology, phytoplankton monitoring, marine mammal stranding, shallow water coral, or deep sea coral. Each focal group had a team that led a hands on activity for the students. This allowed students the opportunity to apply what they have learned through the school year and showcased the exciting work done at NCCOS Charleston. 5/13/2019

15. College of Charleston Summer Research Experience for Undergraduates (REU), Ed Wirth/ Paul Pennington/Katy Chung/Brian Shaddrix, Served as a mentor for Carolina Rios, New York University. Provided a summer research project on benthic injury assessment models. May-July 2019

16. Slocum-Lunz Foundation Annual Board Meeting, Marie DeLorenzo, All South Carolina colleges and universities, Serving 6 yr. term as board member. Slocum-Lunz is a charitable, non-profit corporation whose purpose is the advancement of scientific knowledge and education through the support of students in marine biology and related natural sciences. It awards small research grants to students enrolled in South Carolina institutes of higher education. 4/19/2019

17. Carolinas Society of Environmental Toxicology and Chemistry Meeting Hosted by NCCOS staff Marie DeLorenzo, Pete Key, Katy Chung, College of Charleston, University of SC, Clemson Univ, Catawba, Appalachian State, Served as conference organizers and student presentation judges. Promoted collaborations with environmental scientists in the SC and NC region. 3/15 - 3/17/2019

18. Society of Environmental Toxicology and Chemistry Inclusive Diversity Committee member Marie DeLorenzo, The charge of the Inclusive Diversity Committee is to provide a platform and structure within SETAC North America (SNA) that is inclusive of all people, including underrepresented groups, regardless of ethnicity, sexual orientation, gender identity, socioeconomic status, physical or mental difference, religion, age, or national origin. 11/6/2018

19. College of Charleston Graduate Program in Marine Biology (GPMB) Student Colloquium, Marie DeLorenzo, Judged student presentations, Grice Marine Biology Graduate School of College of Charleston 10/13/2018

20. NOAA Hollings Scholar Mentor, Pete Key, Louisiana State University, Served as a mentor for Joseph Winston. Provided a summer research project on oil bioremediation products. May-July 2018

21. NSF REU - College of Charleston student internship, Marie DeLorenzo, American University Served as a mentor for Cheldina Jean. Provided a summer research project on oil-UV-climate stressors. June-Aug 2018

22. Elizabeth Gugliotti thesis committee/defense, Peter Etnoyer, Marie DeLorenzo, Andrew Shuler, Ren Salgado, College of Charleston Graduate Program in Marine Biology 6/25/18

23. Served as chair of graduate student candidacy exams, Marie DeLorenzo, College of Charleston Graduate Program in Marine Biology, Interaction with local graduate program encourages student involvement in NCCOS research 6/4, 6/5, 6/12 2018

24. Federal Executive Association of the Greater Charleston Area Government Expo, Marie DeLorenzo, Sean Morton, Steve Morton, Sherri Fields, general public and K-12 school groups, Informed public on NCCOS research, created awareness of NOAA science in Charleston, 5/11/18

25. Carolinas Society of Environmental Toxicology and Chemistry Marie DeLorenzo, Pete Key, Katy Chung, Clemson University, College of Charleston, UNC Greensboro, UNC Charlotte, NC State University, Univ. of SC, The Citadel, Appalachian State Univ., Catawba College, Served as conference organizers and student presentation judges. Promoted collaborations with environmental scientists in the SC and NC region, 4/25-4/27 2018

26. Jack and Jill of America, Natasha White, Marie DeLorenzo, Katy Chung, Paul Pennington, Kids and parents visiting NOAA were introduced to marine science and NCCOS research, 3/30/2018

27. University of South Carolina graduate student qualifying exam Marie DeLorenzo,University of South Carolina School of Public Health, Environmental Health Sciences, Fosters collaboration with scientists working on stressor impacts in the academic sector, promotes NCCOS diversity initiative and student involvement, 3/2/2018

28. Student presentation at Society of Environmental Toxicology and Chemistry meeting, Minneapolis, MN, Marie DeLorenzo (NOAA), Cassie Horton, Geoff Scott (USC) University of South Carolina, 11/13/17

29. Gave tour of laboratory for new College of Charleston Graduate Program in Marine Biology students, Marie DeLorenzo, College of Charleston Graduate Program in Marine Biology students, Students learned about NCCOS research, 11/9/17

30. NOAA booth at SCDNR Open House, Marie DeLorenzo, Sherri Fields, Steve Morton, Sean Morton, Natasha White, Cheryl Woodley, Carl Miller, Laura Webster, Pete Key, Katy Chung, Tod Leighfield, Kathy Moore, Wayne McFee, Trey Knott, Public event, Local residents learned about NCCOS research, 10/21/2017

31. Serve as committee member for graduate student, Pete Key and Paul Pennington, College of Charleston, Research guidance for Sarah Kell. June 2017 – present

32. Served as research advisor/committee member for College of Charleston Graduate Student, Paul Pennington (advisor), Pete Key, Marie DeLorenzo, Hannah Rutter Sassman, College of Charleston's Graduate Program in Marine Biology, Contributed to the field of knowledge regarding the reproductive effects of crude oil and dispersants on small estuarine fish, 6/14/17

33. NOAA Hollings Scholar mentor, Pete Key, Served as a mentor for Phil Tanabe, University of Miami. Provided a summer research project on oil dispersants. May - July 2017

34. Charleston of Charleston NSF-REU student Deanna Hausman, Marie DeLorenzo/Paul Pennington, University of Texas at Austin & College of Charleston, Served as a mentor for Deanna on her research project "The Effect of UV Light on Oil Toxicity in the Estuarine Species Palaemonetes pugio" May 31-Aug 2017

35. Mentoring NOAA Hollings Scholar, Marie DeLorenzo/Paul Pennington, St. Mary's College of Maryland, Served as a mentor for Kaitlin on her research project "Effect of Ultraviolet (UV) Radiation on Crude Oil Toxicity in the Estuarine Species, Cyprinodon variegatus" May - July 2017

36. NOAA ECSC partnership, Marie DeLorenzo, Florida A&M University, Served as a co-advisor and NOAA lab host for PhD student Tiffany Baskerville, she successfully defended her dissertation May 25, 2017 Jan 2014-May 2017

37. Presenter and Panelist for “Conservation Forum: Dispatches from the Gulf”, Marie DeLorenzo, Sanibel-Captiva Conservation Foundation3/23/2017

38. Gave instructor presentations in Science of Spills class, Marie DeLorenzo, Maggie Broadwater, OR&R Emergency Response Division, 2/6/2017

39. Guest lecture for Environmental Studies Class, Marie DeLorenzo, College of Charleston, 1/31/17

40. Guest lecture for Environmental Health Class, Marie DeLorenzo, Medical University of South Carolina, 12/7/16

41. Serve as reviewer on the SETAC NA Awards and Fellowships Committee, Marie DeLorenzo, Students and scientists in Society of Environmental Toxicology and Chemistry, 11/9/2016-present

42. Presentation on Toxicity of Shoreline Cleaning Agents in Estuarine Organisms, Marie DeLorenzo, One NOAA Science Seminar Series, 11/16/16

43. MS Degree Candidate College of Charleston, Pete Key served as MS thesis committee member for Rachel Leads, 2015-2018

44. MS Degree Candidate College of Charleston, Marie DeLorenzo and Pete Key served as MS thesis committee members for Sarah Baxter, 2015 - May 2017

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

Peter B. Key
National Centers of Coastal Ocean Science, National Ocean Service, National Oceanic and Atmospheric Administration, Charleston, SC, USA

Katy W. Chung
JHT, Inc. and CSS, Inc. under contract to National Centers of Coastal Ocean Science, National Ocean Service, National Oceanic and Atmospheric Administration, Charleston, SC, USA

J. Blaine West
JHT, Inc. and CSS, Inc. under contract to National Centers of Coastal Ocean Science, National Ocean Service, National Oceanic and Atmospheric Administration, Charleston, SC, USA

Paul Pennington
National Centers of Coastal Ocean Science, National Ocean Service, National Oceanic and Atmospheric Administration, Charleston, SC, USA

Marie E. DeLorenzo
National Centers of Coastal Ocean Science, National Ocean Service, National Oceanic and Atmospheric Administration, Charleston, SC, USA

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

Introduction

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

For this present research, our organism of interest was the planktonic larvae of the grass shrimp. These shrimp inhabit estuaries from Nova Scotia to Texas, play a major role in nutrient cycling, can be prey for many recreationally and commercially valuable fishes, can often be the dominant macrofauna in estuarine creeks, and are used as a model crustacean in toxicity tests (Anderson, 1985; Key et al. 2006). The grass shrimp life cycle is approximately nine months long, and begins with females holding the eggs in their pleopods until hatching occurs. The larvae then swim in the upper water column molting several times until a final molt into a postlarval form (Anderson, 1985; Key et al. 2006). This present research focused on determining the long-term organismal health effects of a short-term exposure. The research previously mentioned has established that UV light can change the toxicity of oil and associated PAHs, but it has not established how it affects the organismal response in the long term after a short term exposure. To further investigate the above issues, newly hatched grass shrimp larvae were exposed for 24 h to an oil sheen with or without 8 hours of UV light and then raised to adult stage in clean seawater with the effects on reproduction observed.

Experimental Procedures

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

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

For those treatments under UV light, measured UV wavelengths were 1.6×10^{-3} W/cm^2 for UV-A as produced by a fixture holding two 54 watt F54T5HO UV-A Plus bulbs. The non-UV wavelengths of 1.8×10^{-6} W/cm^2 for UV-A were produced by a fixture holding two 17 watt F17T8 fluorescent bulbs (standard bulbs used in our toxicity tests). UV measurements were made using an ILT2400 light meter (International Light Technologies, Inc., Peabody, MA) placed at the top of the exposure container in the environmental chamber. The exposure containers were placed 30 cm below the light fixtures.

The larvae were exposed in 250-mL round glass containers that held 200 mL of either clean seawater or seawater with a 1-µm oil sheen. There were 10 larvae per container with three replicates for all treatments. Treatments were conducted concurrently in two environmental chambers at 25°C - one for the UV exposures and one for the non-UV exposures. After 24 h, larvae were placed in clean seawater via pipettes then transferred to well plates. Pipettes that penetrated the sheen to retrieve the larvae did not come into contact with the clean seawater vessel. Each well plate contained six wells. Each well held one larva with 10 mL of clean seawater. Larval development was monitored daily. Molts were recorded and removed, and the number of days to reach postlarval stage was determined. Each larva was fed 60 µL of newly hatched *Artemia* daily. Seawater in the well plates was changed every other day and water quality parameters (dissolved oxygen, salinity, pH, temperature) were measured at that time. This phase of the test was conducted in an environmental chamber at 25°C with 16 h non-UV light:8 h dark. When a larva molted to the postlarva stage, it was moved to a community 19-L

aquarium fitted with mechanical filtration with water temperature at 25°C under a 16 h non-UV light:8 h dark cycle. The shrimp were kept together in their original exposure groups from the postlarval stage until sexed. The shrimp were fed daily *ad libitum* with *Artemia*. Dry weights of a random sample of 10 postlarvae from each exposure group were measured as a further indicator of growth.

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

Water samples were collected to quantify the oil exposure beneath the sheens. Samples for chemical analysis were collected using separate thin oil sheen preparations where a standpipe (Teflon straw) was established in the glass container prior to water and oil additions. After 24h, the water beneath the sheen was collected from the standpipe using a siphon without disturbing the overlying oil layer. Chemistry samples were acidified to a pH of 2 and then transferred into solvent-rinsed 1-L separatory funnels to undergo liquid/liquid extraction. Samples were spiked with isotopically labeled internal standards and then solvent extracted three times with the following solvents, dichloromethane, 50:50 dichloromethane/hexane, and hexane. All solvent fractions were composited and then passed through GF/F paper containing anhydrous sodium sulfate and concentrated in a water bath (40°C) under a stream of nitrogen (14 psi) and solvent exchanged into hexane. Final extracts were further prepared by passing the last hexane fraction through a silica solid phase extract column SPE and then reduced under nitrogen to a final volume of ~1 mL. This final extract was spiked with a recovery standard (p-terphenyl) prior to instrumental analysis on GC/MS. Extracts were then run on an Agilent 6890/5793N GC/MS with split/splitless injector containing an Agilent DB17ms analytical column (60 m × 0.25 mm × 0.25 µm). The mass spectrometer was operated in selected ion monitoring (SIM) mode. Fifty PAHs were analyzed, including both parent and alkylated PAHs (Table 1). Chemical analysis of the 1-µm LSC oil sheen measured 5.26 µg/L of total PAH50 (±4.21 µg/L).

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

Results

Survival of the larvae after 24 h exposure to an oil sheen was not significantly affected ranging from 100% for Control non-UV to 98% for Sheen UV exposures (Table 2). Even after all larvae reached postlarval status, survival was still similar across the treatments staying above 85% (Table 2).

The average day for Control non-UV larvae to reach postlarvae stage was Day 21. At Day 21, only 10% of Sheen UV larvae had become postlarvae while 55% of Control non-UV were postlarvae (Table 2). After all the larvae had become postlarvae, there was a significant difference between the Sheen UV treatment and the other three treatments (Figure 1). The Control non-UV, Control UV and Sheen non-UV shrimp were similar in average number of days to reach postlarval status ranging from 21 to 23 days. The Sheen UV shrimp took a significantly longer time to reach postlarvae – at an average of just over 27 days. The number molts it took the larvae to reach postlarval status were counted and a significant difference was observed as well (Figure 2). It took an average of just under seven molts to become a postlarva for the Control non-UV shrimp while it took over eight molts for the Sheen UV shrimp.

Dry weights ranged from an average of 390.8 µg (±12.2 µg) for Control non-UV up to 419.5 µg (±10.2 µg) for Sheen non-UV exposed shrimp. There was no statistically significant difference among the treatments.

The total number of males, females and male/female pairs from each treatment were consistent among the treatments (Figure 3). The number of males (ranging from 38 for Sheen UV to 45 for Sheen non-UV) was higher than the number of females (ranging from 28 for Sheen non-UV to 30 for Control UV). This sex ratio is similar to grass shrimp field data collected from estuarine creeks in South Carolina, USA (Leight et al. 2005). Even though there were similar numbers for males and females in total for each treatment, our experiment was limited to a maximum of eight pairs for each replicate. As females were the limiting factor, some replicates had less than eight

females so our total number of pairs for each treatment ranged from 18 for the Sheen UV up to 22 for the Control UV.

There were several other parameters that were measured included male and female length, male and female weight, female weight with eggs, egg clutch weight, number of eggs, percent hatch, days to hatch. There was no statistically significant effect on these measured parameters from sheen or UV exposure (data not shown). As soon as seven later later after pairing and up to over a 30 days later, females became gravid. The pairs phase was terminated after 60 days. This was considered more than a sufficient amount of time to mate since a typical *P. pugio* female will produce at least eight broods during a 180-day breeding season (Bauer and Abdalla 2000).

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

Discussion

After 8 h of UV exposure under a 1-µm sheen as newly hatched larvae, grass shrimp development was significantly delayed in the Sheen UV exposed treatment. Grass shrimp larval development has been extensively studied in earlier manuscripts (Sandifer and Smith, 1979; Buikema et al., 1980; Key et al., 1998; Key et al, 2003; Key et al., 2006). These papers point out that molting is one of the most important parameters to measure in crustacean larval life stages. While the normal molting period may be altered by contaminants, which may not affect overall survival, any extension of the larval life stage may lead to increased predation causing a reduction in recruitment (Sandifer and Smith, 1979; Buikema et al., 1980; Key et al., 1998; Key et al, 2003; Key et al., 2006). Thus, more time spent as a larva may equate to more time spent in this planktonic stage, which may equate to more of a chance of becoming a prey item. In addition, the more molts a larva undergoes may cause greater stress on the larva leading to a greater chance of mortality (Anger, 2001; Key et al., 2003).

Identifying the exact components of oil that affect grass shrimp development was beyond the scope of this research, but it is well known that UV light can cause photo-enhanced toxicity of PAHs to invertebrates (Finch et al 2017). For the grass shrimp larvae, the most likely scenario involved absorption of the phototoxic PAH compounds through the gill membrane. The

phototoxic PAHs then form reactive oxygen species creating oxidative stress in the larvae. This in turn causes DNA damage, cell membrane damage, and damage to biomolecules (Finch and Stubblefield, 2016). Keitel-Groner et al. (2020) exposed Northern shrimp (*Pandalus borealis*) larvae to mechanically dispersed North Sea oil for 6 h followed by 30 days of recovery. It was determined that this short term exposure significantly affected long term larval fitness parameters and as these short exposures are seldom reported, the consequences are seldom known (Keitel-Groner et al. 2020). Researchers have pointed out that the aromatic compounds found in oil, especially heterocyclic aromatic compounds and alkyl-substituted PAHs, can contribute to toxicity in grass shrimp (Unger et al. 2008). Photolysis by UV light induces a multitude of reactions, which can produce an array of photochemical by-products. These products include acids, alcohols, esters, ketones, phenols and sulfoxides (Bobra 1992), among others, any of which could induce lethal and sub-lethal effects in grass shrimp. Most research available in the literature has dealt with individual PAH components of oil and their toxicity after UV exposure. Spehar et al (1999) exposed fresh and saltwater aquatic organisms to fluoranthene and UV over a 96h period and found that UV light increased acute toxicity by one to three orders of magnitude. When combined with 15 min of UV exposure, benzo[α]pyrene (BP) caused a high level of DNA lesions in grass shrimp embryos that were slowly repaired (Hook and Lee 2004). Other contaminants have been shown to affect molting in grass shrimp but only after exposures throughout the larval life cycle (Key et al., 1998; McKenney et al., 1998; Key et al, 2003).

Several measured parameters in adults were not significantly different from Control non-UV treatment or the Control UV treatment including growth, egg production, and egg hatching supporting the findings that the effect is based on the interaction of oil sheen and UV light and not a factor of UV light alone. As soon as a week later and up to over a month later, females became gravid. It was found that the rate at which gravid females were produced was significantly slower after 8h of UV exposure under a 1-µm sheen as newly hatched larvae. Others have documented delayed reproduction in female *P. pugio* after chronic contaminant exposure with pesticides (Wirth et al. 2002). Volz et al. (2002) exposed grass shrimp to UV light and the pesticide endosulfan and found that the percentage of gravid females was significantly lower compared to UV controls. However, these exposures were continuous for 50 days during the adult life stage unlike the present research exposures, which were only 24 h oil exposures with 8 h of UV light in the newly hatched larval stage. Any reproductive delays in grass shrimp may affect population size and structure. The effect of a contaminant on sperm or egg quality in adult invertebrates has been previously studied by other researchers, especially in regards to the contaminant modifying the ability to reproduce (Erraud et al. 2019; Lewis and Ford 2012; Matozzo et al. 2008; Sharara et al. 1998). For this present study, it is difficult to determine if the effect of the oil sheen and UV exposure interaction was greater on male reproduction or female reproduction. The exposure did not significantly affect the number of males versus the number of females, but rather the ability to mate or to produce sperm or eggs was probably affected.

Conclusion

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

Data availability

Data are located at NCCOS Charleston Lab and can be obtained from P. Key on request (pete.key@noaa.gov).

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Table 1. The parent and alkylated PAH analytes measured in PAH50

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

Table 2. The % survival of larvae after 24 h exposure, % survival of larvae to postlarval status, and % of larvae becoming postlarvae by Day 21 all after 24 h exposure to four treatments. Note the lag in development at Day 21 of larvae from the sheen treatments as compared to the controls.

Treatment	% Larval Survival after 24 h Exposure	% Survival to Postlarvae	% Larvae becoming Postlarvae by Day 21
Control non-UV	100	98	55
Control UV	100	93	40
Sheen non-UV	98	88	27
Sheen UV	98	85	10

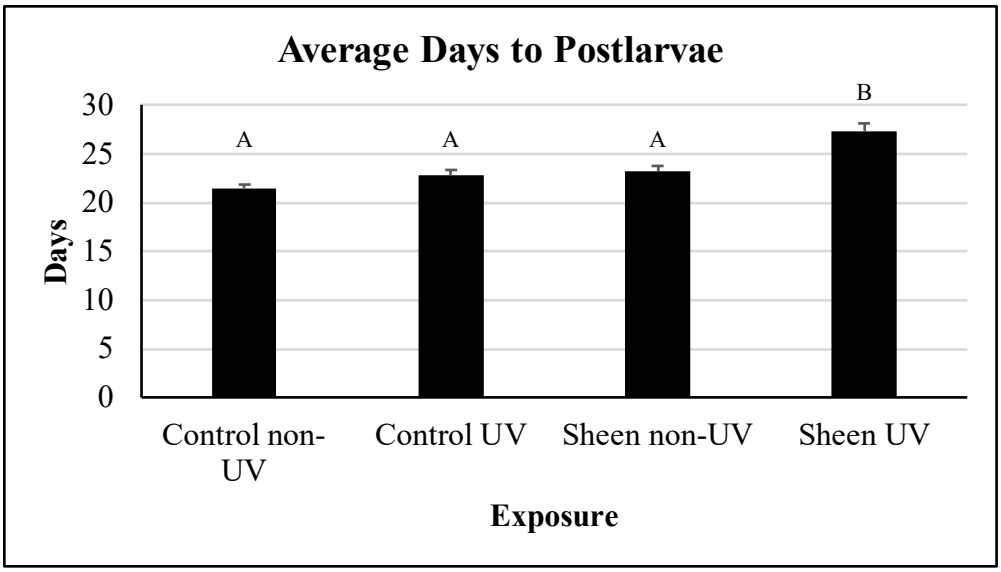

Figure 1. The average number of days for grass shrimp to reach postlarval status in the four exposure groups. Error bars represent standard error of the mean. Treatments denoted by same letter not statistically different from one another ($p<0.05$).

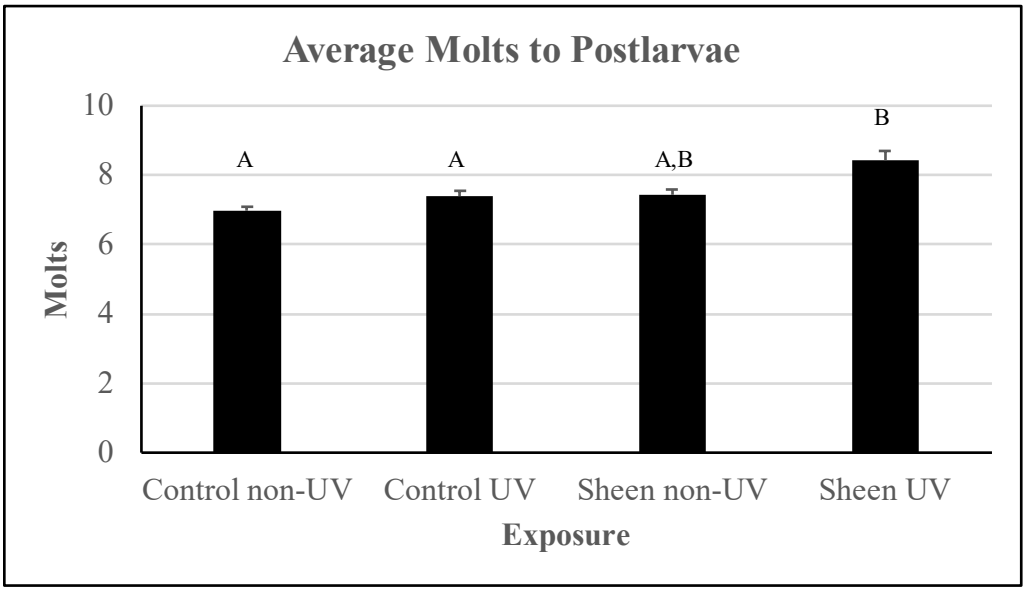

Figure 2. The average number molts for grass shrimp to reach postlarvae status in the four exposure groups. Error bars represent standard error of the mean. Treatments denoted by same letter not statistically different from one another ($p<0.05$).

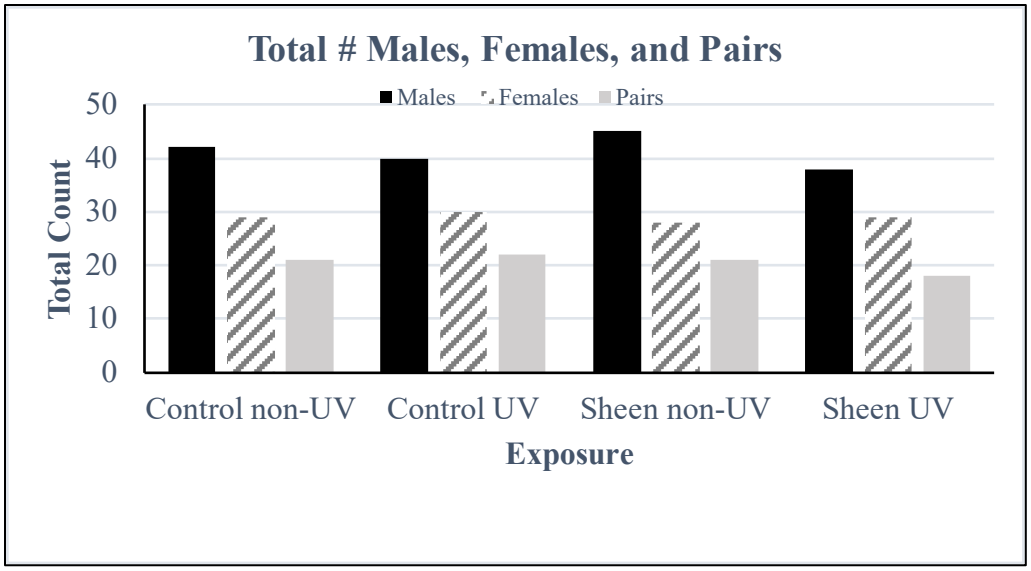

Figure 3. The total number of grass shrimp males, females, and pairs in the four exposure groups.

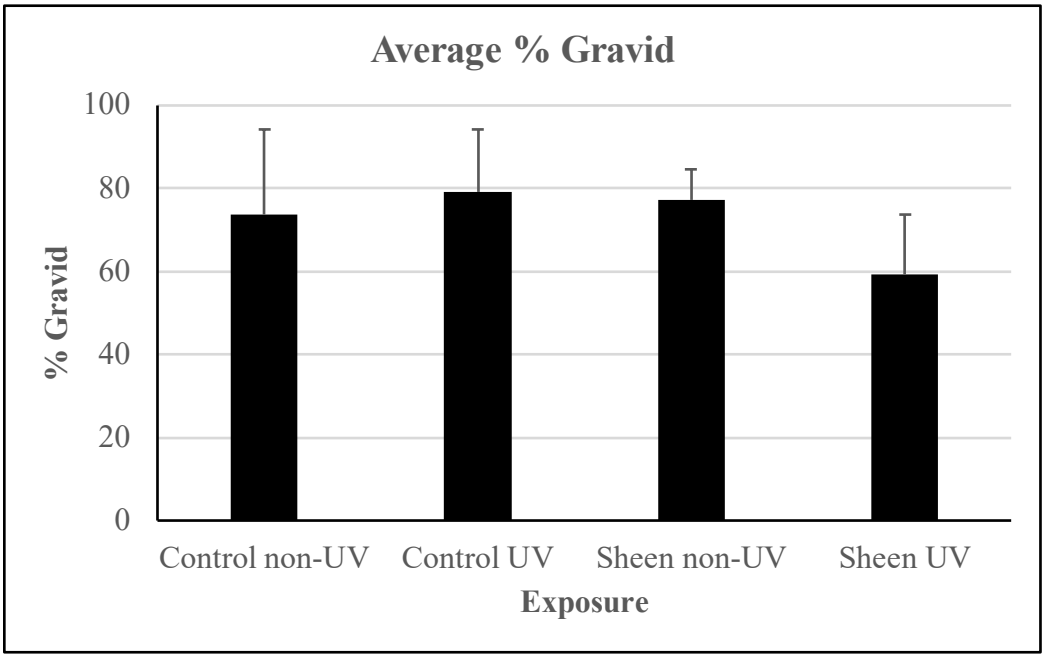

Figure 4. The average percent of female shrimp to become gravid in the four exposure groups. Error bars represent standard error of the mean. There were no statistical differences between the exposure groups.

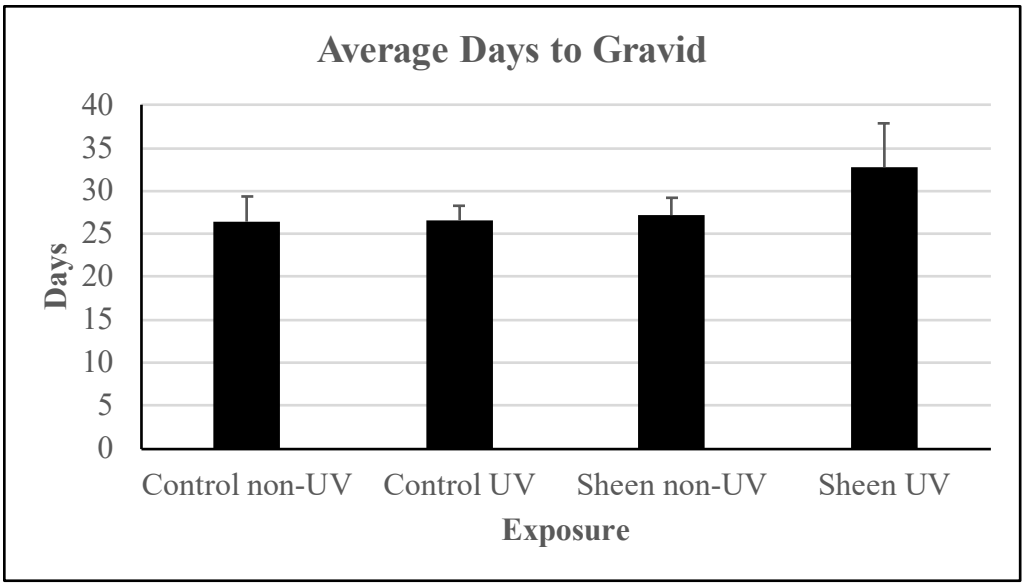

Figure 5. The average number of days it took the females to become gravid in the four exposure groups. Error bars represent standard error of the mean. There were no statistical differences between the exposure groups.

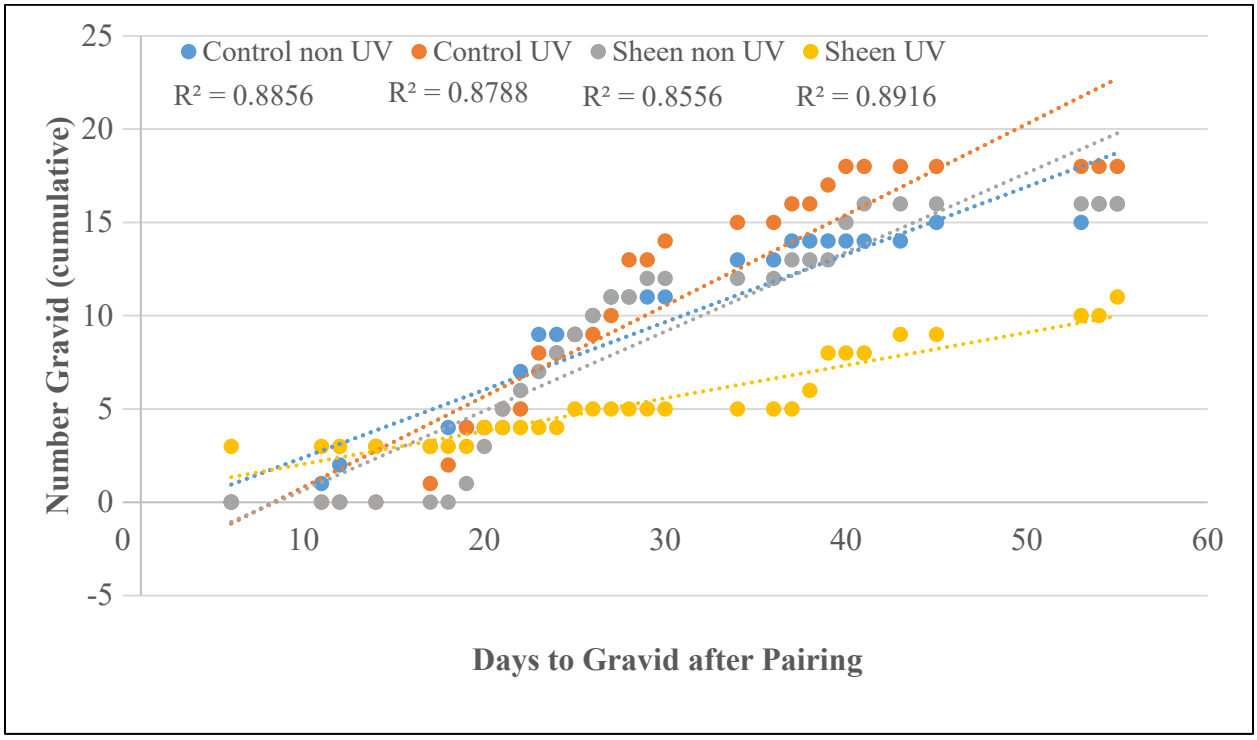

Figure 6. The cumulative number of gravid females versus the number of days it took to become gravid after pairing in the four exposure groups. The slope of the Sheen UV treatment was significantly lower than the slopes of the other treatments ($p<0.0001$).

Comparative Toxicity of Two Chemical Dispersants and Dispersed Oil in Estuarine Organisms

DeLorenzo, M.E.[1], Key, P.B.[1], Chung, K.W.[2,3], Pisarski, E.[2,3], Shaddrix, B.[2,3], Wirth, E.F.[1], Pennington, P.L.[1], Wade, J.[1], Franco, M.[4]

[1]NOAA, National Ocean Service, National Centers for Coastal Ocean Science, Charleston, SC, USA
[2]JHT, Inc., under contract to NOAA, Charleston, SC USA
[3]CSS under contract to NOAA, Charleston, SC USA
[4]College of Charleston, Charleston, SC, USA

Abstract

Chemical dispersants can be a useful tool to mitigate oil spills. This study examined potential risks to sensitive estuarine species by comparing the toxicity of two dispersants (Corexit® EC9500A and Finasol® OSR 52) individually and in chemically enhanced water-accommodated fractions (CEWAFs) of Louisiana Sweet Crude (LSC) oil. Acute toxicity thresholds and sublethal biomarker responses were determined in seven species (sheepshead minnow, grass shrimp, mysid, amphipod, polychaete, hard clam, mud snail). Comparing median lethal (LC_{50}) values for the dispersants, Finasol was generally more toxic than Corexit and had greater sublethal toxicity (impaired embryonic hatching, increased lipid peroxidation, decreased acetylcholinesterase activity). The nominal concentration-based mean LC_{50} for all species tested with Corexit was 150.31 mg/L, compared to 43.27 mg/L with Finasol. Comparing the toxicity of the CEWAFs using the nominal concentrations (% CEWAF), Corexit-CEWAFs appeared more toxic than Finasol-CEWAFs; however, when LC_{50} values were calculated using measured hydrocarbon concentrations, the Finasol-CEWAFs were more toxic. There was greater dispersion efficiency leading to greater hydrocarbon concentrations measured in the Corexit-CEWAF solutions than in equivalent Finasol-CEWAF solutions. The measured concentration-based mean LC_{50} values for all species tested with Corexit-CEWAF were 261.96 mg/L Total Extractable Hydrocarbons (TEH) and 2.95 mg/L Total Polycyclic Aromatic Hydrocarbons (PAH), whereas the mean LC_{50} values for all species tested with Finasol-CEWAF were 23.19 mg/L TEH and 0.49 mg/L Total PAH. Larval life stages were generally more sensitive to dispersants and dispersed oil than adult life stages within a species. These results will help inform management decisions regarding the use of oil-spill dispersants.

Introduction

Following an oil spill, dispersants are applied to alter the chemical composition of oil by decreasing interfacial tension and breaking up oil into particulate-sized droplets (Council 2005). Smaller droplets of oil contain a higher surface area, allowing bacteria to degrade the oil more quickly. The use of dispersants may reduce the overall impact of an oil spill (Lessard and Demarco 2000), however dispersing oil into water may result in increased chemical loading into benthic and coastal habitats (Ramachandran et al. 2004). Current and tidal movement may transport dispersants into sensitive coastal habitats such as mangroves and salt marshes.

Dispersed oil droplets may become trapped and concentrate in semi-enclosed coastal areas (Scarlett et al. 2005).

The 2010 Deepwater Horizon Oil Spill was treated with approximately 7 million liters of the dispersant Corexit® 9500A. Finasol® OSR 52 is another dispersant registered for oil spill response in the U.S., but considerably less is known regarding its toxicity to estuarine species. Corexit is manufactured by Nalco Energy Services, Sugar Land, TX, USA and Finasol is produced by Total Fluides, Paris-La Defense, France. Both dispersant compounds consist of mixtures of petroleum distillates and surfactants, but the exact chemical make-up differs. The list of chemicals in Corexit is publically available (http://blogs.edf.org/health/2010/06/08/presto-corexit%C2%AE-dispersant-ingredients-revealed/), whereas the constituents of Finasol remain undisclosed to the public.

The objectives of this study were to 1) compare the acute toxicity of two oil spill dispersants, Corexit and Finasol, in a suite of common estuarine species; and 2) compare the acute toxicity of dispersed oil preparations (chemically-enhanced water accommodated fractions (CEWAFs) of each dispersant with Louisiana Sweet Crude (LSC) oil) in a suite of common estuarine species.

The species chosen for study are common to southeastern tidal creek estuaries and represent different habitats and trophic levels within the ecosystem. The test organisms included a fish (sheepshead minnow, *Cyprinodon variegatus*), crustaceans (grass shrimp, *Palaemon* [*Palaemonetes*] *pugio* and mysid, *Americamysis bahia*), a gastropod (Eastern mud snail, *Ilyanassa obsoleta*), a bivalve mollusk (hard clam, *Mercenaria mercenaria*), a polychaete (nereid worm, *Neanthes arenaceodentata*), and an amphipod (malacostracan, *Leptocheirus plumulosus*). These seven estuarine species are of ecological and economic importance; contributing important functions such as influencing phytoplankton and nutrient dynamics, serving as prey for commercially and recreationally important fish species, and a providing a source of commercial shellfish revenue (hard clam). For fish, shrimp, clams and snails, the sensitivity of more than one life stage was assessed. Median lethal (LC_{50}) toxicity values were determined for each test organism and a number of sublethal endpoints were measured in some species, including timing and success of embryo hatching, p450 enzyme activity, acetylcholinesterase activity, splenocyte proliferation, and lipid peroxidation activity. The results of this study may aid resource managers' capacity to respond to oil spills by increasing scientific knowledge of the impacts of oil, with and without chemical dispersants, on estuarine salt marsh ecosystems.

Experimental procedures

Test Species and Conditions

C. variegatus, *L. plumulosus,* and *A. bahia* were acquired from Aquatic Biosystems (Fort Collins, CO, USA). *P. pugio* and *I. obsoleta* 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. *M. mercenaria*, were acquired from Bay Shellfish, Inc. (Terra Ceia, FL, USA). *N. arenaceodentata* were obtained from Aquatic Toxic Support (Bremerton, WA, USA).

Seawater (for all testing) was acquired from Charleston Harbor estuary (N 32° 45' 11.52''; W 79° 53' 58.31''), pre-filtered (5 µm), activated carbon filtered, and diluted with deionized water to adjust salinity to 20 ppt. Seawater for the mysid test was UV-sterilized and further filtered to 1

µm and seawater for the grass shrimp embryo test, the larval clam, and larval snail tests was further filtered to 0.22 µm.

Dispersant Testing

All species were tested with Corexit and Finasol, individually, using static renewal exposures. Every 24-h, dead animals were removed, water quality (temperature, salinity, pH and dissolved oxygen) was assessed, and test solutions were renewed. Test chambers consisted of glass jars or beakers (covered and aerated) or 24-well polystyrene plates coated with hydrogel (Corning™) to reduce chemical adherence (Chandler et al. 2004) placed on an orbital shaker (80 rpm). Range finding assays were conducted to determine appropriate dispersant exposure concentrations. For each species and life stage, a definitive test consisting of a seawater control and five nominal concentrations was conducted to determine a median lethal concentration (LC_{50}) for both Corexit and Finasol. Additional test conditions for each species are provided in Table 1.

Dispersed Oil (CEWAF) Testing

Each of the 12 bioassays (fish: embryo-larval and adult; shrimp: embryo, larval, and adult; clam: larvae and juvenile; snail: larvae and adult; mysid; polychaete; and amphipod) performed with the individual dispersants were repeated using CEWAFs of the dispersants in mixture with Louisiana Sweet Crude (LSC) oil. Preparation of the CEWAFs followed methods similar to Hemmer et al. (2011), using low-energy mixing (vortex 25% of the solution height, stirred for 18 hours and allowed to sit for 6 hours). Each CEWAF consisted of 19 L of 20 ppt seawater, 25 g/L of oil, and 1.25 g dispersant/L (a ratio of 1:20 dispersant:oil). The 100% CEWAF was diluted with 20 ppt seawater to achieve additional treatments (50%, 16.7%, 5.6%, 1.85%, 0.62%, 0.21%). Controls consisted of 20 ppt seawater. Test methods were similar to those used for the individual dispersant testing, except that CEWAF testing was conducted using static exposures (Hemmer et al. 2011).

Cellular Bioassays

Sublethal effects on cellular function measured from surviving animals at the end of the 96 h exposure included cytochrome p450 enzyme induction and splenocyte proliferation activity in adult fish; and acetylcholinesterase activity and lipid peroxidation in adult fish, adult shrimp, and adult snails. Cytochrome p450 enzyme induction (based on ethoxyresorufin [EROD]) was measured as an indication of hydrocarbon metabolism in adult fish from the individual dispersants tests and the CEWAF tests. Microsomal fractions of the livers were obtained and measurement of enzyme activity and protein concentration were performed simultaneously using a well plate format adapted from Kennedy and Jones (1994) (DeLorenzo 2012). The splenocyte proliferation assay was performed according to Parent et al. (2011), as a measure of fish immunotoxicity. Assessment of lipid peroxidation activity (LPX) as a measure of cellular oxidative damage was performed for adult fish (liver tissue), adult grass shrimp (whole shrimp), and adult mud snails (tissue removed from shell) from the dispersant alone and CEWAF exposures according to the malondialdehyde method of Ringwood et al. (2003), adapted to microplate format (DeLorenzo et al. 2006). As a measure of nervous system function, acetylcholinesterase (AChE) enzyme activity was assessed using methods of Key et al. (1998) in adult fish brain tissue from the CEWAF exposures and adult grass shrimp (whole shrimp) and adult snails (tissue removed from shell) from dispersant alone and CEWAF exposures.

Chemical Analysis

The different exposure protocols (static-renewal for dispersant alone testing and static for CEWAF testing) were selected based on available chemical analyses. Dispersant solutions were renewed in order to maintain a relatively constant chemical concentration throughout the test since we could not chemically analyze the products. The dispersant-only testing is reported as nominal concentrations of Finasol and Corexit. The CEWAF solutions were not renewed, consistent with methods in Hemmer et al. (2011), but were chemically analyzed to assess hydrocarbon concentrations. The chemical analyses conducted for the CEWAF testing included Total PAH, which was based on a suite of 50 parent and alkylated PAHs (Supplemental Table 1), and total extractable hydrocarbons (TEH) sampled at time (t) = 0 (immediately after dosing) and at t = 6 h, t = 24 h, and t = 96 h for the large volume toxicity tests (adult fish and adult grass shrimp). A time weighted average (TWA) concentration was calculated for Total PAH and TEH using the equation: TWA = (t1c1 + t2c2+ t3c3 + t4c4) / (t1 + t2 + t3 + t4), where t = time and c = concentration at each sampling point. The sample taken immediately after dosing was considered 1 hour, such that the denominator of the equation = 127 h. Water samples (50-500 mL) were collected from each CEWAF test chamber, and replicate samples were composited by treatment and analyzed for TEH and Total PAH according to NOAA SOP CCR-052 (Supplemental Data).

Statistical Analysis

All median lethal concentrations (LC_{50} values) with 95% confidence intervals (CIs), as well as the ten percent effect concentration (LC_{10} values) were determined using SAS Probit Analysis (PROC PROBIT, SAS V.9.4, Cary, NC, USA). Dispersant LC_{50} and LC_{10} values were calculated using nominal exposure concentrations due to the propriety nature of the Finasol product. The CEWAF LC_{50} and LC_{10} values were calculated using nominal concentrations (% CEWAF) and then recalculated using the measured chemistry values (mg/L) for TEH and Total PAH. 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) with Dunnett's procedure for multiple comparisons used to determine which treatments differed significantly from the control.

Results

Water quality for all toxicity tests was maintained within acceptable ranges for dissolved oxygen (≥ 60% saturation), pH (8.0 ± 0.5), temperature (25 °C ± 2), and salinity (20 ppt ± 2). Control survival for all definitive tests met protocol standards (>80% fish and shrimp embryo tests; >90% all other tests). Tests were repeated if the concentration range was either too high or too low to yield a 50% effect concentration. Reference tests using sodium dodecyl sulfate were performed to verify uniformity of response for each batch of field collected test organisms.

Corexit and Finasol toxicity

Table 2 summarizes the dispersant treatments that caused significant mortality in each species. Toxicity values (LC_{10} and LC_{50}) for each species and life stage were calculated using nominal concentrations and are ranked in order of sensitivity (for Corexit) (Table 3). The data indicate that larval life stages were generally more sensitive than adult life stages for the same species, and that Finasol, in general, had significantly greater toxicity (approximately four-fold higher) than Corexit (Table 3). The mean LC_{50} value for all species tested with Corexit was 150.31 mg/L

(range of 9.85 - 702.41), whereas the mean LC_{50} value for all species tested with Finasol was 43.27 mg/L (range of 3.81 - 105.26).

Sublethal effects C. variegatus
There was no significant effect of either dispersant on fish EROD activity (ANOVA Corexit p = 0.4863; ANOVA Finasol p = 0.2598). Mean EROD activity (µM resorufin/mg protein) (± standard error [SE]) in the treatments ranged from 0.404 (± 0.088) to 0.889 (± 0.166). Lipid peroxidation activity in adult *C. variegatus* was also not significantly affected by dispersant exposure (ANOVA Corexit p = 0.6276; ANOVA Finasol p = 0.1708) and the mean MDA levels (nMol/g wet weight) across treatments ranged from 189.79 (± 125.94) to 488.63 (± 268.62).

Finasol exposure significantly impaired *C. variegatus* embryonic hatching success ($p < 0.0001$). Nominal Finasol concentrations of 333 mg/L and 1000 mg/L reduced hatching success by 50% and 80%, respectively, compared to controls. Embryos exposed to 1000 mg/L Finasol took significantly longer to hatch than controls ($p < 0.0001$); 7 days vs. 5 days. No effects on hatching success (p = 0.2055) or time-to-hatch (p = 0.2144) occurred in the Corexit exposures.

Sublethal effects P. pugio
P. pugio embryo hatching success was significantly reduced in nominal Corexit concentrations ≥ 37 mg/L (ANOVA p = 0.0012; >85% reduction in hatching success compared to control) and in nominal Finasol concentrations ≥ 111 mg/L (ANOVA $p < 0.0001$; >79% reduced hatching success compared to control). Finasol (111 mg/L) significantly increased lipid peroxidation activity in adult grass shrimp compared to control levels; indicating a negative effect on cellular membranes. Corexit (1000 mg/L, nominal concentration) also significantly increased lipid peroxidation activity in adult grass shrimp. Larval grass shrimp were the most sensitive *P. pugio* life stage tested for both dispersants. Embryos were the least sensitive life stage tested for both dispersants. AChE activity of Corexit- or Finasol-exposed grass shrimp was not significantly different from controls (ANOVA p = 0.2008) and ranged from mean AChE (nMol/min) (± SE) of 0.2145 (± 0.0135) to 0.2326 (± 0.0282).

Sublethal effects I. obsoleta
There was no significant effect from either dispersant alone on lipid peroxidation activity in adult mud snails (ANOVA Corexit p = 0.9728; ANOVA Finasol p = 0.0929). The mean MDA levels (nMol/g wet weight) across treatments ranged from 96.15 (± 5.91) to 100.09 (± 14.56) in the Corexit exposure and from 72.43 (± 4.51) to 88.93 (± 6.20) in the Finasol exposure.

Nominal Finasol concentrations ≥111 mg/L caused significant AChE inhibition in adult *I. obsoleta* (p = 0.0018). Mean AChE activity was reduced 32-51% in the Finasol treatments compared to control levels. There was no significant effect of Corexit on AChE activity in adult mud snails (p = 0.0627) and mean AChE (nMol/mgP/min) (± SE) ranged from 85.05 (± 8.60) to 107.33 (± 4.90) across treatments.

Dispersed oil (CEWAF) toxicity
CEWAF treatments that caused significant mortality in each species are summarized in Table 4. Toxicity values (LC_{10} and LC_{50}) for each species were calculated using nominal percent CEWAF concentrations and ranked in order of sensitivity for Corexit-CEWAF (Table 5). The nominal

percent CEWAF data indicated that larval life stages were generally more sensitive than adult life stages for the same species, and that Corexit-CEWAF had greater toxicity to the estuarine test species than Finasol-CEWAF (Table 5). Toxicity values were then calculated using the measured chemical concentrations for TEH (Table 6) and Total PAH (Table 7); demonstrating greater toxicity for Finasol-CEWAF than for Corexit-CEWAF. Using measured TEH concentrations, the mean LC_{50} value for all species tested with Corexit-CEWAF was 261.96 mg/L, whereas the mean LC_{50} value for all species tested with Finasol-CEWAF was 23.19 mg/L. Similarly, using measured Total PAH concentrations, the mean LC_{50} value for all species tested with Corexit-CEWAF was 2.95 mg/L, whereas the mean LC_{50} value for all species tested with Finasol-CEWAF was 0.49 mg/L.

Sublethal effects C. variegatus

Exposure to Corexit and Finasol CEWAFs did not significantly alter fish immune function as measured by splenocyte proliferation (ANOVA $p = 0.3876$). Mean splenocyte cell density (fluorescent units [FU] = fluorescence at 485/530 nm) (± SE) ranged from 4462.67 FU (± 417.23) to 9510.44 FU (± 3017.40) across treatments. CEWAF exposure also did not have a significant effect on nervous system function as measured by brain acetylcholinesterase activity (Corexit-CEWAF $p = 0.6205$; Finasol-CEWAF $p = 0.2869$). Mean AChE (nMol/mgP/min) (± SE) in the treatments ranged from 233.87 (± 103.25) to 354.55 (± 40.03).

There was a significant increase in EROD activity for fish exposed to 5.56% and 16.7% Finasol-CEWAF concentrations (ANOVA $p = 0.0032$) and 1.85% Corexit-CEWAF (ANOVA $p = 0.0360$) compared to the control. Mean activity (µmoll/min/µg protein) increased up to five-fold in the Finasol-CEWAF treatments compared to the control, and a maximum nine-fold induction of enzyme activity compared to control was observed in the Corexit-CEWAF exposure.

There was a significant decreasing effect on lipid peroxidation activity in the CEWAF exposed fish to ≥ 1.85% Corexit-CEWAF and to ≥ 16.7% Finasol-CEWAF ($p = 0.0023$). Mean LPX (nMol MDA/mg wet weight) (± SE) in the CEWAF treatments ranged from 847.99 (± 163.12) in the controls to 105.86 (± 116.46) in the highest Finasol-CEWAF concentration and 210.32 (± 168.81) in the highest Corexit-CEWAF concentration.

Only the 100% Finasol-CEWAF negatively impacted embryonic hatching success (7% hatch vs. 93% in the controls; $p < 0.0001$) and embryos exposed to 100% Finasol-CEWAF took significantly longer to hatch than controls (mean hatch time of 9 d vs. 5.5 d in the controls; $p < 0.0001$). Embryos exposed to Corexit-CEWAF ≥ 16.7% had significantly reduced hatching success ($p < 0.0001$) and significantly delayed time-to-hatch ($p < 0.0001$). Hatching success was reduced from 90% in the controls to 62%, 23%, and 3% in the 16.7%, 50%, and 100% Corexit-CEWAF treatments, respectively. Embryos that hatched in the 50% and 100% CEWAFs were not viable (larvae died shortly after hatching). Mean time to hatch increased from 5.5 d in the controls to 9 d in the 50% and 100% Corexit-CEWAFs.

Sublethal effects P. pugio

Grass shrimp embryo hatching success was significantly lower than controls in Corexit and Finasol CEWAF exposures (ANOVA p values < 0.0001). Mean hatching success declined from 94% in the controls to 83%, 61%, and 14% in the Corexit-CEWAF concentrations of 1.85%,

5.56%, and 16.67%, respectively, and from 97% in the controls to 76%, 74%, 74%, and 61% in the Finasol-CEWAF concentrations of 0.62%, 1.85%, 5.56%, and 16.67%, respectively. None of the embryos in the 50% and 100% dispersant CEWAFs hatched.

There was no significant effect of Corexit-CEWAF ($p = 0.3584$) or Finasol-CEWAF ($p = 0.6400$) on grass shrimp AChE activity. Mean AChE (nMol/mgP/min) (± SE) for all treatments ranged from 51.26 (± 3.24) to 74.94 (± 12.29). There was also no significant effect on LPX activity in adult grass shrimp exposed to Corexit-CEWAF ($p = 0.2116$) or Finasol-CEWAF ($p = 0.5472$), although there was a trend toward increasing activity. Mean LPX (nMol MDA/mg wet weight) (± SE) ranged from 206.18 (± 122.34) to 507.16 (± 186.16) in the Finasol-CEWAF and ranged from 338.77 (± 96.54) to 593.58 (± 196.93) in the Corexit-CEWAF.

Sublethal effects I. obsoleta

There was no significant effect on LPX activity in adult mud snails exposed to Finasol-CEWAF ($p = 0.1880$), and mean LPX (nMol MDA/mg wet weight) (± SE) ranged from 66.33 (±27.89) in the control to 104.11 (±11.39) in the highest treatment. Mean LPX activity in the 16.7% Corexit-CEWAF (29.08 ± 6.63) was significantly lower than controls (62.90 ± 15.17; $p = 0.0286$). There was no significant effect of Corexit-CEWAF ($p = 0.7997$) or Finasol-CEWAF ($p = 0.1134$) on adult snail AChE activity. Mean AChE (nMol/mgP/min) (± SE) in the treatments ranged from 70.34 (± 6.55) to 109.28 (± 7.31).

Measured CEWAF Concentrations

Measured chemistry in the CEWAFs included TEH and Total PAH (list of 50 parent and alkylated PAH analytes provided in Suppl. Table 1). Addition of both dispersants to LSC oil chemically enhanced the petroleum signatures detected in the water-accommodated fractions (WAFs). In laboratory testing with undispersed LSC WAFs prior to this study, TEH and measured individual and alkylated PAH concentrations were below detection limits; detection limits were 2 mg/L for TEH and ranged from 5×10^{-7} – 0.107 mg/L for PAHs in the Total PAH. Minimum detection levels were calculated according to Ragland et al. (2014). The t = 0 measured TEH concentrations (mean ± SE) in this study were 1,315 ± 242.47 mg/L in the 100% Corexit-CEWAFs and 67.20 ± 11.00 mg/L in the 100% Finasol-CEWAFs (Table 8). Concentrations of TEH were significantly higher in the Corexit-CEWAFs than the Finasol-CEWAFs ($p = 0.0019$). Total PAH concentrations (mean ± SE) measured in this study were 14.21 ± 1.32 mg/L in the 100% Corexit-CEWAFs and 1.44 ± 0.10 mg/L in the 100% Finasol-CEWAFs (Table 8), and Total PAH concentrations in the Corexit-CEWAFs were also significantly higher than in the Finasol-CEWAFs ($p < 0.0001$).

The hydrocarbon concentrations measured over time in the large volume tests for Corexit-CEWAF and Finasol-CEWAF are shown in Table 9 and Table 10, respectively. The TEH concentrations in the Corexit-CEWAF treatments were 61% and 22% of the initial concentrations after 24 h and 96 h, respectively (mean of all Corexit-CEWAF treatments in Table 9). Similarly, the Total PAH concentrations in the Corexit-CEWAF treatments were 43% and 16% of the initial concentrations after 24 h and 96 h, respectively. Measured hydrocarbon degradation over time was more variable in the Finasol-CEWAF treatments because of limits in detecting the lower concentrations that were present in the Finasol-CEWAFs at the start of the test (Table 10). TEH concentrations in the Finasol-CEWAF treatments averaged 56% of the

initial concentrations after 24 h but were undetectable after 96 h (Table 10). The Total PAH concentrations in the Finasol-CEWAF treatments were 14% and 3% of the initial concentrations after 24 h and 96 h, respectively (Table 10).

The time weighted average (TWA) hydrocarbon concentrations determined for the Corexit-CEWAFs were approximately 28% of the concentrations measured at the start of the experiments (Table 9), whereas the TWA concentrations for the Finasol-CEWAFs were approximately 9% of the initial measured concentrations (Table 10). A comparison of 96h LC_{50} values determined using initial concentrations vs. TWA concentrations for the large volume toxicity tests shows that the TWA calculated LC_{50} values for Corexit-CEWAF were approximately 64% lower than the LC_{50} values calculated using the initial concentrations (Table 11). The TWA calculated LC_{50} values for Finasol-CEWAF were approximately 92% lower than the LC_{50} values calculated using the initial concentrations. Calculations of LC_{50} values using the initial measured concentrations are most likely an underestimation of toxicity because they do not account for chemical loss over the 96 h exposure.

Discussion

The toxicity values available in the literature for Corexit 9500 are in good agreement with those determined in this study. For example, Fuller et al. (2004) reported an LC_{50} of 180 mg/L for *C. variegatus*, while this study reported 153 mg/L for the same species. The response of *C. variegatus* to Corexit is also similar to another estuarine fish (*Fundulus heteroclitus*), which had a 96 h LC_{50} value of 84 mg/L (DeLorenzo 2012). Aurand and Coelho (2005) reported a 96 h LC_{50} value for Corexit with larval (4 d old) *C. variegatus* of 182 mg/L, similar to the value determined for the *C. variegatus* early life stage test in this study of 172 mg/L. A 96 h Corexit LC_{50} value reported for mysids in the literature of 42.0 mg/L (Hemmer et al. 2011) was comparable to 71.61 mg/L reported in this study (32.8 mg/L).

Few ecotoxicity values were available for Finasol OSR 52 prior to this study. A 48 h LC_{50} value of 9.37 mg/L was previously determined for *A. bahia* and a 96 h LC_{50} of 11.66 mg/L for *Menidia beryllina* (USEPA 2003). A 48 h LC_{50} of 24.95 mg/L Finasol was determined for *A. bahia* in this study. The LC_{50} values determined for Finasol with the estuarine species tested in this study ranged from 4.06 mg/L to 177.56 mg/L. The acute toxicity of Finasol was generally 3-5 times that of Corexit for the estuarine species tested.

The most sensitive species tested with both Corexit and Finasol based on acute mortality was the larval life stage of the mud snail, *I. obsoleta*. Larval life stages were generally more sensitive than adult life stages. Embryos were comparatively insensitive to dispersants, which is consistent with previous findings of low permeability of the embryonic coat to other chemicals (DeLorenzo and De Leon 2010; DeLorenzo et al. 2006). In comparison to environmental levels of oil dispersants reported by Kujawinski et al. (2011) which ranged from 10 - 100 µg/L during and after the DWH event, the individual dispersant LC_{50} values reported here are much higher (>3.81 mg/L).

When comparing the toxicity of the two dispersants prepared as dispersed-LSC oil CEWAFs, a different trend in toxicity was observed when using the nominal percent CEWAF to calculate

LC_{50} values, whereby the Corexit-CEWAF was significantly more toxic than the Finasol-CEWAF. The nominal toxicity values do not take into account differences in the amount of oil each dispersant delivered into the seawater. For example, in the adult fish exposure, the 100% Finasol CEWAF Total PAH concentration was 0.94 mg/L and TEH concentration was 37.6 mg/L, whereas the 100% Corexit CEWAF Total PAH concentration was 20.17 mg/L and TEH was 2892 mg/L. The greater bioavailability of oil would account for the greater toxicity seen in the Corexit-CEWAF compared to the same dilutions of Finasol-CEWAF. The LC_{50} values determined for each CEWAF based on measured hydrocarbon concentrations further demonstrate greater toxicity in the Finasol-CEWAF than in the Corexit-CEWAF. For instance, the Finasol-CEWAF was 11-18 times more toxic than the Corexit-CEWAF to the early life stage of *C. variegatus*, based on measured TEH and PAH concentrations. The average hydrocarbon (TEH) concentration measured in the Finasol-CEWAF in this study (67 mg/L) is similar to that reported by Dussauze et al. (2014) of 46 mg/L TPH (total petroleum hydrocarbons). The differences between the relationship between Corexit and Finasol CEWAFs based on nominal and measured hydrocarbon concentrations are likely a function of Corexit being a more effective dispersant and Finasol being inherently more toxic; therefore, the toxicity of the CEWAFs is being driven both by the hydrocarbon concentrations and that of the individual dispersants in a complex mixture.

While the results from this study do not provide a direct comparison between dispersed and undispersed oil toxicity, we can generalize based on previously published studies. The toxicity of mechanically dispersed LSC oil (WAF) has been determined with several of the test species used in this study. Hemmer et al. (2011) reported a 48 h LC50 of 2.7 mg/L TPH for *A. bahia*. Rossi and Anderson (1976) reported a 96 h LC50 of 12.5 mg/L for *N. arenaceodentata*, and Anderson et al. (1974) reported 96 h LC50 values of 200 mg/L for adult *P. pugio* and 29,000 mg/L TPH for adult *C. variegatus*. Compared to the dispersed oil LC_{50} values determined using measured TEH concentrations in this study, dispersed oil would be less toxic to *A. bahia* (37.28 mg/L Corexit-CEWAF and 13.05 mg/LFinasol-CEWAF); less toxic to *N. arenaceodentata* based on Corexit-CEWAF (126.31 mg/L), but equally toxic based on Finasol-CEWAF (12.30 mg/L); more toxic to *P. pugio* (105.40 mg/L Corexit-CEWAF and 26.17 mg/L Finasol-CEWAF); and more toxic to *C. variegatus* (515.56 mg/L Corexit-CEWAF and 28.21 mg/L Finasol-CEWAF).

Bejarano et al. (2014) compared available LC_{50} data for various oils prepared as WAFs versus chemically dispersed with Corexit 9500 (CEWAF) and determined 78% of the CEWAF values were of lower or equal toxicity than WAF values, whereas 12% ranged from 1.55-fold to 8.09 fold greater toxicity. Differences in methods used to prepare WAFs and CEWAFs, particularly the mixing energy level, affects the amount of measured hydrocarbons in solution and thus may complicate comparisons between LC_{50} values.

Several sublethal effects of dispersants alone were identified in this study, such as a reduction in embryonic hatching success in sheepshead minnows and grass shrimp, increased lipid peroxidation activity in grass shrimp, and acetylcholinesterase activity inhibition in mud snails. These results are consistent with previous studies of dispersants on other species. For example, embryonic hatching success of mallard ducks was also significantly impaired by Corexit (Wooten et al. 2011). Corexit 9500 has also been shown to cause oxidative stress, measured by an increase in lipid peroxidation activity, in a mammalian cell line (Zheng et al. 2014).

Acetylcholinesterase inhibition has been seen with other surfactants similar to the surfactant components of Corexit and Finasol. For example, sodium dodecyl sulfate inhibited AChE in daphnia (Guilherminoa et al. 2000) and exposure to sodium dodecylbenzenesulfonate inhibited AChE in the freshwater cladocera *Moina macrocopa* (Martinez-Tabche et al. 1997). These effects indicate potential for chronic effects due to reproductive or neurological impairment. In addition, energy to counter cellular membrane damage may come at the cost of reduced growth or fecundity. Energy is also expended in detoxification processes such as p450 enzyme induction. A previous study found that although LSC oil and a mixture of LSC oil and Corexit induced EROD activity in the mummichog, *Fundulus heteroclitus*, Corexit alone did not significantly induce EROD activity in the fish compared to seawater control (DeLorenzo 2012). The results with *C. variegatus* in this study are consistent with that of *F. heteroclitus*, with no measured effect of either Corexit or Finasol alone on EROD activity, but when oil was present in the exposure (CEWAFs), there was a significant induction of *C. variegatus* EROD activity. There was also a significant decrease in lipid peroxidation activity in fish and snails exposed to dispersed oil, suggesting energy allocation to detoxification.

Overall, Corexit was identified as the more effective and less toxic dispersant. Finasol elicited greater toxicity in the individual dispersant trials, and dispersed lower levels of hydrocarbons into seawater than Corexit in the CEWAF trials. The range of LC_{50} values calculated for the estuarine organisms in this study based on TEH concentrations in the CEWAFs (Corexit-CEWAF of 10 - 1815 mg/L TEH; and Finasol-CEWAF of 0.68 - 90 mg/L TEH) are relatable to TEH concentrations measured in the environment. For example, a mean of 202 mg/L TPH was reported for 66 DWH surface water samples (Sammarco et al. 2013). In the open ocean, the mechanical action of waves and immense water volume dissipate dispersant-formed droplets at a higher rate, but the closed, shallow nature of estuarine, tidal creek, and lagoonal habitats could prevent effective dissolution of dispersants. For this reason, and consistent with current spill response protocols, it is unlikely that dispersants would be applied in coastal or inshore waters. Each spill situation is unique, however, and the results of this study provide response managers with data to guide decisions specific to estuarine habitats. The results of our study demonstrate that dispersant toxicity is compound- and species-specific. Moreover, different dispersants elicit different chemical interactions with oil that will affect bioavailability and toxicity of oil compounds to aquatic species. Should a future oil spill require the use of dispersants, the results of this study will allow managers to make informed decisions regarding the use of Corexit® 9500 or Finasol® OSR 52, particularly when applied to Louisiana Sweet Crude oil.

Data Availability

Dispersant LC50 data are available in the NOAA Chemical Aquatic Fate and Effects (CAFE) database. All data produced by this study as described in this manuscript resides both electronically on NOAA servers and in hard copy. See M. DeLorenzo (marie.delorenzo@noaa.gov) or P. Key (pete.key@noaa.gov) for access.

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Supporting Documents

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Tables

Table 1. Bioassay parameters for each species and life stage tested.

Species/life stage	Age/size	Test Duration	organisms per test chamber / replicates	Volume in test chamber	Temp (°C)	Photoperiod	Feeding
C. variegatus (fish) embryos	48 h post-fertilization	9 d	15/4	300 mL	25	16h L:8h D	Fry: 2 drops 24 h old *Artemia* daily
C. variegatus adult	3.9 - 5.0 cm	96 h	6/3	3.5 L	25	16h L:8h D	none
P. pugio (shrimp) embryo	Stage VI	96 h	24 per well plate /3	2mL per well	28	24h dark	none
P. pugio larvae	24 - 48 h	96 h	10/3	400 mL	25	16h L:8h D	2 drops 24 h old *Artemia* daily
P. pugio adult	2 - 3 cm	96 h	10/3	2L	25	16h L:8h D	none
M. mercenaria (clam) larvae	7 d	96 h	24 per well plate /3	2mL per well	25	16h L:8h D	12,000 cells/mL of *I. galbana* daily
M. mercenaria juvenile	1.0 - 1.2 mm	96 h	30/3	180 mL	25	16h L:8h D	none
I. obsoleta (snail) larvae	≤ 24 h	96 h	24 per well plate /3	2mL per well	25	16h L:8h D	12,000 cells/mL of *I. galbana* daily
I. obsoleta adult	15 - 18 mm	96 h	10/3	400 mL	25	16h L:8h D	none
L. plumulosus (amphipod) juvenile	500 - 710 µm	96 h	10/3	80 mL	25	16h L:8h D	none
N. arenaceodenta	~14 d/ 10-15 mm	96 h	5/3	300 mL	25	16h L:8h D	none

ta (polychaete) juvenile							
A. bahia (mysid)	5 d	48 h	10/3	400 mL	25	16h L:8h D	3-4 drops 24 h old *Artemia* daily

Table 2. Average percent mortality at each nominal exposure concentration for the Corexit and Finasol LC_{50} tests. Asterisks indicate concentrations that were significantly different from the control (ANOVA, followed by Dunnett's test).

Species, Life Exposure Duration	Corexit Nominal (mg/L)	Average Percent Mortality	Finasol Nominal (mg/L)	Average Percent Mortality
Fish, ELS	12	28	12	27
9 d	37	30	37	28
	111*	33	111*	93
	333*	95	333*	100
	1000*	100	1000*	97
Fish, Adult	12	0	12	0
96 h	37*	11	37	0
	111*	17	111*	94
	333*	100	333*	100
	1000*	100	1000*	100
Shrimp, Embryo	12	24	12	24
96 h	37	26	37	32
	111*	33	111*	56
	333*	51	333*	75
	1000*	64	1000*	81
Shrimp, Larvae	4	7	4	0
96 h	8	13	8	10
	16*	23	16*	33
	32*	17	32*	97
	64*	33	64*	100
Shrimp, Adult	12	0	12	23
96 h	37	0	37*	43
	111	13	111*	80
	333*	27	333*	100
	1000*	60	1000*	100
Snail, Larvae	1.4	14	1.4*	21
96 h	4.1*	22	4.1*	53
	12*	52	12*	100
	37*	96	37*	100
	111*	100	111*	100
Snail, Adult	12	0	12	0
96 h	37	0	37	0
	111*	27	111*	77
	333*	93	333*	83
	1000*	100	1000*	100

Table 2 (continued). Average percent mortality at each nominal exposure concentration for the Corexit and Finasol LC_{50} tests. Asterisks indicate concentrations that were significantly different from the control (ANOVA, followed by Dunnett's test).

Species, Life Exposure Duration	Corexit Nominal (mg/L)	Average Percent Mortality	Finasol Nominal (mg/L)	Average Percent Mortality
Clam, Larvae	1.4*	21	1.6	14
96 h	4.1*	39	3.1	10
	12*	49	6.3*	18
	37*	76	13*	44
	111*	100	25*	69
Clam, Juvenile	1.4	18	1.6	11
96 h	4.1*	26	3.1*	36
	12*	29	6.3*	53
	37*	33	13*	98
	111*	100	25*	100
Polychaete, Juv.	12	0	1.4	0
96 h	37	0	4.1	0
	111*	93	12	0
	333*	100	37	6.7
	1000*	100	111*	100
Amphipod, Juv.	12	13	1.4	0
96 h	37*	97	4.1	0
	111*	100	12*	10
	333*	100	37*	100
	1000*	100	111*	100
Mysid, Juvenile	4.1	3.3	4.1	3.3
48 h	12	6.7	12	3.3
	37*	67	37*	100
	111*	100	111*	100
	333*	100	333*	100

Table 3. LC_{50} (95% confidence interval), and LC_{10} toxicity values determined for Corexit and Finasol for each test species. Toxicity values were calculated using nominal exposure concentrations (mg/L). Asterisks indicate a significant difference between Corexit and Finasol LC_{50} values (Wheeler ratio test $p<0.05$).

Species, Life Stage	Corexit LC_{50}	Corexit 95% CI	Corexit LC_{10}	Finasol LC_{50}	Finasol 95% CI	Finasol LC_{10}
Snail, Larvae	9.85*	(7.96 - 11.92)	1.58	3.81*	(3.26 - 4.60)	0.61
Clam, Larvae	16.10	(12.00 – 20.73)	<1.37	15.30	(12.65 - 18.72)	2.21
Amphipod, Juv.	21.43	(17.34 - 26.07)	10.55	22.59	(18.37 - 28.15)	9.57
Mysid, Juvenile	32.80*	(28.46 - 37.02)	15.54	24.95*	(19.40 - 32.74)	15.09
Clam, Juvenile	43.40*	(36.81 - 51.00)	<1.37	5.47*	(4.46 - 6.29)	<1.56
Shrimp, Larvae	64.05*	(54.90 - 74.16)	7.57	18.65*	(16.04 - 22.15)	9.61
Polychaete, Juv.	101.24*	(94.32 - 108.66)	93.77	40.85*	(37.55 - 44.24)	37.68
Fish, ELS	142.26*	(111.50 -	<12.3	37.14*	(20.68 - 60.29)	8.29
Snail, Adult	153.99	(124.76 -	80.76	105.26	(67.17 - 157.18)	39.07
Fish, Adult	162.66	(124.85 -	68.47	105.04	(102.40 -	97.67
Shrimp, Embryo	353.51*	(180.10 -	<12.3	85.99*	(44.00 –	2.98
Shrimp, Adult	702.41*	(471.50 -	122.34	54.17*	(34.65 - 75.48)	<12.3

Table 4. Average percent mortality at each nominal exposure concentrations for the CEWAF LC_{50} tests. Asterisks indicate concentrations that were significantly different from the control (ANOVA, followed by Dunnett's test).

Species, Life Exposure Duration	Corexit CEWAF (%)	Average Percent Mortality	Finasol CEWAF (%)	Average Percent Mortality
Fish, ELS	1.85	21.67	1.85	13.33
9 d	5.56	26.67	5.56	25
	16.7*	98.33	16.7	30
	50*	100	50	23.33
	100*	100	100*	100
Fish, Adult	1.85	11.11	1.85	0
96 h	5.56	0	5.56	0
	16.7	22.22	16.7	0
	50*	100	50*	11.11
	100*	100	100*	100
Shrimp, Embryo	1.85	2.78	1.85	4.17
96 h	5.56	4.17	5.56	2.78
	16.7	19.44	16.7	11.11
	50	18.06	50*	48.61
	100*	36.11	100*	62.50
Shrimp, Larvae	0.62	0	0.62	0
96 h	1.85	3.33	1.85	0
	5.56*	73.33	5.56	3.33
	16.7*	100	16.7*	96.67
	50*	100	50*	100
Shrimp, Adult	1.85	3.33	1.85	0
96 h	5.56*	40	5.56	3.33
	16.7*	93.33	16.7	6.67
	50*	100	50*	36.67
	100*	100	100*	93.33
Snail, Larvae	0.069	31.94	1.85*	43.06
96 h	0.20	37.50	5.56*	84.72
	0.62*	38.89	16.7*	100.00
	1.85*	50.00	50*	100.00
	5.56*	94.44	100*	100.00
Snail, Adult	1.85	0.00	1.85	0.00
96 h	5.56	0.00	5.56	0.00
	16.7*	20.00	16.7*	6.67
	50*	100.00	50*	100.00
	100*	100.00	100*	100.00

Table 4 (continued). Average percent mortality at each nominal exposure concentrations for the CEWAF LC_{50} tests. Asterisks indicate concentrations that were significantly different from the control (ANOVA, followed by Dunnett's test).

Species, Life Exposure Duration	Corexit CEWAF (%)	Average Percent Mortality	Finasol CEWAF (%)	Average Percent Mortality
Clam, Larvae	0.62	11.11	0.62	1.39
96 h	1.85*	83.33	1.85	15.28
	5.56*	80.56	5.56	4.17
	16.7*	98.61	16.7*	100
	50*	91.67	50*	93.06
Clam, Juvenile	0.2*	12.22	0.2	6.67
96 h	0.62*	18.89	0.62*	18.89
	1.85*	34.44	1.85*	24.44
	5.56*	44.44	5.56*	33.33
	16.7*	85.56	16.7*	48.89
Polychaete, Juv.	1.85	0.00	1.85	0.00
96 h	5.56	0.00	5.56	0.00
	16.7*	86.67	16.7	6.67
	50*	100.00	50*	93.33
	100*	100.00	100*	100.00
Amphipod, Juv.	0.2	10	0.62	0
96 h	0.62	13.33	1.85	3.33
	1.85*	10.00	5.56	3.33
	5.56*	63.33	16.7*	30
	16.7*	100.00	50*	100
Mysid, Juvenile	0.62	0	0.62	0.00
48 h	1.85*	33.33	1.85	0.00
	5.56*	66.67	5.56	6.67
	16.7*	100.00	16.7*	80.00
	50*	100.00	50*	100.00

Table 5. LC_{50} (95% confidence interval) and LC_{10} toxicity values for Corexit and Finasol CEWAFs determined for each test species. Toxicity values were calculated using nominal exposure concentrations (% CEWAF). Asterisks indicate a significant difference between Corexit and Finasol CEWAF LC_{50} values (Wheeler ratio test $p<0.05$).

Species, Life Stage	Corexit LC_{50}	Corexit 95% CI	Corexit LC_{10}	Finasol LC_{50}	Finasol 95% CI	Finasol LC_{10}
Clam, Larvae	1.29*	(0.33 – 2.67)	0.11	8.34*	(4.77 - 14.43)	2.15
Snail, Larvae	1.61*	(1.12 - 2.07)	<0.069	2.49*	(1.18 – 3.25)	<1.85
Amphipod, Juv.	2.59*	(1.35 - 3.99)	0.33	19.91*	(16.70 - 28.39)	10.71
Mysid, Juvenile	3.21*	(2.47 - 4.15)	1.15	11.22*	(9.13 - 13.72)	6.19
Shrimp, Larvae	4.59*	(3.63 - 5.23)	2.63	9.63*	(7.93 - 11.69)	6.56
Clam, Juvenile	7.30*	(6.18 - 8.62)	0.33	16.50*	(13.05 – 21.45)	0.46
Fish, ELS	7.72*	(6.38 - 9.08)	0.63	50.68*	(39.01 - 64.22)	0.81
Shrimp, Adult	8.38*	(6.85 - 10.38)	2.23	59.45*	(51.31 - 69.55)	25.95
Polychaete, Juv.	14.64*	(7.50 - 22.94)	4.18	28.85*	(21.72 - 38.14)	18.08
Snail, Adult	21.89	(17.77 – 56.42)	13.10	27.33	(22.79 - 33.30)	17.92
Fish, Adult	26.73*	(19.68 – 39.45)	6.50	67.54*	(57.71 - 79.96)	45.26
Shrimp, Embryo	128.36*	(89.49 – 275.39)	15.98	65.87*	(49.88 - 91.39)	9.83

Table 6. LC_{50} (95% confidence interval) and LC_{10} toxicity values for Corexit and Finasol CEWAFs determined for each test species. Toxicity values were calculated using measured TEH concentrations (mg/L).

Species, Life Stage	Corexit LC_{50}	Corexit 95% CI	Corexit LC_{10}	Finasol LC_{50}	Finasol 95% CI	Finasol LC_{10}
Snail, Larvae	10.39	(6.68 - 13.85)	<2.45	0.68	(0.004 - 1.48)	<3.90
Clam, Larvae	10.80	(2.48 - 22.55)	0.89	7.77	(1.12 - 15.78)	0.98
Amphipod, Juv.	20.22	(10.41 - 32.25)	2.13	6.45	(5.21 - 9.65)	2.91
Mysid, Juvenile	37.28	(28.03 - 49.42)	11.50	13.05	(10.96 - 15.34)	6.44
Shrimp, Larvae	64.88	(51.27 - 73.85)	37.16	13.11	(10.79 - 15.92)	8.93
Clam, Juvenile	84.61	(71.62 - 99.69)	<2.90	7.38	(5.87 - 9.34)	<2.10
Shrimp, Adult	105.40	(85.07 - 131.96)	23.70	26.17	(23.18 - 28.97)	11.92
Polychaete, Juv.	126.31	(78.97 - 176.58)	59.16	12.30	(9.29 - 15.78)	6.70
Fish, ELS	127.97	(102.91-153.78)	<7.50	46.72	(34.21 - 59.41)	<4.40
Snail, Adult	225.05	(178.89 - 592.76)	126.88	26.70	(22.00 - 32.25)	15.26
Fish, Adult	515.56	(413.69-637.34)	169.27	28.21	(24.49 - 32.25)	20.67
Shrimp, Embryo	1815.03	(1265.32 - 3894.26)	225.90	89.72	(67.93 - 124.47)	13.39

Table 7. LC50 (95% confidence interval) and LC10 toxicity values for Corexit and Finasol CEWAFs determined for each test species. Toxicity values were calculated using measured Total PAH concentrations (mg/L).

Species, Life Stage	Corexit LC_{50}	Corexit 95% CI	Corexit LC_{10}	Finasol LC_{50}	Finasol 95% CI	Finasol LC_{10}
Clam, Larvae	0.12	(0.03 - 0.26)	0.01	0.10	(0.06 - 0.19)	0.03
Snail, Larvae	0.30	(0.21 - 0.39)	<0.01	0.03	(0.01 - 0.04)	<0.02
Clam, Juvenile	0.64	(0.54 - 0.75)	<0.01	0.09	(0.06 - 0.13)	0.01
Shrimp, Larvae	0.64	(0.51 - 0.72)	0.37	0.17	(0.14 - 0.21)	0.12
Amphipod, Juv.	0.65	(0.36 - 0.93)	0.11	0.26	(0.19 - 0.53)	0.11
Mysid, Juvenile	0.81	(0.63 - 0.97)	0.28	0.18	(0.15 - 0.22)	0.10
Fish, ELS	1.24	(1.08-1.39)	<0.18	1.11	(0.73 - 1.68)	<0.01
Shrimp, Adult	1.44	(1.21 - 1.76)	0.60	1.25	(0.82 - 1.68)	0.41
Snail, Adult	2.34	(1.90 - 6.09)	1.39	0.34	(0.28 - 0.43)	0.22
Polychaete, Juv.	2.68	(1.60 - 3.76)	1.03	0.40	(0.27 - 0.57)	0.21
Fish, Adult	6.96	(5.55 - 8.62)	2.20	0.66	(0.56 - 0.78)	0.44
Shrimp, Embryo	17.80	(12.41 - 38.20)	2.22	1.14	(0.86 -1.60)	0.17

Table 8. Measured TEH and Total PAH concentrations (mg/L) in the Corexit CEWAF and Finasol CEWAF for each toxicity test at t = 0. CEWAF preparations were used for more than one toxicity test, thus species are reported together based on common CEWAFs.

Species, Life Stage	Corexit % CEWAF	Corexit CEWAF TEH (mg/L)	Corexit CEWAF Total PAH	Finasol % CEWAF	Finasol CEWAF TEH	Finasol CEWAF Total PAH
Fish, ELS	0.62	7.50	0.18	0.62	0.00	0.01
	1.85	18.50	0.45	1.85	0.00	0.04
	5.56	78.80	1.18	5.56	4.40	0.09
	16.7	299.50	2.19	16.7	17.50	0.26
	50	872.00	8.15	50	49.00	1.42
	100	1704.00	12.99	100	83.00	1.54
Fish, Adult	0.62	9.90	0.18	0.62	0.00	0.00
	1.85	27.25	0.51	1.85	0.00	0.00
	5.56	154.20	1.04	5.56	2.10	0.04
	16.7	396.50	5.48	16.7	7.80	0.12
	50	850.00	11.58	50	21.00	0.45
	100	2892.00	20.17	100	37.60	0.94
Shrimp, Embryo	0.62	8.73	0.09	0.62	0.84	0.01
	1.85	26.19	0.26	1.85	2.52	0.03
and	5.56	78.56	0.77	5.56	7.57	0.10
	16.7	235.67	2.31	16.7	22.70	0.29
Shrimp, Larvae	50	707.00	6.93	50	68.10	0.87
	100	1414.00	13.87	100	136.20	1.73
Shrimp, Adult	0.62	3.70	0.17	0.62	0.00	0.01
	1.85	18.10	0.49	1.85	0.00	0.04
	5.56	67.60	1.17	5.56	0.00	0.13
	16.7	215.60	2.61	16.7	7.50	0.36
	50	550.00	7.79	50	21.70	1.58
	100	1150.00	16.63	100	37.00	1.63
Snail, Larvae	0.069	0.00	0.01	0.62	0.00	0.01
	0.20	0.00	0.03	1.85	0.00	0.02
	0.62	2.45	0.10	5.56	3.90	0.07
	1.85	11.00	0.39	16.67	12.40	0.20
	5.56	40.80	1.01	50	43.40	0.67
	100	664.00	19.24	100	74.60	1.44
Snail, Adult	0.62	6.85	0.06	0.62	0.00	0.01
	1.85	13.45	0.17	1.85	0.00	0.02
and	5.56	38.60	0.59	5.56	3.90	0.07
	16.67	166.60	1.78	16.67	12.40	0.20
Clam Larvae	50	451.00	4.90	50	43.40	0.67
	100	589.00	8.82	100	74.60	1.44
Clam, Juvenile	0.2	2.90	0.01	0.2	0.00	0.00

	0.62	9.00	0.05	0.62	0.00	0.00
	1.85	23.10	0.16	1.85	0.00	0.00
	5.56	55.90	0.55	5.56	2.10	0.04
	16.67	199.50	1.41	16.7	7.80	0.12
	50	374.40	4.65	50	21.00	0.45
	100	718.00	9.10	100	37.60	0.94
Mysid, Juvenile	0.62	7.50	0.18	0.62	0.00	0.01
	1.85	18.50	0.45	1.85	0.00	0.04
	5.56	78.80	1.18	5.56	4.40	0.09
	16.7	299.50	2.19	16.67	17.50	0.26
	50	872.00	8.15	50	49.00	1.42
	100	1704.00	12.99	100	83.00	1.54
Polychaete, Juv	0.62	4.20	0.14	0.62	0.00	0.02
	1.85	14.60	0.56	1.85	0.00	0.05
and	5.56	43.20	1.28	5.56	0.00	0.08
	16.67	185.20	2.99	16.67	5.20	0.19
Amphipod, Juv.	50	456.00	6.82	50	19.00	0.84
	100	1000.00	14.09	100	41.20	1.73

Table 9. Time weighted average concentrations for measured TEH and Total PAH in the Corexit CEWAF for the large volume toxicity tests. NM= not measured.

Species, Life Stage	Corexit % CEWAF	Corexit CEWAF TEH (mg/L) Time=0	Corexit CEWAF TEH (mg/L) Time=6	Corexit CEWAF TEH (mg/L) Time=24	Corexit CEWAF TEH (mg/L) Time=96	Time Weighted Average Concentration (mg/L)
Fish, Adult	0.62	9.90	0.00	4.20	0.00	0.87
	1.85	27.25	17.70	13.70	4.10	6.74
	5.56	154.20	71.80	49.60	18.60	28.04
	16.7	396.50	256.00	212.10	69.60	107.91
	50	850.00	1126.00	NM	NM	
	100	2892.00	NM	NM	NM	
Shrimp, Adult	0.62	3.70	3.60	4.40	3.40	3.60
	1.85	18.10	13.10	15.40	2.80	5.79
	5.56	67.60	70.30	52.80	7.60	19.58
	16.7	215.60	121.80	51.40	27.30	37.80
	50	550.00	548.40	NM	NM	
	100	1150.00	818.00	NM	NM	
Species, Life Stage	**Corexit % CEWAF**	**Corexit CEWAF Total PAH (mg/L)**	**Corexit CEWAF Total PAH (mg/L)**	**Corexit CEWAF Total PAH (mg/L)**	**Corexit CEWAF Total PAH (mg/L)**	**Time Weighted Average Concentration (mg/L)**
Fish, Adult	0.62	0.18	0.06	0.03	0.01	0.02
	1.85	0.51	0.36	0.14	0.06	0.09
	5.56	1.04	1.02	1.00	0.25	0.43
	16.7	5.48	4.87	1.86	1.52	1.77
	50	11.58	30.28	NM	NM	
	100	20.17	NM	NM	NM	
Shrimp, Adult	0.62	0.17	0.07	0.04	0.01	0.02
	1.85	0.49	0.23	0.20	0.07	0.10
	5.56	1.17	0.79	0.46	0.24	0.32
	16.7	2.61	1.96	1.74	0.53	0.84
	50	7.79	6.43	NM	NM	
	100	16.63	13.30	NM	NM	

Table 10. Time weighted average concentrations for measured TEH and Total PAH in the Finasol CEWAF for the large volume toxicity tests. NM= not measured.

Species, Life Stage	Finasol % CEWAF	Finasol CEWAF TEH (mg/L) Time=0	Finasol CEWAF TEH (mg/L) Time=6	Finasol CEWAF TEH (mg/L) Time=24	Finasol CEWAF TEH (mg/L) Time=96	Time Weighted Average Concentration (mg/L)
Fish, Adult	0.62	0.00	0.00	0.00	0.00	0.00
	1.85	0.00	0.00	0.00	0.00	0.00
	5.56	2.10	0.00	0.00	0.00	0.02
	16.7	7.80	5.80	0.00	0.00	0.34
	50	21.00	15.40	0.00	0.00	0.89
	100	37.60	NM	NM	NM	
Shrimp, Adult	0.62	0.00	0.00	0.00	0.00	0.00
	1.85	0.00	0.00	0.00	0.00	0.00
	5.56	0.00	2.30	0.00	0.00	0.11
	16.7	7.50	8.50	4.60	0.00	1.33
	50	21.70	22.30	9.40	0.00	3.00
	100	37.00	39.60	23.70	0.00	6.64

Species, Life Stage	Finasol % CEWAF	Finasol CEWAF Total PAH (mg/L) Time=0	Finasol CEWAF Total PAH (mg/L) Time=6	Finasol CEWAF Total PAH (mg/L) Time=24	Finasol CEWAF Total PAH (mg/L) Time=96	Time Weighted Average Concentration (mg/L)
Fish, Adult	0.62	0.00	0.00	0.00	0.00	0.00
	1.85	0.00	0.00	0.00	0.00	0.00
	5.56	0.04	0.01	0.00	0.00	0.00
	16.7	0.12	0.05	0.01	0.00	0.01
	50	0.45	0.16	0.03	0.03	0.04
	100	0.94	NM	NM	NM	
Shrimp, Adult	0.62	0.01	0.01	0.00	0.00	0.00
	1.85	0.04	0.02	0.01	0.00	0.00
	5.56	0.13	0.09	0.03	0.00	0.01
	16.7	0.36	0.26	0.06	0.01	0.03
	50	1.58	0.81	0.18	0.03	0.11
	100	1.63	1.38	0.39	0.07	0.20

Table 11. Comparison of 96 h LC_{50} values (95% confidence interval) determined using initial concentrations (t = 0) vs. time weighted average concentrations (TWA) for the large volume toxicity tests. ND = not determined.

Corexit-CEWAF Exposure in mg/L		
Measured Conc.	Adult Fish 96h LC_{50} (95% CI)	Adult Shrimp 96h LC_{50} (95% CI)
TEH t=0 TEH TWA	515.56 (413.69 - 637.34) 217.78 (ND)	105.40 (85.07 - 131.96) 22.34 (19.07 - 26.41)
Total PAH t=0 Total PAH TWA	6.96 (5.55 - 8.62) 3.55 (ND)	1.44 (1.21 - 1.76) 0.44 (0.36 - 0.54)
Finasol-CEWAF Exposure in mg/L		
Measured Conc.	Adult Fish 96h LC_{50} (95% CI)	Adult Shrimp 96h LC_{50} (95% CI)
TEH t=0 TEH TWA	28.21 (24.49 - 32.25) 0.93 (ND)	26.17 (23.18 - 28.97) 3.74 (3.17 - 4.53)
Total PAH t=0 Total PAH TWA	0.66 (0.56 - 0.78) 0.04 (ND)	1.25 (0.82 - 1.68) 0.12 (0.10 - 0.14)

Ecotoxicity of Perfluorooctane Sulfonate and Fluorine-Free Fire Fighting Foams in Estuarine Organisms

Key, P. B.[1], Wirth, E. F.[1], Chung, K. W.[2], Beck, A.[2], Reiner, J. L.[3], Apeti, D.[4], Johnson, E.[4], DeLorenzo, M. E.[1]
[1]NOAA, National Ocean Service, National Centers for Coastal Ocean Science, Charleston, SC, USA
[2]CSS under contract to NOAA, Charleston, SC USA
[3]NOAA, National Institute of Standards and Technology, Charleston, SC, USA
[4]NOAA, National Ocean Service, National Centers for Coastal Ocean Science, Silver Spring, MD, USA

Abstract

Per- and polyfluoroalkyl substances are a group of fabricated chemicals that includes such compounds as perfluorooctane sulfonate (PFOS) which has been the most extensively produced and studied of these chemicals. These chemicals are very persistent in the environment and have been found in fish, animals and humans where they have the ability to build up and persist over time. PFOS is no longer manufactured in the United States, but they are still produced internationally and can be imported into the United States in consumer goods. As an alternative to PFOS, a number of Fluorine Free Aqueous Film Forming Foam (FF-AFF) surfactant formulations are currently under development and commercially available FF-AFF are being tested to evaluate their ability to meet Department of Defense performance requirements. The main objectives of this research are to characterize the effects of PFOS in larval and adult estuarine shrimp, fish, and oysters; and to develop ecotoxicological data of candidate FF-AFF to fish and aquatic invertebrates. The benefit of this research is well defined, assisting the DOD's environmental research programs and EPA in determining the relative hazard of PFOS and several innovative FF-AFF using a multiple species approach whereby the effectiveness of each product can be weighed against its projected environmental hazard. While other agencies such as EPA may focus on contaminant effects in freshwater organisms, ecotoxicology data gaps for marine and estuarine species should be addressed by NCCOS.

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of fabricated chemicals that includes such compounds as PFOA, PFOS, and GenX. PFAS have been used in a variety of industries since the 1940s including food packaging, commercial household products, and electronics manufacturing. PFAS are toxic and persistent in the environment. In the PFAS group, perfluorooctane sulfonate (PFOS) and perfluorooctanic acid (PFOA) have been the most extensively produced and studied of these chemicals. Both chemicals are very persistent in the environment and have been found in fish, animals and humans where they have the ability to build up and persist over time. PFAS compounds have been found in drinking water but typically localized and associated with a specific facility such as landfills, wastewater treatment plants, and firefighter training facilities. PFOA and PFOS are no longer manufactured in the United

States, but they are still produced internationally and can be imported into the United States in consumer goods such as carpet, leather and apparel, textiles, paper and packaging, coatings, rubber and plastics. (https://www.epa.gov/pfas/basic-information-pfas). Perfluorooctane Sulfonate (PFOS) has been identified as one of the most important of these chemicals due to its multiple health risks (Ahrens and Bundschuh 2014), including immunotoxicity, hepatotoxicity, carcinogenicity, and developmental and reproductive effects in humans (Lau et al. 2007; Wang et al. 2010, 2012). Less is known regarding the bioeffects thresholds for these compounds in marine and estuarine species. While other agencies such as EPA may focus on contaminant effects in freshwater organisms, ecotoxicology data gaps for marine and estuarine species should be addressed by NCCOS.

PFAS has been documented in marine and estuarine waters world-wide (Ahrens and Bundschuh, 2014, Fauconier et al., 2019, Salice et al. 2018, Tian et al. 2019, Yamashita et al. 2005), and PFOS levels have been found to exceed EPA guidelines in Louisiana coastal areas (Salice et al. 2018). Fair et al. (2019) measured PFAS levels in edible fish species sampled from Charleston Harbor, SC, and a number of other studies have documented the presence of PFAS in estuarine fish (Nania et al. 2009, Schuetze et al. 2010, Sedlack and Greig 2012, and Taylor and Johnson 2016). PFAS has also been measured in estuarine invertebrates (de Vos et al. 2008, Kannan et al. 2002, Meador et al. 2016, and Van de Vijver et al. 2003). An NCCOS-GLRI retrospective study including 46 samples from the Great Lakes analyzed for 12 PFAS compounds (PFBS PFHpA, PFHxS, PFOA, PFOS, PFNA, PFDS, PFDA, PFOSA, PFUnDA, PFHxA, PFDoDA), of which 7 (PFDA, PFDoDA, PFNA, PFOA, PFOS, PFOSA, PFUnDA) were detected in mussels. Concentration ranged from 0 – 30 ng/g dry wt with PFOS being the most prevalent, found at 57% of the sites. Most research on PFOS has dealt with its presence in tissue and water, thus we have some understanding of organism exposure, but little data to assess whether these environmental concentrations pose a risk to organism health.

A number of Fluorine Free Aqueous Film Forming Foam (FF-AFF) surfactant formulations are currently under development and commercially available. FF-AFF are being tested to evaluate their ability to meet current Department of Defense performance requirements. These formulations are intended for use as alternatives to PFAS. The PFAS environmental concerns have resulted in new research initiatives aimed at the development of FF-AFF with reduced potential for ecological and human health impacts (Jia et al. 2019). Many of these novel chemicals include variations of siloxanes. These products have proven useful in a variety of industrial uses but generally not as a component of aqueous film forming fire-fighting foams designed to isolate combustible fuel from the atmosphere in the event of a spill or fire. Recently, siloxanes have been suggested as a potential novel class of fluorine-free foams (Hetzer et al. 2014). There is very little data relating to the environmental fate of this class of compounds, which limits our understanding of potential environmental risk associated with proposed new uses for these compounds. This FF-AFF phase of our project is being supported by the Strategic Environmental Research and Development Program (SERDP) under the Department of Defense in collaboration with researchers from National Institutes of Standards and Technology (NIST) and US Army Engineer Research and Development Center (ERDC). ERDC will be responsible for testing with freshwater organisms and that proposed research will not be discussed in this report. NIST is responsible for the chemical analysis of the FF-AFFs. The Ecotoxicology Branch

will be responsible for testing marine organisms and that proposed research is included in this report.

This research is designed to address the critical data gaps necessary to understand the environmental risk of PFOS and novel FF-AFF and ultimately provide for a rank order of the relative risk of the formulations. This research will connect exposure and effects by determining toxicity thresholds for estuarine species. These species are grass shrimp (*Palaemon* [*Palaemonetes*] *pugio*), Eastern oyster (*Crassostrea virginica*), sheepshead minnow (*Cyprinodon variegatus*), mysid (*Americamysis bahia*), a copepod species (to be determined) and a diatom (*Phaeodactylum tricornutum*) which are all sensitive and representative species of estuarine invertebrates and fish. They also serve as food for commercially and recreationally important fisheries.

The main objectives of this research are to develop ecotoxicological data and determine the relative toxicities of PFOS and novel FF-AFF by developing data for acute and chronic toxicity to fish and aquatic invertebrates. It is critical to understand the potential risks (including exposure and hazard) that these formulations may pose to the aquatic environment.

Experimental procedures

Test Species and Conditions
The NCCOS Ecotoxicology Branch has performed extensive risk assessments of pesticides, metals, pharmaceuticals and microplastics on estuarine organisms. The design of this study will follow our standard operating protocol of risk assessments that has been proven successful, which includes bioassays, multi-stressor interactions, biomarkers of exposure, and chemical analysis. Grass shrimp, sheepshead minnow, and oysters for this research are all collected in Charleston County. These long-term reference sites are chosen where pre-exposure to the studied chemicals would not exceed background levels. Mysids, copepods and diatoms will be purchased from a commercial supplier (to be determined). Seawater (for all testing and animal holding/culturing) was acquired from Charleston Harbor estuary (N 32° 45' 11.52''; W 79° 53' 58.31''), pre-filtered (5 µm), activated carbon filtered, and diluted with deionized water to adjust salinity to 20 ppt. Seawater for the mysid test will be UV-sterilized and further filtered to 1 µm. Acute testing for both PFOS and FF-AFF will employ standard 96-h static-renewal toxicity testing protocols (Table 1). Each toxicity test will include 5 treatment levels plus control and each treatment will include 5 replicates for the novel FF-AFF compounds (for which no preliminary toxicity data exists) and 3 replicates for the PFOS compounds (preliminary toxicity data is available). Tests will be carried out in temperature controlled environmental chambers using artificial light. Water quality parameters (salinity, temperature, pH, dissolved oxygen) and survival (or growth inhibition) will be recorded daily.

PFOS Assays
Acute toxicity bioassays with 24-48h old sheepshead minnows and grass shrimp, and 5-7 day old oysters will be conducted to determine median lethal toxicity values for PFOS and to determine sublethal effects such as changes in swimming or feeding behavior. In addition to testing under standard laboratory conditions, multi-stressor interactions will be assessed (e.g., lower salinity,

higher temperature, and ultraviolet light) to determine if these abiotic variables alter the toxicity of PFOS.

PFOS Adult Oyster Assays

Adult oysters were tested for PFOS bioaccumulation and to detect biomarkers of PFOS exposure. Oysters (70-100 mm in length) were collected in the fall and winter seasons from a reference site at the mouth of Leadenwah Creek at North Edisto River on Wadmalaw Island, South Carolina (N 32° 37' 03.9"; W 80° 13' 44.6"). Seawater for testing was collected from Charleston Harbor estuary (N 32° 45ˈ11.52"; W 79° 53ˈ58.31"), filtered (5 µm), UV-sterilized, activated carbon filtered, and diluted with deionized water to 20 ppt salinity. The oysters were scrubbed with 20 ppt seawater to remove algae and barnacles then placed in a controlled laboratory aquatic recirculating system to acclimate at 25°C, 20 ppt salinity, and 16-h light: 8-h dark cycle (16L: 8D). Oysters were fed 10 mL of Shellfish Diet® (Reed Mariculture; Campbell, CA) daily until day of exposure. Oyster length and width were measured and recorded before exposures.

For the bioaccumulation research, 24 oysters were collected and exposed to PFOS in individual glass beakers containing 1 L of two concentrations (0.3 mg/L and 3 mg/L) and a control (0 mg/L). Eight replicates were made for each treatment and control group. Beakers were covered with a clear plastic lid with a hole drilled in it for oxygen exchange. Each exposure concentration and control seawater was replaced every day for 7 days. Mortality was assessed daily and water quality measurements were taken. After 7 days of exposure, half of the oysters from each treatment group were shucked and whole tissue was flash frozen in liquid nitrogen. The remaining four oysters were placed into clean 20 ppt seawater (changed daily) and allowed to depurate for 48 h. At the end of two days depuration, oysters were shucked, flash frozen in liquid nitrogen and stored at -80°C. All 24 oysters were processed for chemical analysis. Additionally, 1 mL water samples were taken for PFOS analysis from each treatment group T=0 and T=24 during the 7 day exposure. Oyster tissue samples and water samples were analyzed for PFOS concentrations using methods previously described by Reiner et al. (2012). The bioaccumulation factor (BAF) was calculated as the measured average PFOS concentration in oyster tissue (ppb wet weight) after 7 days exposure, divided by the measured average PFOS concentration in the water (ppb). Also, the percent PFOS elimination from oyster tissue was calculated for the 7 day exposure experiment. Average total PFOS concentration in oyster tissue (ppb) for each treatment group was subtracted from the average total PFOS concentration in oyster tissue (ppb) after 48 h depuration. This difference was divided by the average total PFOS concentration in oyster tissue (ppb) for each treatment group and multiplied by 100.

For the biomarker assays, each adult oyster was exposed to 1 L of PFOS treatment in 20 ppt seawater (0 mg/L, 3 mg/L, 30 mg/L, 300 mg/L) in a glass beaker. Beakers were covered with a clear plastic lid with a hole drilled in it for oxygen exchange. Oysters were added to the corresponding treatment beaker and placed into an environmental chamber (Percival Scientific) at 25°C and a 16L: 8D light cycle. After 48 h of exposure, oysters were shucked and whole tissue wet weight (wet wt) was recorded. The hepatopancreas (HP) was dissected, weighed, divided into three sections and two of which were frozen in liquid nitrogen for downstream biomarker analysis and the third section was processed immediately for lysosomal destabilization. PFOS 48-h exposure experiments and biomarker analyses were repeated three

times with four or six replicates per treatment group. Additional oysters were collected, not subjected to treatment, and were dissected and analyzed for comparison to controls (T=0) in bioassay tests. For lysosomal destabilization, live cells from *C. virginica* were examined for lysosomal integrity following methods developed by Ringwood et al. (2003). After processing (Ringwood et al. 2003), 50 µL of cells were pipetted onto a microscope slide and at least 50 individual cells were counted per treatment and visually sorted for dye presence in the lysosome (stable) or cytosol (destabilized) at 40x magnification. The percentage of destabilized lysosomes was then calculated. Lipid peroxidation was measured following methods developed by Ringwood et al. (2003). Lipid peroxidation is measured in oyster hepatopancreas tissue as malondialdehyde (MDA) which is a measured end product of cellular membrane damage. After processing the hepatopancreas (Ringwood et al. 2003), aliquots of 300 µL supernatant were plated in triplicate into a clear Corning 96 well plate and read using a µQuant microplate spectrophotometer (Bio-Tek Instruments Inc.). Absorbance readings for each sample was used to determine the amount of MDA in nmol/g (wet wt). The glutathione assay (GSH) methods followed Ringwood et al. (2003), 5, 5'-dithiobis (2-nitrobenzoic) acid-glutathione (DTNB-GSSG) recycling protocol. After processing the hepatopancreas (Ringwood et al. 2003), samples were read via a spectrophotometer UltroSpec 5300 pro (Amersham Biosciences) to obtain GSH measurements expressed as nmol/g (wet wt).

FF-AFF Assays

Acute toxicity testing with FF-AFF compounds will occur with two crustaceans, one bivalve, and one fish species (Table 1). This testing will be for 96 hours and have survival as an endpoint. For the FF-AFF acute testing, protocols will follow those cited in Table 1. Acute tests will include five treatments plus controls. A positive control will also be included. Water quality will be recorded daily. Chronic toxicity testing will occur only with the FF-AFF compounds and will address sublethal toxicity using survival, growth and/or reproduction as endpoints. It is proposed to test each FF-AFF formulation using one invertebrate and one fish species. The selected test organisms will be chosen from those listed in Table 2 and based in part by the relative sensitivity of species acute testing as listed in Table 1 for FF-AFF. The same FF-AFF formulations that are selected for acute testing will be used for the chronic tests and protocols will follow those cited in Table 2. Chronic tests will include five treatments plus controls. A positive control will also be included. Water quality will be recorded daily. For mysids, surviving individuals will be collected at the end of the exposures and the change in mass will be determined. For *C. variegatus*, larval fish will be exposed for 28 days. Endpoints will include growth and survival. Surviving individuals from chronic tests utilizing species of sufficient size for tissue analysis will be collected and archived for future tissue analysis.

Results

PFOS Preliminary Toxicity Assays

Preliminary toxicity data with grass shrimp larvae and sheepshead minnow larvae exposed for 96-h to PFOS show reduced survival starting at the 3 mg/L PFOS concentration with no fish surviving at 30 mg/L (Figure 1).

Preliminary results with oyster larvae PFOS testing showed no significant mortality in after 96-h exposure up to the highest concentration of 30 mg/L (Figure 2). Of the three larval organisms

tested, sheepshead minnow were the most sensitive followed by grass shrimp and oyster. Even though survival was not significantly affected, there was a significant effect on oyster larvae swimming ability at the highest concentration of 30 mg/L (Figure 3). This effect on swimming ability could interfere with normal oyster recruitment, settlement, feeding, and leave larvae more prone to predation. The next step for the acute tests with PFOS will be to establish LC50s for the larval grass shrimp, sheepshead minnow and oyster.

PFOS Adult Oysters Assays

Adult oysters were exposed to PFOS at concentrations up to 300 mg/L for 48 h and there was a significant effect on lysosomal destabilization biomarker at all concentrations (Figure 4). According to Ringwood et al. (2009), the normal range of lysosomal destabilization occurs up to 30% of the cells observed. Control oysters were below the 30% threshold (22%). As seen here, the three exposures had percentages of destabilized (damaged) cells over 60%.

There was no effect on lipid peroxidation levels measured as MDA (Figure 5). One-way ANOVA analysis determined no significant difference between groups. The normal range of MDA in oysters is ≤ 150 nmol/g. Glutathione was analyzed in adult oysters as well (Figures 6a, b). There was no statistical significant difference between treatment groups tested as compared to controls. However, although the majority of oysters fell within the normal acceptable range of 800-1600 nmol/g (wet wt) (Ringwood et al. 2002), there were a higher percentage of oysters below the 800 nmol/g (wet wt) in the 3 mg/L and 30 mg/L exposure groups (Figure 6b). Thus, these oysters were considered to be in a 'concerned' state due to decreased levels of GSH.

PFOS concentrations were measured in the exposure water for both the biomarker assays and the bioaccumulation assay (Table 3a). PFOS concentrations were measured in adult oysters after 7 d exposure to 0.3 and 3.0 mg/L followed by a 2 d depuration period (Table 3b). In the 0.3 mg/L exposure, total PFOS levels concentrated 43 fold over 7d to 12.82 mg/L while in the 3.0 mg/L exposure, total PFOS levels concentrated 84 fold over 7 d to 252.15 mg/L. With this information, a bioaccumulation factor (BAF) was calculated using the measured water concentrations (Table 3a). The BAF for 0.3 mg/L exposed oysters was 50 and the BAF for the 3.0 mg/L exposed oysters was 116 (Table 3b). After the 2 d depuration, total PFOS levels fell to 0.52 mg/L for the 0.3 mg/L and 10.92 mg/L for the 3.0 mg/L concentration representing a 96% elimination for both. When analyzing the oyster tissue, it was found that our PFOS stock consisted of two isomers – a linear isomer and a branched isomer. Oysters were able to uptake both isomers during the 7 d exposure (Figures 7a, b). At 7 d, the linear isomer consisted of 69.36% of the total PFOS in the 3.0 mg/L treatment with the linear isomer comprising 85.3% of the total PFOS in the lower treatment of 0.3 mg/L (Table 3b). However, after the 2 d depuration, the PFOS that remained in the oyster tissue consisted almost solely of the linear PFOS isomer (Figure 7b and Table 3b): 95% of the total PFOS in the 3.0 mg/l treatment and 99% of the total PFOS in the 0.3 mg/L treatment.

Discussion

In the PFAS group, PFOS and PFOA have been the most extensively produced and studied of these chemicals. Both chemicals are very persistent in the environment and have been found in fish, animals and humans where they have the ability to build up and persist over time. PFAS

compounds have been found in drinking water but typically localized and associated with a specific facility such as landfills, wastewater treatment plants, and firefighter training facilities. PFOA and PFOS are no longer manufactured in the United States, but they are still produced internationally and can be imported into the United States in consumer goods such as carpet, leather and apparel, textiles, paper and packaging, coatings, rubber and plastics. (https://www.epa.gov/pfas/basic-information-pfas).

PFOS has been identified as the most important of these chemicals due to its multiple health risks in humans including hepatotoxic, carcinogenetic, and developmental and reproductive effects in humans. PFOS and PFOA have been shown to accumulate in different fish species and numerous invertebrates. A retrospective study of 46 samples from the Great Lakes found seven PFAS compounds detected in zebra mussels. Concentrations ranged from 0 – 30 ng/g dry weight with PFOS being the most prevalent, found at 57% of the sites (Great Lakes Mussel Watch).

The USEPA has set a drinking water advisory of 0.07 ppb for PFOS. A hazardous effect level for freshwater species has been suggested at 1.12 ppb (Salice et al., 2018). Our preliminary results show that, in terms of toxicity to three common estuarine organisms, PFOS toxicity levels are well above these advisory levels. These advisory and hazardous effect levels would seem to be protective of estuarine organisms as well but only in terms of acute toxicity. Additional testing with cellular stress enzymes, or biomarkers, will show chemical and/or physical responses in organisms exposed to contaminants and can be used to measure the effect of contaminants on the health of organisms. Also, abiotic stressors of temperature and salinity can be tested as well to research any changes to LC50s or biomarkers.

The sublethal biomarkers used in this research indicate some cellular stress after PFOS exposure. The lysosomal destabilization assay on adult oyster tissue showed significant results in exposed oysters compared to controls. Lysosomes are intracellular organelles that are involved in many essential functions, including membrane turnover, nutrition, and cellular defense. The lysosome is maintained by a membrane-bound, ATPase-dependent proton pump. Disruption of the proton pump by chemical contaminants can lead to the impairment of vital functions and cell death. Glutathione (GSH) is a ubiquitous tripeptide that is regarded as one of the most important non-protein thiols in biological systems. GSH functions as an important overall modulator of cellular homeostasis, and serves numerous essential functions including detoxification of metals and oxy-radicals. While exposure to pollutants or stressful conditions can result in elevated GSH levels, there is evidence that adverse effects are associated with GSH depletion in marine bivalves, as well as mammalian systems. Lipid peroxidation (LPX), an indicator of damage to cell membranes, occurs when free radicals react with lipids and is a source of cytotoxic products that may damage DNA and enzymes. All three biomarkers will be run with grass shrimp and sheepshead minnow as well as the oysters.

A longer exposure and lower concentrations of PFOS was used to challenge oysters in order to examine the effectiveness of detoxification. Chemical analysis revealed the presence of two PFOS isomers: branched and linear in the oyster tissue. However, following two days of depuration in clean seawater, the linear isomer still remained at high levels whereas the branched

isomer was almost completely eliminated from the tissue. Thus, the amount of PFOS remaining in the tissue is almost exclusively the linear fraction. While these isomers' individual toxicity remain unclear, literature reports the branched isomer is eliminated faster than the linear isomer (Lau, 2015). Though, the process of depuration did eliminate over 94% of PFOS in the tissue, concentrations still remained high with over 10 mg/L reported in the 3mg/L group indicating longer depuration times are needed to further reduce PFOS levels in the tissue.

As the research with PFOS continues, we will work with NOAA's Office of Response and Restoration to submit the toxicity threshold values derived from our study to the Chemical Aquatic Fate and Effects (CAFÉ) database. We will continue to collaborate with NIST, who is conducting the PFOS analysis in water and tissue samples. We will leverage NOAA's Mussel Watch Program data to compare effects thresholds derived from our study to PFOS measurements in field-collected mussels. Great Lake Mussel Watch has submitted a Great Lake Restoration Initiative proposal to EPA's Great Lakes National Program Office for FY21 funding to continue monitoring chemicals of emerging concern with special emphasis on PFAS and its bioeffects on fish and wildlife. As part of this effort, Mussel Watch would monitor mussel metabolomics and other molecular level indicators to determine whether adverse molecular-level bioeffects from exposure to PFAS is contributing to organism stress and trophic transfer of PFAS from colonizing benthic infauna to bivalves, to fish (round gobies). A retrospective analysis of PFAS in mussels collected from all the Great lakes is possible with archived samples.

We will seek additional interactions with the EPA in order to communicate and coordinate our research efforts in order to complement and expand on, rather than be redundant with their ongoing PFAS initiative. Recently, EPA released their PFAS Action Plan, and announced a research initiative on PFAS National Priorities: Research on PFAS Impacts in Rural Communities and Agricultural Operations with the goal "to help communities address the larger issue of PFAS nationwide". In addition to connecting with EPA, we are co-collaborators with NIST and ACOE on a SERDP proposal to study the toxicity of alternative fluorine-free surfactant formulations that are currently under development to replace PFAS chemicals. This additional research would allow us to compare toxicological risk of PFOS levels currently in the environment with that of emerging fluorine-free compounds.

For the FF-AFF portion of this research, this integrates the diverse capabilities of three, well-respected research laboratories (and organizations) that have extensive experience in aquatic toxicity testing, life cycle assessment, and chemical analysis and have contributed to the development and/or standardization of numerous methods. The NOAA team will lead all testing on marine and estuarine species. The researchers from NOAA have an extensive understanding of standard toxicity testing using the proposed organisms. The NOAA team has experience with chemicals that include legacy contaminants, current use pesticides, munitions, nano-materials, and oil spill response formulations (dispersants, surface washing agents and bioremediation agents). It is expected that the toxicity testing efforts surrounding the formulations selected by SERDP will follow a similar research pathway. The National Institutes of Standards and Technology is involved in PFAS and new analytical methods effort, with specific objectives of building collaboration and supporting emerging chemical questions. This work will build on our on-going PFAS-related efforts including development of water-based PFAS reference materials and a suite of AFFF reference materials, creation of a curated PFAS MS library, and

development of PFAS source-tracking methods using non-targeted HRMS. The investigators from the US Army Engineer Research and Development Center (ERDC) will focus efforts on the freshwater toxicity testing with representative, taxonomically diverse test species. This research team has a long history of testing a variety of chemicals across numerous species, most recently having evaluated toxicity of commercially available fluoro-surfactants (e.g., Capstone) and several siloxane-based (fluorine free) replacement compounds including Silwet, 502W, and Glucopon. Recently, the ERDC team has developed capability and methods for testing difficult to test contaminants such as photodegradation of munitions (Kennedy et al 2017), tiered testing of advanced materials and nanomaterials, including novel assessments of toxicity with dynamically changing exposure concentrations (Kennedy et al 2017).

The intended outcomes of this project will be research products delivered to the customer that answer key scientific questions about PFOS and FF-AFF toxicological endpoints. Both the PFOS and FF-AFF research will provide critical data to decision makers for use in evaluations of new technologies and risk-based decisions. For FF-AFF, SERDP will be provided with Interim Reports yearly and a Final Report will be provided to SERDP at the conclusion of the project. Ideally, these project results will be submitted for publication in peer-reviewed journals and will be presented at national and international professional meetings. The research team realizes, however, that there may be proprietary factors that limit the ability to publish the FF-AFF research work in peer-reviewed publications. Ecotoxicological data developed in the proposed research for all FF-AFFF formulations will be transitioned to Army/Navy/Air Force through the Tri-Services Environmental Risk Assessment Work Group (TSERAWG). We will also provide data (toxicity thresholds to be used in OR&R's CAFÉ database and other chemical databases, bioeffects characterizations, and chemical fate analyses), consultation services (data extrapolation and application to field response), and research synthesis (publications and presentations). With NCCOS funding for the PFOS research, we will focus on data gaps as found in the literature. Ecotoxicology Branch will work with Monitoring and Assessment Branch to conduct a hazard assessment using the field collected bivalve PFOS concentrations and the laboratory-derived bioeffects and accumulation data for adult oysters. Based on previous interest by the USACOE PFOS testing, there is potential for an additional collaborative effort. Collectively, the data generated from these research studies will lend quality scientific support for remediation of PFOS contamination and lead to scientifically derived decisions on the ability to use FF-AFF in commercial applications.

Data Availability

All data reported in this manuscript resides both electronically on NOAA servers and in hard copy. See M. DeLorenzo (marie.delorenzo@noaa.gov) or P. Key (pete.key@noaa.gov) for access.

Acknowledgements

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Supporting Documents

This report is based on the following funded projects and draft manuscript.

1. NCCOS discretionary FY2020 project: Ecotoxicology of Perfluorooctane Sulfonate (PFOS) in Estuarine Organisms. Pete Key, Katy Chung, Allisan Beck, Dennis Apeti, Ed Johnson, Marie DeLorenzo
2. Strategic Environmental Research and Development Program (DOD, EPA, DOE) Grant: Ecotoxicity of Fluorine-Free Fire Fighting Foams. Lead PIs: Ed Wirth (NCCOS), John Kucklick (NIST), David Moore (USACE-ERDC). Proposal Number: ER20-A1-1518.
3. Chemical and Biological Effects of Perfluorooctane Sulfonate Exposure in the Adult Eastern Oyster *Crassostrea virginica*. Allisan A. Aquilina-Beck, Jessica L. Reiner, Katy W. Chung, Meaghan J. DeLise, Peter B. Key, Marie E. DeLorenzo. 2020. In NCCOS Charleston internal review.

Table 1. Specific test species with life stage, test compound, endpoint, and protocol for acute (96 h) toxicity testing with PFOS and FF-AFF compounds.

Taxon	Species and Life Stage	Test Compound	Endpoint	Protocol
Invertebrate	*Americamysis bahia* Adult	FF-AFF	Survival	EPA 721-C-16-011 (2016)
Invertebrate	*Palaemon pugio* Larvae Adult	PFOS PFOS	Survival	ASTM E729-96 (2014)
Invertebrate	*Pseudodiaptomus pelagicus, or Acartia tonsa* Adult	FF-AFF	Survival	ASTM WK60640 (2019)
Bivalve	*Crassostrea virginica* Larvae Adult	PFOS, FF-AFF PFOS	Survival	ASTM E724-98 (2012); Adams and Rowland 2002
Fish	*Cyprinodon variegatus* Larvae	PFOS, FF-AFF	Survival	ASTM E729-96 (2014)

Table 2. Specific test species, duration, endpoints, and protocols for chronic toxicity testing with FF-AFF compounds.

Taxon	Species	Duration (d)	Endpoints	Protocol
Fish	*Cyprinodon variegatus*	28	Survival, growth	EPA-712-C-16-008 (2016)
Invertebrate	*Americamysis bahia*	28	Survival, reproduction	EPA-821-R-02-014 (2002)
Diatom	*Phaeodactylum tricornutum*	4	Growth	ISO 10253 (2006)

Table 3.
a. Average measured PFOS concentration in exposure water for the biomarker assay at time = 0h and bioaccumulation assay at times = 0h and 24h. Concentrations shown as ± standard error.

Assay	Time	Nominal PFOS Concentration (mg/L)	Measured PFOS Concentration (mg/L)
Biomarker	T = 0	Control (0)	0.00
		3	1.45 ± 0.044
		30	4.87 ± 0.48
		300	247 ± 17.51
Bioaccumulation	T=0	Control (0)	0.19 ± 0.06
		0.3	0.26 ± 0.02
		3.0	2.16 ± 0.34
	T=24	Control (0)	0.18 ± 0.03
		0.3	0.25 ± 0.02
		3.0	1.91 ± 0.09

b. Average measured PFOS concentrations (total, linear, and branched) in whole oyster tissue for the bioaccumulation assay after 7 day exposure along with the calculated bioaccumulation factor (BAF). Concentrations shown as ± standard error.

PFOS Nominal Water Concentration (mg/L)	PFOS Isomer	Measured PFOS Concentration in Tissue after 7 d exposure (mg/L)	Measure PFOS Concentration in Tissue after 48 h Depuration (mg/L)	Elimination of PFOS from Tissue after 48h Depuration	BAF
Control (0)	Total	0.0104 ± 0.003	0.0044 ± 0.0008		
	Linear	0.0099 ± 0.003	0.004 ± 0.0008		
	Branched	0.0004 ± 0.0001	0.0002 ± 0.0001		
0.3	Total	12.82 ± 20.51	0.52 ± 0.066	96%	50
	Linear	10.94 ± 1.71	0.51 ± 0.066	95%	
	Branched	1.88 ± 0.35	0.0064 ± 0.0012	100%	
3.0	Total	252.15 ± 52.26	10.92 ± 4.78	96%	116
	Linear	174.91 ± 36.55	10.39 ± 4.53	94%	
	Branched	77.24 ± 15.94	0.52 ± 0.24	99%	

Figure 1. Larval grass shrimp and sheepshead minnow survival after 96-h exposure to PFOS.

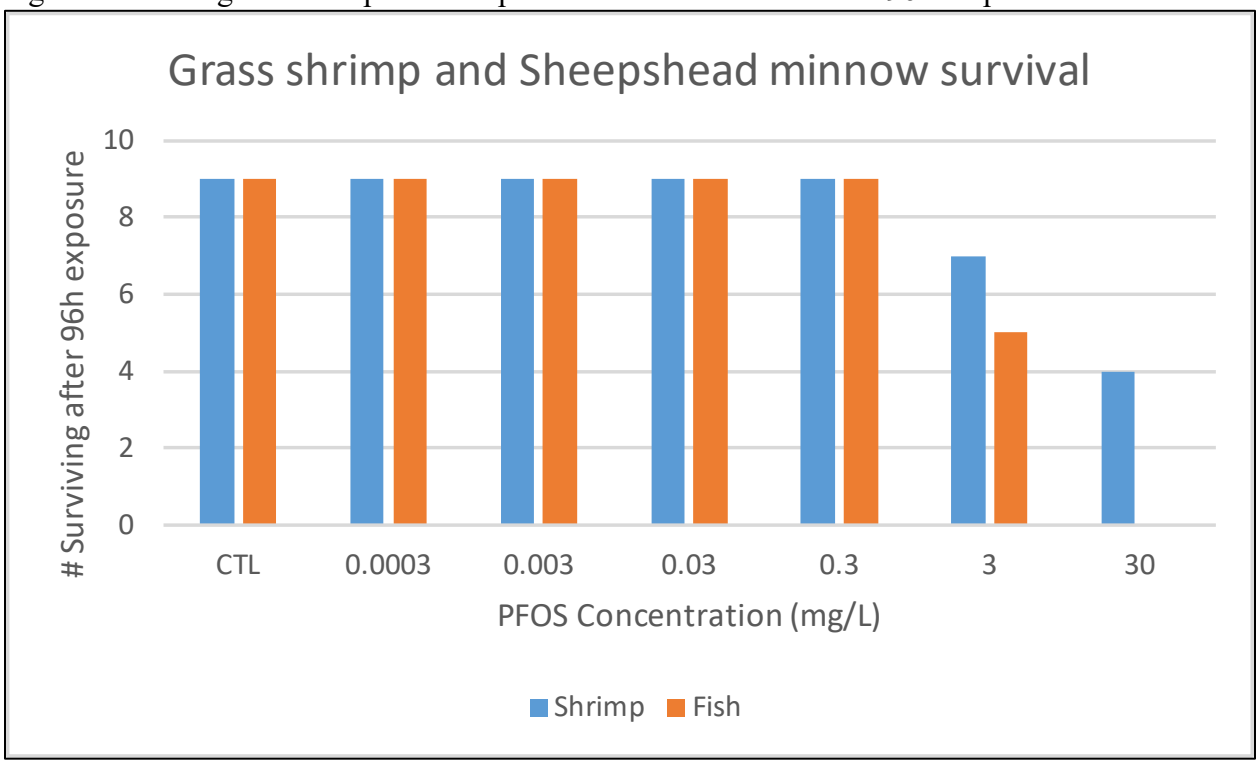


Figure 2. Larval oyster survival after 96-h PFOS exposure.

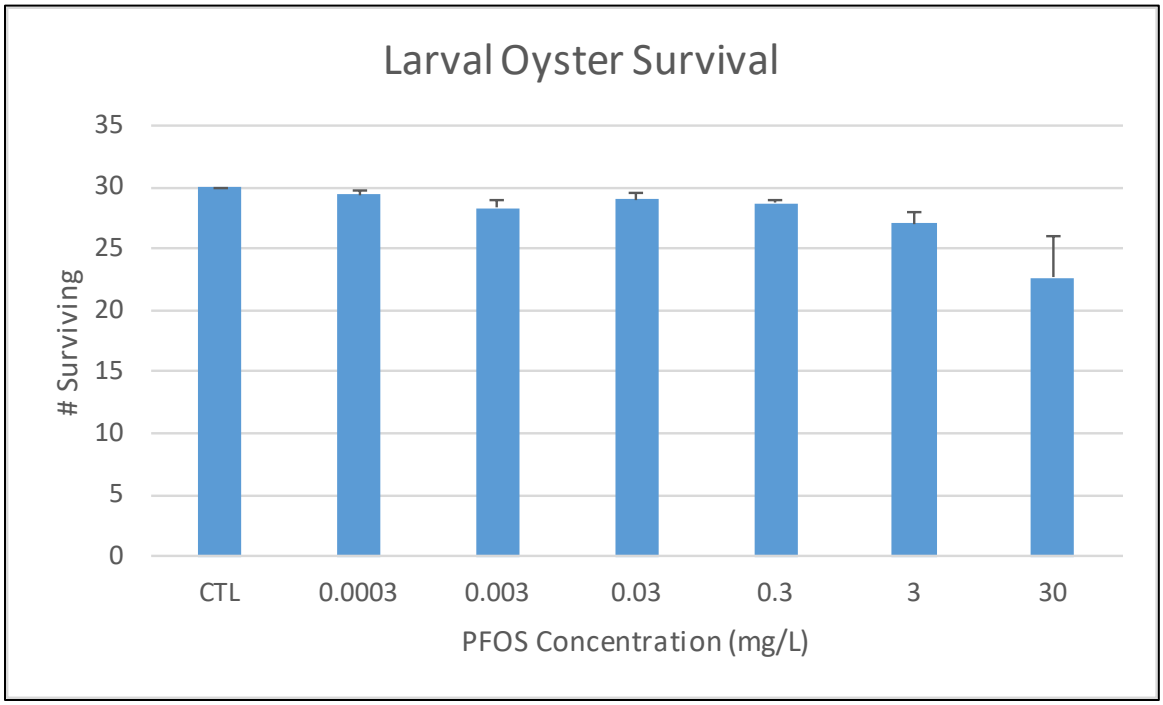


Figure 3. Larval oyster swimming ability after 96-h PFOS exposure.

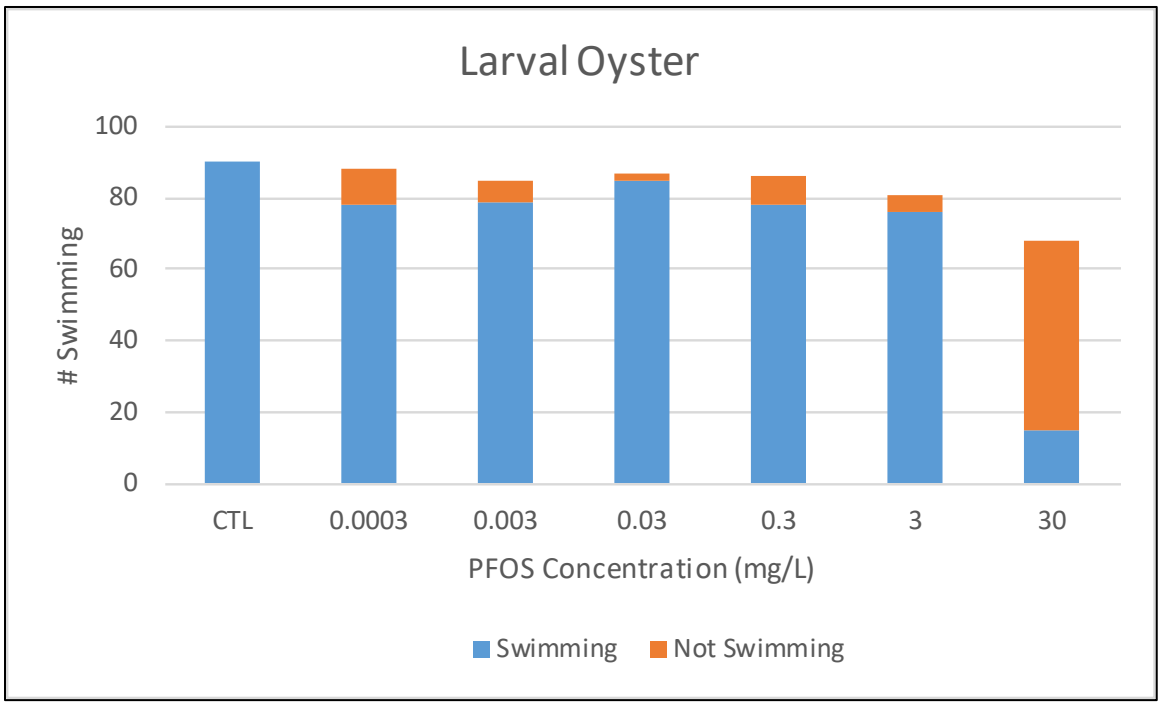


Figure 4. Lysosomal destabilization measured in adult oysters after 48 h exposure to PFOS. The percent of destabilization is graphed against the PFOS concentrations. Different letters represent significant differences between groups, $p<0.0001$ (30mg/L and 300mg/L), $p=0.0003$ (3mg/L).

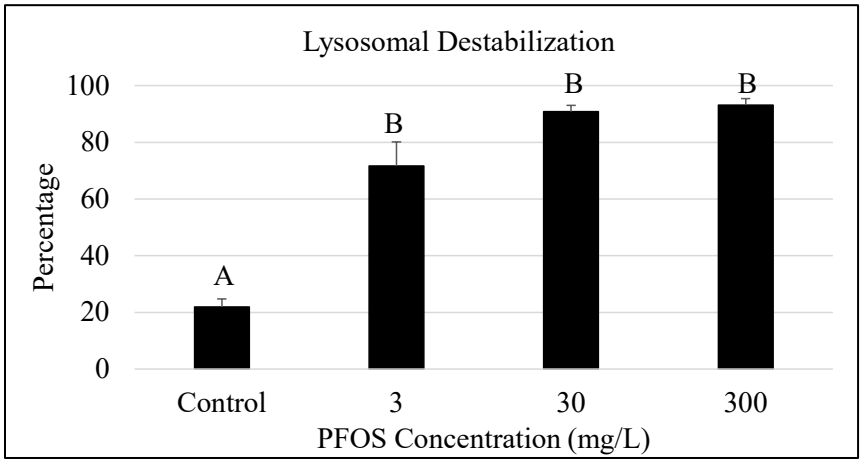


Figure 5. Lipid peroxidation levels in adult oysters after 48 h exposure to PFOS. One-way ANOVA analysis determined no significant difference between groups. The line represents a normal range of MDA at ≤150 nmol/g.

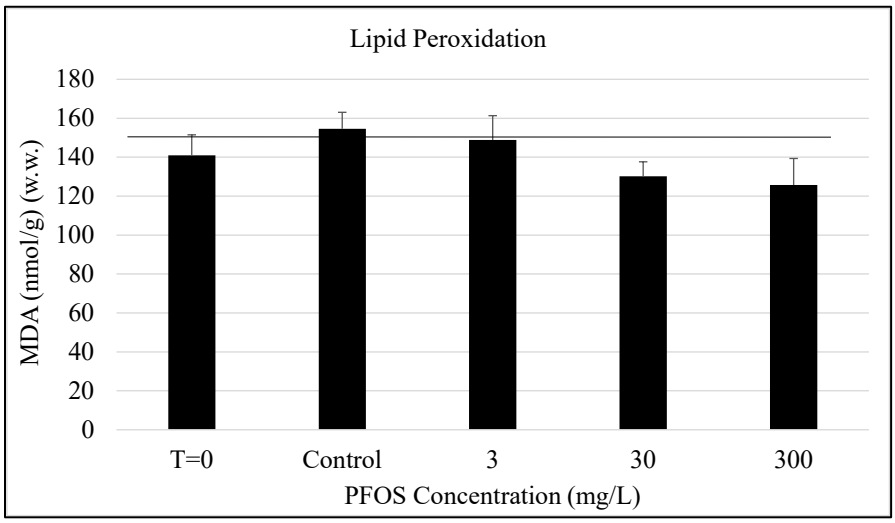
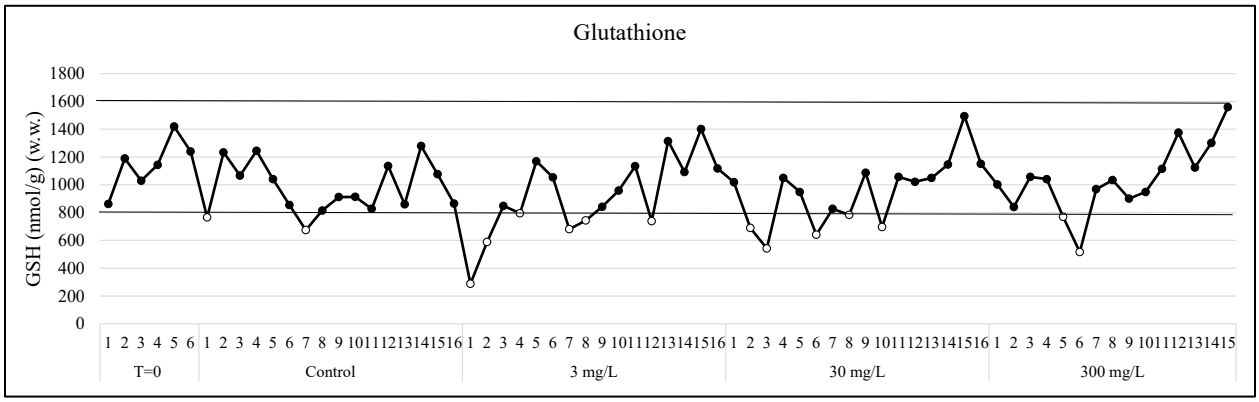


Figure 6.
a. Glutathione levels measured in adult oysters after 48 h exposure to PFOS. One-way ANOVA analysis determined no significant difference between groups. Most oysters fell within in acceptable normal ranges of 800-1600 nmol/g. Empty circles (o) represent oyster glutathione levels < 800 mg/L.



b. Analysis of oysters that fall within the 'concerned' range of <800 nmol/g (empty circles in Figure 6a) are three to four times higher in the 3 mg/L and 30 mg/L treatment groups compared to controls and T=0. T=0 are oysters not subjected to treatment conditions.

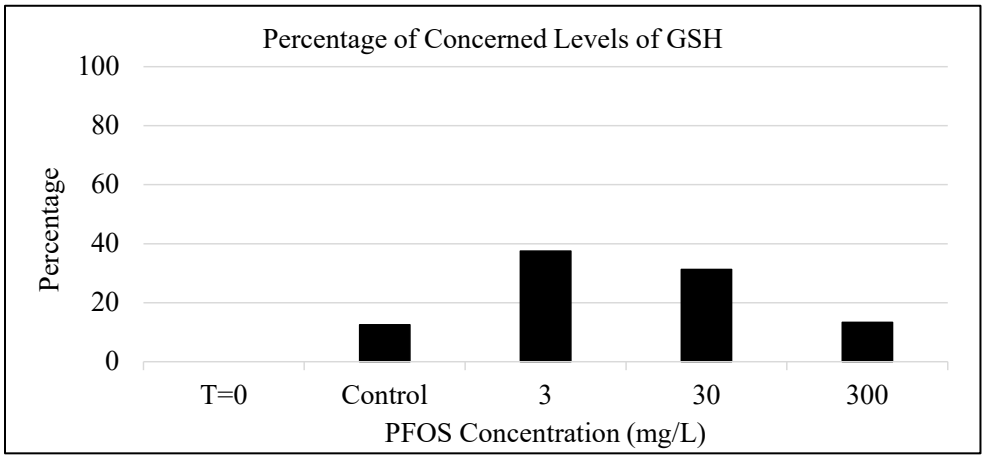
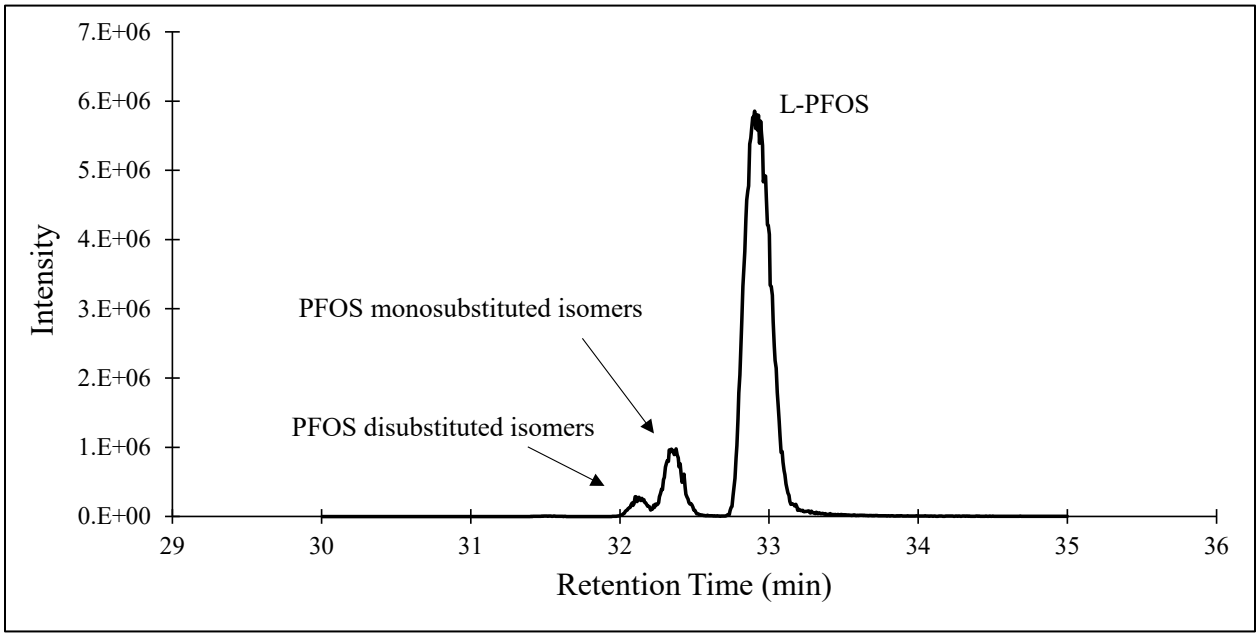
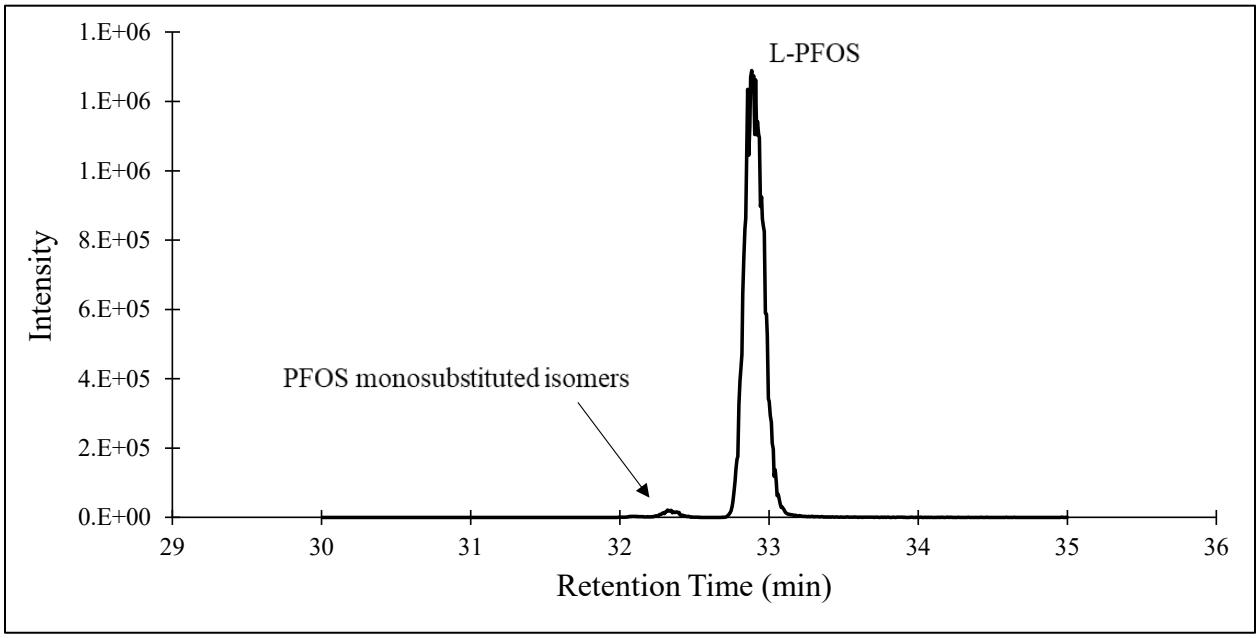


Figure 7. Extracted ion chromatogram of PFOS (transition 499 > 80) in oyster (*C. virginica*) tissue samples. Graphs are of a single oyster representative of their respective groups.

a. PFOS uptake in an adult oysters after 7 d exposure to 0.3 mg/L. Graph shows both PFOS isomers present: branched PFOS isomers (monosubstituted and disubstituted) and linear PFOS (L-PFOS) isomer.



b. PFOS depuration in an adult oyster after 2 d in clean seawater. Graph shows both PFOS isomers present: branched PFOS isomer (monosubstituted) and linear PFOS (L-PFOS) isomer.



Title: Efficacy and Ecotoxicological Effects of Shoreline Cleaners in Salt Marsh Ecosystems

DeLorenzo, M.E.[1], Key, P.B.[1], Wirth, E.F.[1], Pennington, P.L.[1], Chung, K.W.[2], Pisarski, E.[2], Shaddrix, B.[2], Baxter, S.[3], Fulton, M.H.[1]

[1] NOAA/NOS/NCCOS; Stressor Detection and Impacts Division
[2] CSS Inc., under contract to NOAA/NOS/NCCOS; Stressor Detection and Impacts Division
[3] College of Charleston, 66 George St, Charleston, South Carolina 29424

Abstract

Salt marsh ecosystems are sensitive habitats that may be susceptible to oil and oil spill mitigation chemicals during clean up. This research 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.

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.

Experimental Procedure

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.

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).

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).

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).

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

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).

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.

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).

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).

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).

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.

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.

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).

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.

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).

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).

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)).

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.

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.

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).

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.

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).

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.

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 (LC50) was determined after 96 h exposure (Table 5).

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.

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).

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 ($p <0.0001$).

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).

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).

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).

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).

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).

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).

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).

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%.

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).

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.

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.

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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Supporting Documents

This report is based on the following funded projects and documents.

1. Baxter, S.E., DeLorenzo, M.E., Key, P.B., Chung, K.W., Beckingham, B., Fulton, M.H. (2018) Toxicity Comparison of the Shoreline Cleaners Accell Clean® and PES-51® in Two Life Stages of the Grass Shrimp, *Palaemonetes pugio*. *Environ Sci Poll Res* 25(11):10926-10936. DOI: 10.1007/s11356-018-1370-2.
2. DeLorenzo, M.E., Key, P.B., Wirth, E.F., Pennington, P.L., Chung, K.W., Pisarski, E., Shaddrix, B., Baxter, S., Fulton, M.H., 2017. Efficacy and ecotoxicological effects of shoreline cleaners in salt marsh ecosystems. NOAA Technical Memorandum NOS NCCOS 232. 110 pp.
3. NCCOS Discretionary Project: Ecological Effects of Shoreline Cleaners FY15, $235,000

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)	

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

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
6-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)
4-d	TEH	0	0.47	0	0.61	0
0-d	TEH	0	0.31	0	0.86	0.39
2-h	PAH50	0.584	96.4	106	112	97.2
6-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)
4-d	PAH50	0	6.80	4.31	11.3	4.37
0-d	PAH50	0	3.21	1.57	7.77	4.29

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					

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) *+

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%

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

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

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)*

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)

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

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)*

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.

	Tile		Water	
Treatment	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

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 %

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				

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

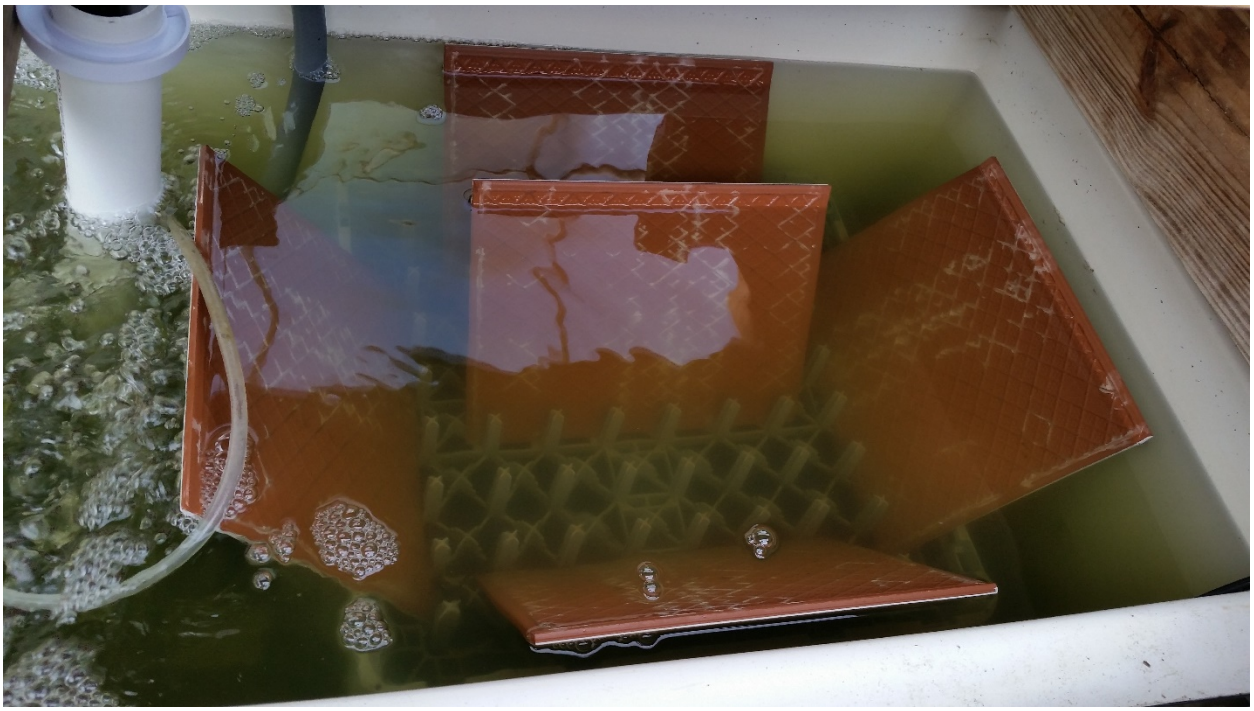

Figure 2. Ceramic tiles in place in the bottom sump of a mesocosm system.

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

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

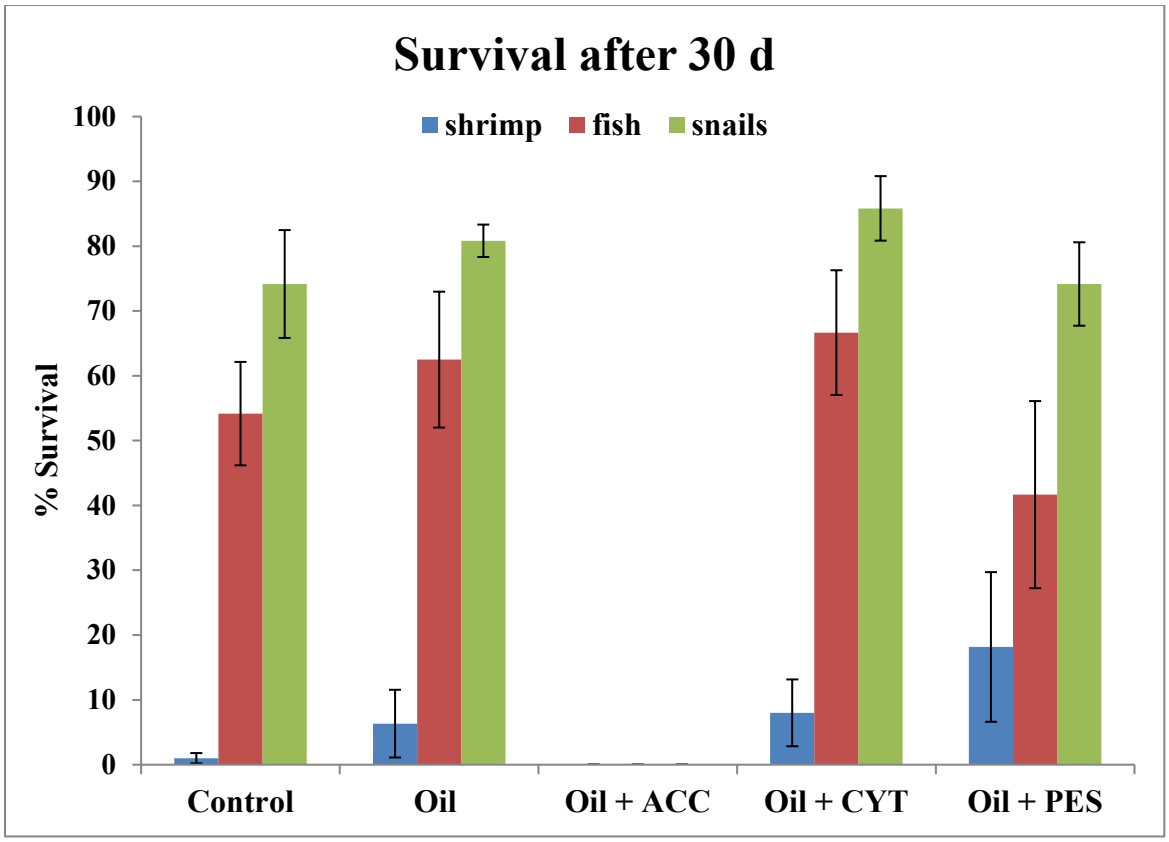

Figure 5. Survival of fish, shrimp, and snails after 30 d in the mesocosm treatments.

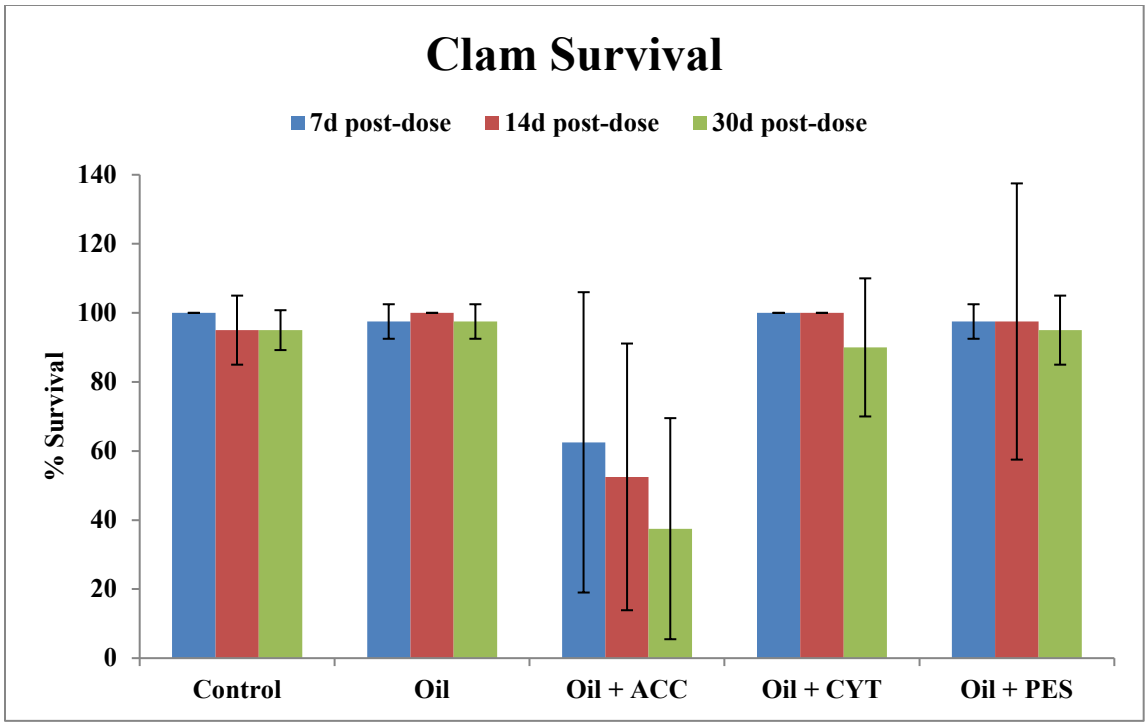

Figure 6. Survival of juvenile clams after 30 d in the mesocosm treatments.

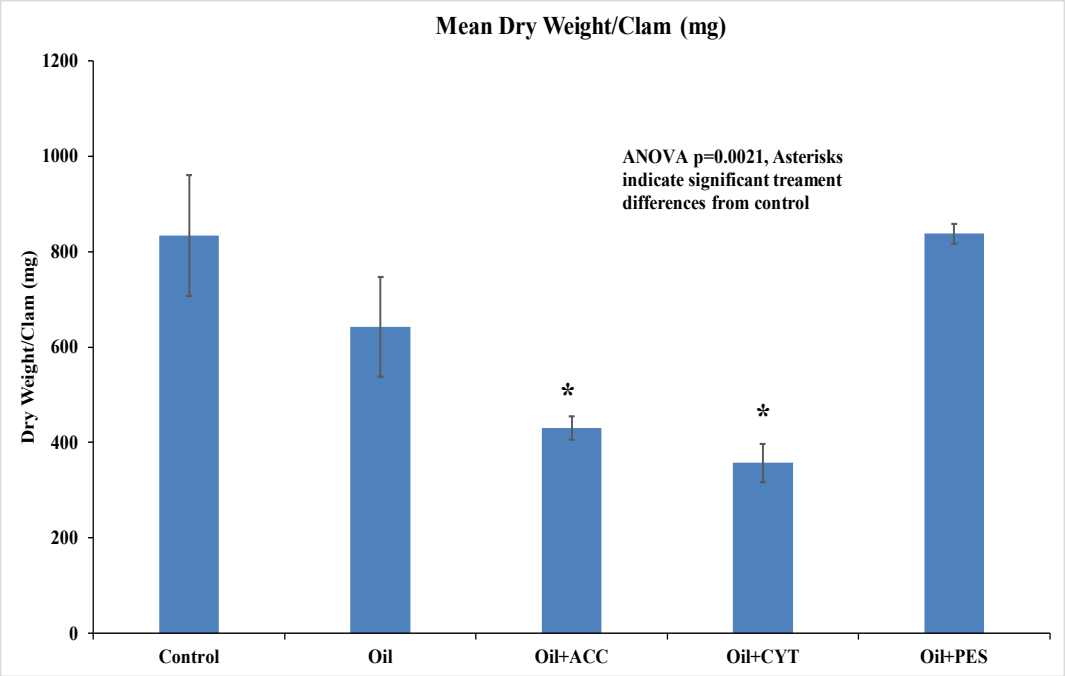

Figure 7. Juvenile clam dry weight after 30 d in the mesocosm treatments.

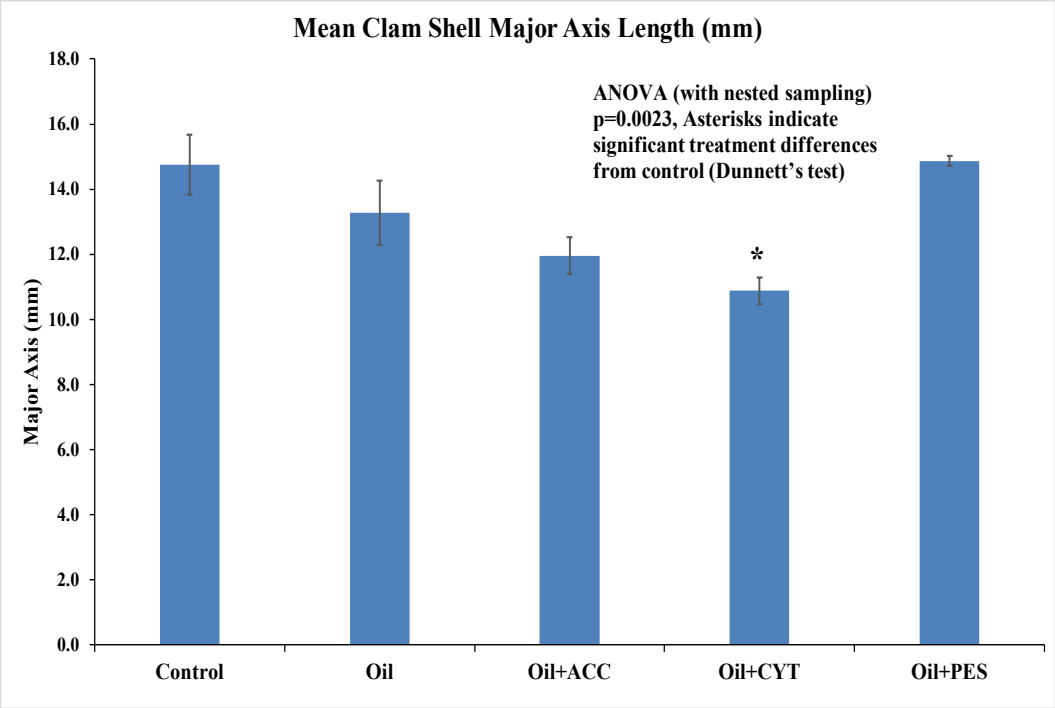

Figure 8. Juvenile clam shell major axis length after 30 d in the mesocosm treatments.

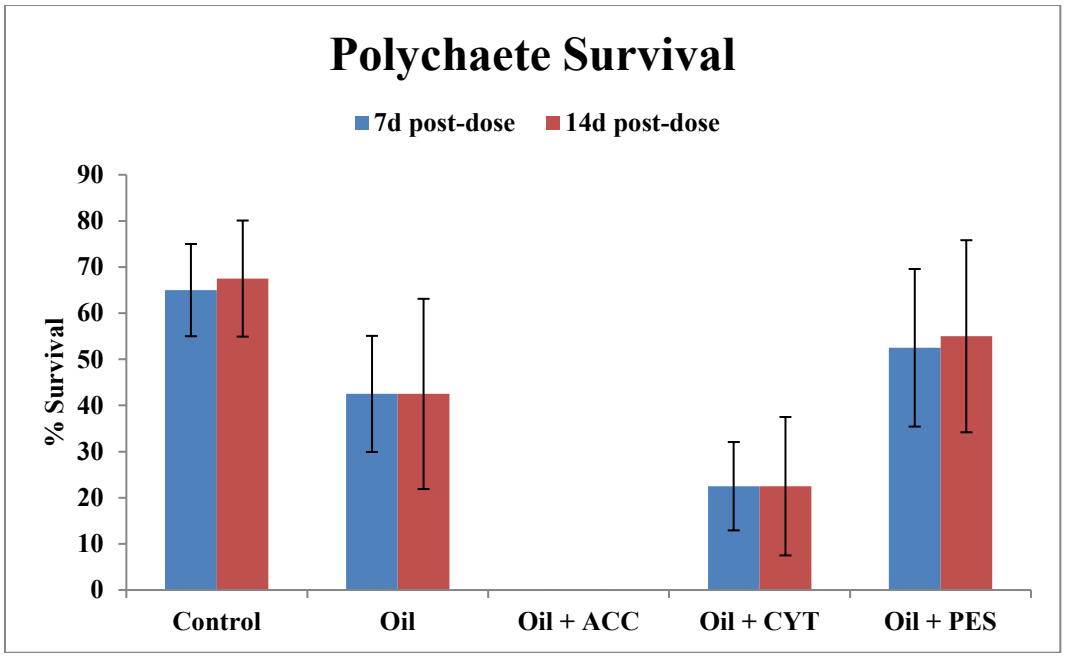

Figure 9. Survival of polychaetes after 7d and 14d in the mesocosm treatments.

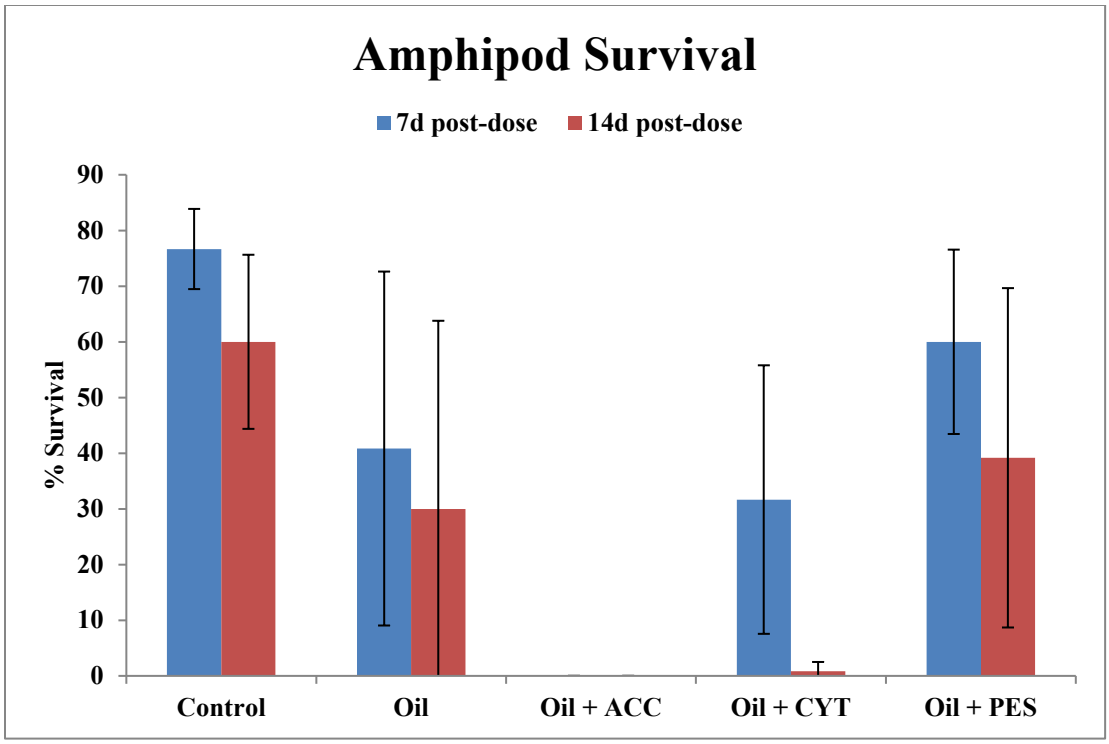

Figure 10. Survival of amphipods after 7d and 14d in the mesocosm treatments.

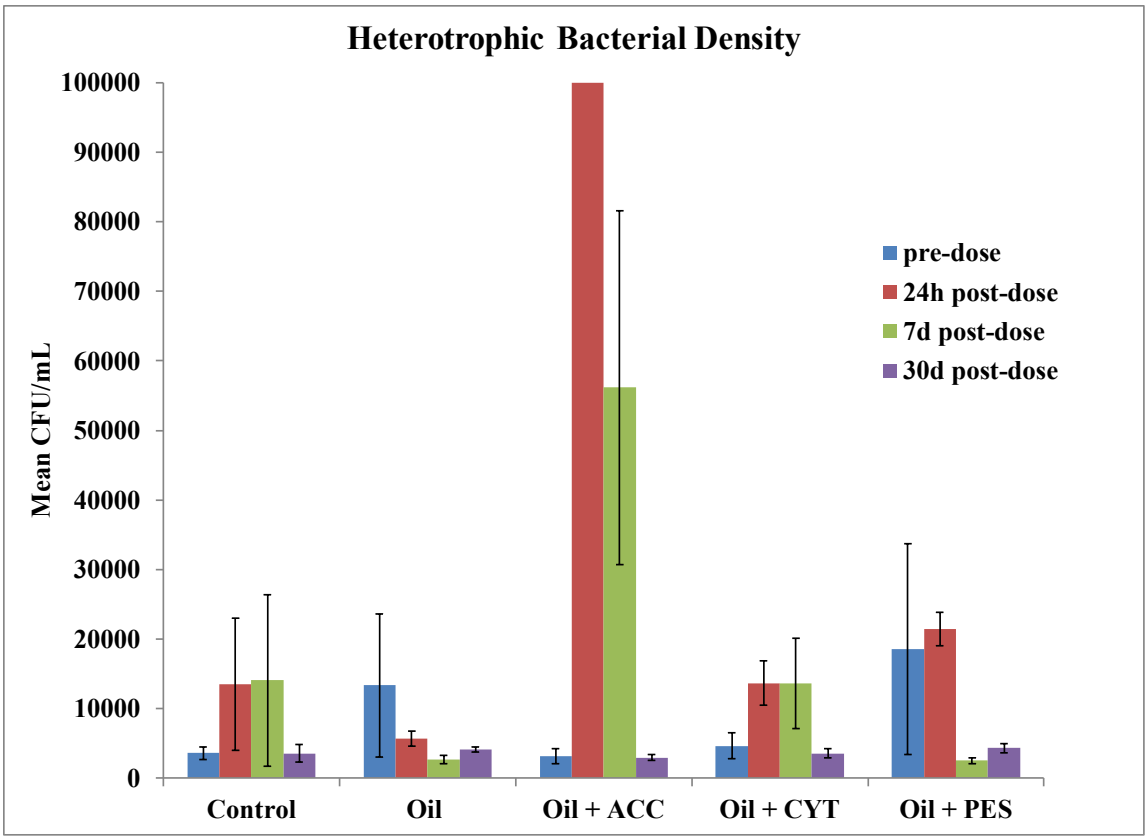

Figure 11. Heterotrophic bacterial densities measured at each timepoint in the mesocosm treatments.

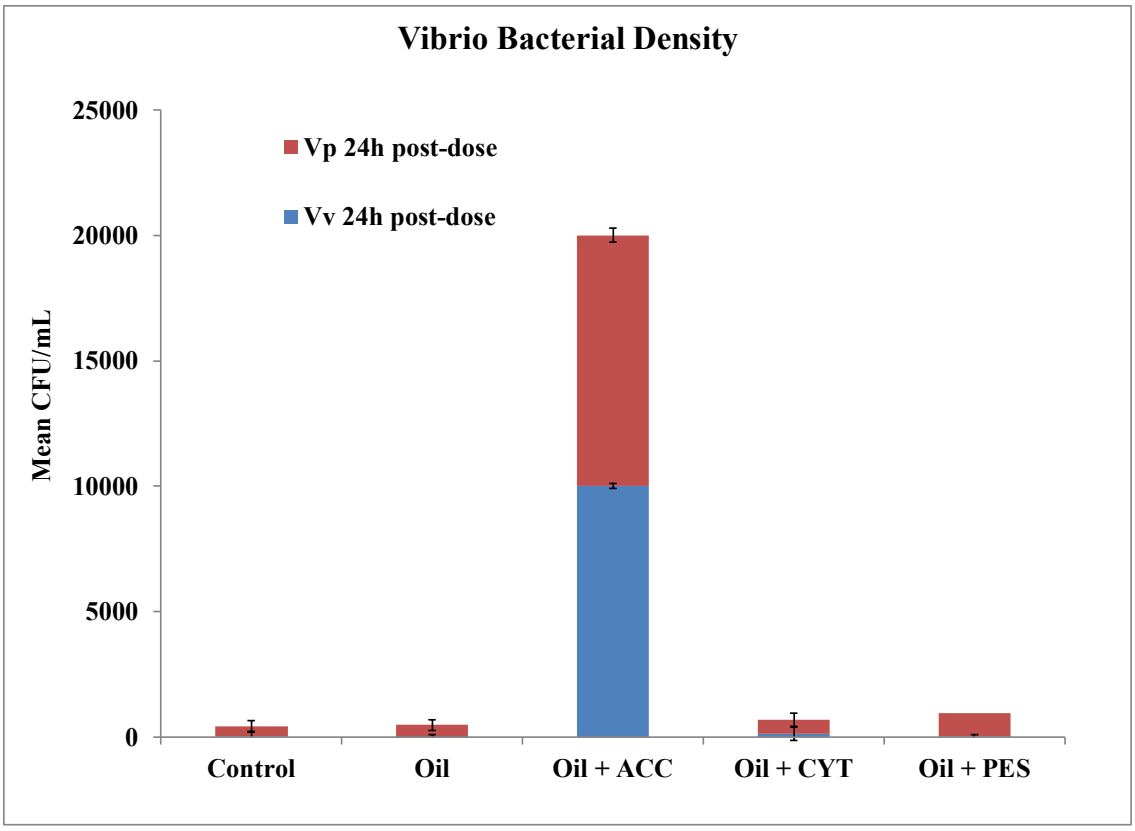

Figure 12. Densities of *Vibrio parahaemolyticus* (Vp), and *Vibrio vulnificus* (Vv) measured 24h post-dose in the mesocosm treatments.

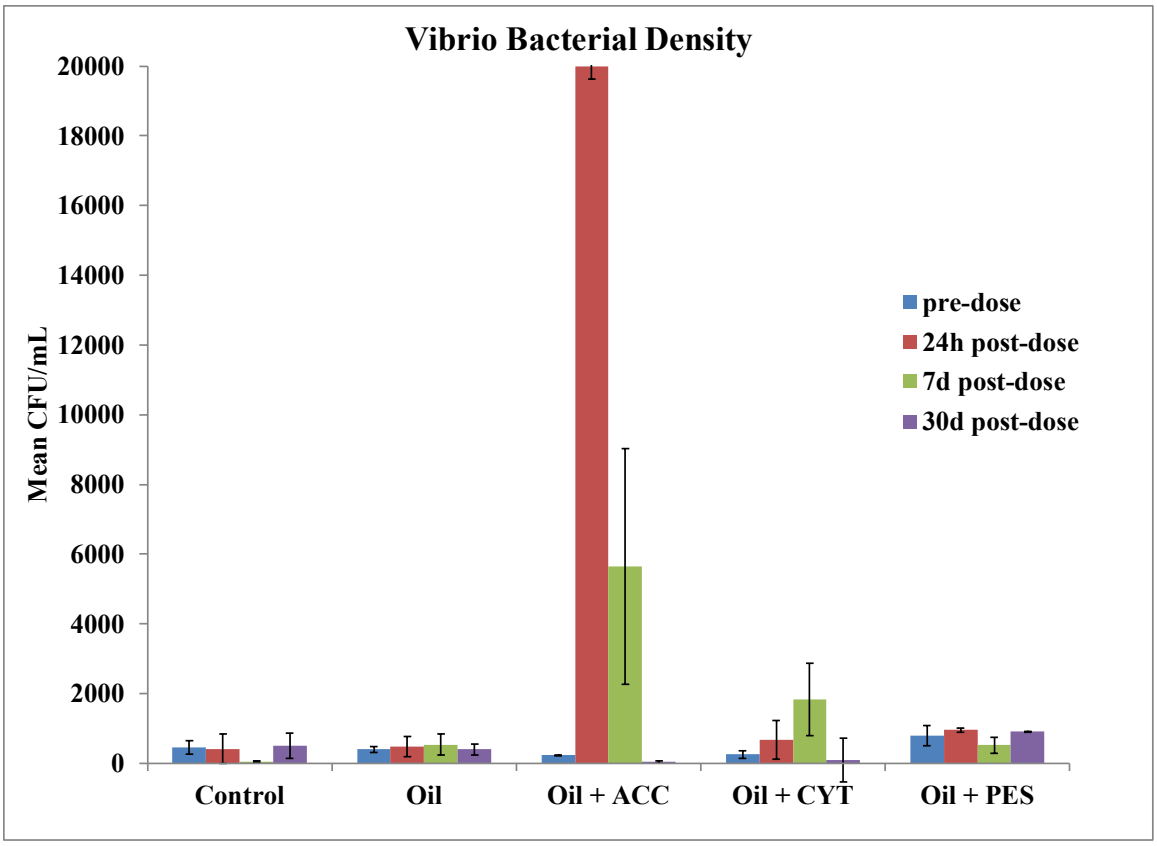

Figure 13. Total *Vibrio* bacterial densities (sum of *V. parahaemolyticus* and *V. vulnificus*) measured at each time-point in the mesocosm treatments.

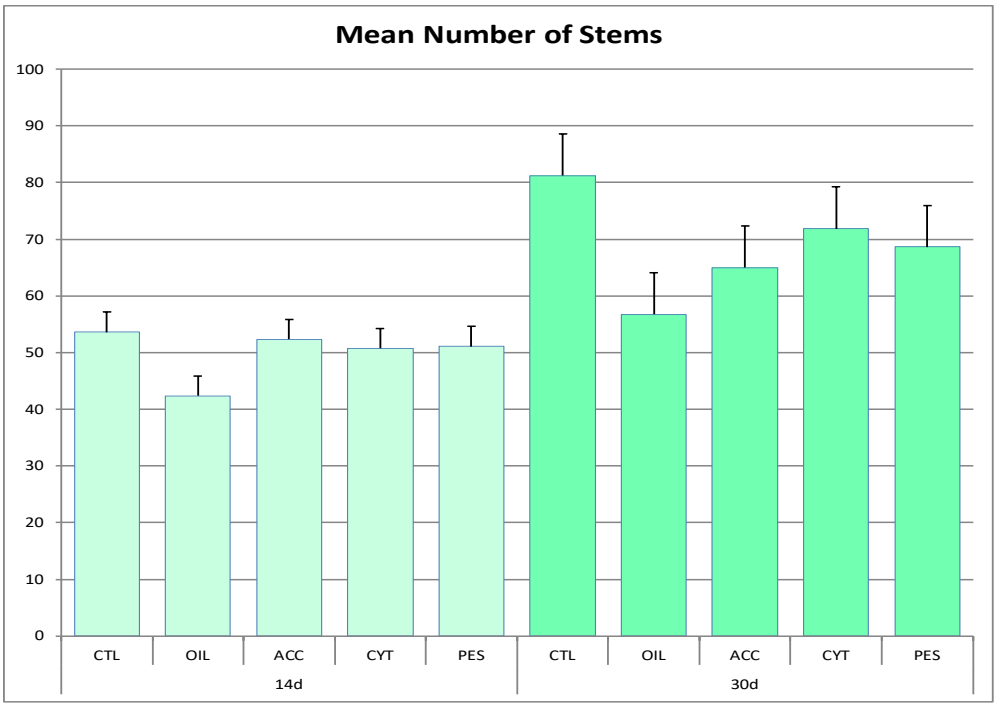

Figure 14. Mean number of *Spartina* stems measured in each mesocosm treatment. Repeated Measures ANOVA, no significant effect of treatment (p=0.1861).

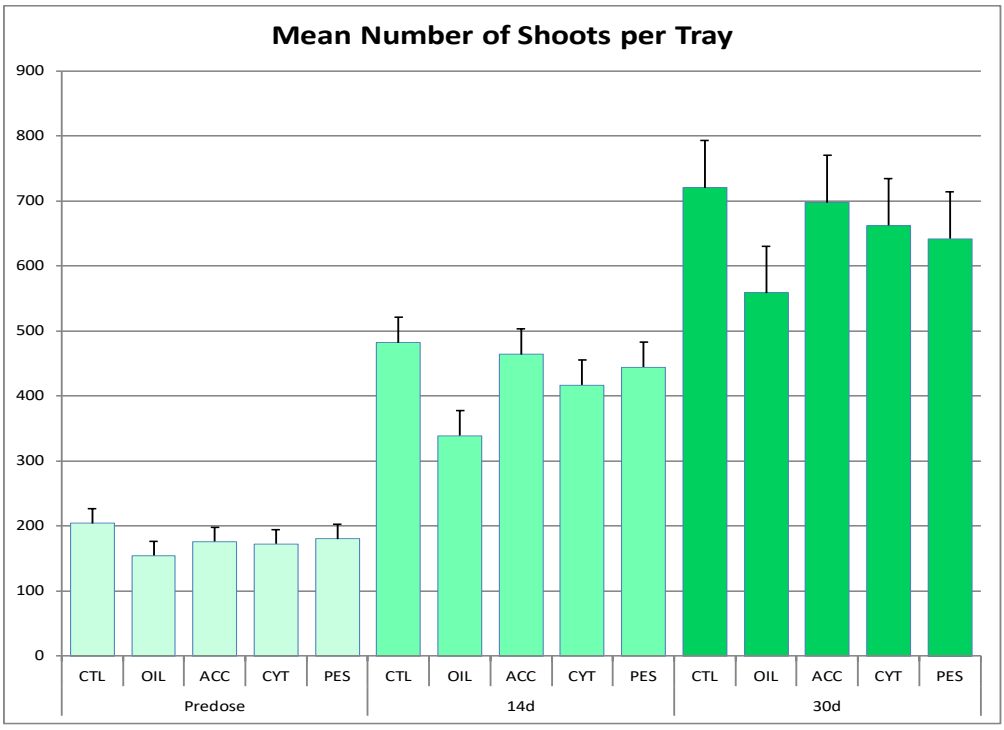

Figure 15. Mean number of *Spartina* shoots measured per tray in each mesocosm treatment. Repeated Measures ANOVA, no significant effect of treatment (p=0.3644).

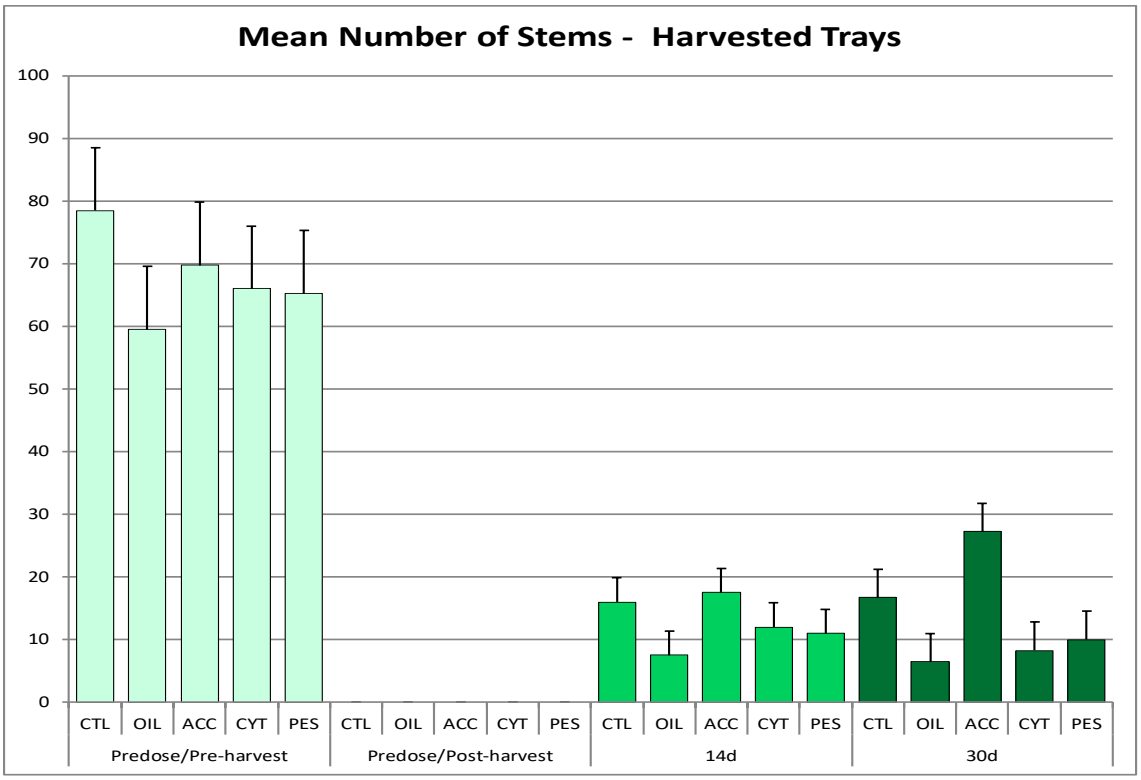

Figure 16. Mean number of *Spartina* stems in the harvested trays of each mesocosm treatment. Repeated Measures ANOVA, no significant effect of treatment (p=0.3616).

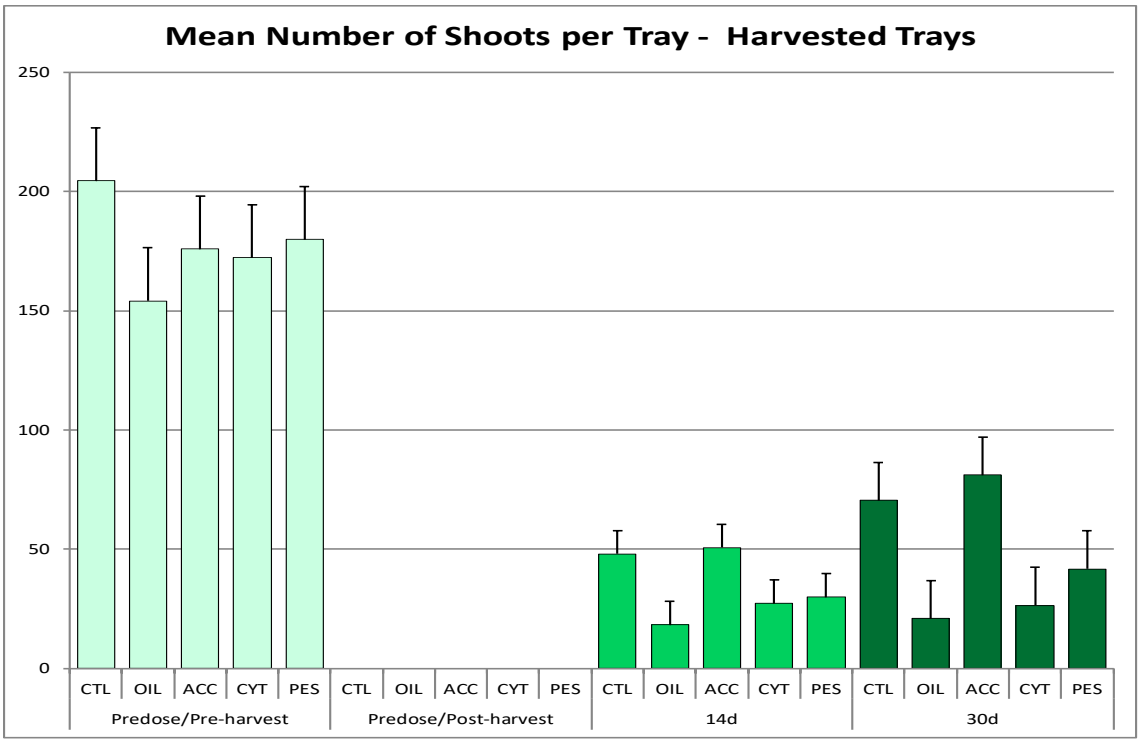

Figure 17. Mean number of *Spartina* shoots in the harvested trays of each mesocosm treatment. Repeated Measures ANOVA, no significant effect of treatment (p=0.0784).

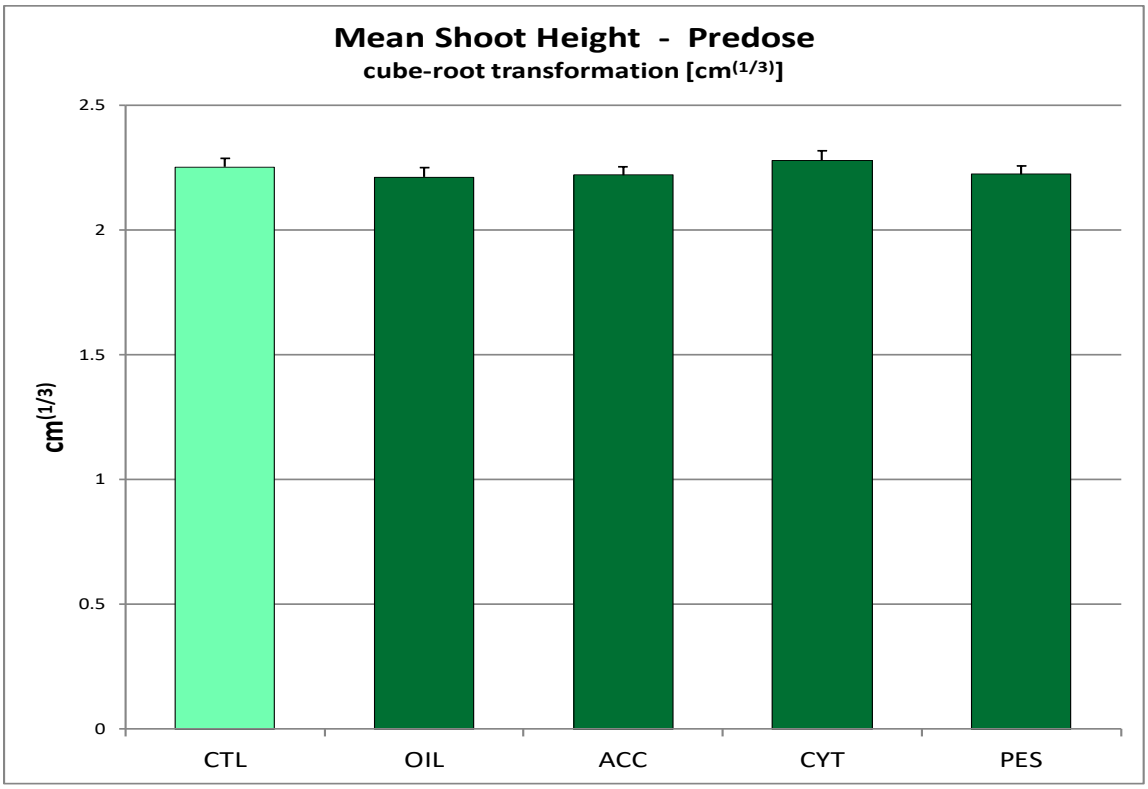

Figure 18. Mean *Spartina* shoot height measured in each mesocosm treatment pre-dose. One-way ANOVA (with nested sampling) p=0.6453.

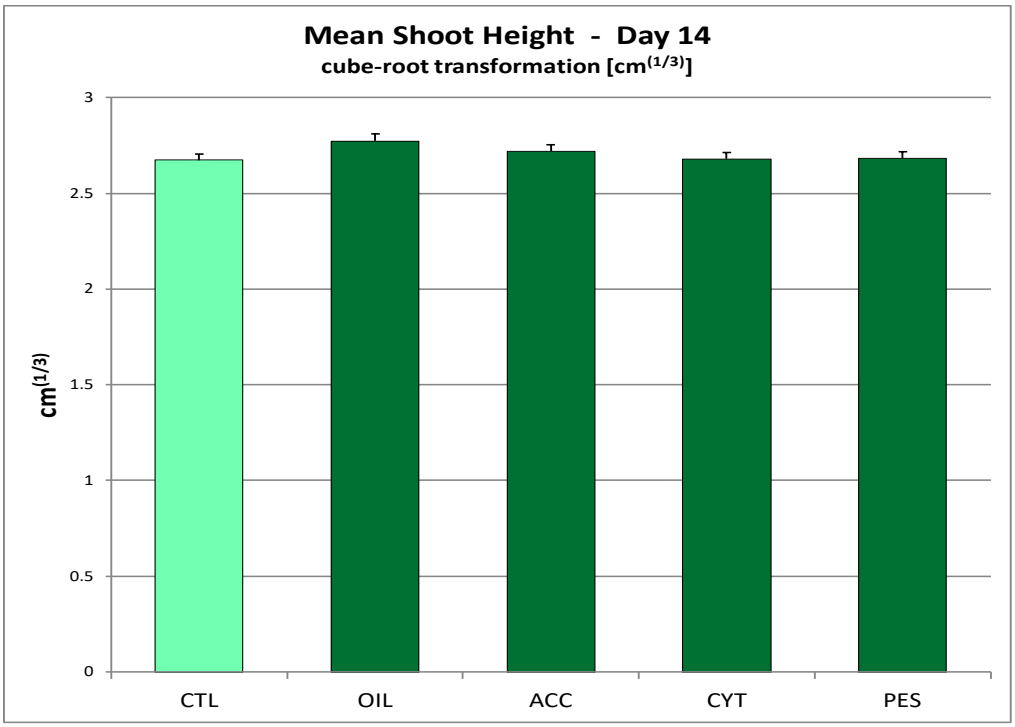

Figure 19. Mean *Spartina* shoot height measured in each mesocosm treatment 14 d post-dose. One-way ANOVA (with nested sampling) p=0.2789.

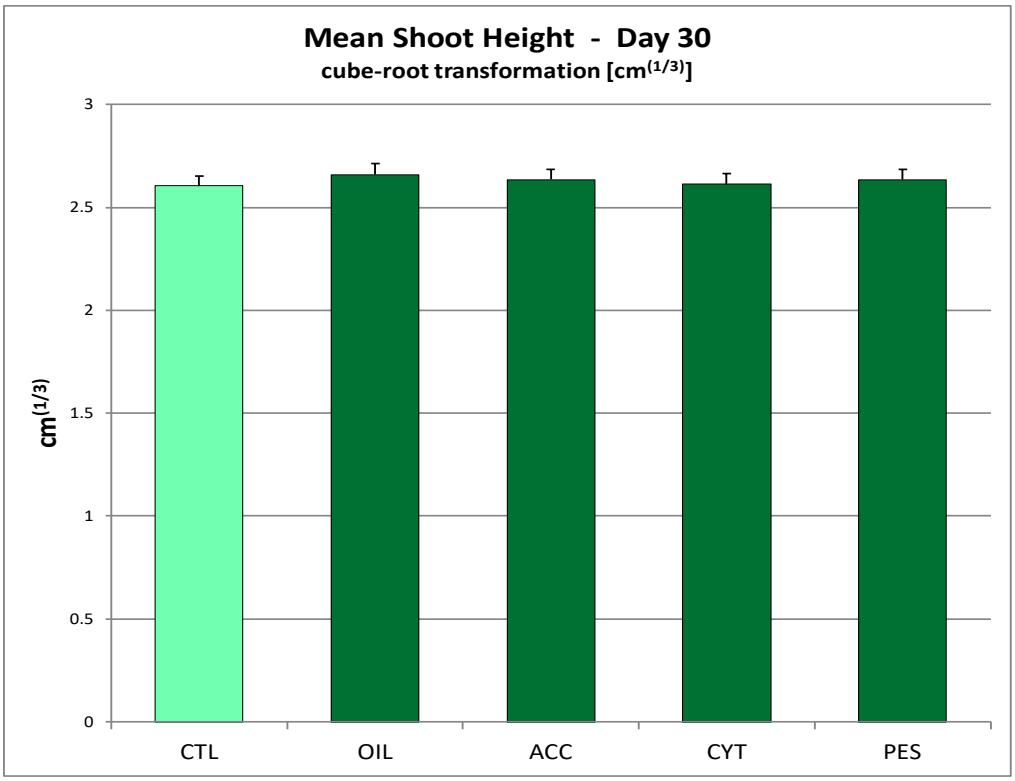

Figure 20. Mean *Spartina* shoot height measured in each mesocosm treatment 30 d post-dose. One-way ANOVA (with nested sampling) p=0.9501.

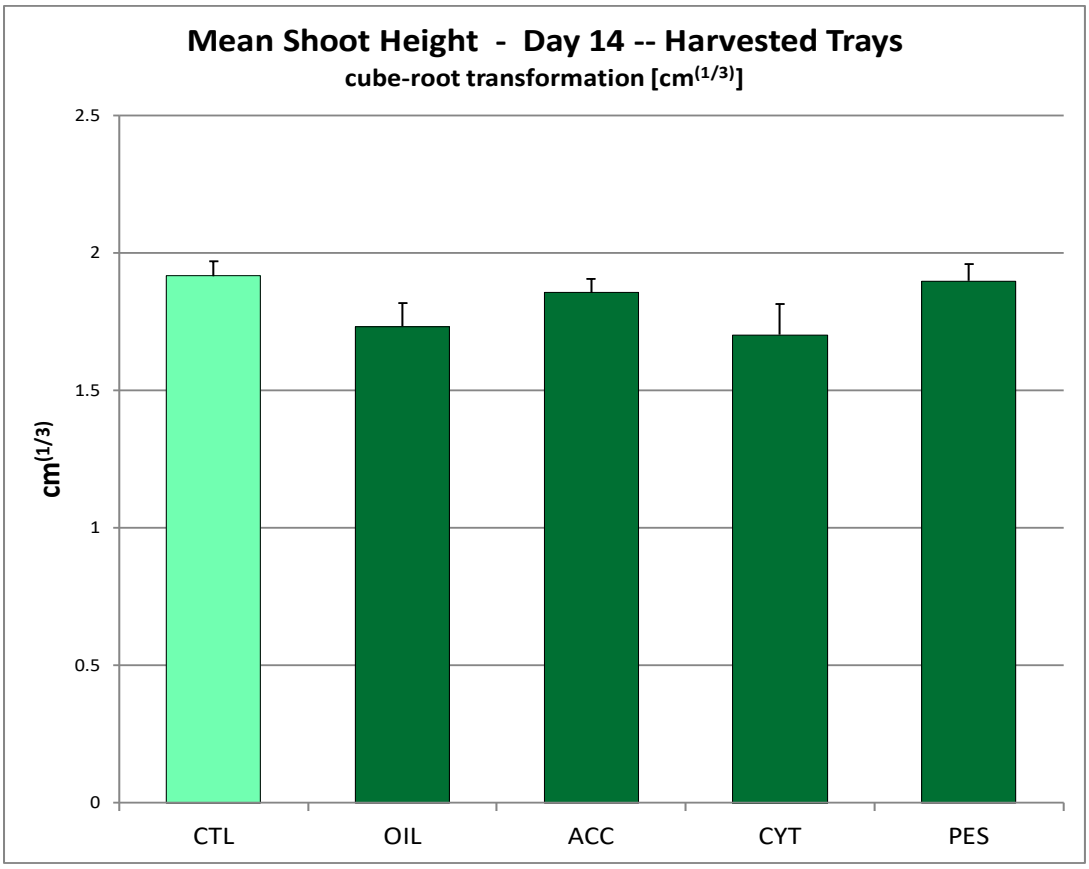

Figure 21. Mean *Spartina* shoot height measured in the harvested trays of each mesocosm treatment 14 d post-dose. One-way ANOVA (with nested sampling) p=0.2768.

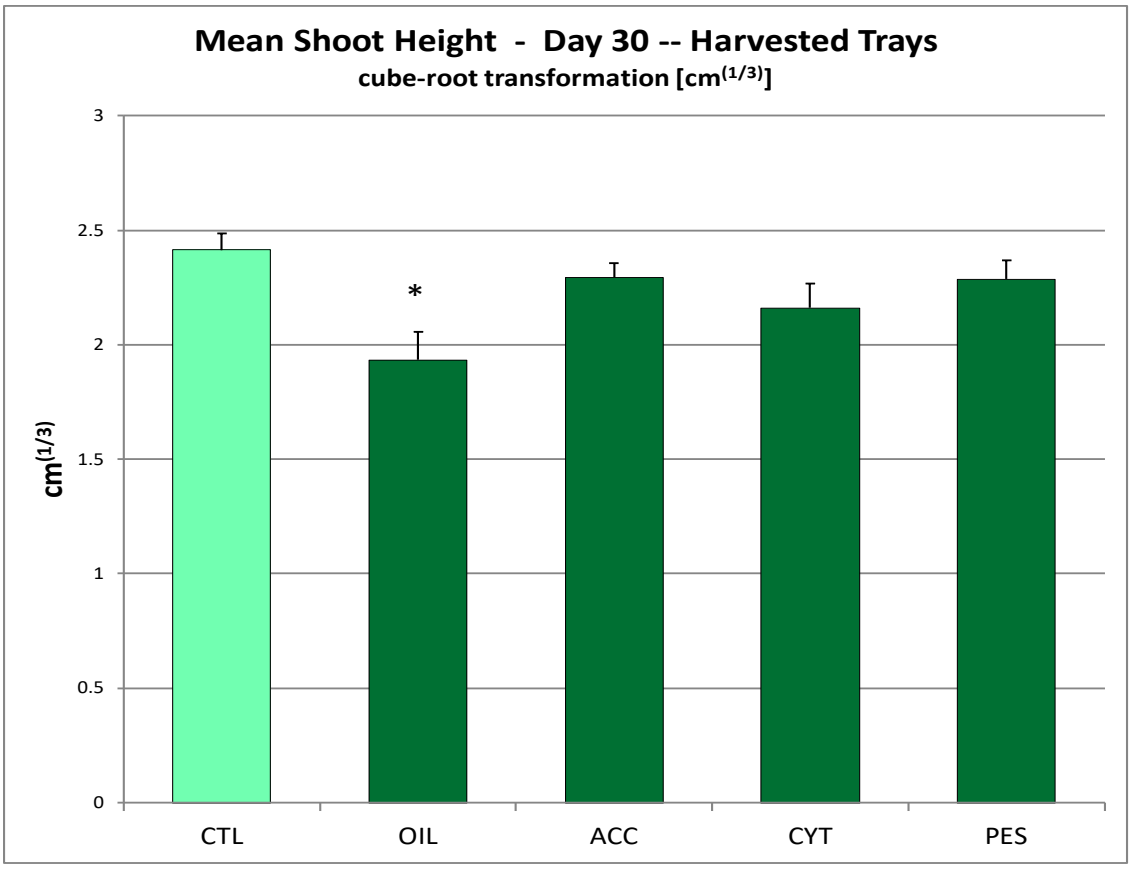

Figure 22. Mean *Spartina* shoot height measured in the harvested trays of each mesocosm treatment 30 d post-dose. One-way ANOVA (with nested sampling) p=0.0425, Dunnett's test oil treatment significantly different from control (p=0.0143).

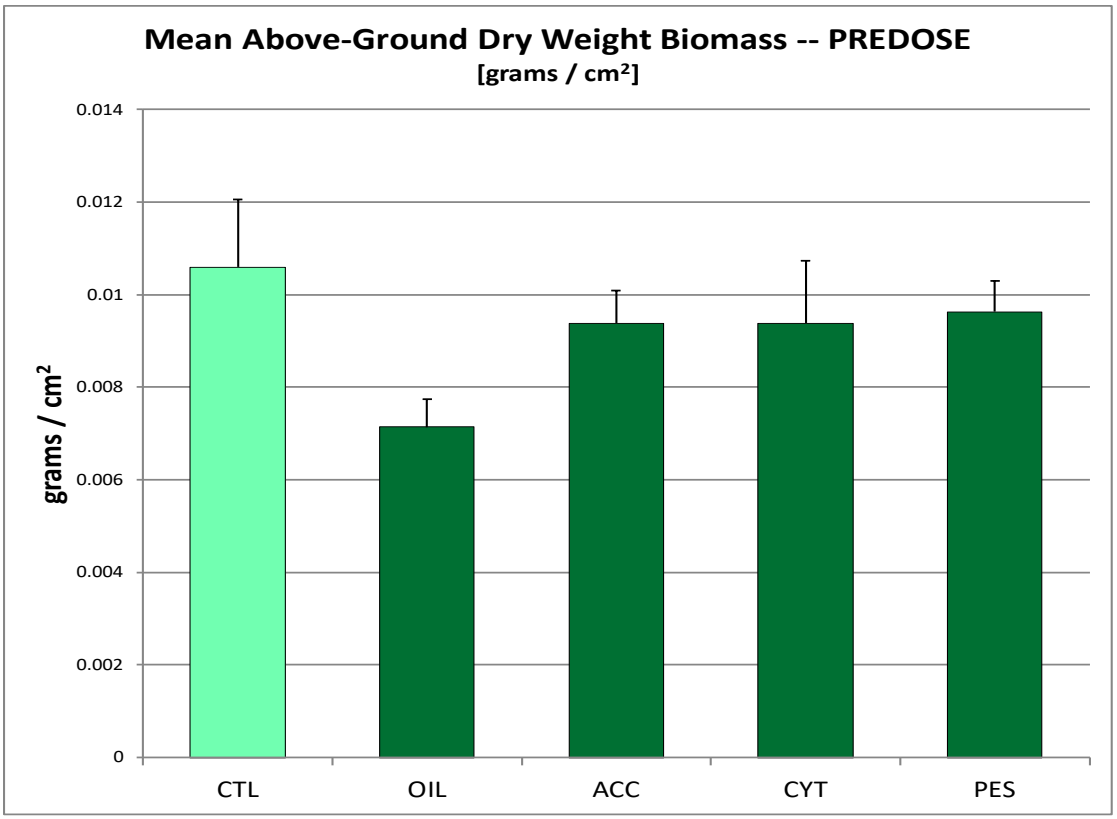

Figure 23. Mean *Spartina* above ground biomass measured in each mesocosm treatment pre-dose. One Way ANOVA p=0.2434.

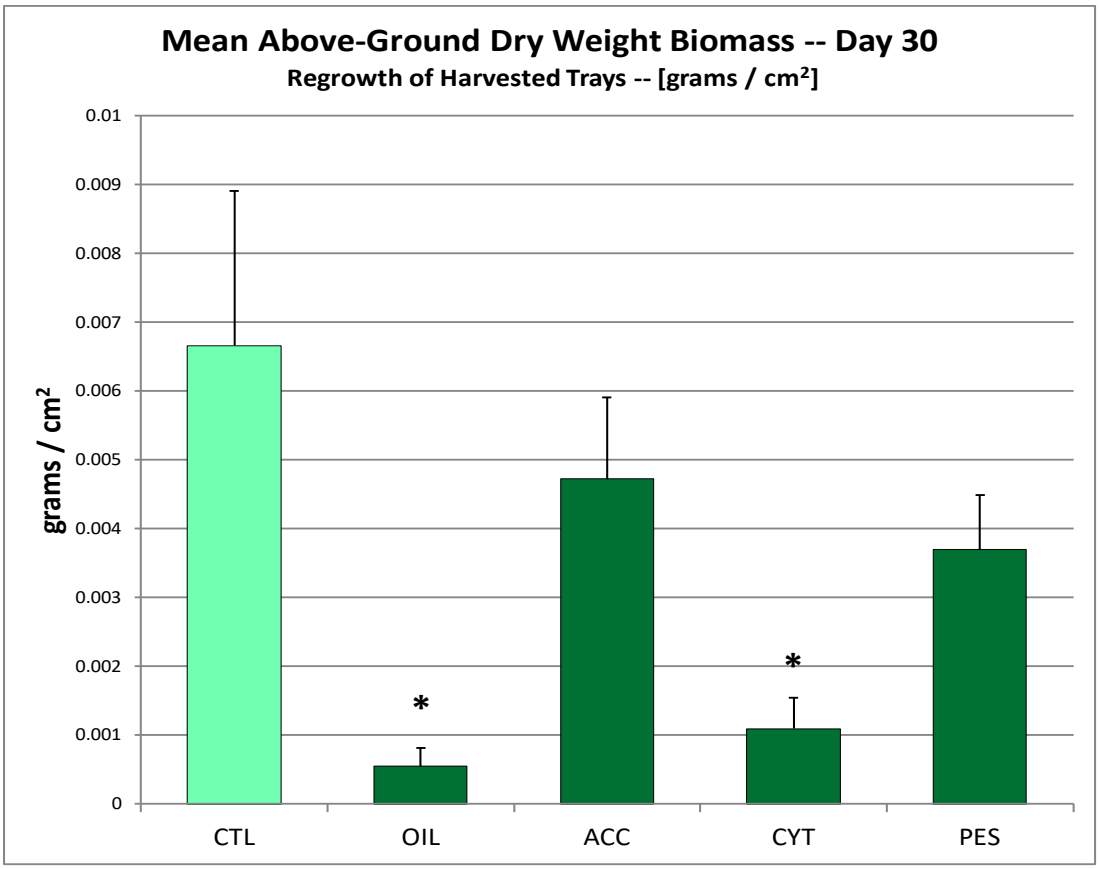

Figure 24. Mean *Spartina* above ground biomass measured in each mesocosm treatment 30 d post-dose. One way ANOVA p-value = 0.0146, Dunnett's test CYT (p=0.0179) and Oil (p=0.0095) treatments significantly different from Control.

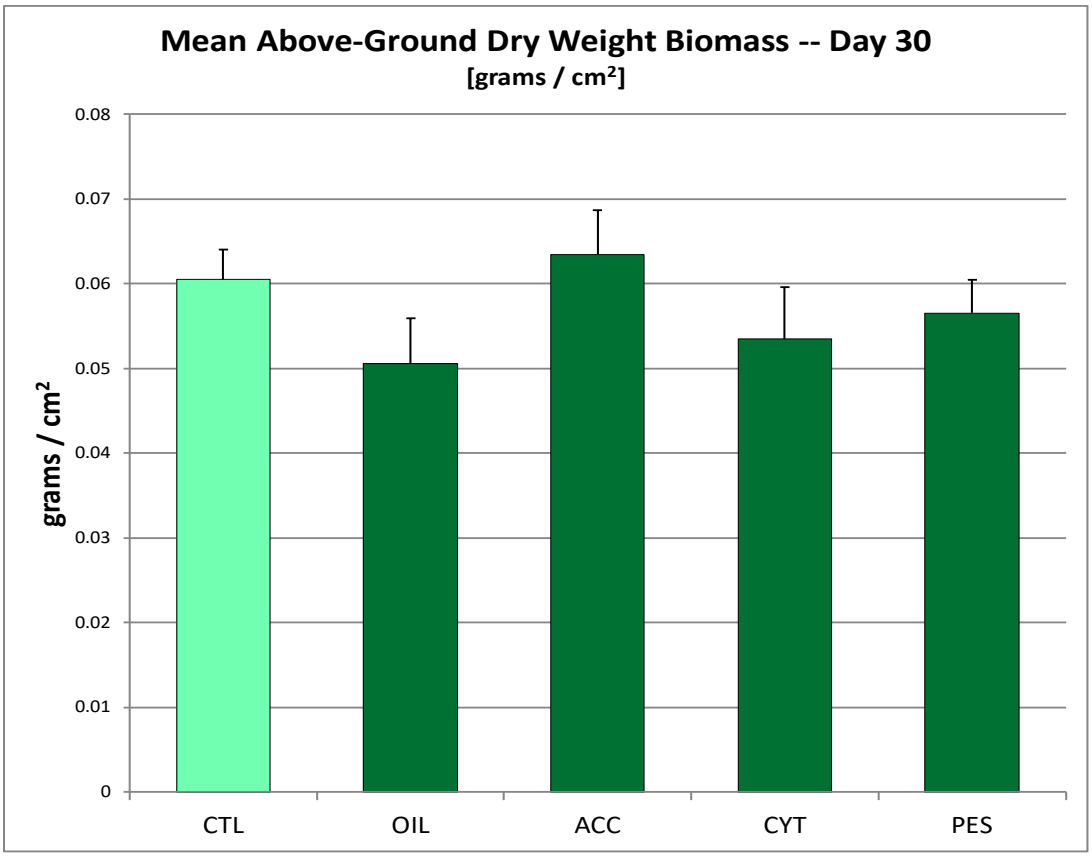

Figure 25. Mean *Spartina* above ground biomass (dry weight) measured in each mesocosm treatment 30 d post-dose. One way ANOVA (with nested sampling) p-value= 0.7004.

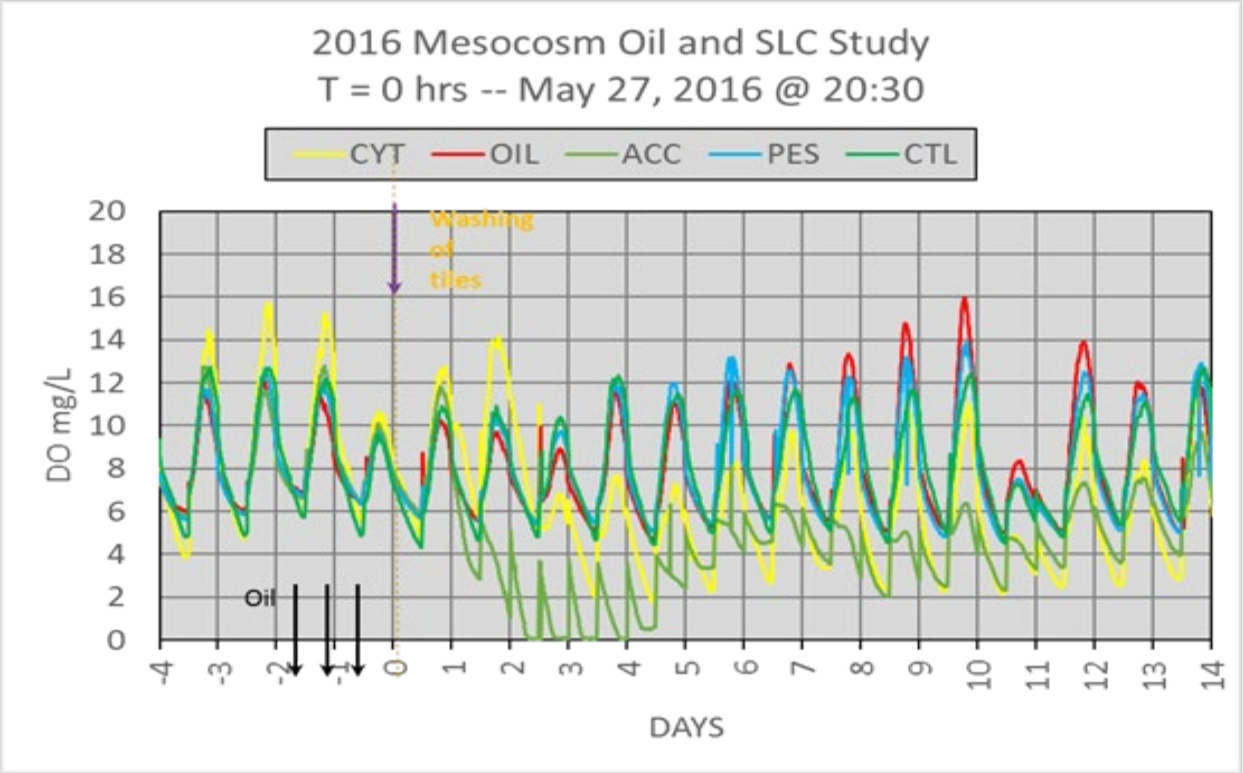

Figure 26. Water column dissolved oxygen concentration measured in the mesocosm treatments.

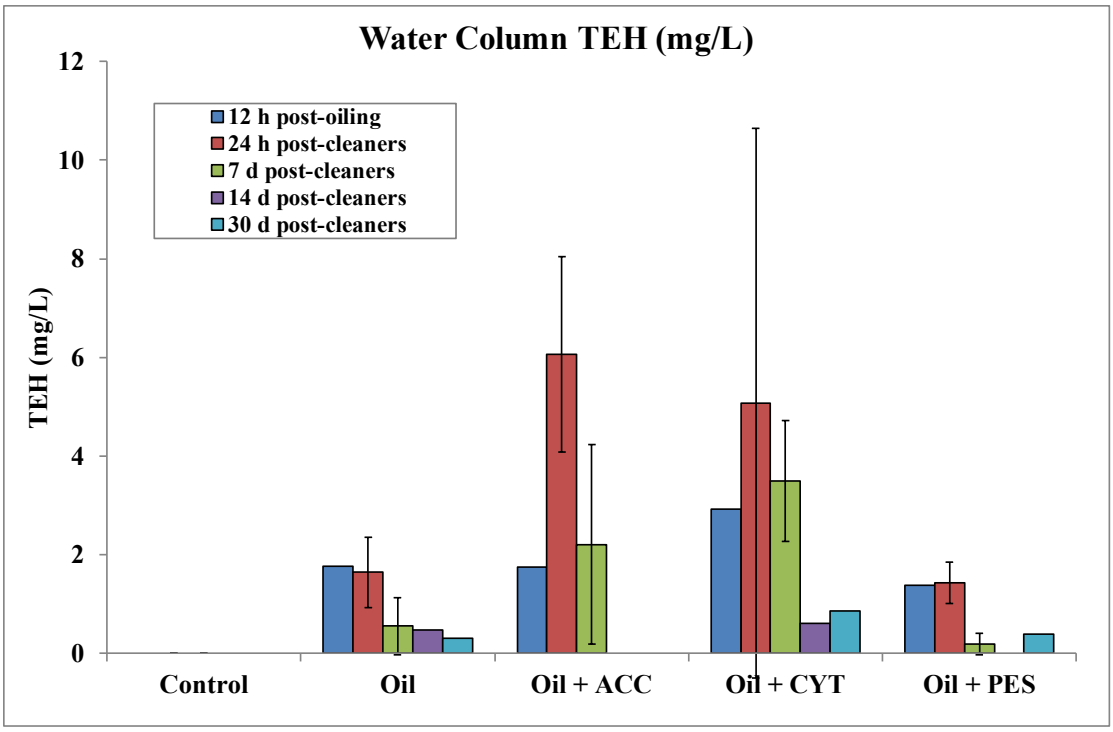

Figure 27. Water column Total Extractable Hydrocarbon (TEH) concentration (mg/L) measured at each time-point in the mesocosm treatments.

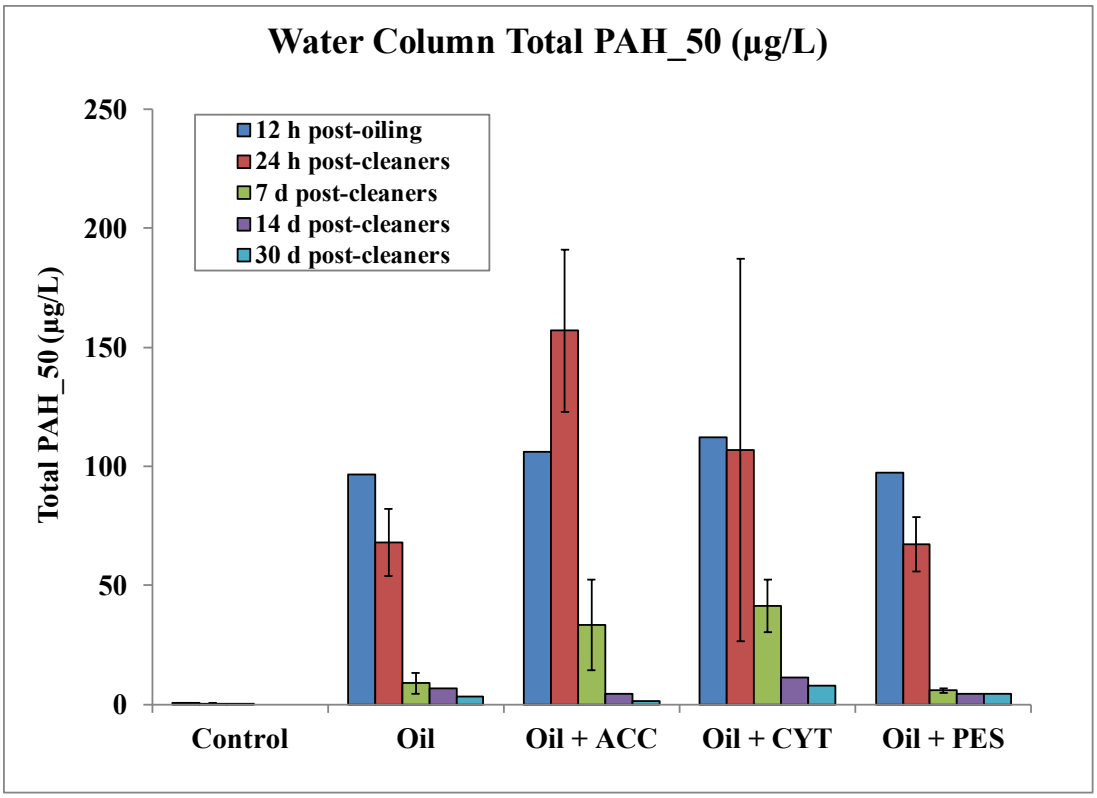

Figure 28. Water column Total PAH concentration (µg/L) measured at each time-point in the mesocosm treatments.

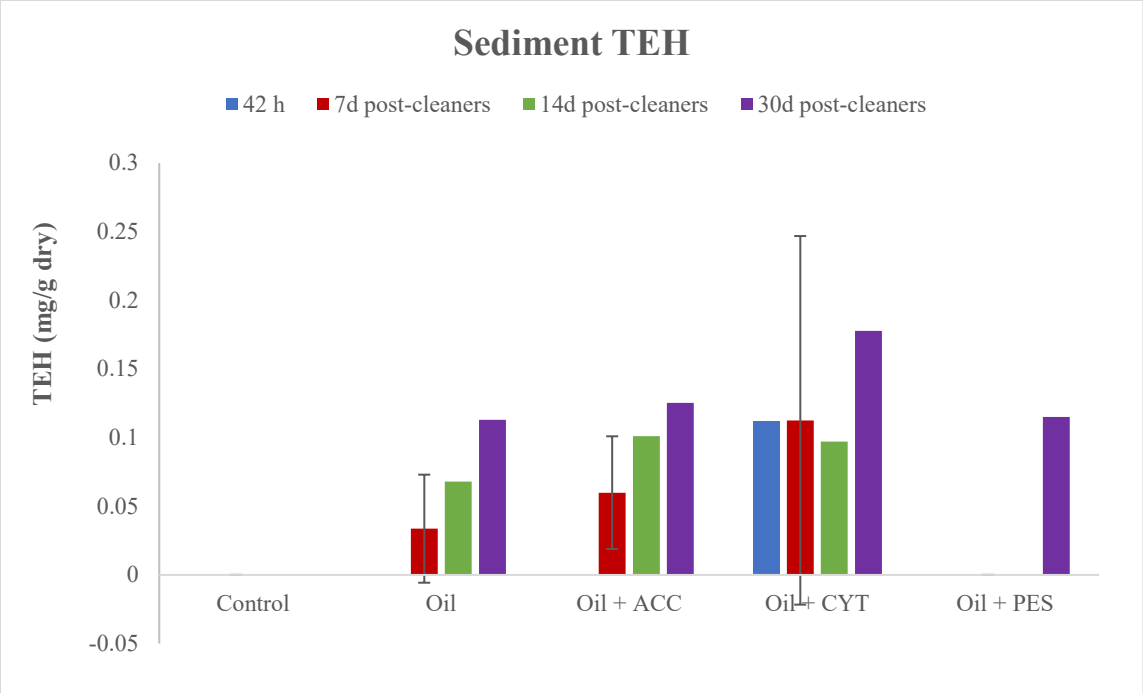

Figure 29. Sediment Total Extractable Hydrocarbon (TEH) concentration (mg/g dry weight) measured at each time-point in the mesocosm treatments.

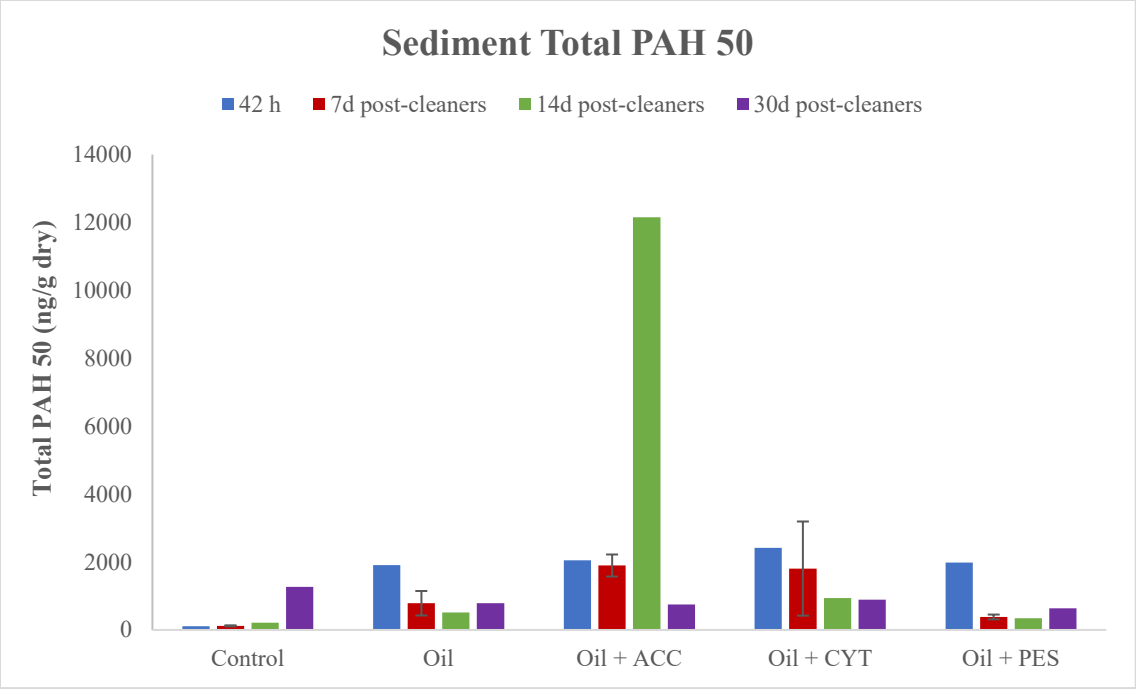

Figure 30. Sediment Total PAH concentration (ng/g dry weight) measured at each time-point in the mesocosm treatments.

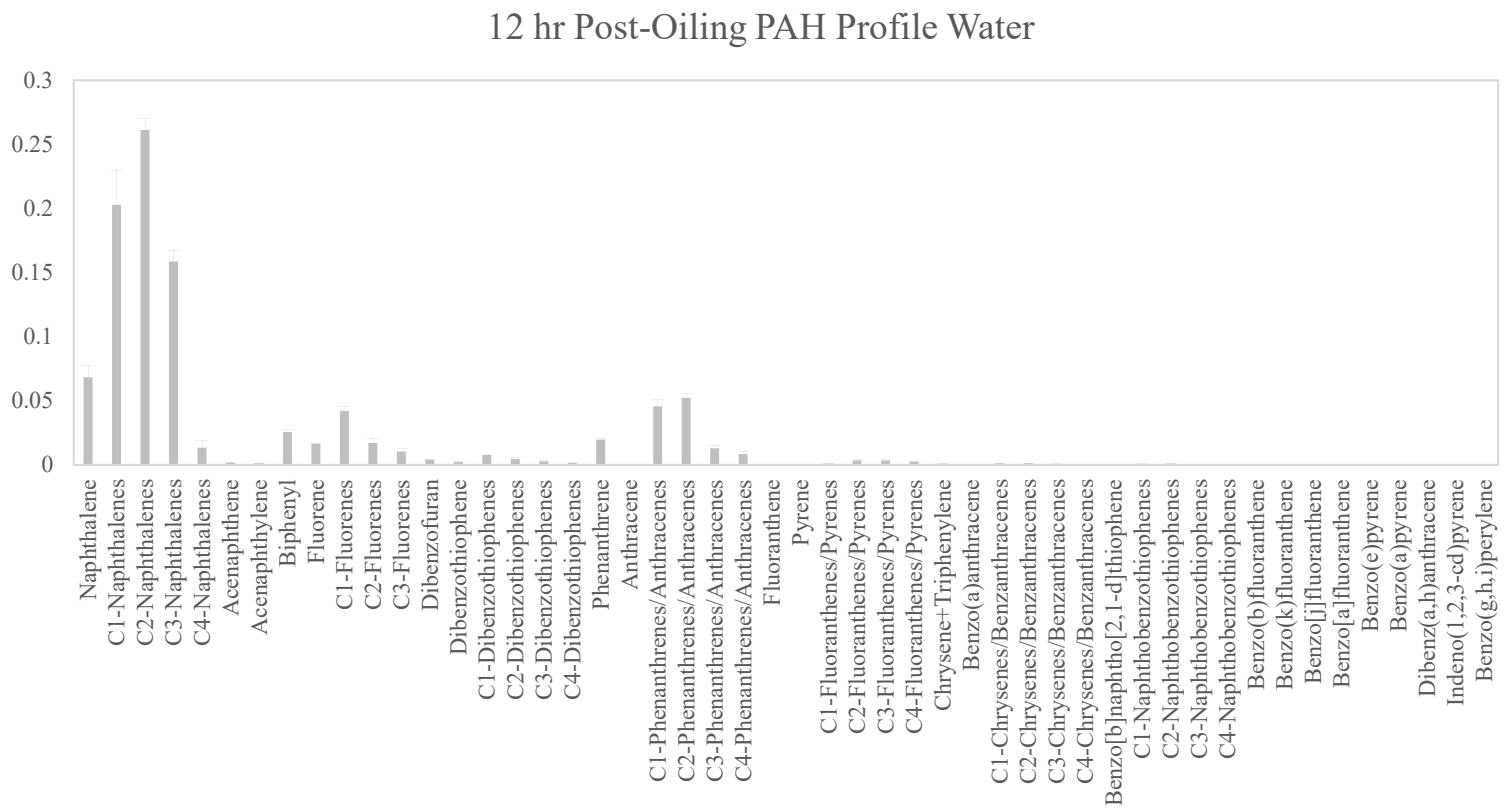

Figure 31. Average (with standard deviation bars) PAH profile in composite water samples from all treatments (OIL, ACC, CYT and PES). Composites were taken 12 h after the last dose of oil but prior to the application of shoreline cleaners. PAH proportions shown here and in subsequent figures were obtained by dividing individual PAH concentrations by TPAH 50 concentrations.

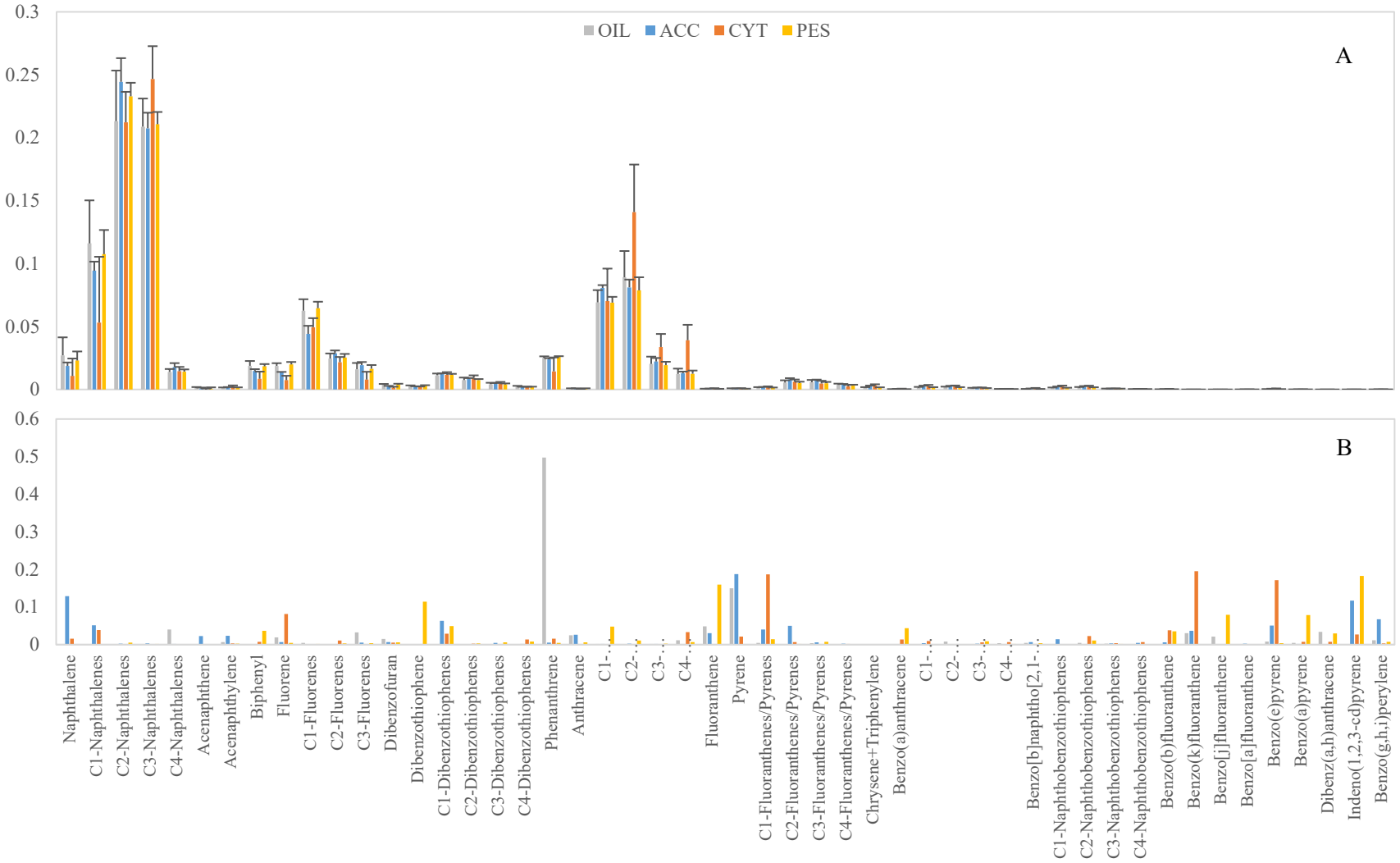

Figure 32. PAH profiles at 24 h post shoreline cleaner application in both water (A) and sediment (B).

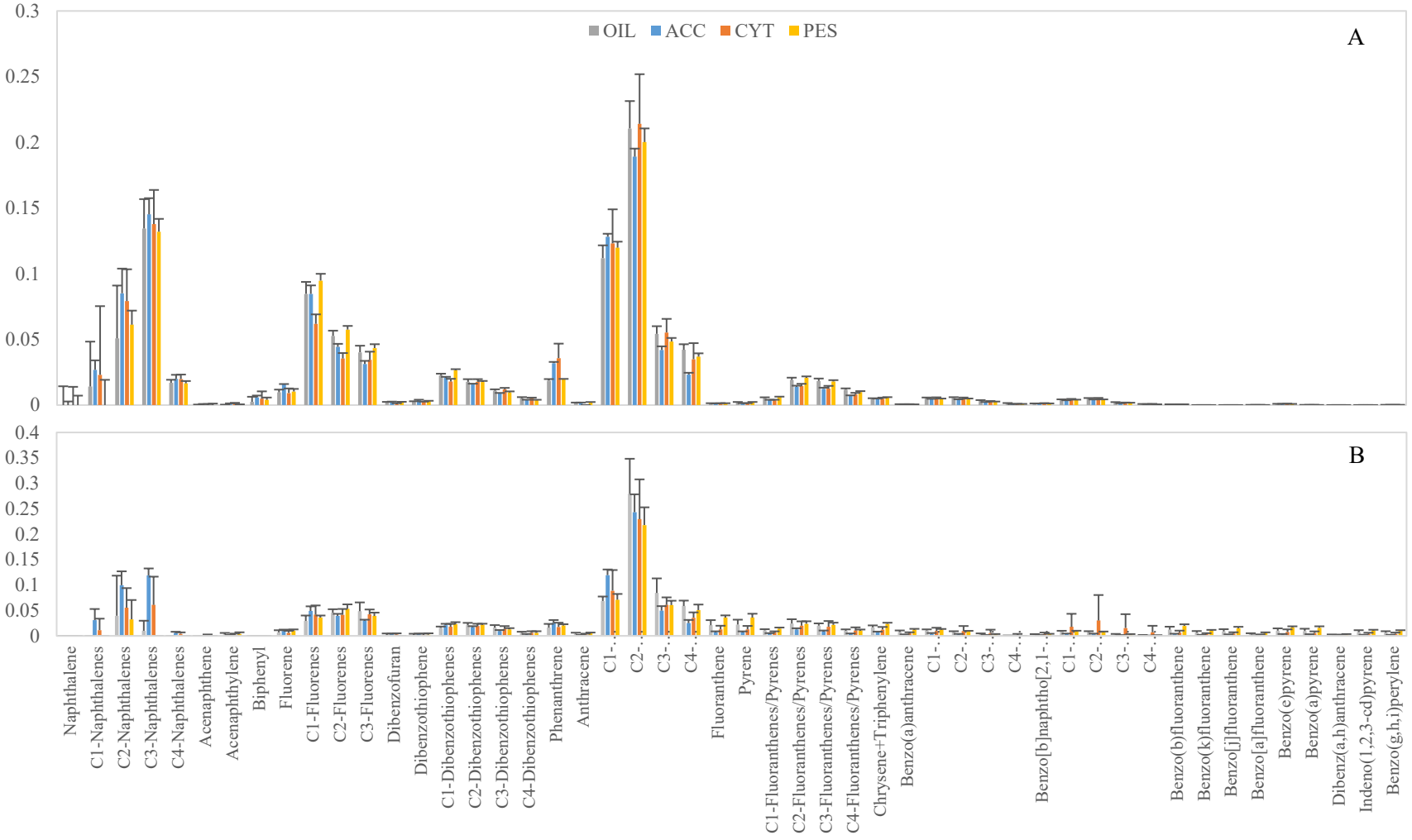

Figure 33. PAH profiles at 7 d post shoreline cleaner application in both water (A) and sediment (B).

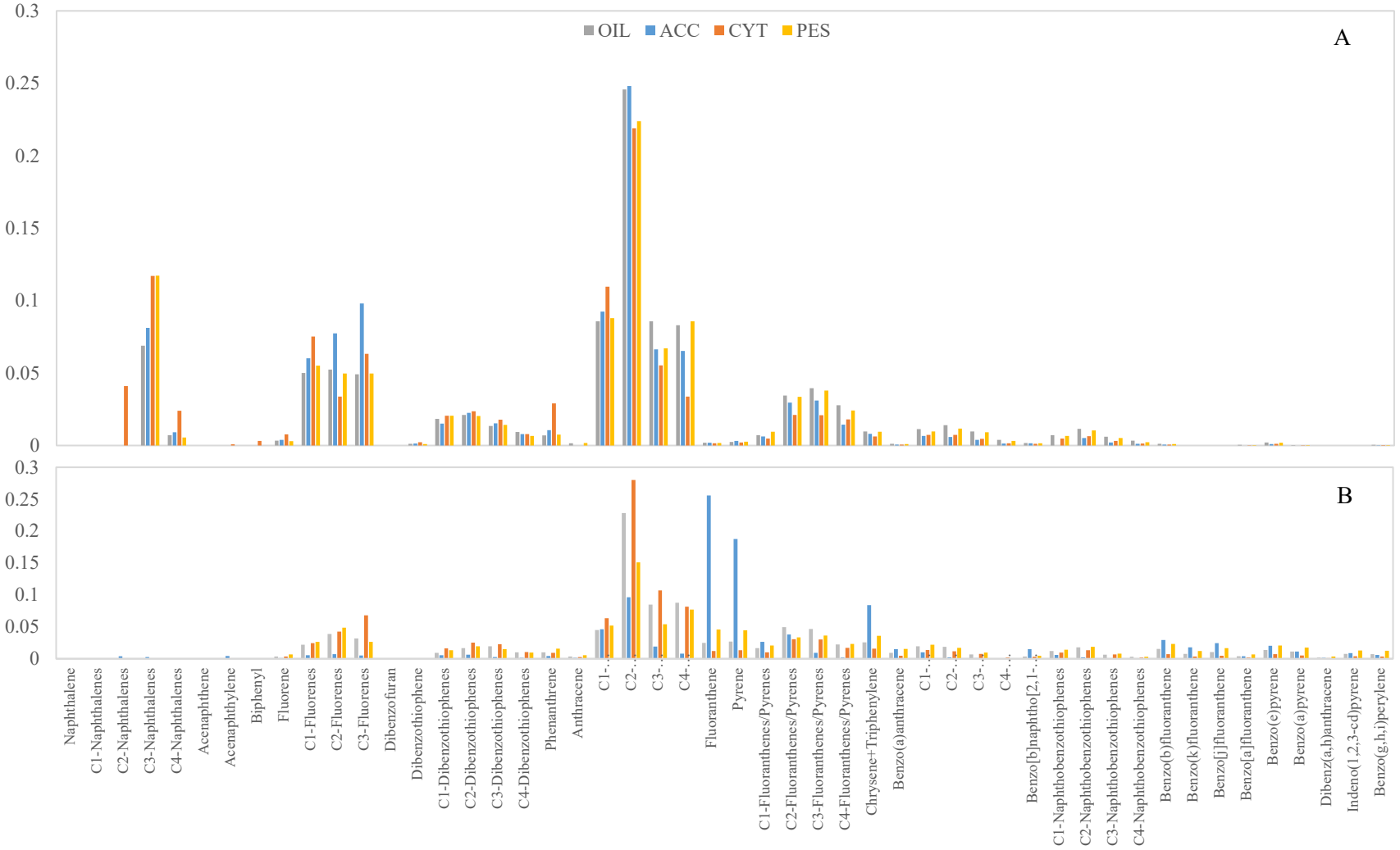

Figure 34. PAH profiles at 14 d post shoreline cleaner application in both water (A) and sediment (B).

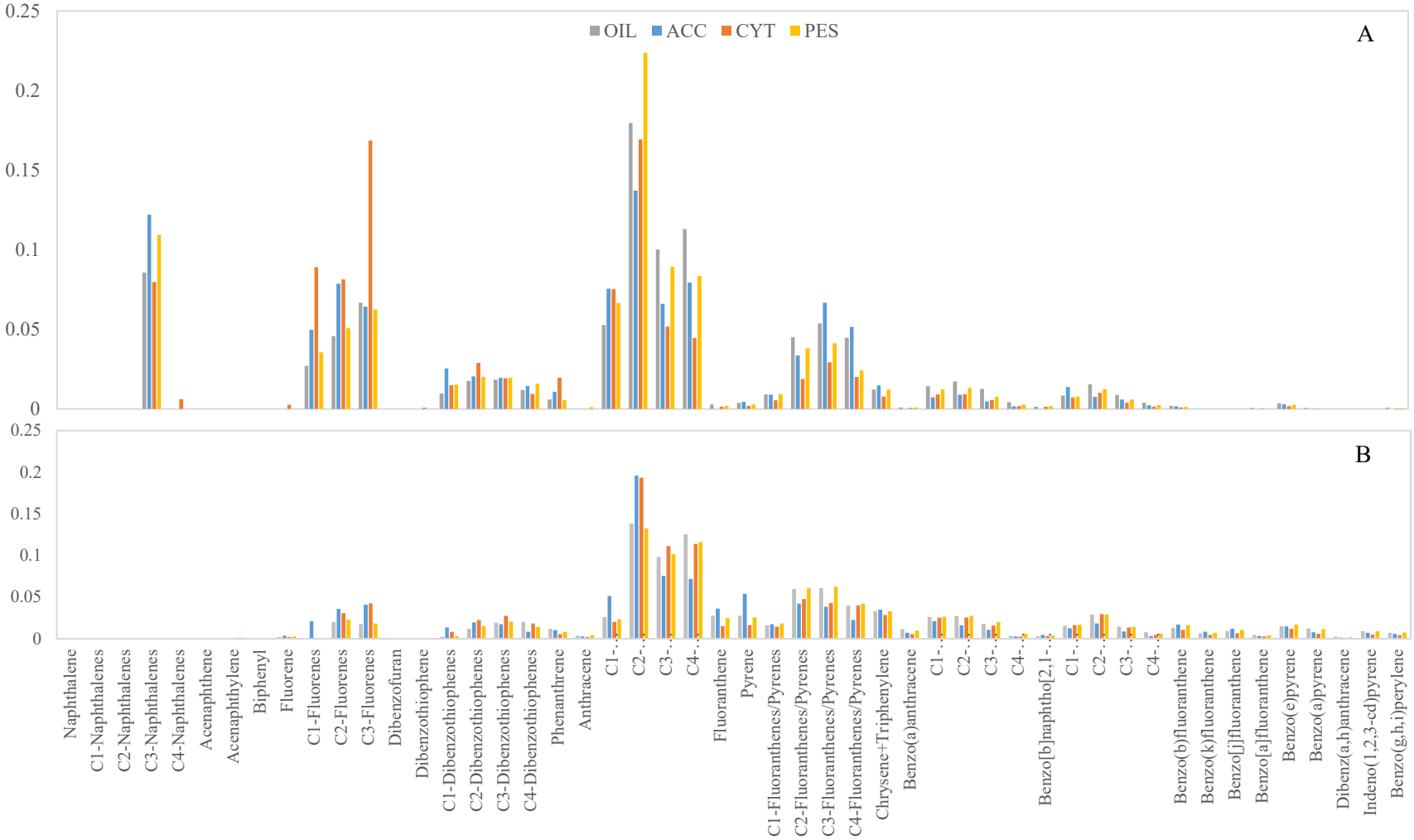

Figure 35. PAH profiles at 30 d post shoreline cleaner application in both water (A) and sediment (B).

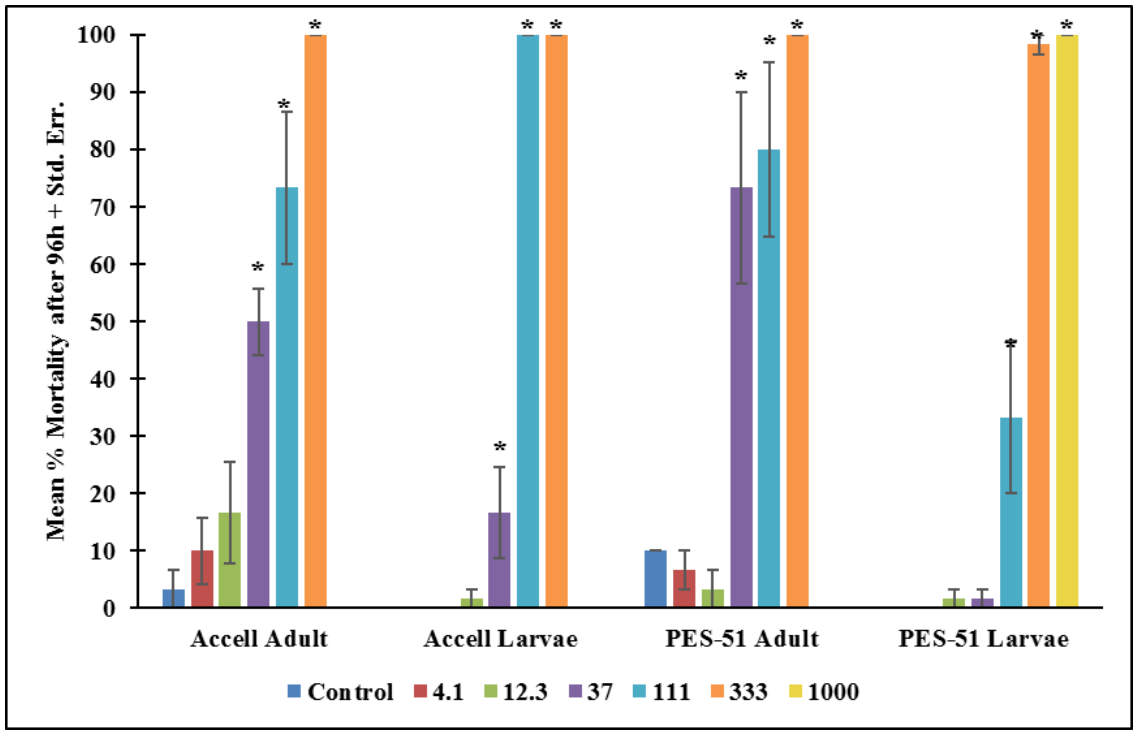

Figure 36. Adult and larval grass shrimp mortality after 96 h laboratory exposure to shoreline cleaners (ppm) only**.** Larval shrimp were exposed to PES-51 from 12.3 - 1000 ppm. Asterisks (*) indicate significant differences from the control (ANOVA $p<0.0001$, Dunnett's test).

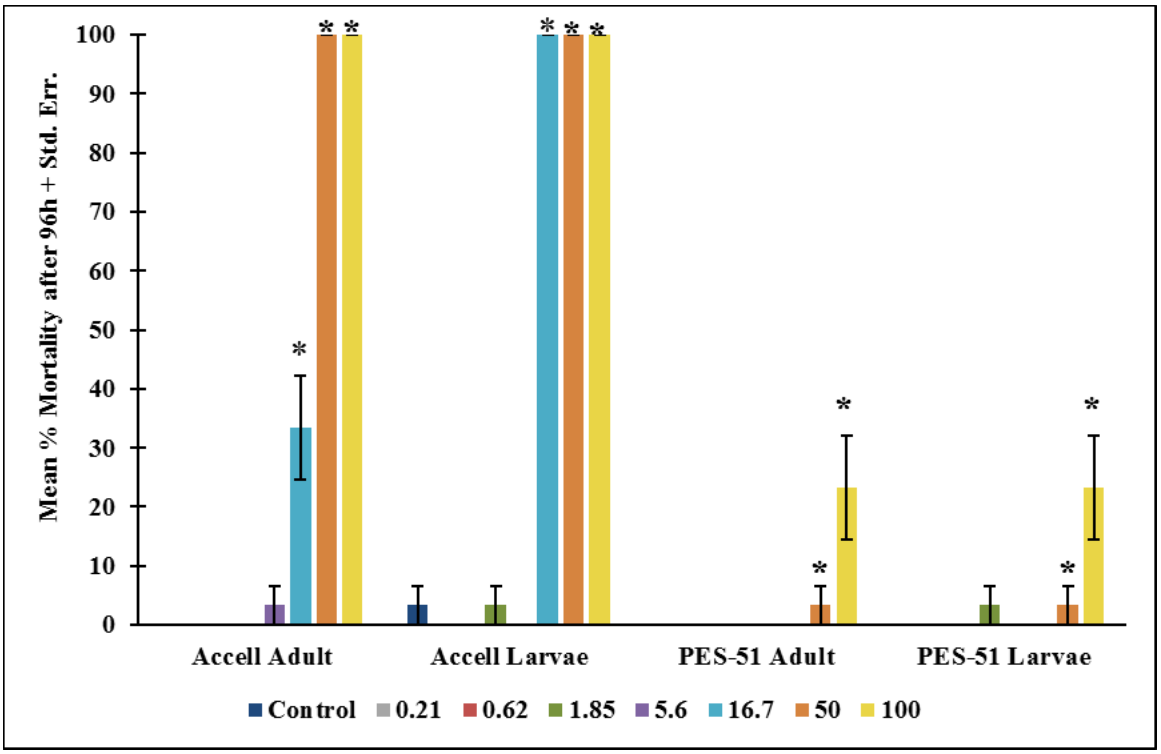

Figure 37. Adult and larval grass shrimp mortality after 96 h laboratory exposure to shoreline cleaner-CEWAF (% CEWAF). Asterisks (*) indicate significant differences from the control, Dunnett's test (ANOVA: Accell adult $p<0.0001$; Accell larvae $p<0.0001$; PES-51 adult $p=0.0029$; PES-51 larvae $p=0.0056$).

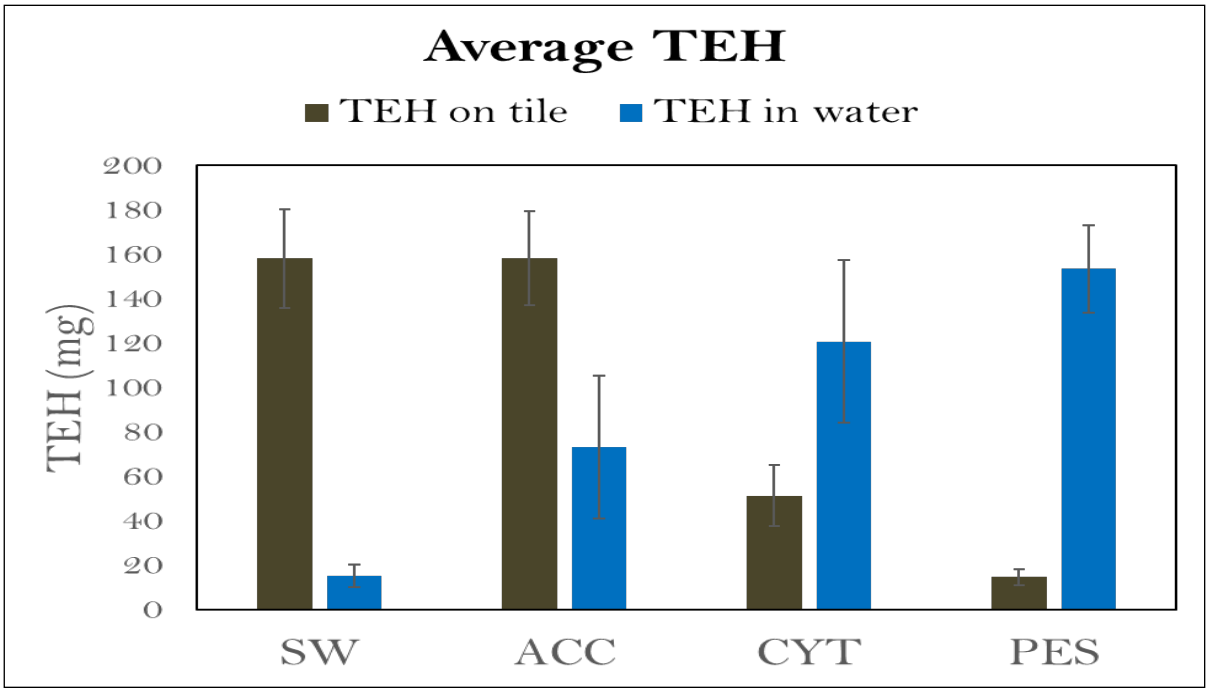

Figure 38. Average TEH in rinse water after SLC application and washing of oiled tiles. The rinsate included all oil related products in the water, floating oil was not separated from the water rinse.

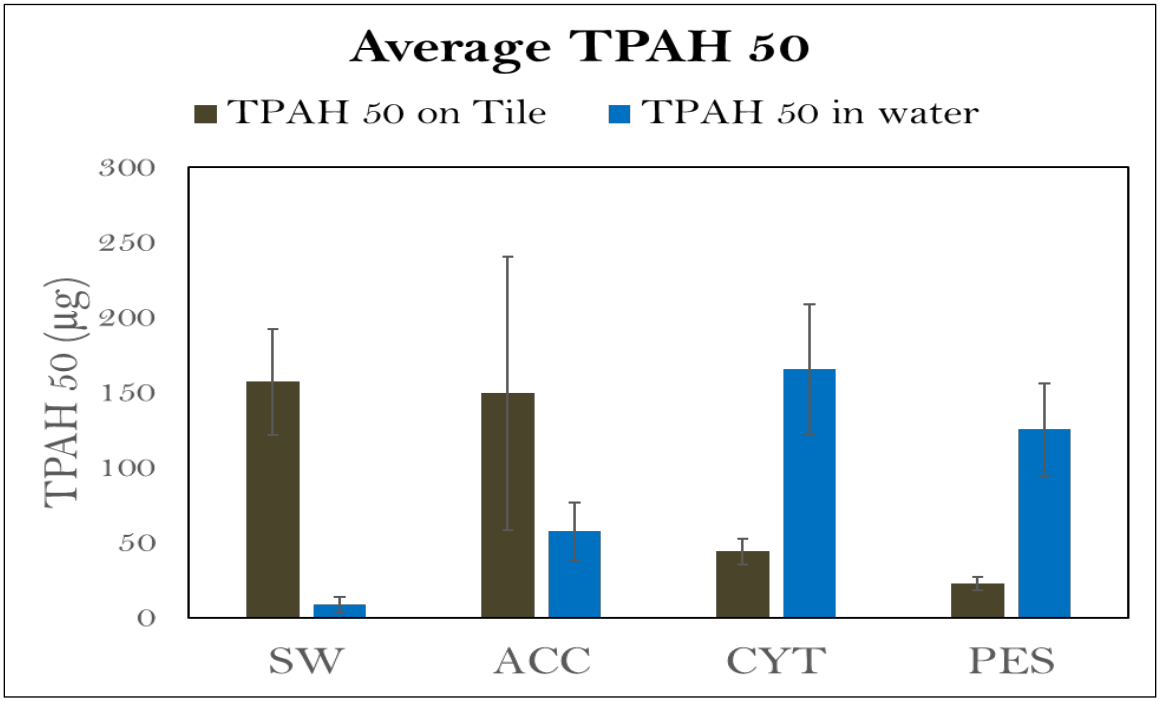

Figure 39. Average Total PAH50 in rinse water after SLC application and washing of oiled tiles. The rinsate included all oil related products in the water, floating oil was not separated from the water rinse.

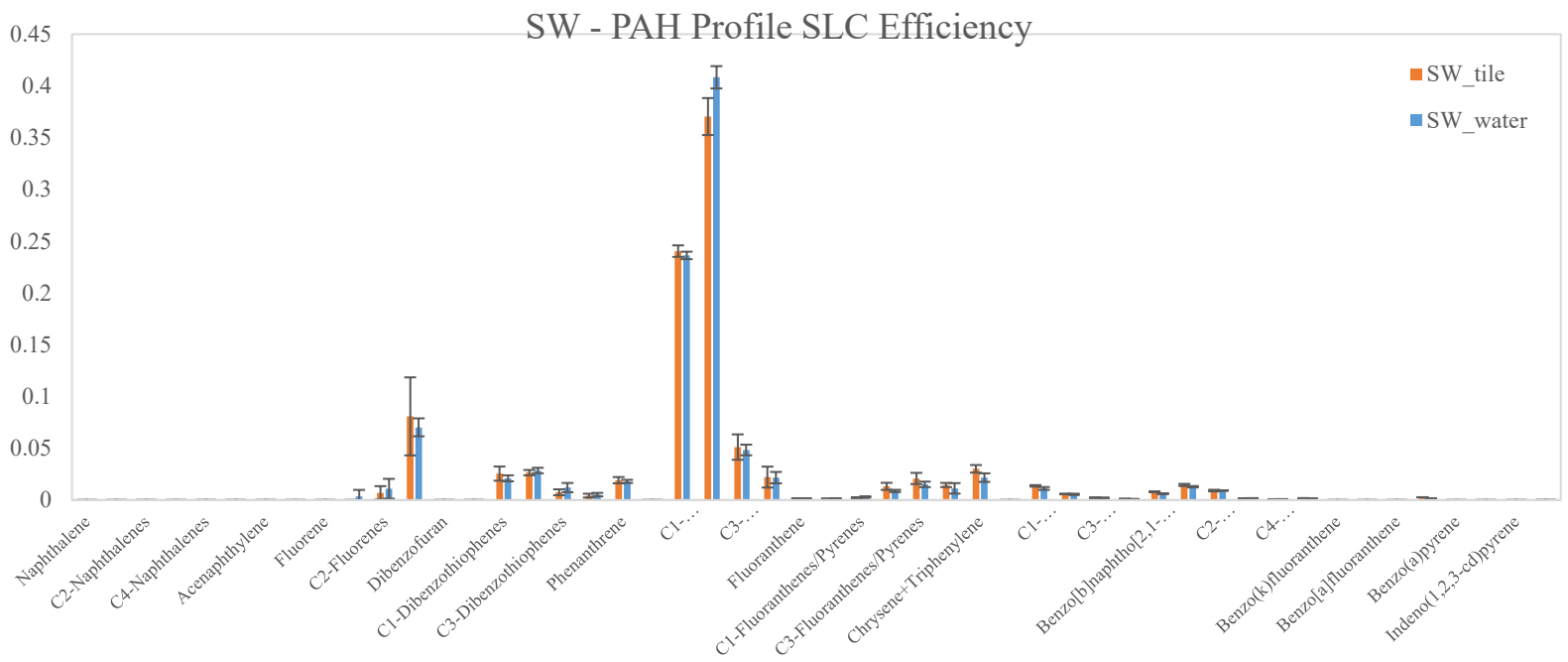

Figure 40. PAH profiles for oil remaining on the tile (orange) after SLC application/rinsing and oil in the rinsate (blue) for the seawater only protocol.

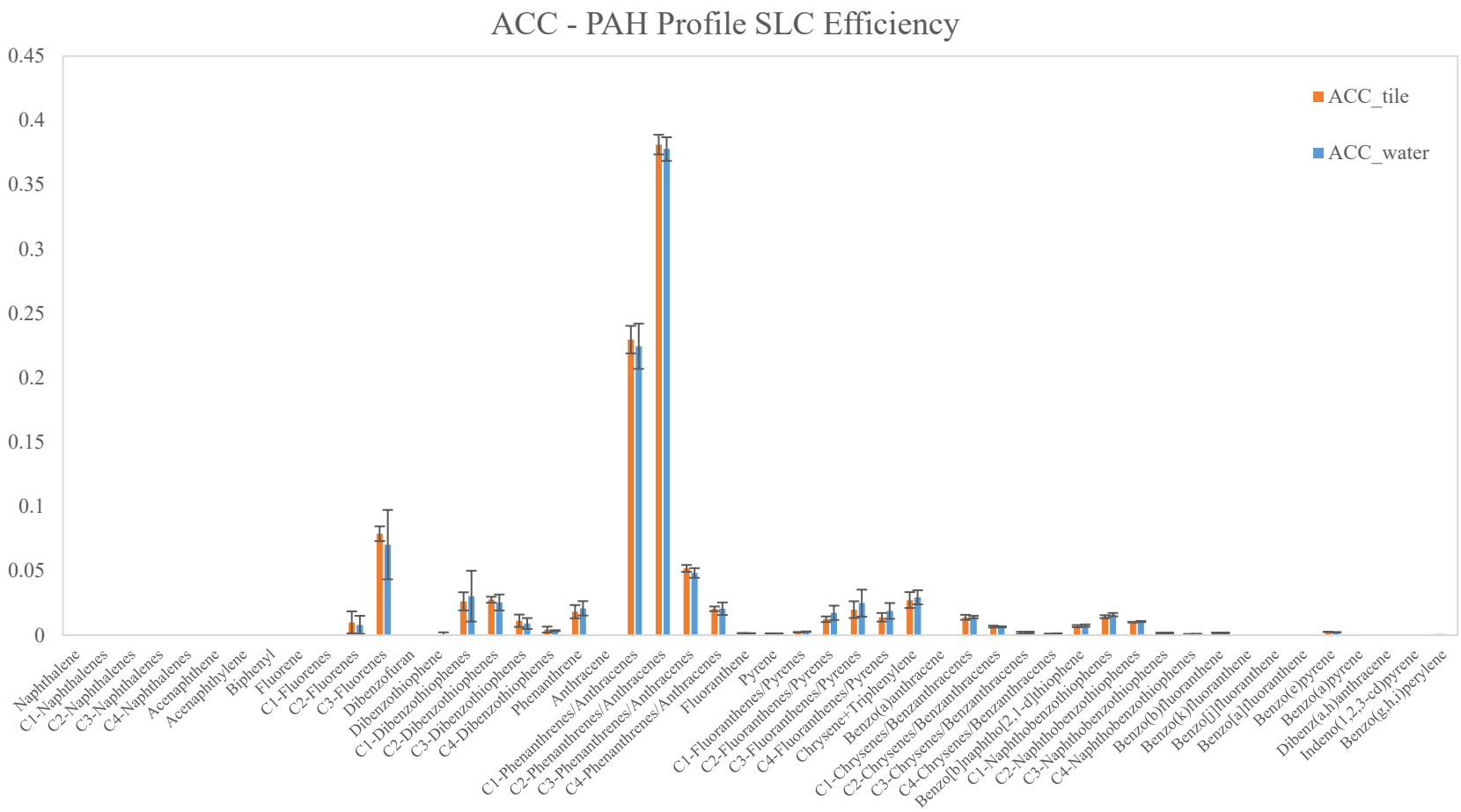

Figure 41. PAH profiles for oil remaining on the tile (orange) after SLC application/rinsing and oil in the rinsate (blue) for Accell.

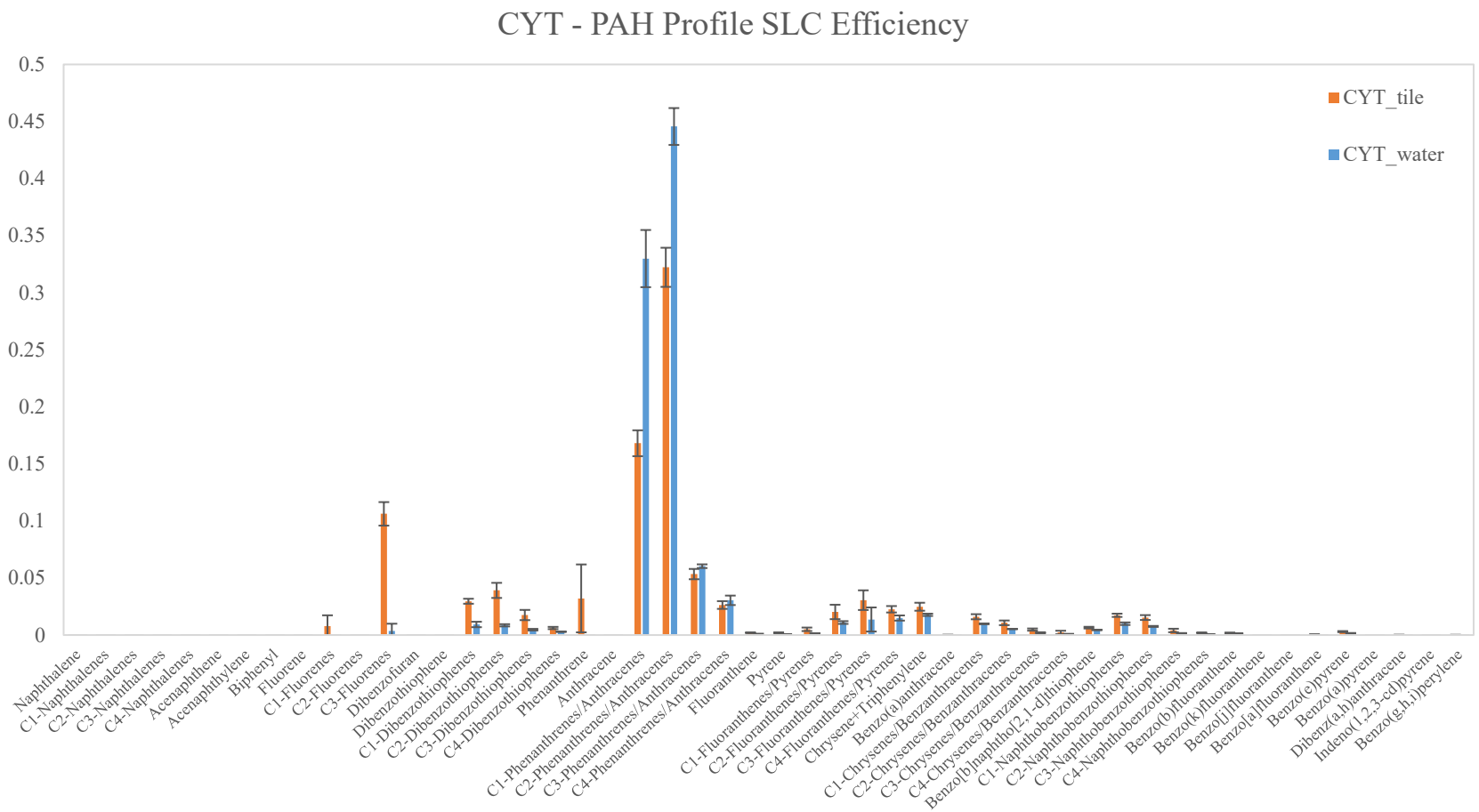

Figure 42. PAH profiles for oil remaining on the tile (orange) after SLC application/rinsing and oil in the rinsate (blue) for CytoSol.

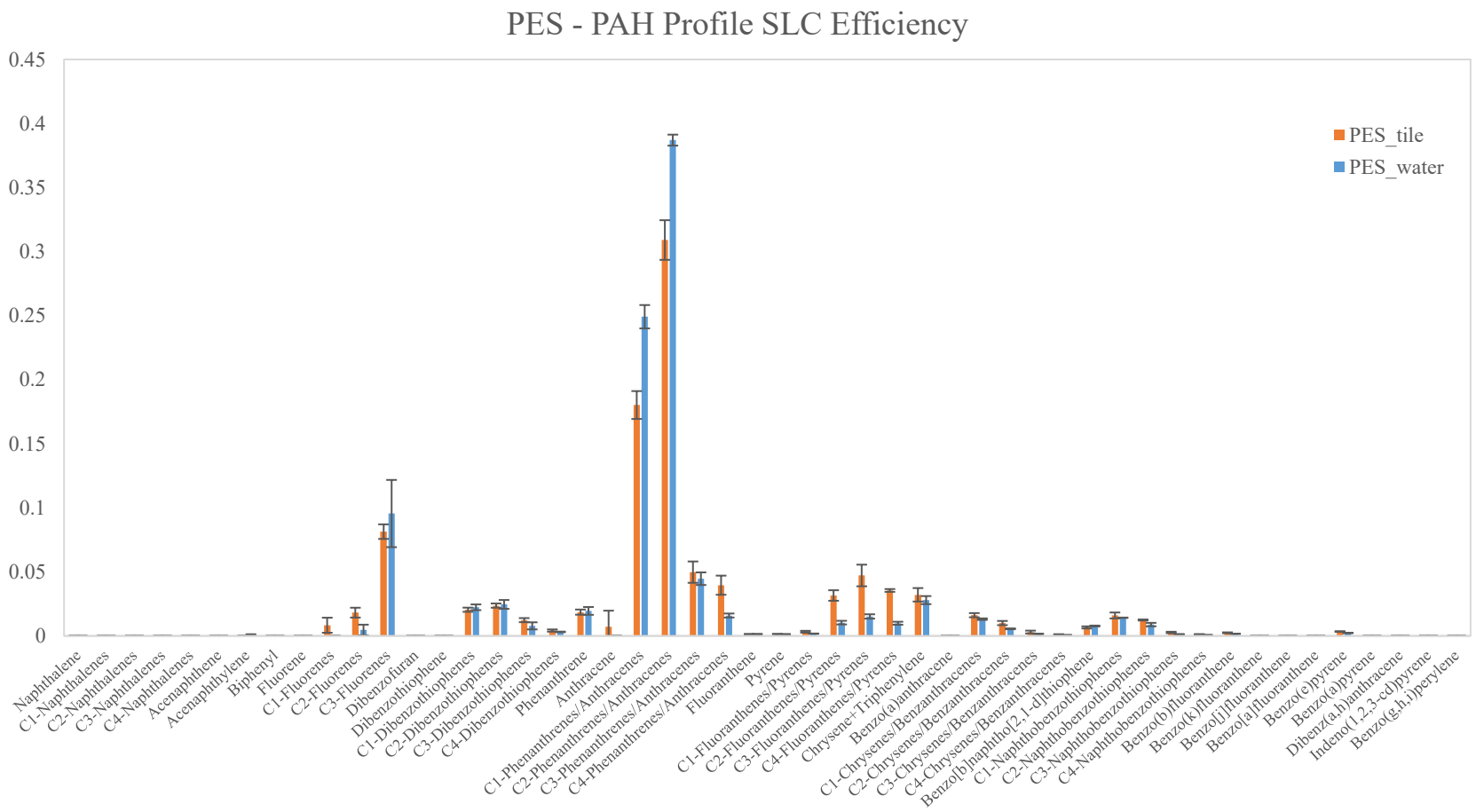

Figure 43. PAH profiles for oil remaining on the tile (orange) after SLC application/rinsing and oil in the rinsate (blue) for PES-51.

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

Title: Defining Protocols for Replanting as an Oil Spill Response Tactic in Coastal Marshes

Pennington, P.L.[1], Wirth, E.F.[1], Daugomah, J.W.[1], Chung, K.W.[2], Pisarski, E.[2], West, J.B.[2], Ramirez, J.L.[3], and DeLorenzo, M.E.[1]

[1] NOAA/NOS/NCCOS; Stressor Detection and Impacts Division

[2] CSS Inc., under contract to NOAA/NOS/NCCOS; Stressor Detection and Impacts Division

[3] College of Charleston, 66 George St, Charleston, South Carolina 29424

Childs, C.[4], Zengel, S.[5], and Michel, J.[5]

[4] NOAA/NOS/ORR Emergency Response Division

[5] Research Planning, Inc.,1121 Park Street, Columbia, SC 29201

Abstract

The Office of Response and Restoration (OR&R) and the National Centers for Coastal Ocean Science (NCCOS) are conducting a joint investigation to refine protocols for planting native marsh grasses as an oil spill response tactic in coastal marsh environments. Coastal marshlands are ecologically critical areas that provide essential food, refuge and nursery habitat. They are also highly sensitive to oil spills and exceedingly difficult to clean up. Many of the techniques used to clean other types of oiled shorelines can cause additional damage in marshlands and are not viable treatment options in these sensitive environments. During the Deepwater Horizon oil spill, NOAA investigated and implemented a wide variety of clean-up tactics in the most heavily impacted coastal marshes in Louisiana. Subsequent monitoring and investigations revealed that one of the most beneficial tactics employed was to replant native marsh grasses in the impacted areas. While this tactic shows potential, methods for replanting as an oil spill response treatment have not been defined or optimized. This investigation utilizes NCCOS's marsh mesocosm facility in Charleston, SC to simulate an oiled coastal marsh. The oiled mesocosms will be re-planted with selected treatment regimes and their recovery will be followed over a 2-year period. This project will seek to assess the recovery of structure and function of replanted marsh grasses and compare different clean-up treatments relative to unoiled reference conditions. The project will also seek to determine how replanting influences weathering and degradation of the oil. The initial round of experiments will also compare the relative viability of replanting using local, field-collected transplants vs. commercially available grasses obtained from regional nurseries.

Introduction

Oil spills are global incidents where oil is released into the environment by tankers, offshore oil rigs, or pipelines. These events differ in oil type, extent of oiling, biological characteristics of the environment where the spill occurred, and the season of occurrence (Michel & Rutherford, 2014). Oil spills spread into coastal environments and can disrupt every part of the food web. Organisms in these environments can be directly or indirectly affected due to the community structure within coastal ecosystems.

In 2010, the Deepwater Horizon explosion and oil spill became the largest marine oil spill in the United States. An estimated 4.9 million barrels of crude oil dispersed into the Gulf of Mexico for 87 days (Summerhayes, 2011). This event has had lasting impacts by threatening marine ecosystems, including Southeastern wetland environments. It directly impacted smooth cordgrass, *Spartina alterniflora*, in salt marsh ecosystems by spreading oil coverage along 796 kilometers of shoreline (Michel et al., 2013). There have also been other devastating oil spills such as the Exxon Valdez in 1989 which spilled 260,000 barrels of crude oil into Alaskan waters and the MV Prestige in 2002 which is the largest environmental disaster to occur in Portugal and Spain (Marcos, Aguero, Garcia-Olivares, & Luis De Pablos, 2004).

In September of 2002, the M/V Ever Reach spilled 12,500 gallons of intermediate fuel oil, IFO 380, into the Cooper River in Charleston, South Carolina (Mccay, Rowe, Ward, & Forsythe, 2006). Although significantly smaller than DWH and Exxon Valdez, it had an observed impact on wildlife (Mccay, 2006). After the spill, 21 brown pelicans were treated and released. Other organisms were considered to be injured during the event including birds, marine mammals, sea turtles, and subtidal fish and invertebrates. However, they were only able to estimate loss of birds. The report did not consider the salt marsh plants, although the model maps showed that the oil was dispersed from the Cooper River to Folly Beach and surrounding areas which contain salt marshes throughout (Mccay, 2006; Mccay, Rowe, Ward, & Forsythe, 2006). Even at this scale of spill, there were observed negative impacts on the ecosystem and other implications that were not taken into consideration such as the unique structure of salt marshes and the complex interactions that occur there.

Salt Marsh Ecosystems

Salt marshes are ecologically rich environments that provide ecosystem services that benefit society such as nursery grounds for fish and shellfish, , carbon sequestration, and resources for fisheries (Frey & Basan, 1978; Mitsch, Gosselink, Anderson, & Zhang, 2009; Perkins, Ng, Dudgeon, Bonebrake, & Leung, 2015).. They can also protect shores from erosion, buffer coasts from storms, and naturally filter pollutants from the environment (Mitsch et al., 2009). In 2010, it was estimated that 123.3 million people, or 39% of Americans lived near the coast. This number is also expected to continue to increase to 47% by 2020 (NOAA, 2018). Coastal urbanization can negatively impact these ecosystems by having more boat traffic, which increases the chance of oil spills.

Along the Eastern United States seaboard, *S. alterniflora* is the dominant coastal plant species. The presence of this species in coastal environments physically modifies the

environment to be functional for other species and provides them with habitat and energy through trophic interactions. They have a strong role in structuring communities making them a foundation species. The zonation of plants in the low and high marsh are a defining characteristic of salt marshes (Bertness et al., 2014). Other important salt marsh plants include *S. patens* (saltmeadow cordgrass), *Juncus roemerianus* (blackneedle rush), *Salicornia virginica* (glasswort), and *Iva frutescens* (marsh elder). Ecologically, *S. alterniflora* are poor competitors that are displaced by other marsh plants and are left to reside in the low marsh where they are exposed to fluctuating salinities, desiccation, and inundation (Bertness, 1991). These qualities allow them to combat erosion and provide nursery and habitat for marine organisms. Studies have shown that oil spills have different physical effects on salt marsh plants. *Spartina alterniflora* can be very tolerant of oil compared to *S. patens* which tends to be more sensitive (Lin & Mendelssohn, 1998; Michel & Rutherford, 2014).

No. 6 Fuel Oil

No. 6 fuel oil is a heavy refined oil product used by barges, ships, and tankers. These ocean traveling vessels burn the oil to use as fuel. It is produced by combining heavy residual oil with lighter oils. When dispersed in water, it forms dark, thick slicks with large amounts of oil and can float, suspended in the water column, or sink. These oils vary in viscosity, but the most viscous oils can form tarballs and patches of oil instead of slicks. This oil is persistent and can travel hundreds of miles as tarballs by winds and currents (NOAA, no date).

The general way that this type of oil affects salt marsh vegetation is by covering or smothering plant stems, leaves, and soil (Michel & Rutherford, 2014). It generally has low amounts of acutely toxic compounds but can have long-term impacts when there is re-oiling, thick layers of oil on the sediments, and whole plant coverage (Baker, 1973).

No. 2 Fuel Oil

No. 2 fuel oil is a 'diesel fuel' product and goes by many different names including red diesel, high-sulfur diesel, off-road diesel, and marine-grade diesel. It lighter than No. 6 fuel oil but has a higher sulfur content than the more refined diesel that is used in automobiles and trucks on roadways in the United States.

Restoration of Salt Marshes

Restoration of salt marshes are commonly performed to mitigate the effects of increased urbanization. Restoration is often performed by replanting *S. alterniflora* or other marsh plants that increase the re-establishment of other organisms' populations that were disrupted. One aim of these restoration efforts is to combat erosion, provide filtration of water, and provide habitat for other organisms. The eastern U.S. have implemented extensive marsh restoration programs that utilize mass production of *S. alterniflora* (Craft et al., 2003; Garbisch, et al., 1975). "Seeds to Shoreline" is a South Carolina statewide restoration program that educates K-12 teachers and students about salt marshes. The program allows for classrooms to grow *S. alterniflora* and transplant their seedlings into restoration sites (Bell, 2020).

Prior to replanting a marsh, oiled plants (or dead mats) are often removed via cutting and/or raking (Rutherford, 2019). The oiled plant biomass is then hauled away. This method serves to remove much of the heavy oil since the emulsified oil tends to stick to the plant material. In heavily oiled situations, this practice is necessary to expose areas of open sediment where restoration efforts (replanting) could then take place.

The source of plants for restoration can have a significant effect on the outcomes of long-term restoration efforts. Trait variation of different source plants can increase the success of a restoration project. For a project that has a goal of decreasing soil erosion, the source plant with higher belowground biomass would be preferred. These traits are also dependent on abiotic characteristics of the environment such as nutrient availability (Bernikn et al., 2018). Locally sourced *S. alterniflora* was reported to outperform commercially available *S.* in survivorship and growth after two seasons in a South Carolina marsh after transplantation (Beck & Gustafson, 2012).

Using transplants of *S.* to restore oiled wetlands has great potential for success. Lin and Mendelssohn (1998) determined that *S. alterniflora* can be transplanted into contaminated marsh with south Louisiana crude oil concentration ≤ 100 mg g- and ≤250 mg g- for *S. patens*. Recovery of these plants is dependent on the toxicity of the oil and weathering processes that occur after the spill. When exposed to No.2 fuel oil *S. alterniflora* had high tolerance, with detrimental effects to total biomass production at oil ≥ 228 mg g- dry soil (Lin et al., 2002). Another benefit of using *S. alterniflora* transplants in oiled marshes is that they also facilitate the degradation of oil. This could be used as a type of phytoremediation by using the plants to aid in the clean-up of oil (Lin & Mendelssohn, 1998; 2008). Research with different kinds of oils would be beneficial to determine if this phytoremediation and transplant of *S. alterniflora* can be used for other types of oil spills as well.

There are two main methods for planting *S. alterniflora* into salt marshes. The most popular method is a containerized plug or pot planting. In this method, a plant is raised in a nursery in a small planter pot (usually 2” x 2” square). Once the plant is established and mature, the plug is removed from the pot and placed into a pre-made hole of the same size along the shoreline to be restored. The same method can be used for field transplants. *S. alterniflora* plugs are collected from an uncontaminated field site using a standard garden bulb or plant extractor. This will yield a plant with an intact core of about 2” to 3” in diameter that can be planted directly into an area to be restored.

The other method that we would like to consider is that of a ‘bare-root’ planting (PSU, 2016). In this scenario, the sediment (or soil) is carefully removed from the plant plug or core by gently washing with water until only roots and stems remain. This plant can then be planted directly into the restoration area into a pre-made hole. Bare-root plantings are a common practice for some fruits and ornamental plants in residential gardening and commercial growing operations. The main advantage is that one does not have to transport the mass of sediment from nurseries or source areas to restoration areas. When dealing with hundreds or thousands of plants, the weight of the sediment or soil can be significant. Another important aspect that makes this method appealing for environmental restoration work is that unwanted materials such as

foreign soils (with terrestrial microbes or microbes from other aquatic systems) can be eliminated.

Overarching Project Objective:

To determine oiling and/or clean-up treatment conditions where the re-planting of saltmarsh vegetation would be a viable option.

Initial Pilot Studies

In a series of shorter trials through either laboratory- or mesocosm-based studies, we will test the viability of replanting local wild transplants versus commercial nursery stock in oiled systems using cordgrass, *S. alterniflora*. As part of these initial tests, we will also consider how best to optimize planting factors such as plant density, the use of fertilizers or lack thereof, the use of bare root plants vs. containerized plants, the use of different plant varieties or cultivars, and/or the use of mixed varieties or mixed species plantings. Using laboratory and pilot mesocosm studies, we will determine at what oiling levels (re-)plantings shall be expected to survive and thrive. The results of these initial tests will be used to formulate the experimental design for the longer mesocosm test.

Pilot Study Objective 1: Determine the oil slick thickness (µm) of No.6 Fuel Oil at which adverse effects and mortality will be observed in *S. alterniflora* and *S. patens*.

Pilot Study Objective 2: Determine the growth of above-ground and below-ground biomass of *S. alterniflora* at different nutrient levels using commercial fertilizer.

Pilot Study Objective 3: Determine the oil slick thickness (µm) of No.2 Fuel Oil at which adverse effects and mortality will be observed in *S. alterniflora*

Pilot Study Objective 4: Determine which cultivar source of *S. alterniflora* , local wild transplants or commercial nursery stock will have greater survivorship and better growth performance for replanting in oiled sediments.

Mesocosm Study:

In a heavily oiled *S. alterniflora* marsh mesocosm (with fuel oil), we propose to test the effectiveness of several re-planting strategies. This study will assess the success (or failure) of the strategies over a two year period. Objectives 1 & 2 below will be performed simultaneously.

Mesocosm Study Objective 1: We plan to focus on the following three clean-up tactics: (1) leaving the marsh vegetation intact, (2) cutting and removing the marsh vegetation, (3) comparing nursery raised plants vs. wild transplants, and (4) bare-root plantings vs. containerized (plugs) plantings. Following oiling and clean-up; we will quantify how re-planting impacts the recovery of vegetation structure and function compared to oiled controls (unplanted) and reference/unoiled conditions.

Mesocosm Study Objective 2: In a heavily oiled *S. alterniflora* marsh mesocosm, we propose to examine how the various clean-up protocols and re-planting scenarios influence weathering and degradation of oil in marsh surface waters and marsh sediments. Samples will be collected and analyzed for residual PAHs (polycyclic aromatic hydrocarbons) and TEH (total extractable hydrocarbons).

Experimental Procedure

Pilot Study Objective 1: Range Finding Study and Pilot Study with No. 6 Fuel Oil

A greenhouse microcosm experiment was set up at the NOAA National Centers for Coastal Ocean Science lab in Charleston, South Carolina, USA. *S. alterniflora* and *S. patens* 2-inch plugs were ordered from Environmental Concern located in St. Michael's, Maryland. The sediment used for the experiment was collected from Wadmalaw Island, SC from the West Branch of Leadenwah Creek (32°38'52.0"N 80°13'19.3"W). This is a known reference site for the research that NOAA NCCOS performs. The sediment was collected in the low marsh at the edge of the bank and sieved through a 3 mm sieve to remove benthic macrofauna. Using a scoop, the sediment was deposited into 16 oz. cups with one plant plug. Three cups were placed into a 40 X 27 X 18 cm box and filled with 5µm filtered, 20 ppt seawater from Charleston Harbor. The water was kept at the sediment-air interface. Throughout the experiment deionized water was added to a fill line to ensure salinity was maintained at 20 ppt due to evaporation. Each box had air tubing set up to allow for one aquarium air stone to be placed in each box. Plants were then allowed to acclimate for 2 months. Starting measurements for stem density and stem lengths were taken at the beginning of the acclimation period and again at the end of the acclimation period to calculate a pre-treatment growth rate.

The experimental design was a randomized block design with 6 treatments and 3 replicates per treatment for each species (Figure 1). The nominal No. 6 Fuel Oil slick thickness treatments were a control (no oil), 25.6 µm, 64 µm, 160 µm, 400 µm, 1000 µm. Each treatment had 3 replicates per treatment, n=54 plugs per species. Oil was adjusted to room temperature to allow for ease of handling. The treatments were dispensed to the containers by mass rather than volume due to the stickiness of the oil to the glassware. A known volume of oil was weighed in a graduated cylinder and dispensed into a container. The cylinder was then re-weighed to determine the amount of oil transferred by difference. The mass of oil dispensed into the treatments was used to calculate the actual thickness of oil in each treatment. One plug (cup) from each replicate was removed after 28-d for assessment of above-ground and below-ground biomass. A second cup had the standing crop cut to observe regrowth beyond 28-d. Those cuttings were frozen for future hydrocarbon analysis. The third and final cup from each box was left undisturbed for growth monitoring.

The amount of regrowth after 180-d was measured. Water quality was monitored throughout the acclimation period and experiment using a YSI Professional Plus and YSI 556 MPS. Leaf samples were cut from *S. alterniflora* with razor blades and whole stem samples were cut from *S. patens* from every cup at time 0d, 7d, 14d, and 28d. The samples will be stored in a -80 °C freezer until they can be analyzed. Stem density and stem height was measured at 0d and 28d. Aboveground and belowground biomass was measured at the end of the experiment.

<u>Plant Responses</u>

Plant responses that will be measured are mortality, stem density, stem length, stem mortality, chlorophyll, aboveground biomass, and belowground biomass. The stems will be quantified for mortality, stem density, and stem height. Aboveground and belowground biomass

will be taken by separating the two sections, cleaning the roots of soil, and storing samples in a -80°C freezer until analyzed. The biomasses will be weighed to get a wet weight, oven dried at 70°C and weighed again for dry weight. The leaf and stem samples for chlorophyll analysis will be performed using a fluorometer. If allotted for, PAM assays will be performed on leaf samples to collect additional plant chlorophyll and productivity measurements.

Oil Chemistry

A sample of No. 6 Fuel Oil will be characterized for PAHs using gas chromatography mass spectrometry (GC/MS). This will be performed in the NOAA NCCOS Organic Chemistry Lab at Hollings Marine Lab Charleston, South Carolina. To prepare for extraction the sample will be diluted with toluene. A solid phase extraction will be performed with silica as a sorbent (500mg/3cc) and hexane and dichloromethane as eluant solvents. The samples will be concentrated using a Turbovap concentration workstation at 40C under a gentle stream of nitrogen (~14 psi). The samples will be measured on a Agilent GC/MS (6890/5973), operated in selected ion monitoring mode, containing a DB-17ms analytical column (60 m x 0.25 mm x 0.25 um) and split/splitless injector and analyzed using MSD ChemStation software.

Chlorophyll Extraction

Frozen leaf and stem samples will be ground in a mortar and pestle using sieved (500µm) silicate. The procedures for chlorophyll extraction will be performed as previously described by Biber (2007). Chlorophyll will read on a 10AU Field and Lab Fluorometer. The chlorophyll will be standardized to leaf area.

Pilot Study Objective 2: Growth of Spartina under difference nutrient concentrations.

For this study, *S. alterniflora* seeds were collected from the Leadenwah Creek Site and allowed to germinate in a greenhouse over the winter. The seedlings were planted into 2" containers with a commercial potting soil and allowed to grow from January 2020 until June 2020. The potting soil contained a very minimal amount of fertilizer (0.1-0.08-0.06 :: N-P-K). In June 2020, they were repotted into 4" pots with the same kind of potting soil for fertilizer dosing. The experiment is currently underway and consists of 90 pots.

There are three treatments with 30 replicates each: a control (low nutrients), a mid nutrient dose, and a high nutrient dose. The mid nutrient dose matched the application rates used by Cagle et al. 2020. The high dose was double that of the mid dose. Fertilizer dosing was performed with a commercial garden fertilizer (Osmocote® Plus 15-9-12 :: N-P-K) that consists of slow release capsules. The capsules were placed directly on top of the soil.

Using methods previously described, the growth of the plants will be assessed weekly for 28-d. Stem density and maximum stem heights will be determined. At the end of the 28-d the plants will be harvested for above-ground biomass determination and the below-ground biomass (roots/rhizomes) will be separated from the soil for root image analysis.

Pilot Study Objective 3: Range Finding Study with No. 2 Fuel Oil

For this Objective, local wild transplant and nursery raised plants will be used. The local wild transplants of *S. alterniflora* will be collected from a local field site such as Leadenwah Creek, Wadmalaw, SC at the NOAA reference site. Sediment will also be collected at this site and sieved as previously described. Commercial nursery grown *S. alterniflora* will be purchased from Environmental Concern located in St. Michael's, Maryland.

The greenhouse will be set-up with four mesocosm tanks as in Pennington 2007 and DeLorenzo et al. 2017 to allow for the oil and water level to cover a significant portion of the standing during simulate tidal flux. Some modifications will be made. Rather than using the standard sediment trays (used by Pennington et al. 2007 and DeLorenzo et al. 2018), each plant will be placed into 4" planter pots and supported by PVC pipes (4" in diameter and 6" tall). Each mesocosm will have 36 pots: 12 pots will have local wild transplants of *S. alterniflora* , another 12 pots will have nursery raised *S. alterniflora* , and the remaining 12 will have sediments only (no grass plantings) and will be used for Pilot Study Objective 4.

The systems will be dosed at a range of No. 2 Fuel Oil levels based on sheen or slick thickness. There will be a control system with no fuel oil and three dosed systems (low, medium, and high). The actual levels are still to be determined. The duration of the exposure will be 96 hours.

Spartina alterniflora growth and biomass measurements will be determined as described previously. The plants will be measured for stem density, stem height, above and belowground biomass, and chlorophyll after 96 hour exposure. Leaf samples for chlorophyll analysis will be collected every 24 hours from each individual pot by randomly selecting a leaf. These will be stored in freezer until analysis. Hydrocarbons (PAHs and TEH) will also be determined as described previously.

Pilot Study Objective 4: Replanting into sediments contaminated with No. 2 Fuel Oil

The previous dosing study affords us the opportunity to dose sediments with high amounts of No. 2 Fuel Oil to look at replanting success. After Pilot Study Objective 3 has concluded, the remaining 12 cups with oiled sediment in each of the four systems will have new plugs of *S. alterniflora* transplanted into them. There will be a combination of bare-root plantings and containerized (plug) plantings. The growth success of the plants will be monitored using the growth determination methods as previously described. The experiment will run for 28d or longer. The tide cycle will consist of 2 low and 2 high tides within 24 hours for the duration of the experiment.

Plant growth measurements will include stem density, stem height, mortality, chlorophyll, aboveground biomass, and belowground biomass. The stems will be quantified for mortality, stem density, and stem height after 28 d. Aboveground biomass, belowground biomass, and chlorophyll *a* will be measured as previously described. Every week, leaf samples will be measured with a PAM fluorometer. Time permitted, oil samples from each treatment will be collected to determine the total PAHs and total extractable hydrocarbons for each oil slick thickness.

Data Analysis

Statistical analysis will be conducted using Microsoft Excel, version 16.0, SAS statistical software (SAS Institute) and RStudio (R Studio Team, 2015). The growth rate of the plants (stem density and stem height) during the acclimation and experimental periods will be calculated. A t-test will be performed to determine whether the two growth periods were significantly different from one another. Nested ANOVAs will be performed to determine the effect of plant source on growth performance of each species. Another analysis that could be performed is a Probit analysis to determine LC50 or EC50 values, depending on the outcomes of the data.

Mesocosm Study Objectives 1 & 2 (performed simultaneously)

Setup

Each mesocosm system consists of two tanks, one upper and one lower in accordance with procedures outlined in NOAA Technical Memorandum NOS NCCOS 62 (Pennington et al. 2007) and as modified in DeLorenzo et al. 2017). The 20 systems used in this study are enclosed in a greenhouse, which incorporate natural light and temperature conditions (Figure 1).

The specific setup of the experiment units follow that of Key et al. 2014. The lower tank, or sump, provides tidal water to the upper tank via a pump set to a timer. The tide is semi-diurnal, so twice daily seawater is pumped into the upper tank (mesocosm) from the lower tank (sump) to simulate a flood tide. The seawater is dispensed into the mesocosm tanks (443 L each) several months 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. Four 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. 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 (*S. 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 (6 trays in each 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.

Two to three months following the sediment collections, *S. alterniflora* marsh grass plugs (approx. 7.5 cm in diameter) were collected from the same site using a standard garden plant/bulb extractor. Two plugs were placed into each of the three *S. alterniflora* sediment trays. *Spartina alterniflora* was allowed to grow in the tank systems for at least 2 months prior to dosing.

Experimental Design of Mesocosm Study

All systems will be oiled with same amount of fuel oil with the exception of control units. At the conclusion of the pilot studies, we will choose the fuel oil type (No. 2 or No. 6) and the oiling level (or dosing amount) to use. In all likelihood, we will be uing No. 2 Fuel Oil based on preliminary results from Pilot Study Objective 1. The system will be dosed by sheen or slick thickness on the surface of the water. The objective is to dose high enough such that we have a complete kill of the *S. alterniflora* in the systems. We will also decide whether not it will be beneficial to use a fertilizer amendment based on the results of Pilot Study Objective 2.

Table 1 shows the experimental design for the study. The 20 mesocoms units will be randomly assigned to four treatment groups. There will be a Control group that receives no fuel oil. The other three treatments groups (TRT A-C) will all receive the same dose of fuel oil. Again, the dose will be enough to cause a complete kill of the *S. alterniflora* . The differences in the TRT groups will represent the *S. alterniflora* replanting strategies employed. TRT A will only receive oil and no replanting of any kind. TRT B will receive oil and will be replanted with local transplants at a period of time (to be determined) after the oiling event has occurred. TRT C will be similar except that it will receive plants raised by a commercial nursery. Further, for TRTs B & C, *S. alterniflora* replanting will be split within the systems using two different planting methods. The first will be a bare-root replanting. Sediments will be gently washed away from the source plant (either local transplant or nursery plant) leaving the bare roots and stems. Those plants will be planted into the systems. The second method will be a containerized planting (or plug planting). We will keep the source sediments intact such that the roots are disturbed as little as possible prior to planting. The whole plug with intact sediments will be planted into the systems.

Study Timeline

The established mesocosm system will be allowed to ‘grow-in’ for at least 2-months and stabilize prior to dosing. Once the systems are dosed with fuel oil, they will be monitored for at least 2 weeks to observe the dieback of the *S. alterniflora* standing crop. After the dieback is complete (ideally within 2-4 weeks), the dead standing biomass of *S. alterniflora* will be harvested from the systems (including the control). Based on the mesocosm conditions and presence of remaining fuel oil we will then make a decision as to when replanting should occur. The plan is two replant within 1 month after the harvesting the dead *S. alterniflora* standing biomass, but that is subject to change based on the conditions of the systems. After replanting, the systems will monitored regularly for a period of 18 months to 2 years to assess the success of the various replanting tactics.

Plant Responses

Plant responses that will be measured are mortality, stem density, stem length, stem mortality, chlorophyll, aboveground biomass, and belowground biomass. The stems will be quantified for mortality, stem density, and stem height. Aboveground and belowground biomass will be taken by separating the two sections, cleaning the roots of soil, and storing samples in a -80°C freezer until analyzed. The biomasses will be weighed to get a wet weight, oven dried at 70°C and weighed again for dry weight. The leaf and stem samples for chlorophyll analysis will be performed using fluorometric techniques If possible, PAM assays will be performed on leaf samples to collect additional plant chlorophyll and productivity measurements.

Oil Chemistry

Water and sediment samples for chemical analysis of total extractable hydrocarbons (TEH) and polycyclic aromatic hydrocarbons (PAHs) will be collected at various intervals after oiling. To quantify PAH and TEH, water samples will be 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) will include a blank, TEH spike (10 mg) and PAH spike (400 ng). All samples will be spiked with PAH and TEH internal standards and mixed thoroughly. There will be 18 deuterated PAH internal standards (d8-naphthalene, d10-1-methylnaphthalene, d8-acenaphthylene, d10-acenaphthene, d10-fluorene, d8-dibenzothiophene, d10-phenanthrene, d10-anthracene, d10-fluoranthene, d10-pyrene, d12-benz[a]anthracene, d12-chrysene, d12-benzo[b]fluoranthene, d12-benzo[k]fluoranthene, d12-benzo[e]pyrene, d12-benzo[a]pyrene, d12-perylene and d12-benzo[g,h,i]perylene [Cambridge Isotope Laboratories, Inc. Tewksbury, MA]) and 2 TEH internal standards (d26-dodecane and d42-eicosane perylene [Cambridge Isotope Laboratories, Inc. Tewksbury, MA]).

Samples will be solvent extracted three times with the following solvents, dichloromethane, 50:50 dichloromethane/hexane and hexane. After extraction, samples will be 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 will be cleaned-up using silica Solid Phase Extraction (SPE) (3 mL/0.5 g [Phenomenex Torrence, CA]) and spiked with a recovery standard (d14-p-terphenyl [Cambridge Isotope Laboratories, Inc. Tewksbury, MA]) prior to instrumental analysis on GC/MS.

Sediment samples (top 1-2 cm) will be collected from the mesocosm upper tanks using solvent rinsed metal spatulas. Sediments will be 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 will be extracted under pressure using Accelerated Solvent Extraction (ASE200) (Dionex Inc.) with dichloromethane:acetone (1:1 v/v). The extracts will be reduced in volume to 2mL under nitrogen and passed through a Biobead column via Gel Permeation Chromatography (GPC) to remove interferences. Additional clean-up can be achieved by using silica SPE. The final volume will then be exchanged under nitrogen to hexane and the extracts analyzed for both PAH and TEH.

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

Chlorophyll Extraction

Frozen leaf and stem samples will be ground in a mortar and pestle using sieved (500µm) silicate. The procedures for chlorophyll extraction will be performed as previously described by Biber (2007). Chlorophyll will read on a 10AU Field and Lab Fluorometer. The chlorophyll will be standardized to leaf area.

Results and Discussion

This study is currently a work in progress and any results presented are preliminary. Table 2 displays the status of the study objectives up to this point. Only preliminary results from Pilot Study Objective 1 are presented at this time.

Pilot Study Objective 1: Range Finding Study and Pilot Study with No. 6 Fuel Oil

We tested two species of marsh grass (*S. alternifora* and *S. patens*) under static conditions. Plants were in 16 oz cups submerged to the sediment surface in bins with aerated seawater (20 ppt). Five different slick thickness (76.8 to 3000 µm) of Fuel Oil were applied plus controls. The initial duration of the study was 28 days. We documented change in stem counts, shoot height, and biomass (after 28 days). Preliminary data from this pilot for No. 6 Fuel Oil showed no significant differences in treatments versus controls up to the 3000 µm slick thickness (Figure 3, Figure 4, Figure 5, and Figure 6) after 28-days post-dose.

We also followed the regrowth of plants for an additional 45 days (73 days post dose) from plants cut at the 28-d time point. Figures 7 & 8 show that there is a reduction in the regrowth in terms of numbers of new stems after 45 days in both species especially at the higher concentrations. However, the significance of this trend is still being investigated and these results should be considered preliminary.

Conclusion

This multi-component study is, in part, currently in progress and also on-hold due to COVID-19. Therefore, we cannot reach any definitive conclusions at this time. It appears that No. 6 fuel oil had no effect on *Spartina* spp. under the conditions that we tested up to a slick thickness of 3000 µm. However, during the regrowth period it does seem that growth was reduced in the higher treatments. Based on these results we have decided that No. 6 Fuel Oil will not be used in the mesocosm test. In all likelihood, we will switch to using No. 2 Fuel Oil pending the results of the remaining pilot studies.

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Table 1: Mesocosm Study Experimental Design

Treatment	TRT A			TRT B			TRT C			Control		
Oil	Fuel Oil No. 2			Fuel Oil No. 2			Fuel Oil No. 2			None		
Plant Source for replanting	None			Local Transplants			Nursery Plants			None		
	Replicate mesocosms A-E			*Replicate mesocosms A-E*			*Replicate mesocosms A-E*			*Replicate mesocosms A-E*		
		Trays (1-6) split within each rep			Trays (1-6) split within each rep			Trays (1-6) split within each rep			Trays (1-6) split within each rep	
		Trays 1-3	Trays 4-6		Trays 1-3	Trays 4-6		Trays 1-3	Trays 4-6		Trays 1-3	Trays 4-6
	A	None	None	*A*	bare root	plug	*A*	bare root	plug	*A*	None	None
	B	None	None	*B*	bare root	plug	*B*	bare root	plug	*B*	None	None
	C	None	None	*C*	bare root	plug	*C*	bare root	plug	*C*	None	None
	D	None	None	*D*	bare root	plug	*D*	bare root	plug	*D*	None	None
	E	None	None	*E*	bare root	plug	*E*	bare root	plug	*E*	None	None

Table 2 -- Current Status of Project Objectives

Objective	**Description**	**Status**
Pilot Study Objective 1:	Range Finding Study and Pilot Growth Study with No. 6 Fuel Oil	Complete – results preliminary
Pilot Study Objective 2:	Growth of *Spartina* under difference nutrient concentrations.	In progress
Pilot Study Objective 3:	Range Finding Study with No. 2 Fuel Oil	On-hold due to COVID-19
Pilot Study Objective 4:	Replanting into sediments contaminated with No. 2 Fuel Oil	On-hold due to COVID-19
Mesocosm Study Objective 1:	Long-term assessment of oiled-marsh replanting strategies	System setup is complete, but the study itself is on-hold due to COVID-19
Mesocosm Study Objective 2:	Short- and Long-term Hydrocarbon analysis	System setup is complete, but the study itself is on-hold due to COVID-19

Figures:

Figure 1 -- Picture of greenhouse microcosm set-up for S. patens. Each box contains 3 cups with an individual plant plug. There are 18 boxes for 6 treatments with 3 replicates each.

Figure 2 -- Individual mesocosm test systems enclosed in the greenhouse, NCCOS laboratory, Charleston SC

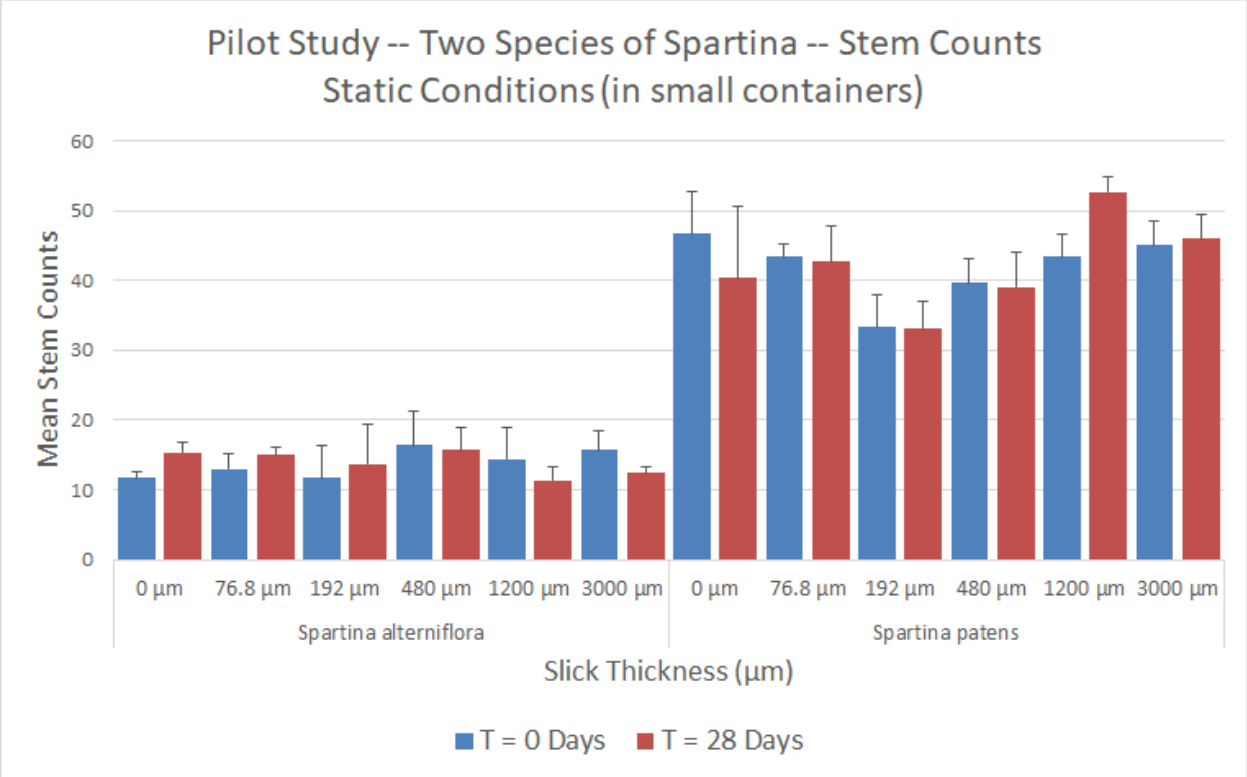

Figure 3 -- Pilot Study Stem Counts

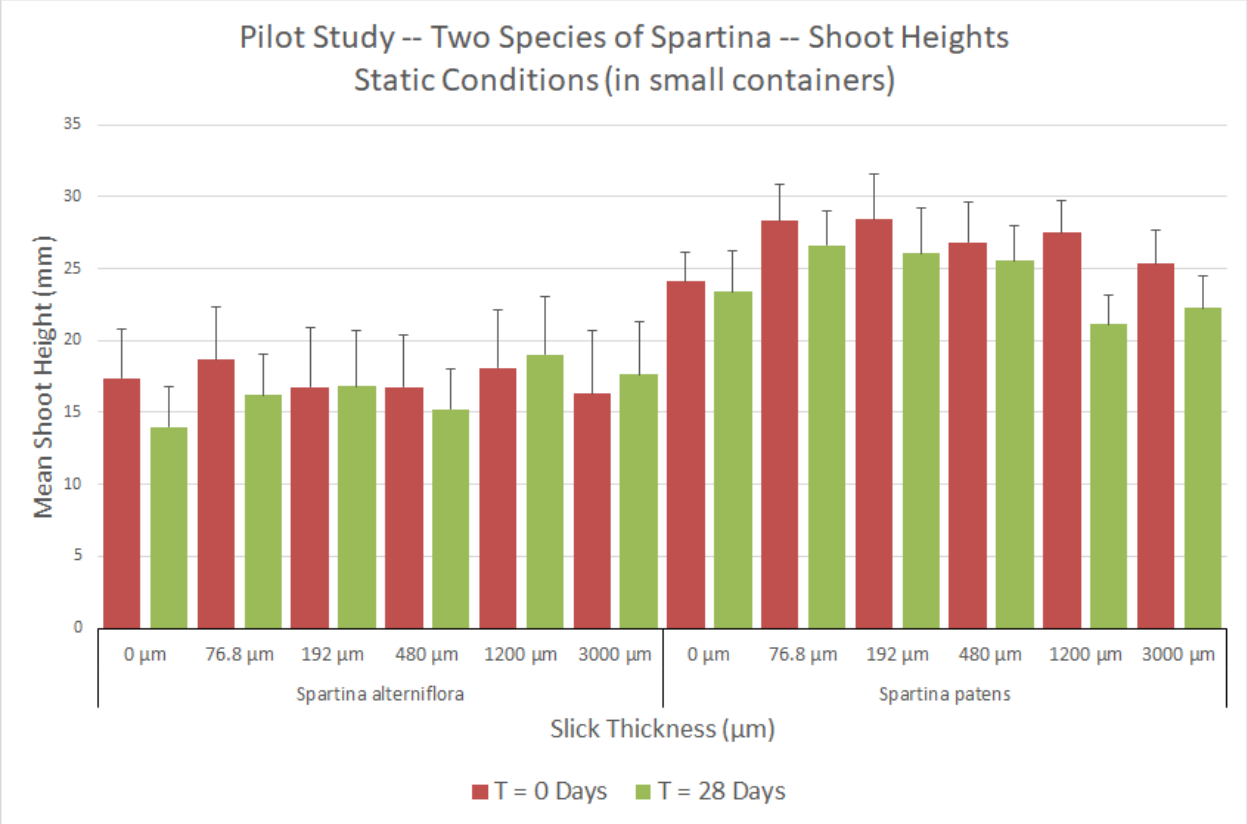

Figure 4 -- Pilot Study Shoot Heights

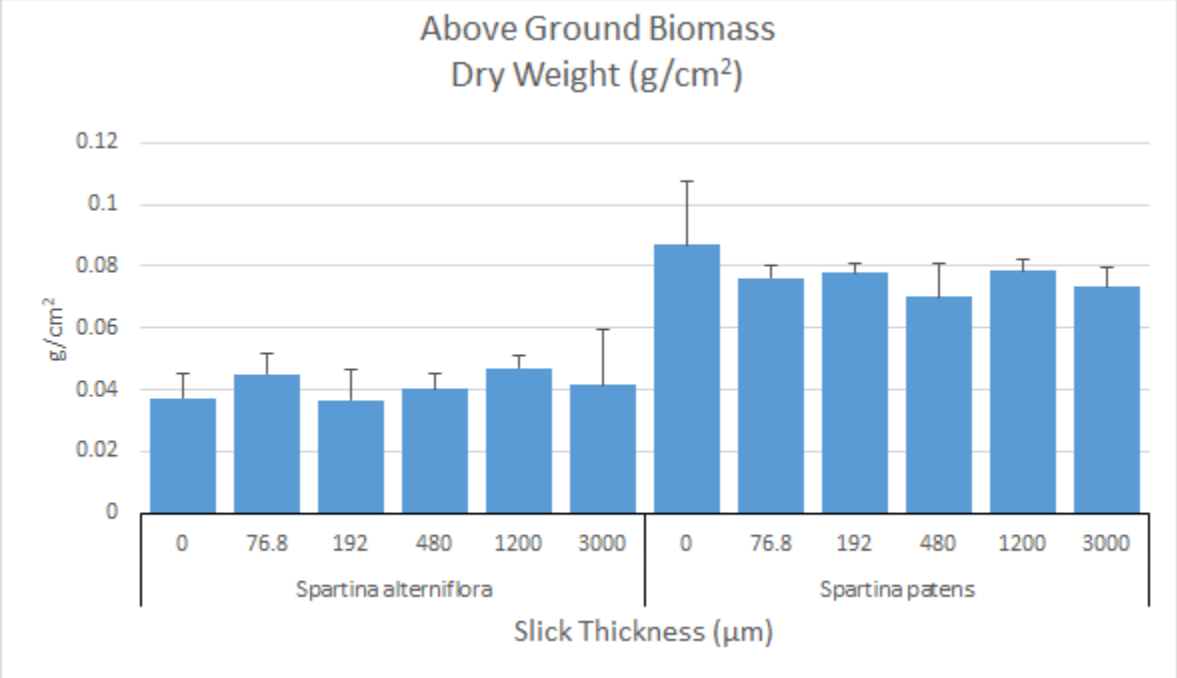

Figure 5 -- Pilot Study Above-Ground Biomass

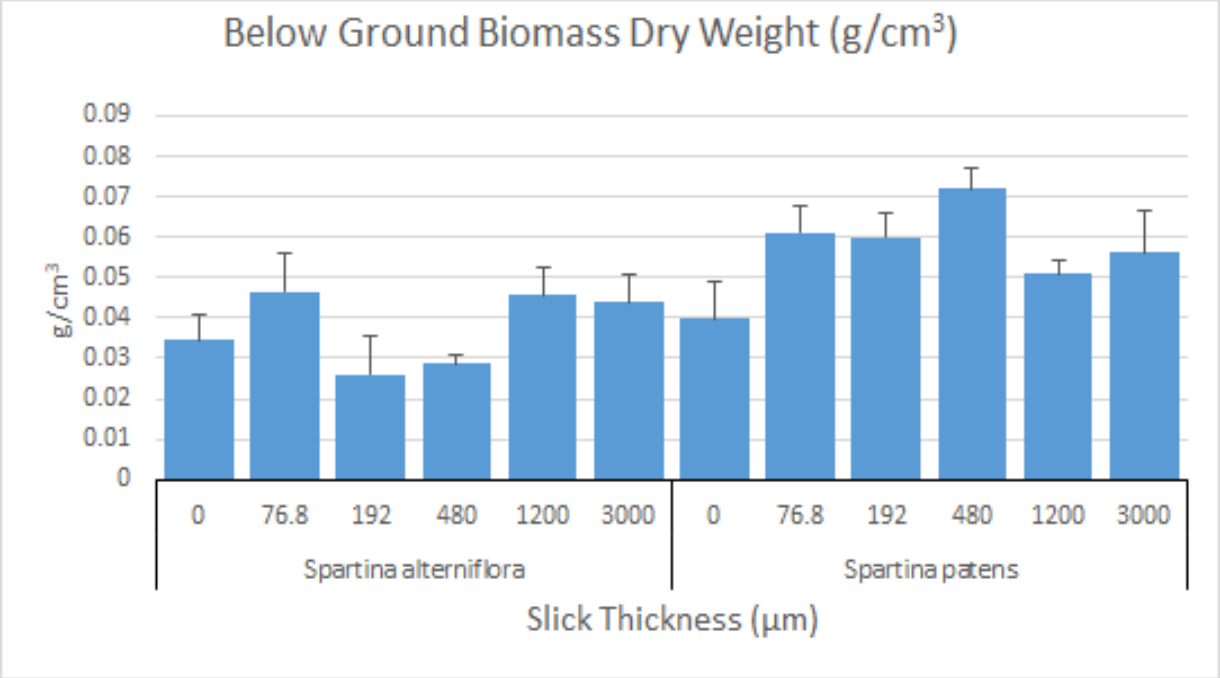

Figure 6 -- Pilot Study Below-Ground Biomass

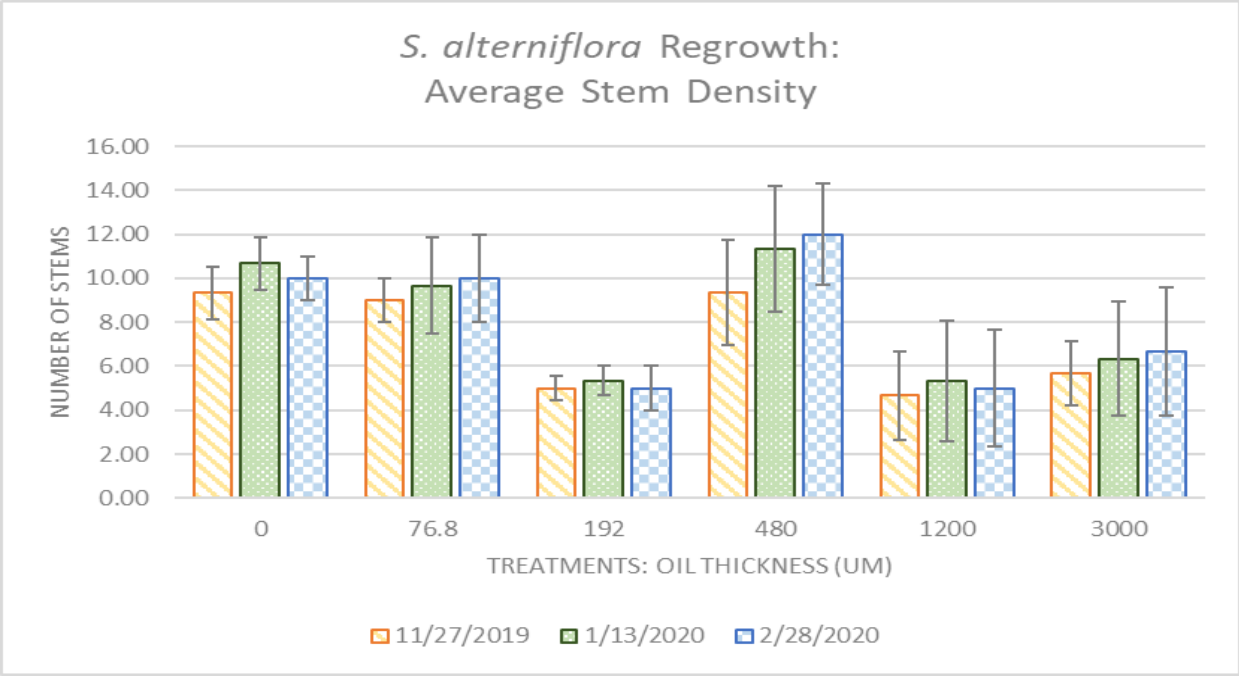

Figure 7 -- *S. alterniflora* regrowth -- stem density

Figure 8 -- *S. patens* regrowth -- stem density

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

Field-based mesocosms: *in situ* deployments for assessing impacts of chemical spills in coastal areas

Paul Pennington
National Centers of Coastal Ocean Science, National Ocean Service, National Oceanic and Atmospheric Administration, Charleston, SC, USA

Pete Key
National Centers of Coastal Ocean Science, National Ocean Service, National Oceanic and Atmospheric Administration, Charleston, SC, USA

James Daugomah
National Centers of Coastal Ocean Science, National Ocean Service, National Oceanic and Atmospheric Administration, Charleston, SC, USA

Blaine West
JHT, Inc. and CSS, Inc. under contract to National Centers of Coastal Ocean Science, National Ocean Service, National Oceanic and Atmospheric Administration, Charleston, SC, USA

Marie DeLorenzo
National Centers of Coastal Ocean Science, National Ocean Service, National Oceanic and Atmospheric Administration, Charleston, SC, USA

Abstract

Field-based mesocosms are useful tools for assessing toxicity at field locations. Also called *in situ* deployments or *in situ* bioassays, they can be particularly useful for assessing toxicity in the wake of oil or chemical spills in nearshore areas. The primary limitation is the readiness of the protocols and equipment at the actual time of a spill. A prolonged time lag in the deployment of field-based mesocosms after a spill can result in the loss of valuable ephemeral data. NOAA's Office of Response and Restoration, Assessment and Restoration Division (OR&R ARD) has identified this as a research need and will collaborate with NCCOS Charleston's Ecotoxicology Branch to develop protocols that are 'ready to implement' in the event of future spills. To address that need, this project will accomplish the following: (1) select two to four appropriate test species that are common to both Gulf of Mexico and the Southeast United States coasts (early and juvenile life-history stages will be considered to allow for growth observations over several weeks); (2) fabricate deployment apparatus for quick implementation at affected field sites; (3) conduct initial trials at a reference site near NCCOS Charleston where fauna are deployed in field cages to assess survival rates and growth; and, (4) make further refinements to the protocols and perform field trials at one or more sites in the Gulf of Mexico.

Introduction

Field-based mesocosms are useful tools for assessing toxicity at field locations. Also called *in situ* deployments or *in situ* bioassays, they can be particularly useful for assessing toxicity in the wake of oil or chemical spills in nearshore areas. Field-based mesocosms are portable structures built to hold aquatic invertebrates or fish that are placed in an aquatic ecosystem to assess human impacts on that ecosystem. The structures are built of a mesh material to allow for water flow into and around the structure. In an estuarine environment, such a structure would be placed in response to a human induced impact such as an oil or chemical spill. These field-based mesocosms are used since chemical analysis of water or sediment in the concerned location may not give enough information that a contaminant is affecting the surrounding biota. The organisms in the field-based mesocosm would serve as an indicator of any harmful conditions present in that location and also an indicator of the length of time a harmful condition may be present.

Studies using caged invertebrates and fish *in situ* have been carried out by several researchers for a variety of reasons. *In situ* bioassays, in which caged fish and shrimp were placed in estuarine creeks for 96 hours to monitor pesticide runoff, were first used by NCCOS Charleston personnel over 30 years ago (Scott et al. 1999 ; Fulton et al. 1996). Crane et al. (1995) placed amphipod species in cages for 28 days in a freshwater stream to monitor pesticide runoff and a sewage effluent outfall. Frodge et al (1995) caged freshwater fish for 72 hours in lakes in Washington State to observe any mortality related to dissolved oxygen concentrations. Schulz (2003) placed a caged amphipod species in rivers in South Africa to assess survival after agricultural field runoff. Other research has shown that more than just mortality can be measured using field-based mesocosms. Burton et al. (2005) used 10 different freshwater invertebrate and fish species to measure survival, growth, feeding, and chemical uptake at several sites in Ohio. Researchers in Spain placed caged *Daphnia* in streams receiving runoff from agriculture fields and noted severe effects on grazing rates and inhibition in several enzyme biomarkers (Barata et al., 2007).

The research with field-based mesocosms has used a variety of materials and structure shapes depending on the organism being deployed. For an epibenthic amphipod, Schulz (2003) used polyethylene jars (100 x 90 mm) with two holes cut into the side wall covered with stainless-steel mesh. Shaw et al. (1996) used cylindrical cages constructed from HDPE Nalgene bottles cut and sectioned with nylon mesh to hold insect nymphs and amphipod larvae. To hold brown shrimp and white shrimp species, Rozas et al. (2014) constructed 0.89 m^2 cages using nylon mesh netting. The studies with the different organisms and cage materials point to the variety of choices that can be made with field-based mesocosms. Crane et al. (2007) reviewed the advantages and disadvantages of 14 different *in situ* approaches. Some advantages of the *in situ* deployments were improved capturing of fluctuating exposures and more cost effective than laboratory methods; while disadvantages were the potential of highly variable results and potential requirement to feed the caged organisms (Crane et al. 2007).

With all this in mind, it was proposed by NOAA's Office of Response and Restoration, Assessment and Restoration Division (OR&R ARD) to build on field and laboratory toxicity studies that were key to the Deepwater Horizon oil spill nearshore assessment. ARD nearshore

Natural Resource Damage Assessment (NRDA) specialists proposed to partner with NCCOS Charleston's Ecotoxicology Branch to develop protocols to assess oil exposure and injury to nearshore fauna in field-based mesocosm studies. During actual spill events, there is a time limitation in developing appropriate studies, potentially resulting in loss of valuable ephemeral data. As field studies were foundational in the Deepwater Horizon oil spill NRDA claim, these field-based mesocosm studies are recommended for future assessments by OR&R oil spill investigators. OR&R proposes to work with NCCOS to develop protocols that are ready to implement in the event of future spills, thus reducing time to implementation and potential loss of information during the immediate aftermath of spills. This proposed work will be a continuation of a line of research that the Ecotoxicology Branch begun in 2011 in response to the Deepwater Horizon oil spill, including assessing the effects of crude oil and dispersants in salt marsh lab-based mesocosms.

The primary objective of this project is to provide standard protocols for assessing impacts of oil and other chemical spills using field-based mesocosms (*in situ* deployments). The project will involve the research and development of applicable designs and protocols drawing on the previous work of others to develop a system that is 'ready to implement' and applicable to several different geographies. The end users of this protocol will be NOAA, NOS, OR&R ARD, and other agencies that are involved in oil and chemical spill assessment. The end-result will be a product (protocol and apparatus) that can be used post-spill to collect valuable ephemeral toxicity data.

This is a two-year NCCOS discretionary funded project. The Year 1 product is a proof-of-concept (feasibility) report outlining the prototype apparatus, selected test species, basic methodology, and proof of control survivorship resulting from reference field trials. In Year 1, the following questions will be answered.

1. What are the potential target test species (invertebrates and small forage fish) to be considered and the best life history stages (adult, juvenile, and or larvae) to use?

2. What are the endpoints to be considered (mortality, survivorship, growth, reproductive potential, or other sublethal endpoints)?

3. Can the test organisms be collected from local waters or will they need to be obtained from a commercial provider or aquaculture operation?

4. How will the appropriate and accessible deployment and reference sites be selected (taking into account depth, tidal range, current velocity, salinity, and bottom substrate)?

5. What is the best type of cage, enclosure, or corral needed for each species to be tested?

6. Will individual organisms need to be tracked over time or caged individually?

7. How many replicates need to be deployed at site to achieve adequate statistical power? Can we demonstrate repeatability?

In Year 2, one of the main objectives will be to choose the best apparatus and deployment methodology by testing at a local reference site and then to build a set for deployment in the Gulf

of Mexico. OR&R ARD and others within NCCOS will be consulted to select suitable deployment sites along the Gulf of Mexico coastline. Once a suitable site is selected, one or more deployments of the recently devised protocol will be performed to test the transferability of the apparatus and protocol to another system. A protocol or Standard Operating Procedure (SOP; Appendix A) will be provided as a final product along with a complete set of deployment cages to OR&R ARD.

Experimental Procedures

For the test deployments, it was decided to use two different shapes of field-based mesocosms. One was a square, cube-shaped structure built from scratch, and the other was a round, cylindrical structure adapted from a commercial source. The cube frame was built with ¾ inch PVC pipes and fittings to obtain a size of 50 cm x 50 cm x 50 cm (volume = 0.125 m^3). The frame was then covered on all sides with black plastic mesh with openings of 3 mm x 4 mm (5 mm diagonal). The mesh was attached to the frame via plastic cable ties. This cage design was based on previous field deployments of caged animals by NCCOS Charleston personnel (Scott et al., 1999; Fulton et al. 1996; Figure 1). The cylindrical mesocosm was 61 cm in diameter and 61 cm tall (volume = 0.178 cubic meters). It was purchased from a commercial website (www.catchlivebait.com) and is listed as a bait-holding cage (described as *Offshore Bait Pen 24"* on website). It was made of heavy duty galvanized, stainless steel coated with PVC and a mesh size of 2.5 cm x 2.5 cm. To have the same mesh size as the square cage, it was covered over with the same black plastic mesh with an opening size of 3 mm x 4 mm (5 mm diagonal) as the cube frame (Figure 2). All plastic mesh was purchased from Memphis Net & Twine (www.memphisnet.net). Three cages were made of each shape for a total of six cages. For the purposes of testing the *in situ* cages, the organisms used were the grass shrimp *Palaemon (Palaemonetes) pugio* and the mummichog *Fundulus heteroclitus*.

The first set of cages held grass shrimp and were deployed in June 2019. The shrimp were collected at the deployment reference site (Leadenwah Creek as mentioned below) using the same size grass shrimp that are used in laboratory toxicity tests (15 - 25 mm). For each cage, grass shrimp were collected by push net, sorted for size and placed in the cages. There were 100 grass shrimp placed in each cage. Cages were placed on the creek bottom and anchored with rebar (Figure 3). The cylindrical cages were placed vertically on the creek bottom. The cages were placed where they would remain under water at low tide. For this first deployment, the cages were retrieved after 96 hours. Shrimp were not fed other than what was available in the water flowing through the cage. The reference site for our test deployments was Leadenwah Creek on Wadmalaw Island, SC (N 32° 38' 51.00"; W 080° 13' 18.05"), a tidal tributary of the North Edisto River.

Cages were cleaned before the next deployment in which mummichogs were used. This deployment occurred seven days after retrieval of the previous grass shrimp deployment. These fish were collected by minnow trap from Doghouse Creek (N 32°44'46.0", W 079°54'12.6"), a tidal tributary of the North Edisto River 10 miles from the deployment site. As with grass

shrimp, only those fish that corresponded to the size used in laboratory toxicity tests were placed in the cages (45 - 75 mm). There were 25 fish placed into each cage. As the fish were larger than the shrimp, they required more space in which to live therefore less fish were used. Cages were placed in the same location as the grass shrimp, fish were not fed, and cages were retrieved after 96 hours. The results from this first deployment (given in detail in the Results section) indicated that the round cages were a better choice for the grass shrimp than the square cages. Therefore, the second deployment only used the round cages.

The second deployment of shrimp and fish at the reference site occurred in October - November 2019 for a longer period of time (7 to 21 days, collected at 7 day intervals) and to see if there would be any seasonal effect on survival. Only the round cages were used. Grass shrimp, 100 shrimp per cage collected as before and the same size as before, were the first to be deployed. Cages were placed in the reference creek as before. Cages were cleaned after the final shrimp cage was retrieved on Day 21, then fish were added as before (25 fish per cage collected from the same site and of the same size as before). The results from this second deployment (given in detail in the Results section) indicated that the round cages were the correct choice but the mesh size for the grass shrimp needed to be smaller.

For the third deployment in February 2020, the cages were modified to prevent grass shrimp from escaping. A tighter black plastic mesh was used to cover the cages: 1.6 mm x 1.6 mm (2.6 mm diagonal). Also for this deployment, three new round cages for fish only were built (as described previously) so that shrimp and fish could be deployed at the same time. As before, grass shrimp (100 per cage) and mummichog (25 per cage) were collected, added to the cages, and placed in the reference location. At this third deployment, both caged shrimp and fish were in the field simultaneously for up to 21 days (collected at 7 day intervals).

For the fourth deployment in May - June 2020, grass shrimp (100 per cage) and mummichog (25 per cage) were placed at the same reference site in the same cages as previously described. This was considered the spring deployment to test for seasonal effects from higher air and water temperatures. At this deployment, the overall cage design was considered complete with room for slight modifications if necessary. At this fourth deployment, both caged shrimp and fish were in the field simultaneously for up to 21 days. This was the last deployment to date.

For complete instructions on how to construct the fish and shrimp field-based mesocosms see Appendix A.

Results

The first series of deployments occurred in coastal South Carolina with two different types of exclusion cages with two different estuarine species to assess control survivorship and growth. During the fieldwork, the logistical procedures were determined such as deploying the cages onsite, monitoring organisms during the exposure, and retrieving the organisms and cages at the end of the exposure. A time range for the duration of the deployments was also determined. Along with deployment of the cages, a YSI datasonde was deployed that continually measured

the following water quality parameters: dissolved oxygen (DO) in % and mg/L; salinity in ppt; temperature in degrees Celsius; and pH.

The results of the first deployment along with associated water quality parameters are seen in Tables 1a and b. For the round cages, grass shrimp retrieval ranged from 83 to 103 shrimp. For the square cages, grass shrimp retrieval ranged from 48 to 74 shrimp. No dead shrimp were found in either set of cages. From this initial deployment, it was seen that the round cages performed better for grass shrimp than the square cages in terms of retrieval/survival. After the first deployment, all fish except for one were retrieved from the round cages (Table 1a). All fish except for three were retrieved from the square cages (Table 1a). Water quality parameters were not considered extreme for a June timeframe compared to other years at the reference site (Table 1b; Blaine West, NCCOS Charleston personal communication). The conclusion from the results of the round versus square cages were that round cages were the best to use due to the survival of grass shrimp.

The results of the second deployment along with associated water quality parameters are seen in Tables 2a and b. The first shrimp cage was retrieved after seven days with 50 shrimp missing (Table 2a). The second cage was retrieved after 14 days with 66 shrimp missing (Table 2a). The third cage was retrieved after 21 days with 68 shrimp missing (Table 2a). At the shrimp cage retrievals, shrimp were seen passing through the mesh. After seven days, the first fish cage was retrieved and only one fish was missing (Table 2a). After 14 and 21 days, the remaining cages were retrieved with only one fish missing (Table 2a). Water quality parameters were not considered extreme for an October-November timeframe compared to other years at the reference site (Table 2b; Blaine West, NCCOS Charleston personal communication). The conclusion from this deployment was that the mesh size for the grass shrimp cages (at the same size as the fish) was too large and the shrimp were able to escape.

The results of the third deployment along with associated water quality parameters are seen in Tables 3a and b. After seven days, grass shrimp retrieval was 93 out of 100 and all mummichogs were found (Table 3a). After 14 days, grass shrimp retrieval was 97 out of 100 and all mummichogs were found (Table 3a). After 21 days, grass shrimp retrieval was 78 out of 100 and all mummichogs were found (Table 3a). Water quality parameters were not considered extreme for a February timeframe compared to other years at the reference site (Table 3b; Blaine West, NCCOS Charleston personal communication). The conclusion of deployment #3 was that the grass shrimp cage mesh size was successful in retaining the majority of grass shrimp for at least 21 days. Based on laboratory toxicity tests, 80% survival in control organisms at the end of the test duration is considered a valid test. It was also concluded that feeding the organisms beyond what was available in the water column was not necessary for 21-day survival.

The results of the fourth deployment along with associated water quality parameters are seen in Tables 4a and b. After seven days, grass shrimp retrieval was only 42 out of 100 and all mummichogs but one were found (Table 4a). The shrimp cage was heavily silted (5 to 10 cm of sediment on bottom of cage) compared to the mummichog cage. After 14 days, grass shrimp retrieval was 86 out of 100 and all mummichogs were found (Table 4a). After 21 days, grass shrimp retrieval was 96 out of 100 but the mummichog cage was missing (Table 4a). The seven-

day retrieval was the lowest count of shrimp to date using the smaller mesh size. No dead bodies were found in the cage, which is not unusual as grass shrimp are cannibalistic. The cages were deployed during a week with heavy rain including Tropical Storm Bertha, which may have caused impingement on the cage mesh, but the other shrimp cages were not affected. Water quality parameters were not considered extreme for a May-June timeframe compared to other years at the reference site (Table 4b; Blaine West, NCCOS Charleston personal communication). Heavy silting of the shrimp cage at seven days was also observed which may have contributed to low dissolved oxygen and/or crowding due to less square cm living space (Figure 4). Also of note in all three grass shrimp cages was the presence of 20 to 30 juvenile *P. pugio* less than 10 mm in size that would not have been added at the initial deployment. These were young of the year shrimp that would not have been present at the February or October deployments. These smaller shrimp were able to penetrate the mesh, but installing a finer mesh would not be feasible as greater fouling would probably occur (Figure 4). The lost cage after 21 days was perhaps due to the heavy rains experienced in this deployment. High water levels and greater water volumes could have dislodged the rebar that anchored the cage in the sediment, and the cage could have rolled down the creek with the tide and current. Another possibility is that the people could have tampered with the cage, dislodging it from the rebar, or it simply could have been stolen. The anchoring system using rebar will be adjusted in future deployments to prevent accidental loss of a cage or to make it more difficult to tamper with. The next set of cage deployments will be in August 2020.

Discussion

The answers to the questions formulated for Year 1 are as follows along with their corresponding discussion:

1. What are the potential target test species (invertebrates and small forage fish) to be considered and the best life history stages (adult, juvenile, and or larvae) to use?

Two common species occurring along the East Coast of the US and along the coast of the Gulf of Mexico are the grass shrimp, *Palaemon* (*Palaemonetes*) species, and killifish, *Fundulus* species. Due to their abundance, ease of capture, and prior lab and fieldwork experience, these species were chosen (Key et al. 2006; Abraham 1985). For the purposes of testing the *in situ* cages, the grass shrimp *P. pugio* and the mummichog *F. heteroclitus* were used. It was also decided to use only the adult life stage of both species. Testing with larval and juvenile stages may follow after testing with adults is complete.

2. What are the endpoints to be considered (mortality, survivorship, growth, reproductive potential, or other sublethal endpoints)?

Survivorship is the main endpoint considered as growth in organisms already at the adult stage is difficult to measure. Reproductive potential can only be measured during certain times of the year. All surviving organisms can be saved for later analysis for biomarkers of contaminant exposure, such as enzyme analysis and reactive oxygen species inducement, or chemical analysis of contaminant uptake.

3. Can the test organisms be collected from local waters or will they need to be obtained from a commercial provider or aquaculture operation?

Using *Palaemon* species and *Fundulus* species for the East Coast and Gulf of Mexico allows for locally collected shrimp and fish to be used. These organisms are native to the East Coast and Gulf of Mexico and are locally abundant (Key et al. 2006; Abraham 1985). It is the intent to use locally collected organisms for all locations in which the mesocosms will be placed. For field-based mesocosms on the West Coast, use of local crustaceans and fish will be determined.

4. How will the appropriate and accessible deployment and reference sites be selected (taking into account depth, tidal range, current velocity, salinity, and bottom substrate)?

The deployment sites will be determined on a case by case basis depending on where an oil spill has occurred. The site should be located where tidal fluctuations will not allow the cages to be exposed at low tide.

5. What is the best type of cage, enclosure, or corral needed for each species to be tested?

The results showed that a round cage design that allowed for free-swimming grass shrimp and mummichog was the best option. This cage was even more desirable considering that the base cage could be purchased already built. Adding appropriate sized mesh to the base design allowed modifications to be made according to the size and type of organisms to be caged. It must be stated that the mesh sizes used for the deployments with grass shrimp and mummichog were chosen for the size class used. These mesh sizes may not be appropriate for all fish and shrimp and may be adjusted depending on the size of the organism to be caged.

6. Will individual organisms need to be tracked over time or caged individually?

The deployments as seen in Tables 1-4 and described above show that having free-swimming grass shrimp and mummichog in a field-based mesocosm can be successful. Due to the number of shrimp and fish involved, a tracking device for each organism would not be feasible. Also, since survival was considered successful in these free-swimming trials, having the shrimp and fish individually caged does not seem necessary.

7. How many replicates need to be deployed at site to achieve adequate statistical power? Can we demonstrate repeatability?

Standard laboratory toxicity tests use a minimum of three replicates for each exposure concentration. With this in mind, we chose to have three replicates of the field cages. Each field cage will be able to hold the maximum density of organisms depending on the size of the organism.

Conclusion

The cages for grass shrimp and mummichog seem to be the correct size to ensure survivability at 80% or higher at up to 21 days deployment. While smaller shrimp seem to enter the cage during deployment, their presence did not seem to interfere with survival of the larger shrimp placed in the cage, nor with assessment of the caged shrimp survival. Having a mesh size smaller than 2.6

mm (diagonal) for the shrimp cage was not seen as a viable option due to possibility of easier fouling. Fouling, which is growth of algae, barnacles, or debris impinged on the mesh, can lead to partial or total blockage of water flow through the cage. This prevents the organisms from having complete exposure to any contaminant present in the estuary and decreases survival due to low oxygen stress.

The larger mesh size on the mummichog cages did not lead to a finding of outside organisms present inside the cage. Mummichogs are voracious predators and would have eaten any organism small enough to enter through the mesh. Aside from a fish cage being missing, the survival of the fish was no less than 96% throughout the four deployments.

Going forward, this project will continue to test the field-based mesocosms during all seasons of the year using the round cage iterations with the appropriate mesh sizes for shrimp and fish to get a fuller understanding of any seasonal effects. We have collaborated with Dr. Paul Montagna of University of Texas at Corpus Christi to test these field-based mesocosms along the Texas coast in the Gulf of Mexico. Due to COVID-19 and the ban on NOAA travel, this testing has been delayed until further notice

Data availability

Data are located at NCCOS Charleston Lab and can be obtained from P. Pennington (paul.pennington@noaa.gov) and B. West (blaine.west@noaa.gov) on request.

Acknowledgements

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Supporting Documents

1. This report is based on the following funded NCCOS discretionary FY18 project: Field-based mesocosms: in situ deployments for assessing impacts of chemical spills in coastal areas. Paul Pennington, Blaine West, James Daugomah, Pete Key

2. Appendix A. Field Mesocosm (Construction and Deployment) Standard Operating Protocol. NCCOS Charleston. James Daugomah, Blaine West. 2020.

Table 1.

a. Field-based mesocosm cage deployment #1. This first field deployment was three round cages and three square cages each of grass shrimp and mummichog for 96 hours. Grass shrimp and mummichog were deployed at separate times using the same set of cages.

Organism	Date Retrieved	Round Cage	Square Cage
		# alive out of 100	# alive out of 100
Grass Shrimp	6/17/2019	96	66
	6/17/2019	83	48
	6/17/2019	103	74
		# alive out of 25	# alive out of 25
Mummichog	6/28/2019	24	24
	6/28/2019	25	24
	6/28/2019	25	24

b. Water quality parameters for deployment #1 spanning the 96 hours for each deployment of grass shrimp and mummichog.

Grass shrimp	DO (%)	DO (mg/L)	Salinity	pH	Temperature (°C)
Average	66.09	4.74	14.22	7.1	28.19
Minimum	36.9	2.7	1.04	6.32	23.87
Maximum	137.6	9.02	22.91	7.76	35.16
Range	100.7	6.32	21.87	1.44	11.28
Mummichog	**DO (%)**	**DO (mg/L)**	**Salinity**	**pH**	**Temperature (°C)**
Average	62.63	3.98	24.93	7.43	31.68
Minimum	27.1	1.83	20.11	7.16	28.61
Maximum	115.1	7.08	26.43	7.7	35.20
Range	88	5.25	6.32	0.54	6.59

Table 2.

a. Field-based mesocosm cage deployment #2. This second deployment only used round cages. The first cage was retrieved after 7 days, the second cage after 14 days and third cage after 21 days. Grass shrimp and mummichog were deployed at separate times using the same set of cages.

Organism	Date Retrieved	Round Cage
		# alive out of 100
Grass Shrimp	10/22/2019	50
	10/29/2019	34
	11/5/2019	32
		# alive out of 25
Mummichog	11/5/2019	24
	11/12/2019	26
	11/19/2019	24

b. Water quality parameters for deployment #2 spanning the 21 days for each deployment of grass shrimp and mummichog.

Grass shrimp	DO (%)	DO (mg/L)	Salinity	pH	Temperature (°C)
Average	69.37	5.09	28.47	7.36	22.66
Minimum	44.4	3.27	9.58	6.95	14.94
Maximum	103.5	7.57	32.47	7.64	27.81
Range	59.1	4.3	22.89	0.69	12.86
Mummichog	**DO (%)**	**DO (mg/L)**	**Salinity**	**pH**	**Temperature (°C)**
Average	76.27	6.16	29.5	7.47	17.71
Minimum	45.8	3.27	13.28	7.14	9.99
Maximum	104.9	9.37	31.47	7.74	27.81
Range	59.1	6.1	18.19	0.6	17.82

Table 3.

a. Field-based mesocosm cage deployment #3. The first cage was retrieved after 7 days, the second cage after 14 days and third cage after 21 days. Grass shrimp and mummichog were deployed simultaneously in separate cages.

Organism	Date Retrieved	Round Cage
		# alive out of 100
Grass Shrimp	2/4/2020	93
	2/11/2020	97
	2/18/2020	78
		# alive out of 25
Mummichog	2/4/2020	25
	2/11/2020	25
	2/18/2020	25

b. Water quality parameters for deployment #3 spanning the 21 days. Since grass shrimp and mummichog were deployed at the same, only one set of parameters are shown.

Grass shrimp and Mummichog	DO (%)	DO (mg/L)	Salinity	pH	Temperature (°C)
Average	87.08	7.81	20.8	7.76	14.54
Minimum	61.3	5.15	3.47	6.94	8.65
Maximum	109.8	9.8	26.41	8.65	21.30
Range	48.5	4.65	22.94	1.71	12.65

Table 4.

a. Field-based mesocosm cage deployment #4. The first cage was retrieved after 7 days, the second cage after 14 days and third cage after 21 days. Grass shrimp and mummichog were deployed simultaneously in separate cages.

Organism	**Date Retrieved**	**Round Cage**
		# alive out of 100
Grass Shrimp	5/27/2020	42
	6/3/2020	86
	6/10/2020	96
		# alive out of 25
Mummichog	5/27/2020	24
	6/3/2020	25
	6/10/2020	Cage Missing

b. Water quality parameters for deployment #4 spanning the 21 days. Since grass shrimp and mummichog were deployed at the same, only one set of parameters are shown.

Grass shrimp and Mummichog	**DO (%)**	**DO (mg/L)**	**Salinity**	**pH**	**Temperature (ºC)**
Average	64.23	4.59	16.4	7.11	27.91
Minimum	24.9	1.75	1.88	6.36	21.03
Maximum	124.8	8.26	24.48	7.66	34.43
Range	99.9	6.51	22.6	1.3	13.39

Figure 1. The square cage used in deployment #1.

Figure 2. The round cage used for all deployments.

Figure 3. Deployment #1 showing one each of the square and round cages anchored at the reference site. Two more of each shape were placed adjacent to those in the figure. At the top right in the figure is the datasonde used for continuous measurements of water quality parameters. All other deployments with all round cages looked similarly.

Figure 4. Silting and fouling on the field-based mesocosm after 21 days deployment at Leadenwah Creek, SC. Silting is seen as the dark, black material at the bottom of the cage. Fouling is seen as the lighter, brown material covering the mesh.

Appendix A. Field Mesocosm (Construction and Deployment) Standard Operating Protocol

ECOTOX/SOP00-078
Created on 6/17/2020 Revised on6/24/2020

Title: **FIELD MESOCOSM (CONSTRUCTION AND DEPLOYMENT)**

Author:	Blaine West, James Daugomah	Date:
Program Manager:	Peter B. Key	Date:
Branch Chief:	Marie E. DeLorenzo	Date:

1.0 OBJECTIVE

To develop in situ deployments or in situ bioassays to rapidly assess mortality in a specific estuarine site.

2.0 HEALTH AND SAFETY

No specific PPE is needed for cage construction but care should be taken to prevent puncture wounds from the sewing needle. For field work personnel should wear strong, thick footgear designed for wetland work. In cold weather, when establishing a new site, or when walking a site that has oyster beds, hip waders or wet suits should be worn. A float plan should be filled out on google drive and shared with the working group when performing the field deployments. For more details on working in coastal wetlands refer to Ecotox SOP 029.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Individuals familiar with tidal creek habitats should supervise this sampling procedure.

4.0 REQUIRED AND RECOMMENDED MATERIALS

24” Cylindrical cage
Plastic Mesh (1/4”or 7mm,
1/8” or 5mm, 1/16” or 2.6mm)
Cable ties 4 inch, and 18 inch
Scissors
Thick rubber Boots
Waders or Wetsuit
50 lb dacron fishing line
4 ft Rebar (at least 2 per cage)
Forceps with a 45^0 tip
45 mm straight carpet needle
49 mm curved upholstery needle
3/4” schedule 40 pvc pipe
Write in the rain field notebook

5.0 PROCEDURE

5.1 Site Establishment

- A preselected reference site should be established for each region (i.e. Southeast, Gulf Coast, Pacific Northwest) that will be used for mortality assessments.
- Ensure both reference and contaminated sites are both deep enough for the cage to remain submerged at low tide and shallow enough to access by foot to minimize gear loss
- The site must also be suitable to ensure the cage bottom is in full contact with the sediment during the deployment

5.2 Cage Construction

Materials

- The cages used are cylindrical 24" Offshore bait pens with pvc coated 1" galvanized steel mesh and are available from catchlivebait.com (Actual measurement is 61cm tall and 61 cm in diameter).
- The mesh is a UV stabilized polyethylene available from Memphis net and twine. They are listed as "Plastic netting standard duty". The vendor lists three sizes in standard units at 1/4", 1/8" and 1/16". The mesh is used for the outside cover of the *Fundulus* cage and the *Palaemonetes* cage. The mesh sizes in this document will be referred to in metric as the measured diagonal length of 7 mm, 5 mm, and 2.6 mm.

Cutting the pieces for the cage (Fish and shrimp cages)

- Using the appropriate mesh size (1/8" for *Fundulus* or 1/16" for *Palaemonetes*) for the desired organism, cut the piece for the external circumference of the cage (approximately 215 cm x 72 cm). This will allow for a 4-5 cm overhang on top and bottom of the cage.
- Cut mesh for the bottom and top of cage (75 cm^2). This piece should be two layers with the interior mesh being the size appropriate for the organism to be contained, the outside mesh should be the rigid 1/4" mesh that will be in contact with the sediment.

5.2.1 Fish Cage Construction

- Remove existing wire lid and bungee strap.
- Put first layer of 5 mm mesh around outside circumference of cylinder (approximately 215 cm x 72 cm) this will allow for a 4-5 cm overhang on top and bottom of the cage.
- Secure the external circumference piece of mesh to the stock cage using 5-7 18" cable ties (Figure 1).

Figure 1

- The 4-5 cm overhang should be pressed around the top/bottom edges to form a secure double layer around the circumference edge when the bottom mesh is attached (Figure 2).

Figure 2

- 18" cable ties should be used to secure the 75 cm^2 mesh to the top and bottom edge by inserting from the outside of the cage and finished on the bottom, not the side (Figure 3).
- Once the 18" cable tie is initially inserted, a pair of forceps is useful to turn the tip of the cable tie at a 90 degree angle to come out of the side mesh, then turn it back through the bottom mesh to complete. Each cable tie should be closed to lock and pulled tight as you work around the edge.

Figure 3

- Secure top and bottom cage pieces using 18” cable ties.
- The top of the cage should be secured in place and the opening for access cut with scissors.

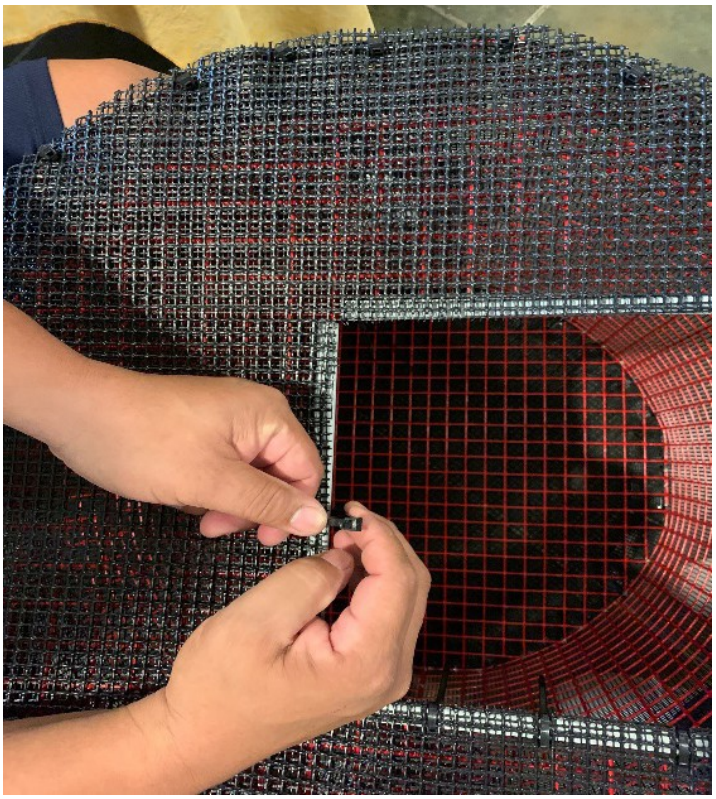

Figure 4

Assembly of the access lid opening

- Cut an opening on top of cage mesh matching the existing door opening of stock cage (Figure 4).
- Cut two layers of mesh in a square so that it overlaps the existing opening by 3-4 cm, the

mesh size will depend on organisms deployed (Figure 5).

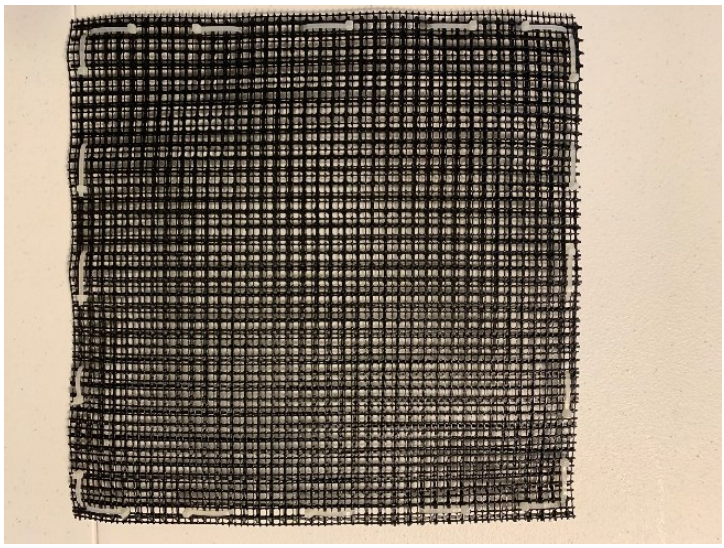

Figure 5. A double mesh lid secured with 4" cable ties

- Secure the two pieces of lid together with 4" cable ties using 5 on each edge around all 4 sides of the lid (Figure 5).

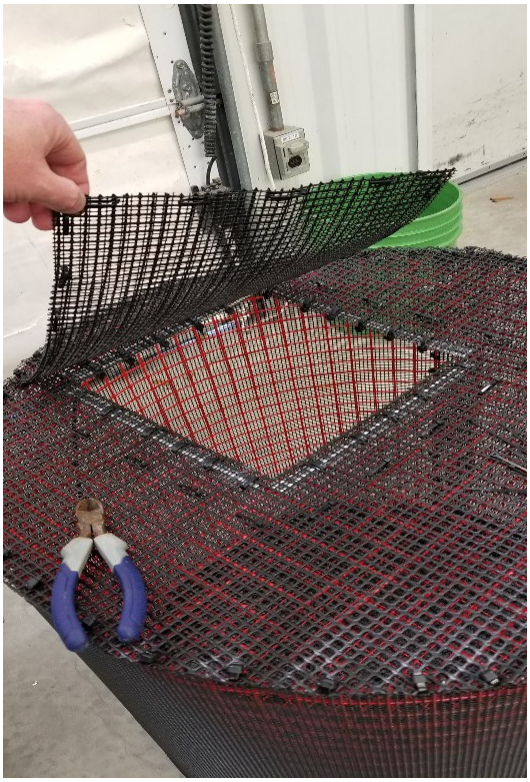

Figure 6

- Secure the mesh to the stock cage around the opening (Figure 4).

- Secure the edge of the lid closest to the outer edge of the cage (Figure 6).

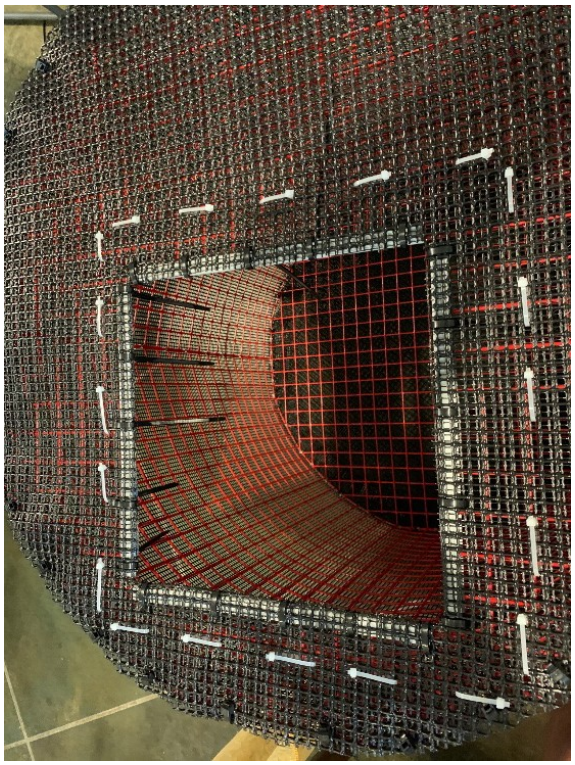

Figure 7

- Place five 4" cable ties on each edge of the lid spaced at equal distance (Figure 5). These cable ties will be connected with those on the cage. Once the animals have been counted into the cages these will serve as connection points for an additional 4" cable tie that will secure the lid (Figures 7-8).

Figure 8

- Two ¾" PVC tubes should be cut to 60% of the height of the cylinder.
- Drill four holes at equal distant on tube large enough to push a medium sized cable tie through.

- Insert cable ties through both sides (this step eases the attachment process).
- Attach the tube through external mesh of cage so that it is flush with the bottom and anchored by the metal of the cage skeleton (Figure 9).

Figure 9

- 4' pieces of rebar should be bent at a 90 degree angle using a sledgehammer and bench vise for securing cages to the sediment (Figure 10).

Figure 10

5.2.2 Shrimp Cage Construction

- The order in which the 2.6 mm mesh size is secured is the same as in the fish cage. This section only highlights the steps that are uniquely different in the construction of the shrimp cages.
- Secure one end of the side mesh to the cage using a running stitch with double strung 50 lb test dacron fishing line and a 45 mm straight carpet needle.
- Next roll the mesh around the cage and overlap it, then secure the other end using the same technique as the first edge (Figure 11).

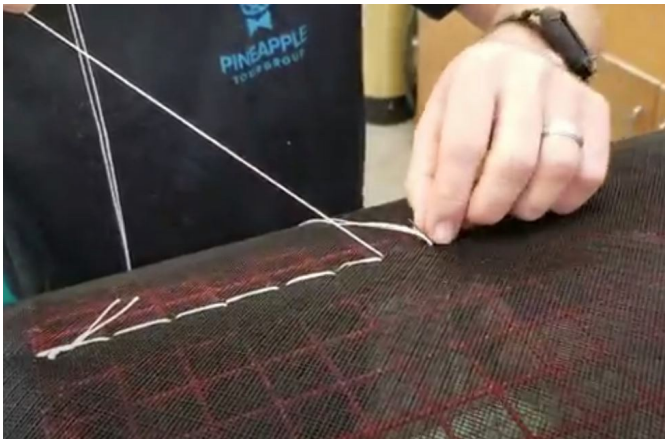

Figure 11

- Fold the excess side mesh to the end of the cage and place on top of the 75 cm^2 mesh cutouts for the top/bottom (small mesh facing inward and larger mesh out to be in contact with sediment).
- Secure the center of the lid before starting to prevent the edge from getting off center when sewing around the edge (especially when working alone). If working with a partner you can have one person hold the bottom while the other sews.
- Using an overcast stitch with a curved upholstery needle, sew approximately 8 inch sections to secure the top/bottom in place (Figure 12).
- Stretch the mesh across the top of the cage as you work around the edge to keep the mesh as tight as possible and repeat the 8 inch sections until complete, it is helpful to trim the excess mesh ahead of the stitch to access the edge of the cage forsewing.
- When the edge is complete finish the stitch by turning it through the same hole twice and using a fishermans knot through the loop created with the second turn to fully secure the thread (Figure 13).
- Trim off all excess mesh.
- Measure the distance from the top to bottom and cut a piece of the thick coarse mesh used for the bottom to fit over the exterior and prevent damage to the fine mesh. Pull tight and secure with zip ties. This piece will also serve as the attachment point for the pvc mounting tubes.

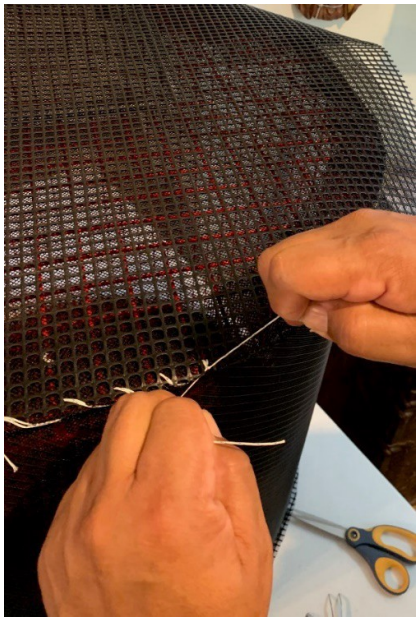

Figure 12

Figure 13

- Assemble the lid in the same manner as the fish cage except slide a piece of the 2.6 mm mesh between the two larger mesh pieces (Figure 14).
- Cut 3 lid covers, 1 each for 3 mesh sizes. Place the smallest mesh size in the middle. The largest mesh size on exterior. Place cable ties (n=5) on each side of lid, to secure three pieces in place.

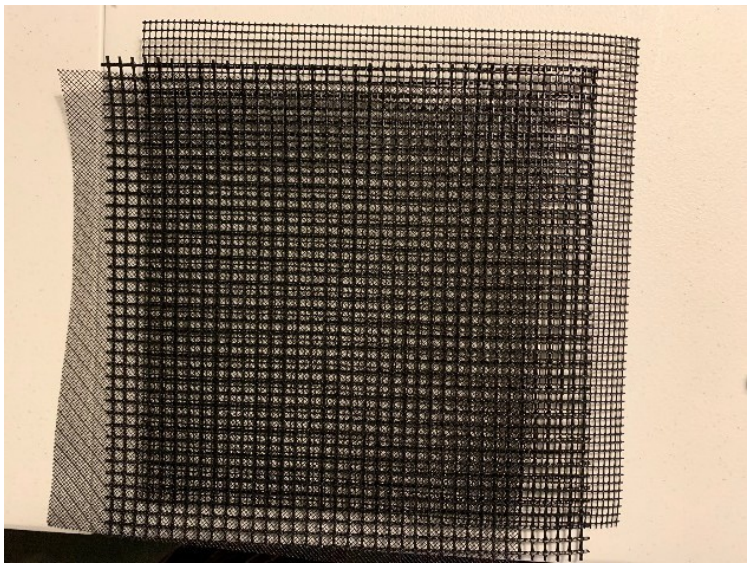

Figure 14

5.3 Field Deployment

5.3.1 Collection of Test Organisms

- Along the Atlantic and Gulf of Mexico Coasts, it is suggested to use *Fundulus* species for the fish and *Palaemon* (*Palaemonetes*) species for the shrimp.

  These cages as described here are made specifically for *Fundulus* in the range of 40-60 mm and *Palaemon* 20-30 mm. For other regions, organisms need to be in these size ranges in order for the method to work as designed.
- Organisms should be collected from a known uncontaminated site before dispersing to a potentially impacted site.
- Test animals can be collected a day or two ahead of deployment date and held in the laboratory if there is not enough time or it is thought that not enough can be captured in one day for testing.
- Fish species can be collected using minnow traps (or other suitable method depending on the region).
- Shrimp can be collected using a dip net.

5.3.2 Deploying Animalsand Securing the cages

- Prior to deploying animals, the deployment site or sites should have been fully vetted at low and high tides to properly place the cages.
- Animals should be pre-counted (25 *Fundulus* sp. per cage and 100 *Palaemonetes* sp. shrimp per cage) into buckets depending on the number of cages desired to help organize dispersal.
- Carefully pour the buckets into the cages and secure the top with 5 cable ties per side.
- Arrange the cages in the water close together so they can be easily located but far enough apart so water can flow around them unobstructed.
- Make sure the bottom of the cages are in contact with the sediment, then secure them by pushing the 4' pieces of rebar through the pvc tubes and as far down into the sediment as possible (at least two tubes with rebar should be used but more can be mounted depending on environment) (Figure 15).

Figure 15

5.3.3 Retrieving Cages and Animals

- At the time of desired endpoints, cages should be carefully removed from the water.
- Retrieval should be done at or near low tide in order for the cages to be found most easily.
- Working with one cage at a time, remove the rebar and carry the cage to an edge or sandbar so that there is enough water to keep the animals slightly submerged while counting (Figure 16).

Figure 16

- Remove the lid by cutting the cable ties on three sides and if there is silt on the bottom carefully attempt to rinse as much of it out into the water as possible.
- The contents of each cage should be carefully counted into buckets either using small nets or by hand in case of an error so it could possibly be recounted.
- Note all counts, cage conditions and weather in the field notebook.
- After all animals are counted they can either be returned to the water or brought back to the lab for further analysis.

6.0 QUALITY CONTROL/QUALITY ASSURANCE

Cages should be thoroughly inspected before each deployment for damage in the mesh or seams coming apart so that animals cannot escape. Cages should be tagged and numbered to monitor for individual defects that cannot be seen. If a single cage consistently performs poorly it should be removed from service.

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Title: Analysis of Floating Oil Under UV Light at Different Environmental Conditions: A Pilot Study

Emily C. Pisarski[1], Edward F. Wirth[2]

[1] CSS Inc., under contract to NOAA/NOS/NCCOS; Stressor Detection and Impacts Division
[2] NOAA/NOS/NCCOS; Stressor Detection and Impacts Division

Abstract

Oil spills are subject to many environmental weathering processes that alter the chemical and physical properties of oil. Photo-oxidation is one weathering process, whereby the products formed during the photolysis of oil have been shown to be more polar, and potentially more soluble in water. Understanding how oil weathers under solar irradiation can improve modelling of oil fate and transport during spills. This study examined chemical and physical changes that occur in floating oil exposed to ultraviolet light (UV-A) at different temperatures. Laboratory exposures of floating oil (Louisiana Sweet Crude) were prepared in beakers of seawater on an orbital shaker platform, with manipulations of light (UV-A or fluorescent light) and temperature (10, 21 and 30°C). Samples were collected (6h, 24h, 48h, and 7d) and the oil was photographed to examine physical changes. Changes in chemical composition were analyzed by gas chromatography mass spectrometry to examine differences in hopanes, steranes, polycyclic aromatic hydrocarbons (PAHs) and total extractable hydrocarbons (TEH). Oil exposed to UV light became more viscous and formed “tar-ball” like substances whereas oil exposed to fluorescent light remained less viscous and more sheen-like. Generally, no differences were observed between light treatments for the hopane/sterane and TEH data, however, oil exposed to UV light was observed to have a greater loss of individual PAHs when compared to oil without UV light exposure. Results from this study will be used to improve models that forecast the fate of floating oil in the environment and inform future directions for additional research.

Introduction

Oil spills in the environment continue to remain a common occurrence. The National Oceanic and Atmospheric Administration's Office of Response and Restoration (NOAA OR&R) estimate that, on average, they respond to roughly 150 oil and chemical spill events each year (OR&R, 2017). While not all oil spills are of the same magnitude as the Exxon Valdez oil spill in Prince William Sound in 1989, which released almost 257,000 barrels, or the Deepwater Horizon (DWH) oil spill in the Gulf of Mexico in 2010, which released 3.1 million barrels of oil, they all have detrimental impacts on the environment (OR&R, 2017).

Responding to aquatic oil spill events can be very challenging and the response measures that are employed are often dependent upon several factors including their location (e.g.

proximity to land versus open ocean, closeness to sensitive habitats, etc.), type of oil, and environmental conditions (e.g. wind, wave action, sunlight exposure, etc.). To complicate matters further, upon release into the environment, oil is subject to various weathering processes. These weathering processes can be physical, chemical, or biological in nature, and include volatilization/evaporation, biodegradation, dissolution, dispersion, settling, emulsification, spreading, and solar radiation/photo-oxidation (Stout and Wang, 2007; Jonker et al., 2005; Bacosa et al., 2018).

The Office of Response and Restoration (OR&R) has developed various models to help aid in their response to spill events. Two of these models are GNOME, or the General NOAA Operational Modeling Environment, and ADIOS, or Automated Data Inquiry for Oil Spills. The primary use of GNOME is to allow the user to forecast the potential trajectory of a spill due to factors including winds, tides, currents, and spreading (OR&R, 2017). The model also includes some limited weathering data, related to evaporation and dispersion, which will also factor into the trajectory of the spill. The model provides both a "best guess" trajectory, and a "minimum regret" trajectory. The "best guess" trajectory makes the assumption that the wind data that the user input remains the same throughout the spill scenario. The "minimum regret" trajectory factors in uncertainty in wind and current data.

The ADIOS tool is primarily used to examine how oil weathers by taking spill and environmental conditions into account, as well as the proposed cleanup action (OR&R, 2017). Currently, ADIOS only models for five days, and primarily focuses on evaporation, dispersion and droplet formation, and how that impacts physical properties such as density, viscosity and water content (OR&R, 2017). One of the major limitations of the model is that it does not account for other weathering processes such as photo-oxidation.

Some of the research performed after the DWH oil spill event found that the weathered oil contained large proportions of oxygenated hydrocarbons (Aeppli et al., 2018). These oxygenated species are a result of solar irradiation, and while it was initially thought that the bulk of photo-transformation of oil occured in the days/weeks/month after an oil spill, current research indicates that photo-oxidation is primarily happening in the first few hours after an oil spill (Ward et al., 2018). Once thought to be a minor component in oil weathering, photo-oxidation is becoming an increasingly more important process for the fate of oil, however there is still a large knowledge gap in understanding oil photo-oxidation. Through discussions with the Emergency Response Division (ERD) of OR&R, the Ecotoxicology Branch at the National Centers for Coastal Ocean Science (NCCOS) conducted a pilot study to better understand the effects of solar irradiation on surficial oil slicks. The primary goal of this pilot study was to gain a better understanding of how solar irradiation alters the physical and chemical properties of oil, and if environmental factors (e.g. temperature) influence the chemical and physical properties of irradiated oil.

The results of this study will be used to support a new database that OR&R is in the process of developing. Their proposed database will be a compilation of various physico-chemical properties in both fresh and weathered oil (e.g. density, viscosity, hydrocarbon

composition). Data from this database will be used to enhance existing fate and transport models, as well as assist in cleanup/remediation efforts for future oil spills.

Experimental Procedure

Laboratory Setup

All floating oil exposures were performed in the same environmental chamber located at the NCCOS lab in Charleston, SC, USA. Seawater from the Charleston Harbor, Charleston, SC, USA was piped into the chamber. Prior to use, the seawater was settled for 72 hr, filtered to ~30 µm through a mixed media bed filter, and then filtered to 10 µm through an additional cartridge filter. Afterwards, the seawater was sterilized by a 150W UV light to reduce bacterial growth. The chamber allowed for temperature manipulation so that exposures could be run and maintained at 10, 21, and 30°C.

Light fixtures were hung from the ceiling and contained either ultraviolet A (UV-A, AgroMax 4 foot T5 UV-A Plus bulb, HTG Supply; 300-420 nm) or fluorescent bulbs (GE 54 watt 48 inch miniature Bi-pin T% 4100 K white fluorescent). There were two sets of light fixtures, each holding two bulbs apiece, for each light treatment. Orbital shaker platforms (Benchmark Orbi-Shaker XL, 46 x 46 cm; Lab Companion SK-600, 45 x 45 cm), which were used to ensure that there was movement throughout the exposures, were placed underneath the light fixtures and held at a constant rotation (70 rpm) for all test exposures. The distance between light fixtures and orbital platform was ~45 cm. Light fixtures and their respective orbital shaker platforms were separated by the use of black plastic sheeting on all four sides. Overhead room lights were not used/turned off during the floating oil exposures. Photographs of the experimental setup are in Figures 1 and 2.

Test Exposures

Test exposures were run at 10, 21, and 30°C. All test exposures were run for seven days, with a photoperiod of 12 h light followed by 12 h dark. The UV light intensity was measured periodically throughout all three exposures using a UV-VIS spectrometer (Ocean Insight, formerly Ocean Optics, FLAME S-UV-VIS ES with cosine corrector; 200-850 nm). Spectrometer calibrations and light readings were processed using OceanView software. The absolute irradiance of the spectral output for the UV-A lights is found in Figure 3. The average light reading at 380 nm was 0.059 ± 0.002 mW/cm^2 in the UV treated samples, with a total UV-A integrated dose of 2.55 mW/cm^2. This light reading is representative of a spring day in the southern United States (Bridges et al., 2018). The test exposure consisted of 600 mL glass beakers containing 100 mL of seawater. Louisiana Sweet Crude (LSC) oil was applied as a surface slick by using an Eppendorf Repeater pipettor; 200 uL of LSC was added to the beaker. There were 16 beakers per light treatment, set up for each test, which was enough to cover four sampling timepoints, with four replicate samples for each timepoint. The placement of beakers

on the orbital shaker platform was randomized for each experiment, using a random number generator.

Samples for chemical and physical analysis were taken at 6 h, 24 h, 48 h and 7 d for physical and chemical analyses. Physical changes to the oil slick were documented through the use of photography (Cannon EOS Rebel T5i). The oil slick was photographed aerially from a fixed distance/zoom (30 cm/33mm lens) using a tripod. Care was taken not to disturb the slick as they were transported from the orbital shaker platform to the photography location. After the photographs were taken, samples were collected for chemical analyses. For three of the replicates, the entire sample (water + oil slick) was collected into 250 mL wide mouth, solvent rinsed amber bottles. The beaker was rinsed with dichloromethane three times (~10 mL/rinse), to remove any residual oil, and added to the sample. For the fourth replicate, the water underneath the slick was siphoned away and subsequently collected through teflon tubing into a 250 mL wide mouth amber bottle. Care was taken during the siphoning process to avoid collecting any of the surficial slick. After siphoning, the water level was marked on the collection bottle so that the water volume could be accurately recorded at a later date. Thirty milliliters of dichloromethane was added to the sample to preserve the sample until liquid/liquid extraction could be performed. All samples were stored in a +4 ℃ refrigerator until extraction.

Chemical Analysis

All samples (water+slick and water only) were extracted by liquid/liquid extraction as detailed in Reddy and Quinn (1999). Briefly, a suite of isotopically labeled hydrocarbon internal standards were added to samples (Table 1). Samples were then sequentially extracted with 30 mL of dichloromethane, 1:1 dichloromethane:hexane, and hexane. Solvent extracts were composited (total volume = 90 mL), filtered through anhydrous sodium sulfate, concentrated, and solvent exchanged to hexane using a TurboVap II (water bath 40℃, nitrogen 14 psi). Extracts (1.0 mL) were then fractionated using silica solid phase extraction (SPE) cartridges (3 mL/500 mg). Samples were loaded onto the SPE cartridges, and then immediately eluted with two column volumes of hexane (fraction 1 or F1), followed by two column volumes of 1:1 dichloromethane:hexane (F2), and then two column volumes of methanol (F4). All solvent fractions were concentrated using a TurboVap LV (water bath 40℃, nitrogen 8 psi). Extracts were transferred to 2 mL amber autosampler vials (final volume = 0.5 mL), and stored in a -20℃ freezer until instrumental analysis.

Fractions were run on an Agilent gas chromatograph mass spectrometer (GC/MS 6890/5973) using a split/splitless injector operating in splitless mode, and a DB17ms analytical column (60m x 0.25mm x 0.25um). The mass spectrometer was operated under electron impact ionization and selected ion monitoring modes. Analyte classes analyzed, and reported in this paper, include hopanes and steranes (F1), PAHs (F3), and TEH (F3). Data for hopanes and steranes was acquired from F1 first, after which F1 and F2 were combined to acquire the PAH and TEH data. The F4 fraction, which contains the more polar components of oil, was not

analyzed during this experiment, but instead stored to be analyzed at a later date. Additionally, water only samples, to this date, have not been analyzed and will not be reported in this paper.

Prior to data acquisition, the instrument was calibrated with standards ranging from 0.5-2500 ng (hopanes/steranes), 0.1-10,000 ng (PAHs), 0.2-20.4 mg (TEH). Coefficient of determination was ≥ 0.995 for all analytes of interest. The calibration curve for hopanes and steranes was developed by using a standard reference material (SRM) from the National Institute of Standards and Technology (NIST), SRM 2266 (Hopanes and Steranes in 2,2,4-Trimethylpentane). The TEH calibration curve was made by diluting measured masses of LSC oil, and extracting the calibration curve through silica SPE. The PAH calibration curve was developed using a suite of PAH mixes made internally by the Chemical Contaminants Research (CCR) group.

Chromatographs were analyzed by MSD Chemstation software (Agilent Technologies, Inc. ver E.02.02.1431). The method detection limit (MDL) was determined according to methods detailed in Ragland et al. (2013). Metadata and data reports were managed by an internally developed laboratory information management system (NCCOS ChemLIMS; https://admin.coastalscience.noaa.gov/chemlims/login.aspx).

Statistical Analysis

Statistical analyses were performed using the JMP software program (version 12.1.0). Prior to analysis, data were evaluated to make sure that it met the assumptions of normality in order to run parametric statistics. P-values were considered statistically significant at an alpha level less than 0.05.

Quality Assurance/Quality Control

A series of reagent spikes and reagent blanks were run with each experiment. Data quality guidelines are standard across all projects within the Chemical Contaminants Research Program. For reagent spikes, spiked analyte recoveries must be within 80-120% of the expected concentration for at least 80% of the analytes. Reagent blanks were used in the MDL calculation (described above). If reagent blanks contained measurable quantities of hydrocarbon analytes, that value was subtracted from the samples associated with the reagent blank.

Results

Physical Changes

The photographs obtained from each exposure/treatment/timepoint are shown in Figures 4-6 (10, 21, and 30°C respectively). The photos from the 10°C exposure at day seven (Figure 4) were lost prior to computer backup and are therefore not shown; all other exposures show a complete set of photographs documenting the observed physical changes at each of the four measured time points. Qualitative analysis of the photographs show that regardless of temperature, tarball-like substances formed in all of the UV light treatments. The early stages of

tarball formation (i.e. oil consolidating into a "blob" and migrating towards the sidewalls of the beaker) was seen as early at 48 h (Figures 4 and 5), however, the actual formation of the tarballs formed somewhere between 48 h and 7 d. Sampling between 48 h and 7 d was not part of the original design, thus we have no evidence to document when the exact moment of formation occurred, and if it differed between temperatures. In the no UV exposures, tarball formation was only documented in the 30℃ exposure (Figure 6). However, qualitatively, this was just the early stage of formation as the blobs were loosely formed and if disturbed would break apart.

Chemical Changes

Measured chemistry was taken at each of the four time points (6 h, 24 h, 48 h and 7 d) and included the analysis of hopanes and steranes, PAHs and TEH. The PAH data is reported as PAH50, which is the sum of the 50 parent and alkylated PAHs detailed in Table 2. Likewise, the hopanes and steranes that were measured are also summed for data comparisons (biomarkers). Figures 7-9 show the TEH measurements from each exposure (10, 21, and 30°C respectively). Within each exposure, TEH values were generally consistent (Figures 7-9). Student t-tests were run at each time point, to compare measured TEH in each light treatment. No significant differences were observed between light treatments at any of the time points in the 21 or 30°C exposures. In the 10℃ exposure, a statistical difference was observed at the 7 d time point ($p=0.0027$), but not in any of the other time points in this exposure. The measured TEH in the UV treatment at 7 d was significantly higher than the measured TEH in the no UV treatment.

The biomarkers data is shown in Figures 10-12 (10, 21, and 30℃, respectively). As observed in the TEH data, the biomarkers remained relatively consistent throughout all time points in each exposure. Student t-tests were run as each time point to compare measured biomarker data in each light treatment. No significant differences were observed between any of the treatments/time points in the 10 and 30°C exposures. In the 21℃ exposure at 7d, the biomarkers in the no UV treatment were significantly higher than the UV treatment ($p=0.034$).

The PAH50 data is plotted in Figures 13-15 (10, 21, and 30℃ respectively). In all three exposures, PAH50 concentration declined throughout the duration of the experiment. As with the other measured chemistry, Student t-tests were run at each time point to compare measured PAH50 concentrations in each light treatment. There were no significant differences observed between light treatments in the 21°C exposure. In the 10 and 30℃ exposures, significant differences were only observed at 48 h (10°C; $p=0.0172$) and 7 d (30℃; $p = 0.0127$). In both of these instances, the PAH50 concentration in the no UV treatment was significantly higher than the UV treatment.

In addition to analyzing total concentrations of different hydrocarbon classes, we also analyzed changes in individual PAHs throughout each exposure. PAHs are thought to be one of the more susceptible classes of hydrocarbons to be transformed by solar irradiation, due to the presence of chromophores (D'Auria et al., 2008). This analysis was done by calculating the percent loss of PAHs in both the UV and no UV treatments. Percent loss, which is detailed in Prince et al. (2003), was calculated according to the formula in Figure 16, and is determined

using PAH: hopane ratios from both fresh oil and the weathered oil samples taken throughout the experiment. PAHs are normalized to the 17α(H)21β(H)hopane because biomarkers in general have been shown to be highly resistant to weathering processes and thus useful for understanding how the other components in oil change (Prince et al., 2003; Wang et al., 2007). To better understand if and how the percent loss (% loss) differed between treatments, the difference in percent loss was calculated (% loss no UV - % loss UV) and plotted in order of increasing molecular weight and degree of alkylation (Figures 17-19; 10, 21 and 30℃ respectively). From these figures, it is observed that the vast majority of values are negative, indicating that there was more loss in PAHs in the UV treatment than in the no UV treatment.

Discussion

Physical Analyses

Data generated from the photographic portion of this experiment showed that when oil was exposed to UV light, tarballs formed, regardless of temperature. In the no UV treated oil, tarball formation was only observed in the highest temperature (30℃), indicating that temperature also factors into tarball formation. Creating tarballs in a laboratory setting has previously been unsuccessful, and being able to create them, at least on a small scale, can be useful in allowing us to better understand the mechanisms behind tarball formation and their impact on the environment (OR&R, 2017). Broadly speaking, tarballs are defined as having a hard and crusty outer shell that encompasses a more fluid-like interior; thus tarballs are thought to contain both weathered (exterior shell) and fresh (interior) oil. After an oil spill event, they are likely to wash up on shore, even in areas not in the vicinity of the spill. One of the many concerns with tarballs is that it is largely unknown how much energy is needed to break them apart, and since their interior is mainly fresh oil, if they break apart, they have the potential to oil additional areas.

Chemical Analyses

In all exposures, the biomarker and TEH chemistry remained relatively consistent. This result is not unexpected. Biomarkers are a class of hydrocarbon compounds in oil that have been found to be very resistant to weathering processes (Prince et al., 2003; Wang et al., 2007; Garrett et al., 1998). The fact that our biomarker measurements did not show a decrease over time supports other studies that have looked at biomarkers in weathered oil. Measuring biomarkers is important as they are not only useful identifiers of oil source, but they are also useful in understanding the degree of weathering that has occurred in oil (Prince et al., 2003; Wang et al., 2007). As for the TEH data, TEH (or total petroleum hydrocarbons/TPH) is essentially a bulk measurement for all of the hydrocarbons in oil. The measurement is a baseline integration of the chromatogram, typically from C10-C44, rather than an integration of a single peak, thus the measurement includes both resolvable peaks and the unresolved complex mixture (UCM). The

shape and/or composition of the UCM tends to change as oil weathers, however those changes may not always be readily apparent when looking at the total ion chromatogram (TIC) or even the measured TEH. In a study by Prince et al. (2003), the TICs were compared for oil was either kept under dark conditions or exposed to UV light, and visually, there was no obvious difference between the chromatograms. It wasn't until the authors examined those same samples through thin layer chromatography that they were able to see differences in the different hydrocarbon classes. The authors found that in the UV exposed oil, there were higher proportions of resins and polar compounds than in the oil without UV light exposure, while the proportion of aromatics was lower in the UV exposed oil (Prince et al., 2003). The authors hypothesized that the aromatic compounds were being transformed into polar or resin compounds. Thus in our study, additional chemical analyses such as thin layer chromatography can elucidate if there are compositional changes occuring, and if those compositional changes are altered by changes in environmental factors, such as temperature.

In all exposures, the PAH50 concentration decreased over time. This result was expected as PAHs are highly susceptible to weathering processes including volatilization and photo-oxidation (Stout and Wang, 2007; Jonker et al., 2005). Generally speaking, the light to mid molecular weight PAHs, are more susceptible to volatilization (Latimer and Zheng, 2003; Howard et al., 2005; Stout and Wang, 2007), whereas the mid to heavy molecular weight PAHs as well as PAHs with a higher degree of alkyl substitution (C3, C4 compounds) are more susceptible to photo-oxidation (Howard et al., 2005; Garrett et al., 1998; Lee, 2003). While PAH50 concentrations were generally not significantly different between light treatments, we did observe in the percent loss plots (Figures 17-19), that there was generally a greater loss of PAH analytes in the oil that was exposed to UV light. However, it is important to note though that for the purpose of this analysis, the replicate chemistry data was averaged when calculating the percent loss, and based on the formula and design on the experiment, we were unable to include any sort of standard deviation in this measurement thus the plots only show the general trend of the data.

During the 10 and 21°C exposures (Figures 17 and 18), it was also observed that the greatest difference in percent loss between the light treatments usually occurred at 48 h, and not at 7 d. We hypothesize that this may be related to when the tarball forms as a "skin-like" feature surrounded it, which would potentially slow weathering processes. The formation of "skins" on oil have been previously reported and they are thought to be a result of surface aggregation of the heavier and more polar constituents of oil, such as resins and possibly photo-transformed compounds (Fingas, 2011). In contrast, in the 30°C exposure, we observed that there were a lot more instances where the difference in percent loss was greater in the no UV treatment, and there was no clear trend on when the greatest difference in percent loss occurred. Additionally, this was the only exposure where we observed the beginning of tarball formation in the no UV treatment. Since skin formation is potentially related to decreases in oil weathering, we think that based on the time at which the tarball started forming in the UV and no UV treatments could be responsible for the lack of clear trend in differences in PAH depletion.

The results of the physical changes documented in this study demonstrated that UV light is a factor in tarball formation. The results from the chemical analyses, however, were not as clear on how UV light alters the chemical properties of oil and reasons for this include factors such as oil type and measured chemistry. For example, the oil used in this study was Louisiana Sweet Crude oil, which contains roughly 1-2% PAHs (Boehm et al., 2016). As stated previously, PAHs are one of the more likely hydrocarbon classes to undergo photo-oxidation, however, they are susceptible to other weathering processes as well, and their low proportion in this oil type makes it difficult to ascertain the true influence of photo-oxidation. Additionally, as this was a pilot study, the decision was made not to heavily invest in new instrumental platforms or methodologies, so we chose to use chemical analyses that were already well established in our chemistry program, but were not entirely suited to understanding the effects of UV light on oil chemistry.

The information generated from this pilot study was extremely useful as a starting point for further investigations into the interaction of UV light and floating oil. A phase two of this study, entitled "Measuring the photo-oxidation of floating oil" has been proposed and awarded through the NCCOS discretionary proposal process for fiscal year 2020. In collaboration with ERD/OR&R the study objectives include 1) measure changes to bulk physical properties of oil (e.g. density, viscosity) under UV exposure 2) measure changes in chemical composition of the water accommodated fraction when oil is exposed to UV light 3) characterize the relationship between UV intensity and time to tarball formation and 4) use the experimental design developed for the pilot study and apply it to another oil type (intermediate fuel oil/IFO). We propose to accomplish these objectives by using a similar experimental design setup (e.g use of environmental chamber, manipulation of environmental factors such as temperature) as used in this study and enhancing our chemical analyses to measure additional hydrocarbons including saturates, asphaltenes and resins, and employing additional techniques such as thin layer chromatography-gas chromatography, fourier-transform infrared spectroscopy (FTIR), and liquid chromatography tandem mass spectrometry (LC-MS/MS). These techniques will allow us to quantify a greater range of compounds, such as oxygenated/more polar species, which are thought to be formed during photo-oxidation. The data generated will be used by OR&R to aid in the development of their new weathered oil database. The database will be a compilation of physico-chemical properties of several oils, which can be used to aid in active spill response, spill response planning, and environmental impact analysis.

Data Availability

All of the raw chemistry data for this project is located in the Chemistry Information Management System, also known as ChemLIMS, which is an NCCOS administrative application, housed on the Microsoft Azure Cloud. The ChemLIMS database is only accessible to NCCOS personnel, and is maintained by the NCCOS Web and App support team. Photographs taken throughout the duration of the study are located in both the NCCOS shared "projects" drive, under the CCR swap folder as well as on the Google share drive. Both locations access is restricted to NCCOS personnel only.

Acknowledgements

The authors would like to thank Dalina Viveros, Carl Childs, and Chris Barker with the Emergency Response Division at the Office of Response and Restoration for their collaboration on this project and the proposed future work. Funding for this project was provided through the NCCOS Discretionary Proposal Process in FY 2018-2019.

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Supporting Documents

Standard Operating Procedures (SOPs) for the methods detailed in this study can be found here. Specific SOPs used in this study with their corresponding titles are found in Table 3.

The preliminary results of this study have been presented at the Society of Environmental Toxicology and Aquatic Chemistry conference (Toronto, Ontario, Canada November 3-7, 2019) and SDI Bioeffects workshop (November 2019), and can be found here.

Tables

Table 1: List of internal standards used for the quantitation of PAHs, TEH, and hopanes and steranes.

Internal Standards		
d_8-naphthalene	d_{10}-anthracene	d_{12}-benzo[a]pyrene
d_{10}-1 methylnaphthalene	d_{10}-fluoranthene	d_{12}-benzo[e]pyrene
d_8-acenaphthylene	d_{10}-pyrene	d_{12}-perylene
d_{10}-acenaphthene	d_{12}-benz[a]anthracene	d_{12}-benzo[ghi]perylene
d_{10}-fluorene	d_{12}-chrysene	17b(H), 21b(H)-hopane
d_8-dibenzothiophene	d_{12}-benzo[b]fluoranthene	d_{41}-n-eicosane
d_{10}-phenanthrene	d_{12}-benzo[k]fluoranthene	d_{26}-n-dodecane

Table 2: List of all of the compounds analyzed for in this study. PAH50, is the sum of all parent and alkylated PAHs in this table.

Parent PAH	**Alkylated PAH**	**Hopanes/Steranes (Biomarkers)**
naphthalene	C1-naphthalenes	17α(H), 21β(H)-22S-homohopane
biphenyl	C2-naphthalenes	17α(H), 21β(H)-22R-homohopane
acenaphthene	C3-naphthalenes	17α(H), 21β(H)-30-norhopane
acenaphthylene	C4-naphthalenes	17α(H), 21β(H)-hopane
fluorene	C1-fluorenes	17α(H)-22,29,30-trisnorhopane
dibenzofuran	C2-fluorenes	ααα 20R 24R-ethylcholestane
dibenzothiophene	C3-fluorenes	ααα 20R cholestane
phenanthrene	C1-dibenzothiophenes	αββ 20R 24R ethylcholestane
anthracene	C2-dibenzothiophenes	αββ 20R 24S methylcholestane
fluoranthene	C3-dibenzothiophenes	αββ 20R cholestane
pyrene	C4-dibenzothiophenes	C27ββS
benz[a]anthracene	C1-phenanthrenes/anthracenes	C29ββS
benzo[b]naphtho[2,1-d]thiophene	C2-phenanthrenes/anthracenes	Ts

chrysene+triphenylene	C3-phenanthrenes/anthracenes	
benzo[b]fluoranthene	C4-phenanthrenes/anthracenes	
benzo[k]fluoranthene	C1-fluoranthenes/pyrenes	
benzo[j]fluoranthene	C2-fluoranthenes/pyrenes	
benzo[a]fluoranthene	C3-fluoranthenes/pyrenes	
benzo[a]pyrene	C4-fluoranthenes/pyrenes	
benzo[e]pyrene	C1-chrysenes/benzanthracenes	
dibenzo[a,h]anthracene	C2-chrysenes/benzanthracenes	
indeno[1,2,3-c,d]pyrene	C3-chrysenes/benzanthracenes	
benzo[g,h,i]perylene	C4-chrysenes/benzanthracenes	
	C1-naphthobenzothiophenes	
	C2-naphthobenzothiophenes	
	C3-naphthobenzothiophenes	
	C4-naphthobenzothiophenes	

Table 3: Standard operating procedures (SOPs) used in this study. The entire SOP can be found here.

SOP Number	**Title**
CCR002	Organic Glassware Cleaning
CCR006	TurboVap II Workstation
CCR041	Integration of Sample Chromatograms
CCR042	Integration of Procedural Blanks for MDL Calculation
CCR047	Sample Receipt
CCR053b	Liquid/Liquid Extraction and Cleanup of PAH/TEH/Biomarkers from Seawater
CCR061	Azure ChemLIMS database
CCR076	Extended PAH Suite Quantitation

Figures

Figure 1: General experimental setup for floating oil exposures. All UV exposures took place on the right side of the room and no UV exposures took place on the left. The black plastic sheeting surrounded all four sides, and was pinned shut during exposures. Light readings using a spectrometer were taken periodically in the no UV exposure to ensure that there was no cross contamination from the UV lights.

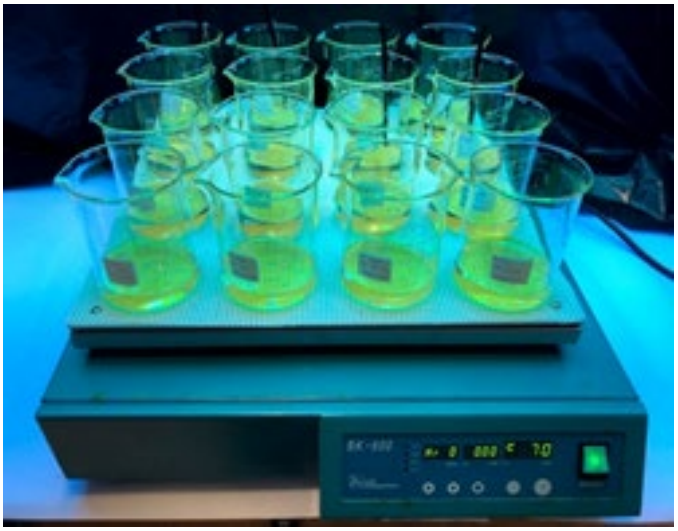

Figure 2: Examples of experiment setup for no UV (left) and UV (right) floating oil exposures.

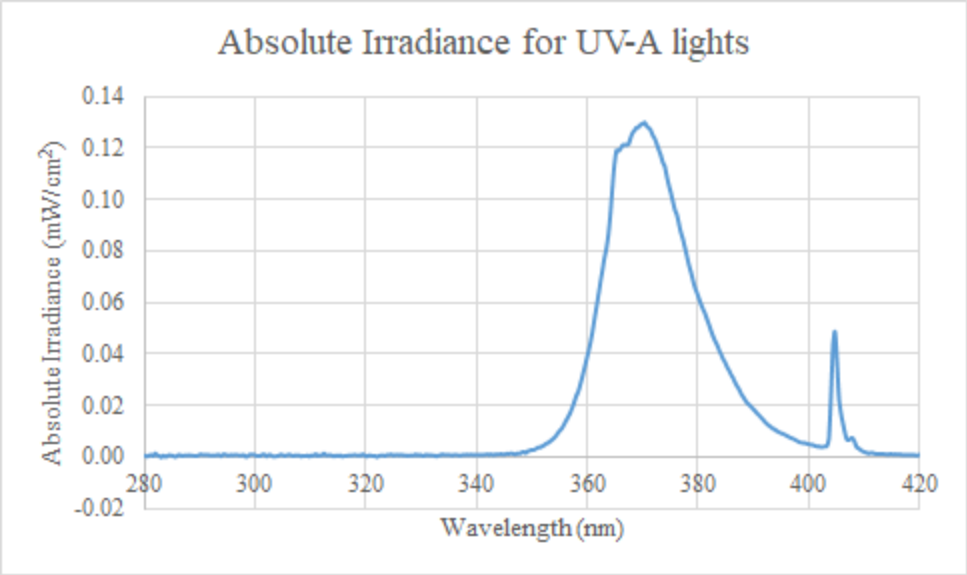

Figure 3: The absolute irradiance by wavelength for the UV-A lights used in this study. All light readings were measured using an Ocean Optics FLAME spectroradiometer.

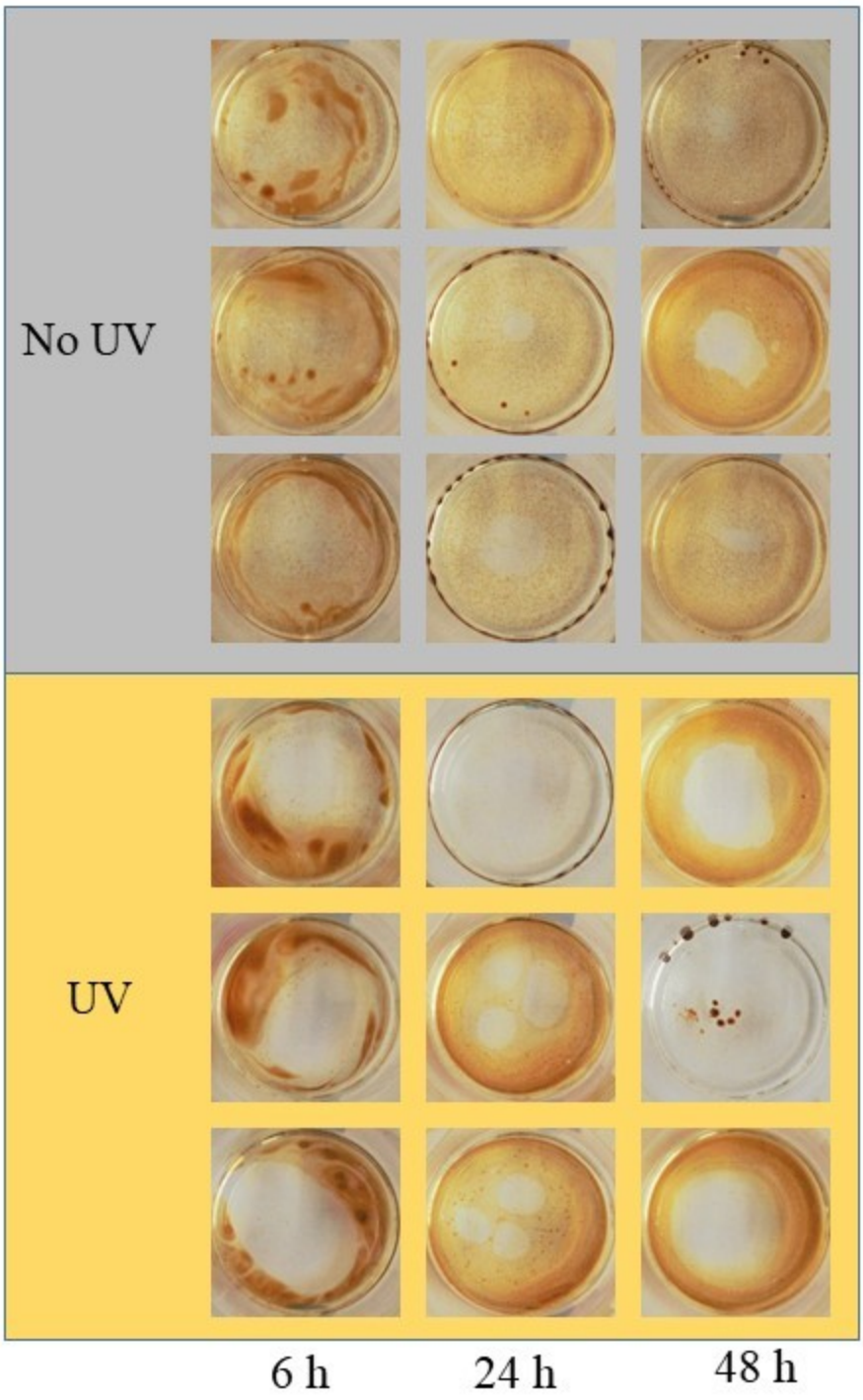

Figure 4: Photographs of floating oil slicks from the 10°C experiment. Samples from the no UV exposure are on the top (gray) and the UV samples are on the bottom (yellow). Time increases from left to right. Photographs from 7 d are not shown.

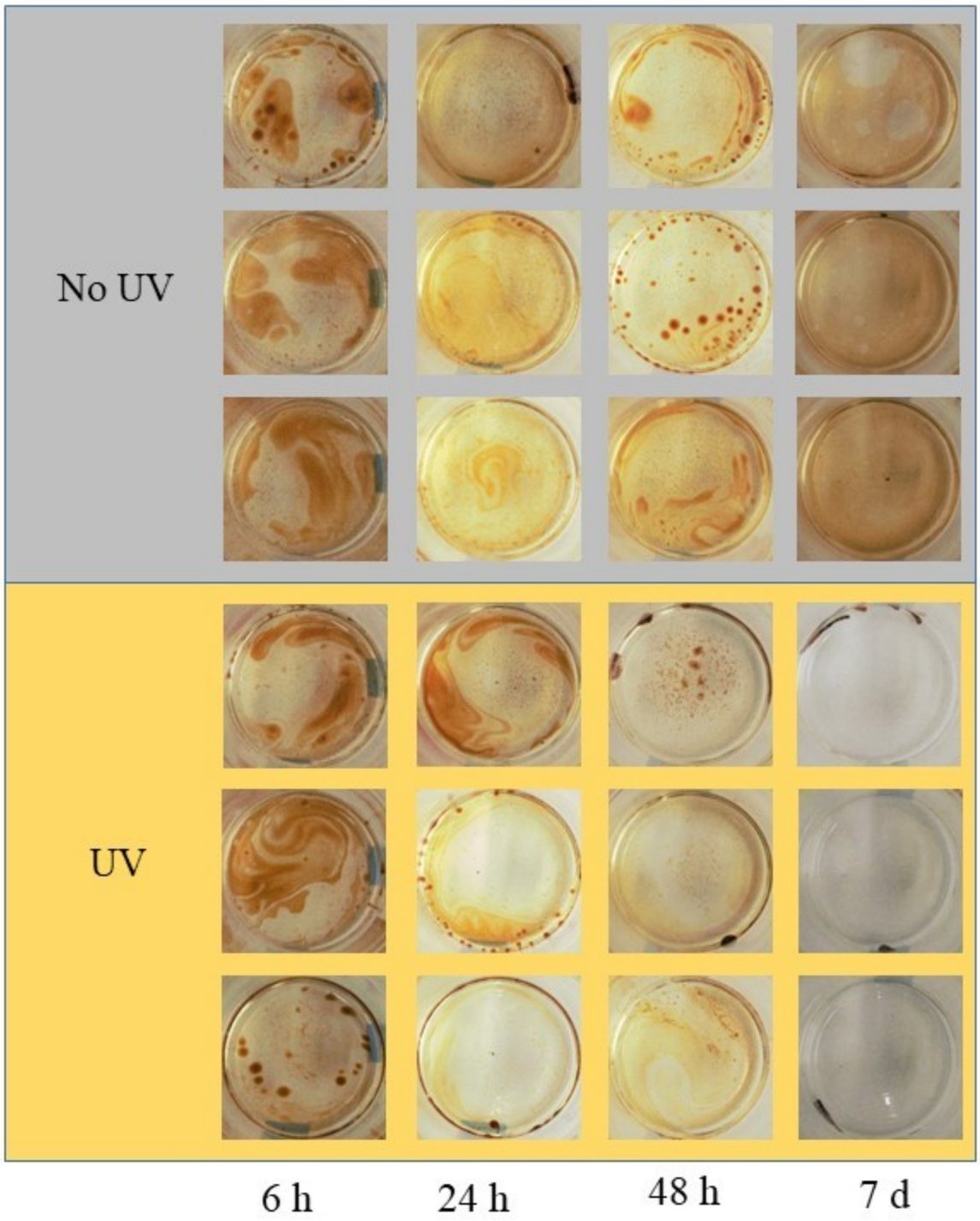

Figure 5: Photographs of floating oil slicks from the 21°C experiment. Samples from the no UV exposure are on the top (gray) and the UV samples are on the bottom (yellow). Time increases from left to right.

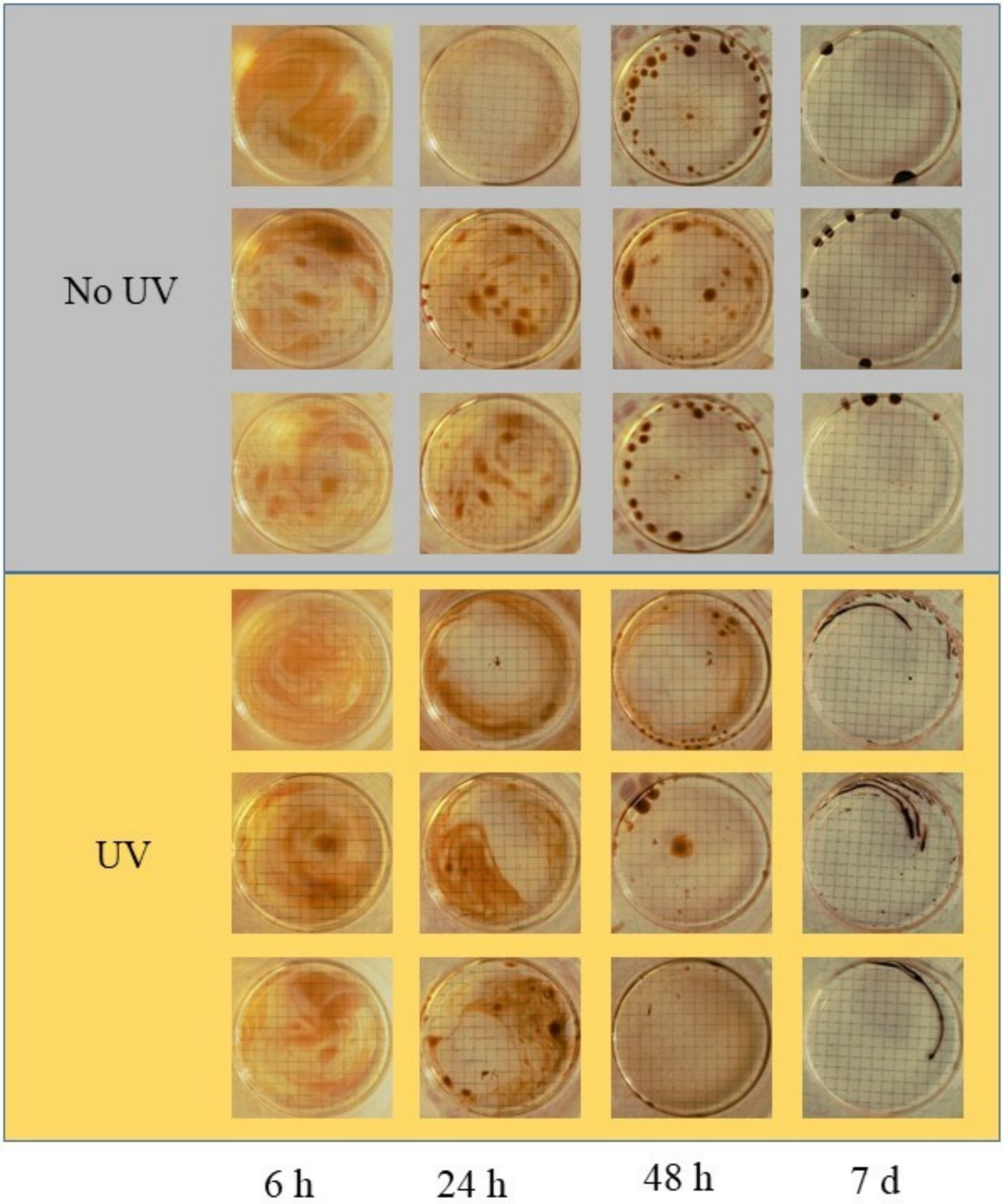

Figure 6: Photographs of floating oil slicks from the 30°C experiment. Samples from the no UV exposure are on the top (gray) and the UV samples are on the bottom (yellow). Time increases from left to right.

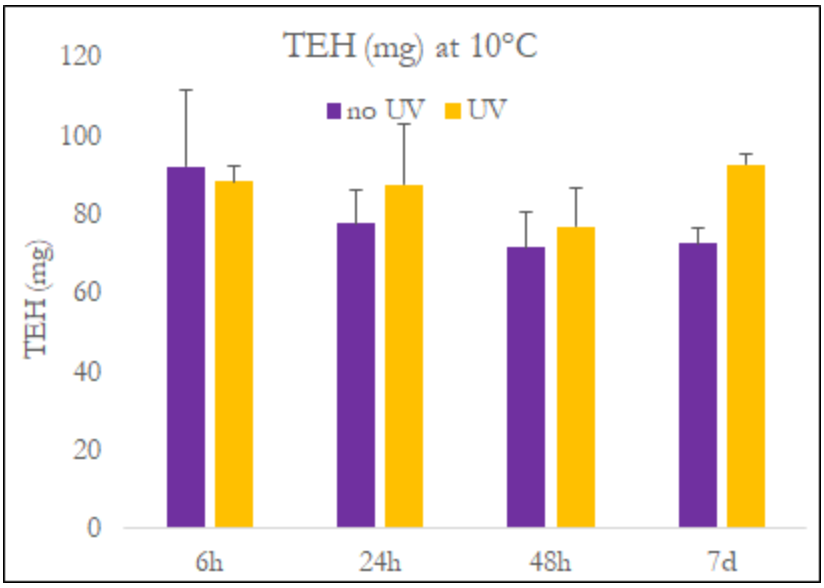

Figure 7: Average TEH measurements from the 10°C exposure. Error bars are one standard deviation.

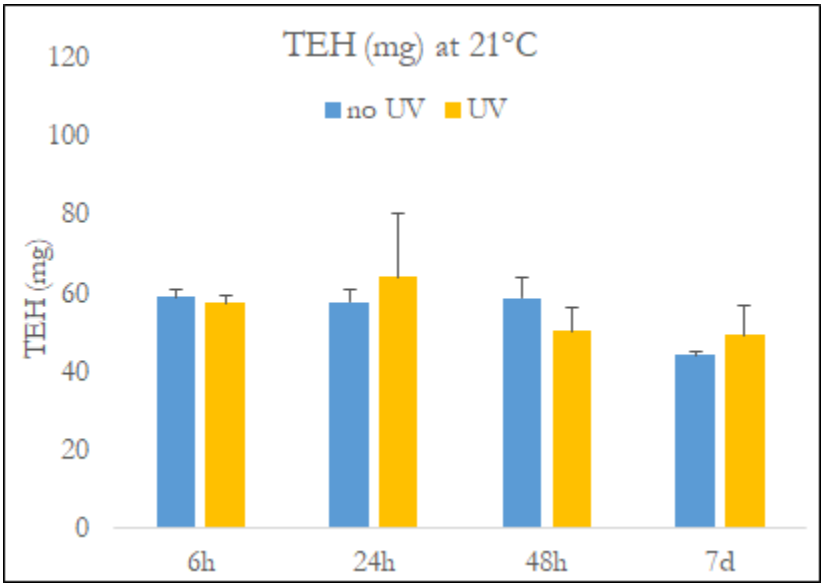

Figure 8: Average TEH measurements from the 21°C exposure. Error bars are one standard deviation.

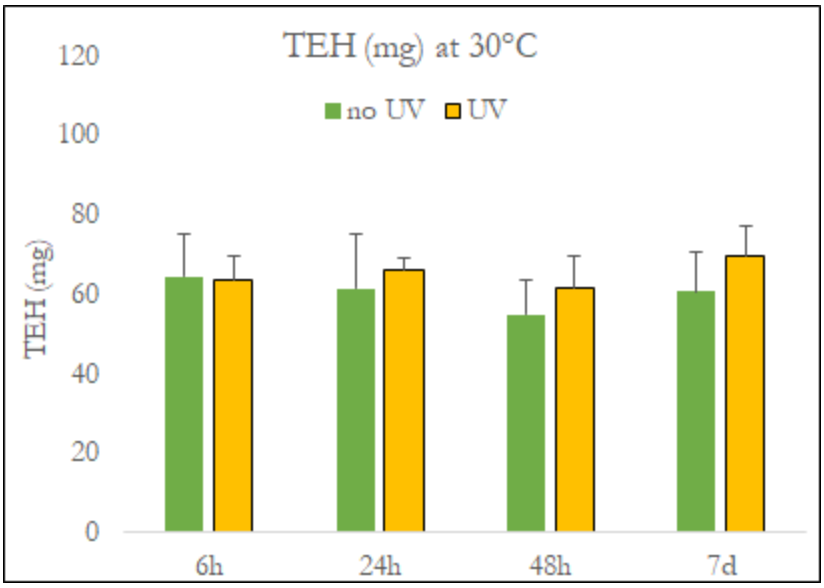

Figure 9: Average TEH measurements from the 30°C exposure. Error bars are one standard deviation.

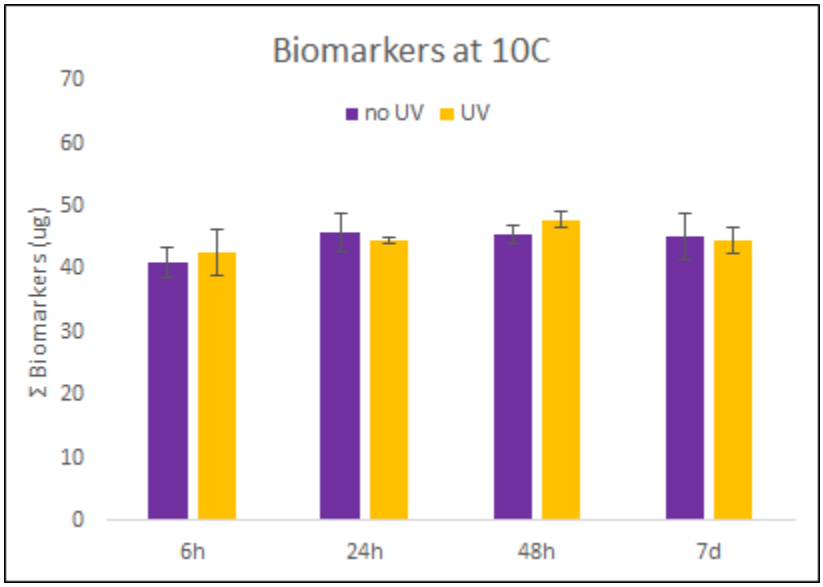

Figure 10: Average biomarker measurements (sum of 13 hopane and sterane compounds) from the 10°C exposure. Error bars are one standard deviation.

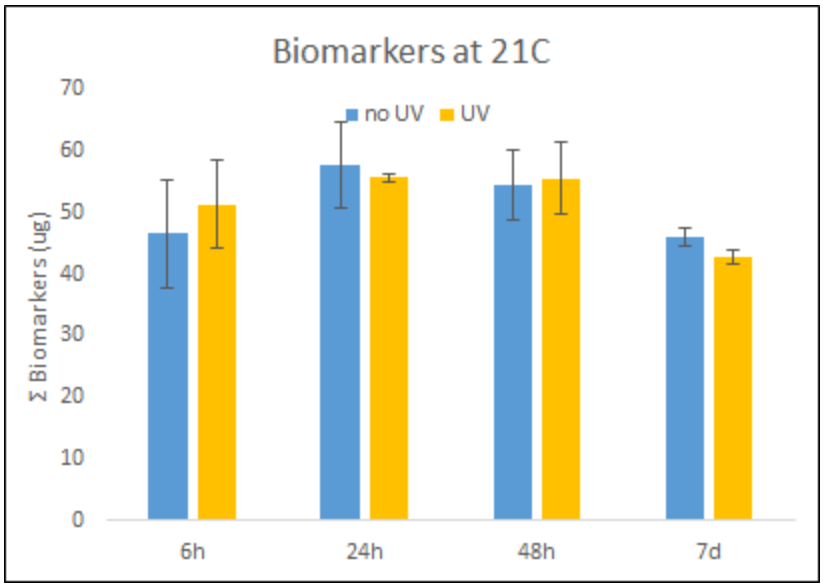

Figure 11: Average biomarker measurements (sum of 13 hopane and sterane compounds) from the 21°C exposure. Error bars are one standard deviation.

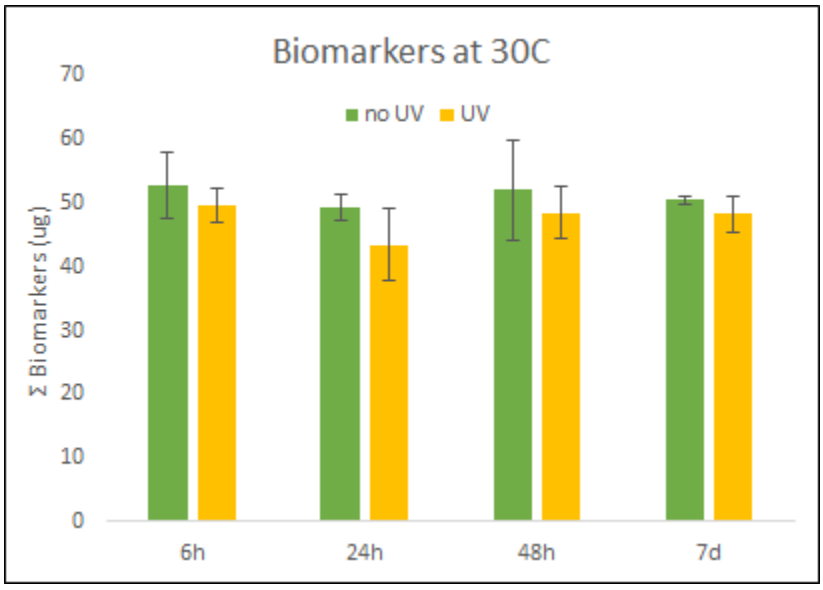

Figure 12: Average biomarker measurements (sum of 13 hopane and sterane compounds) from the 30°C exposure. Error bars are one standard deviation.

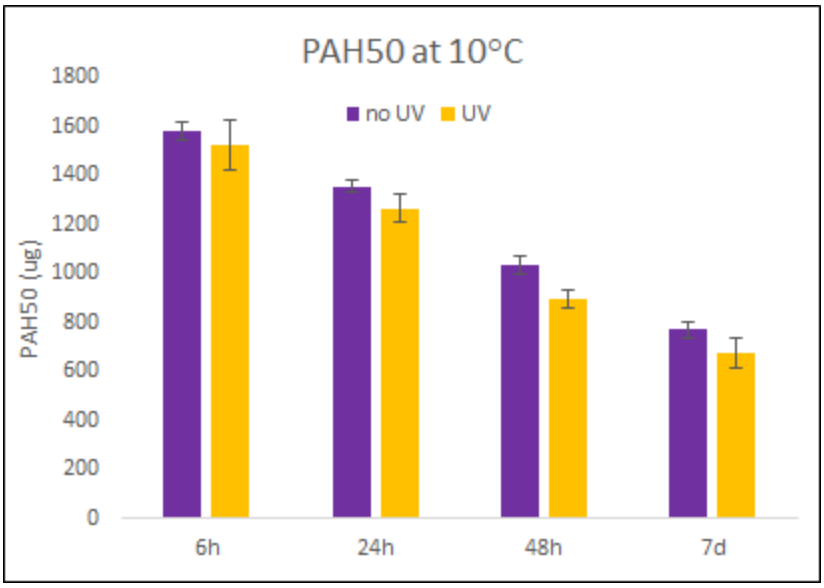

Figure 13: Average PAH50 measurements from the 10°C exposure. Error bars are one standard deviation.

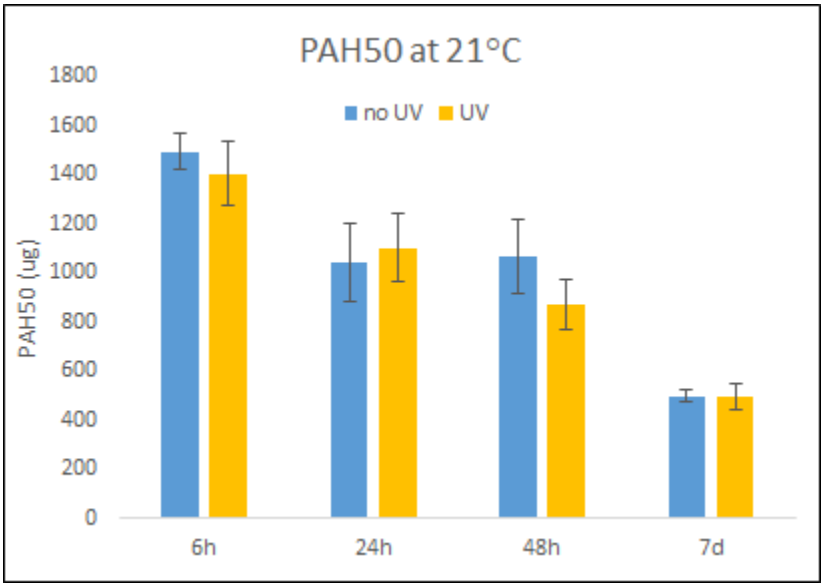

Figure 14: Average PAH50 measurements from the 21°C exposure. Error bars are one standard deviation.

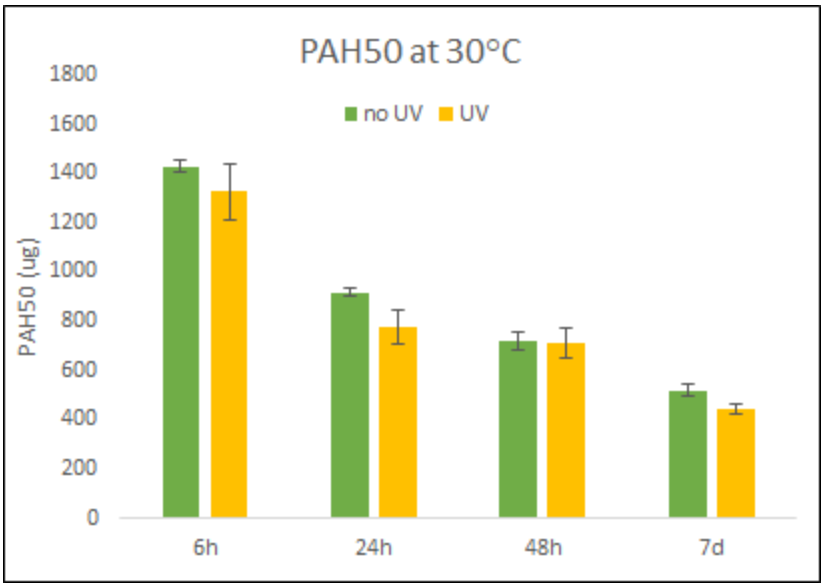

Figure 15: Average PAH50 measurements from the 30°C exposure. Error bars are one standard deviation.

$$\%\ loss = \frac{\left[\left(\frac{A_0}{H_0}\right) - \left(\frac{A_t}{H_t}\right)\right]}{\left(\frac{A_0}{H_0}\right)}\ x\ 100$$

H_0 = 17α(H)21β(H)hopane concentration in fresh oil
A_0 = PAH concentration in fresh oil
H_t = 17α(H)21β(H)hopane concentration at desired time point/treatment
A_t = PAH concentration at desired time point/treatment

Figure 16: Percent loss formula from Prince et al., (2003). Used to calculate the loss of PAH analytes in both the UV and no UV treatments. The percent loss was calculated in relation to fresh oil.

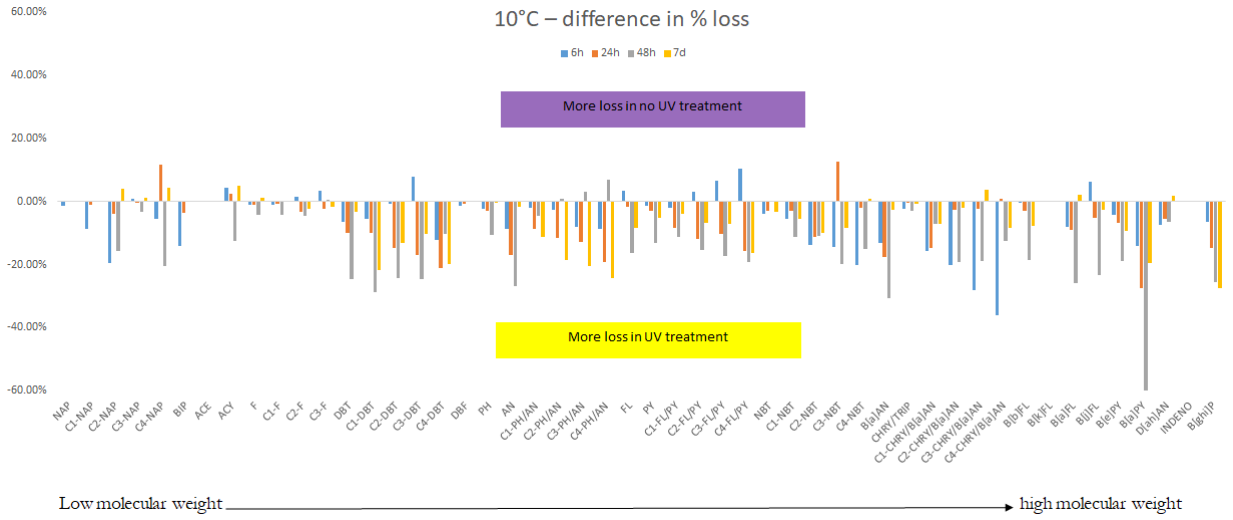

Figure 17: The difference in percent loss between no UV and UV light treatments in the 10°C exposure at all four time points. Values that are negative indicate that there was more loss of the PAH in the UV treated oil than in the no UV treated oil. PAHs are arranged from left to right, in order of increasing molecular weight and degree of alkyl substitution.

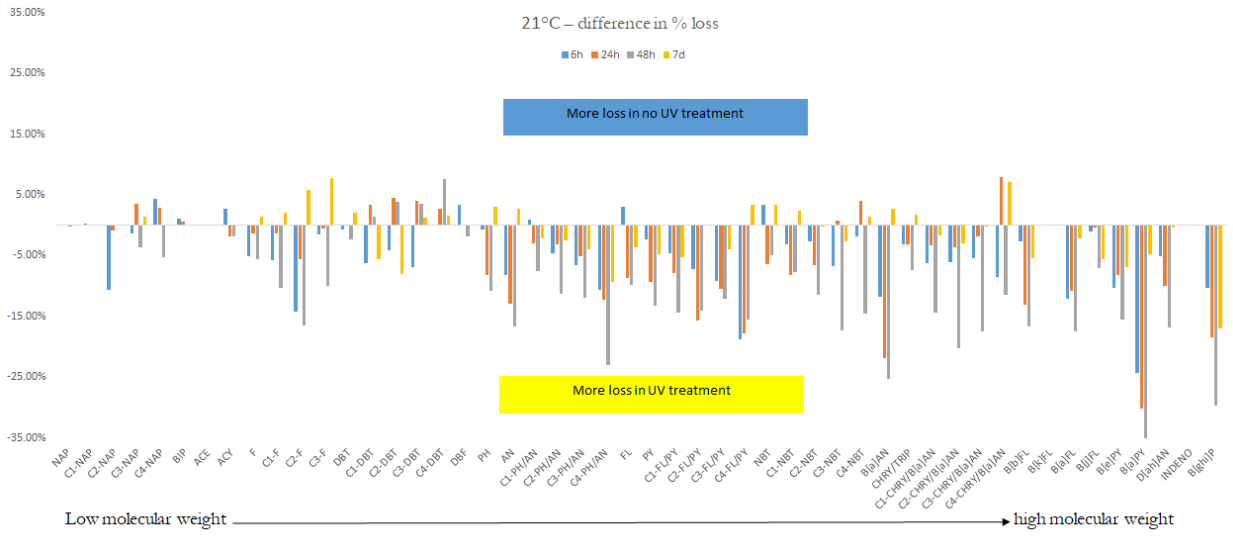

Figure 18: The difference in percent loss between no UV and UV light treatments in the 21°C exposure at all four time points. Values that are negative indicate that there was more loss of the PAH in the UV treated oil than in the no UV treated oil. PAHs are arranged from left to right, in order of increasing molecular weight and degree of alkyl substitution.

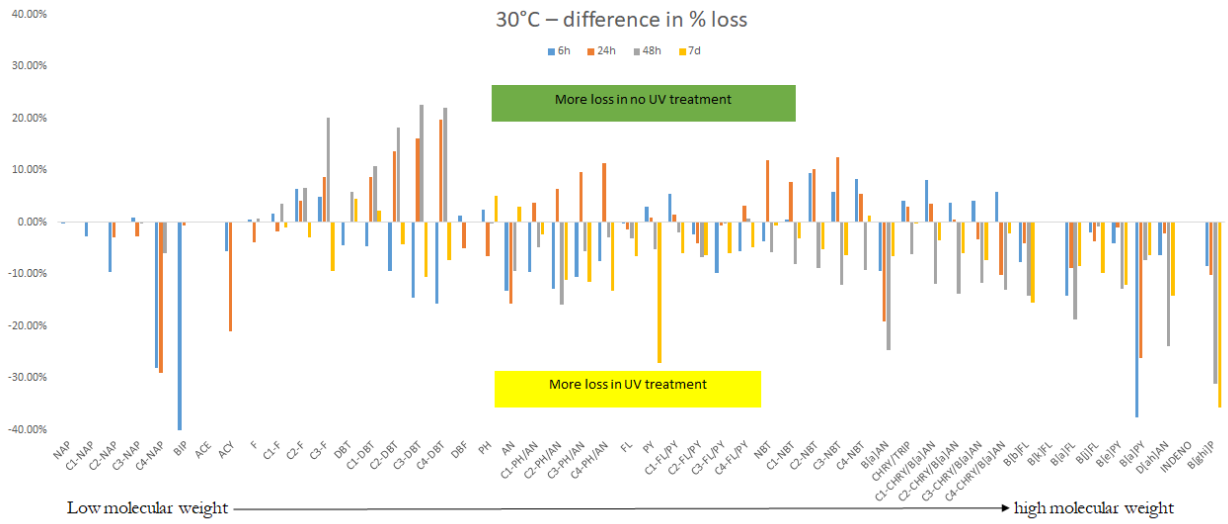

Figure 19: The difference in percent loss between no UV and UV light treatments in the 30°C exposure at all four time points. Values that are negative indicate that there was more loss of the PAH in the UV treated oil than in the no UV treated oil. PAHs are arranged from left to right, in order of increasing molecular weight and degree of alkyl substitution.

Comparison of Chemical Contaminant Measures Using CLAM, POCIS, and Silicone Band Samplers in Estuarine Mesocosms

Ed Wirth[1*]
Brian Shaddrix[2]
Peter Key[1]
Marie DeLorenzo[1]
Paul Pennington[1]
Dave Whitall[3]

[1] Ecotoxicology Branch, NCCOS Charleston, SC 29412
[2] CSS, Inc., Ecotoxicology Branch, Charleston, SC 29412
[3] Monitoring and Assessment Branch, NCCOS, Silver Spring, MD
Corresponding author (*)

Abstract

Sampling chemical contaminants in the water column using discrete grab samples is dependent on time, chemical input, tide, flow, and precipitation. Thus, a discrete water sample represents a "snapshot". Additionally, large volumes of water may be required in order to quantify chemicals and this may not be logistically feasible. Other integrators of contamination (e.g. sediment and bivalve chemistry) have been used to address chemical concentrations in light of the limitations associated with discrete sampling. However, not all analytes accumulate in sediments or tissues and some ecosystems may not have appropriate sediments (e.g. sandy sediment) or bivalve populations. Thus, multiple sampling platforms have been engineered and in this study, we attempt to understand the benefits of POCIS (Polar Organic Chemical Integrative Sampler), silicone bands, and the CLAM (Continuous Low Level Aquatic Monitoring). This experiment utilized six estuarine mesocosm systems to test the response of passive (POCIS, Silicone Bands) and active sampling (CLAM) technologies in comparison with traditional grab sampling methodologies for triclosan, bifenthrin and pyrene. Concordance (r_c) analysis between grab and CLAM data resulted in excellent agreement for all chemicals (r_c ranged from 0.84-0.97). Passive samplers (POCIS and SB) were able to collect these three contaminants, although expected differences in extracted mass based on increasing dose was not observed with triclosan. Dose dependent differences for bifenthrin and pyrene were observed. Converting passive sampler results to aqueous concentrations still requires laboratory determination of various rate constants, but these passive platforms may be useful in determining the presence of contaminants across a broad spatial area.

Introduction

Environmental monitoring of anthropogenic contaminants in water and sediments is a critical factor in determining the potential impact or risk these chemicals pose in an ecosystem. To regularly track the distribution and understand the concentrations of these pollutants, experimental and monitoring designs can quickly become complex and expensive using historical sample collection and extraction techniques. These historical field based protocols are based on discreet 'grabs' of the environment and these protocols often require extensive time, field staff, field gear and whole sample transport logistics. At best, this type of discrete sampling allows for detailed laboratory extraction and analysis protocols resulting in a concentration from that sample that represents the chemical concentrations at a given point in time but limits the understanding of how concentrations change over time based on factors such as flow or changing input (Vrana et al., 2005; Stuer-Lauridsen, 2005). To allow more efficient and comprehensive field sampling of chemicals in ambient waters, researchers have leveraged widely accepted passive air quality monitoring practices for occupational and environmental exposure (Figure 1; from Zabiegala et al., 2010) and have begun to develop passive monitoring tools for assessing chemicals in water. Over the past 20-25 years, passive monitoring in water has grown in acceptance.

Monitoring contaminants in water using passive sampling devices (PSD) has addressed the costly and logistically complicated hurdles associated with discrete sampling; PSD deployment is often simpler and less time consuming, the PSD is often smaller and PSDs are generally able to estimate time weighted average (TWA) concentrations (Kot-Wasik et al., 2007). While identified disadvantages of PSDs have been described to include the inability to monitor short-term changes in chemical concentration, the need for predetermined calibration of devices and sampling rates, and changes in sampling efficiency, the advantages of PSDs often serve monitoring programs well by reducing field costs, producing TWA concentrations that are more likely appropriate for understanding the environmental status of a location (Kot-Wasik et al., 2007). Using PSDs to evaluate contaminant distribution and concentrations in waters has become more accepted by regulatory agencies; even to the point where the European Union has legislated anthropogenic chemical management using, in part, data generated using PSDs (Zabiegala et al., 2010).

Passive sampling devices are often classified as either non-equilibrium (or linear) and equilibrium based devices. As a rule, these PSDs are based on first-order uptake kinetics where the PSD samples the adjacent environment in a linear pattern until equilibrium is achieved (Figure 2). Regardless of how the PSDs are classified, the mass (and TWA concentration) of chemicals can be calculated as noted in Vrana et al., (2005) and updated by Larsen et al. (2009).

Equation 1: (Larsen et al., 2009) $C_w = (N_t/mK_{sw}*(1/1-e^{-Rs\ t/mKsw})$

Where C_w is the TWA water concentration in ng/L, N_t is the measured mass of chemical in ng, m is the mass of the PSD, K_{sw} is the sampler/water partition coefficient, t is time in hours and R_s is the laboratory measured sampling rate in L/h.

There have been numerous reviews of PSDs used in aquatic environments (Stuer-Lauridsen 2005; Vrana et al., 20065; Zabiegala et al., 2010) and factors related to data quality (Roll and Halden, 2016; Joyce and Burgess, 2018) and those reviews are helpful in understanding the historical timeline of PSD development. When evaluating chemical contaminants in marine waters, we need to be very careful about what sampling approach we take since concentrations of pollutants in water can change quickly due to tides, currents and freshwater inputs. In order to understand and evaluate the breadth of current sampling technology, we choose to highlight three sampling paradigms in addition to the benchmark process where a discreet grab sample is collected and analyzed. In order of increasing complexity, we selected silicone wristbands (Smalling et al. 2018), Polar Organic Chemical Integrative Sampler (POCIS) (Alvarez et al., 2010) and the Continuous Low-Level Aquatic Monitoring (CLAM) (Aqualytical Inc.).

Silicone bands as a PSD is a relatively new technique. Initial uses of SB may be traced to being used as a personal dosimeter for a wide range of compounds (O'Connell et al., 2014 a, b). Silicones, in general, have been used in several applications including stationary phases in gas chromatography analytical columns, solid phase microextraction, and stir bar sorptive extraction, thus, they have demonstrated the ability to absorb a wide variety of organic compounds (Seethapathy and Gorecki, 2012). To date, silicone bands have been used to measure exposure to PAHs, PPCPs, phthalates, pesticides, and industrial compounds (O'Connell et al. 2014), organophosphate and brominated flame retardants (Hammel et al. 2016; Hammel et al. 2018), and nicotine (Quintana et al. 2019). Silicone bands have primarily been used for monitoring contaminant exposure in humans (i.e. humans wear the silicone bands for a period of time, after which the bands are extracted and analyzed), however, more recently, silicone bands have been deployed in the environment to understand pesticide exposure in frogs (Swanson et al. 2018). The use of silicone bands is advantageous as they are cheap (individual bands often cost less than $0.25 each), come in a variety of sizes and colors, can be modified into different configurations, and are widely available.

The POCIS device was developed in the early 2000's in response to identified limitations when using Empore disks that highlighted the limited range of chemicals that can be associated with the disk as well as issues related to performance (Alvarez et al., 2004). In order to better sample polar organic chemicals in the water, the USGS developed the POCIS device using a sampling matrix that targeted a wider range of polar environmental contaminants and was able to estimate the TWA of dissolved contaminants in the water (Petty et al., 2002 and Alvarez et al, 2004). Initially, the sampling matrix used in POCIS was ENV+, a resin that targets polar organic chemicals but more recent POCIS use include HLB (hydrophilic/lipophilic balance) as this

sampling matrix allows this PSD platform to accumulate a wider range of hydrophilic and lipophilic chemicals (Alvarez et al., 2010).

CLAM units are small submersible extraction samplers that were designed and manufactured by Aqualytical, Inc. (Louisville, KY; https://aqualytical.com). Each submersible uses U.S. EPA approved solid phase extraction media for the quantitative analysis of trace organics in water. Combined with in-situ low flow rate extraction sampling, CLAM units can be deployed for hours or days capturing targeted contaminants of interest during environmental events. Each unit can quantitatively sample up to 100 L of water thereby lowering the detection limits of analytical equipment during instrumental analysis. Water is pumped through a pre-filter and sorbent filter initially to reduce surface adsorption of analytes to tubing and internal components. Multiple applications can be used with this technology including sampling marine environments, watersheds, agricultural runoff, and drinking water systems. Logistically, CLAM units are also beneficial when sampling in remote locations where shipping costs are kept to a minimum for filters versus traditional bulk water shipping.

Evaluating the distribution and concentrations of environmental pollutants remains an important facet of risk management. Historical PSDs, and PSDs embracing newer technology, have created an opportunity for NOAA to re-imagine chemical monitoring. The objective of this study is to evaluate three passive (or passive sampling based) samplers; directly comparing these PSD results with traditional grab samples under typical estuarine conditions found along the Gulf of Mexico and southeastern Atlantic coast.

Experimental Procedures

Mesocosm Background/System

The marine marsh simulation (mesocosm) units located at the NCCOS facility (Figure 3a) in Charleston, SC were used to monitor chemical behavior under environmentally relevant conditions. Briefly, nine mesocosm systems located in a greenhouse were used to evaluate four chemical measurement protocols described below. Each mesocosm system included an upper and lower tank and followed the designs found in Pennington et al. (2007). These systems generally followed a natural photoperiod of 16h:8h light:dark and maintained roughly 25 °C mean temperature. The lower tank provided a reservoir that mimics a diurnal tidal influx where seawater is pumped into the upper marsh tank. Each mesocosm system contained approximately 445 L of seawater and associated containers with field collected sediment and commercially obtained *Spartina alterniflora* grass. These systems were set-up and allowed to condition in the greenhouse for approximately five weeks prior to testing.

Selected Chemicals

Three chemicals (triclosan, bifenthrin and pyrene) were selected for examination during this experiment based on the variability of chemical classes, water solubility and toxicity to coastal invertebrates. Triclosan (purchased from Sigma-Aldrich) is an antibacterial and antimicrobial agent that can be found in consumer products such as detergents and is often found in environmental samples. This emerging contaminant has a reported LC50 of 305 µg/L for adult grass shrimp, *Palaemon* (*Palaemonetes) pugio*, (and 1.54 µg/L for larvae; DeLorenzo et al., 2008) and the reported Log_{10} octanol-water partition coefficient (Log K_{ow}) is 4.79 (Bester, 2005; NIET, 2012). Bifenthrin is a pyrethroid insecticide that is known to be extremely toxic to marine invertebrates with a reported Log K_{ow} = 6.0 (Hansch et al., 1995) and an LC50 in adult grass shrimp of 0.02 µg/L (Harper et al., 2008). Finally, pyrene is a 4-ringed polycyclic aromatic hydrocarbon often resulting from incomplete combustion. Pyrene has a Log K_{ow} = 4.88 (Hansch et al., 1995) to 5.08 (Lide, 1997). Grass shrimp mortality was reported to be ~60% at 1 mg/L and 20% at 100 µg/L (Oberdörster et al., 2000; Alden and Butt, 1987). This experiment generally targeted a nominal "low" dose (100, 0.02 and 2.2 µg/L respectively for triclosan, bifenthrin and pyrene) that was based estimated LC5 data and then a nominal "high" dose 2-4 times the low dose (200, 0.08, 8.8 µg/L respectively).

Study Design and Sampling Plan

We choose three sampling platforms to evaluate chemical contaminants in marine waters in addition to the accepted sampling benchmark, discrete sampling (Figure 3b). For the first 28-day dosing exposure, each mesocosm system was dosed with triclosan, bifenthrin and pyrene at the nominal low dose concentrations. There were three replicates randomly assigned for each sampling platform (POCIS, SB, and CLAM). POCIS and SB were deployed on day 0 and collected after 28-days. Water quality (salinity, temperature, dissolved oxygen and pH) were recorded daily in each mesocosm system and was continuously measured in one randomly selected mesocosm for each treatment. Weekly, discreet water and sediment samples were collected and composited from each treatment. CLAM units were deployed for up to 8 h and on retrieval, the volume of water pumped through the unit (and HLB cartridge) was calculated. At each CLAM retrieval, a discreet water sample was collected. After the 28-day low dose experiment, the systems were allowed to re-equilibrate and settle for seven days and then the high dose experiment was conducted in the same manner.

Sampling Protocols

Sediment Sampling

Sediments were collected in the field from a known reference site (Leadenwah Creek), and transported to the laboratory where they were sieved and placed in sediment trays for each mesocosm tank. A pre-dose sediment aliquot was collected prior to dosing in order to achieve baseline quantitative results. Sediment samples were collected in pre-cleaned 118 mL jars at t=0, t=24 h, t=7 d, t=14 d, t=21 d, t=28 d time points (6 total), throughout the course of each dosing experiment. Since each mesocosm was dosed in the same manner, all time points were composite sediment collections with each passive PSD treatment except for the samples on day 14 where all mesocosms were sampled individually. This was to assess quantitatively whether all other treatments needed to be composited within each time point, or collected individually in the interest of time and materials costs used moving forward throughout the experiment. Once collected, samples were transported back to the laboratory and stored in a -40 °C freezer awaiting chemical extraction protocols.

Sediment extraction was accomplished using verified and validated protocols (CCR-004 and CCR-005) using Accelerated Solvent Extraction (Dionex ASE 200). Samples were weighed out individually and wet weights were recorded in grams out to two decimal places for accuracy. An aliquot of each sample was also removed from each jar, weighed, recorded, and baked in an oven at 105 °C overnight for dry weight determinations. The sediments were then added to mortar bowls along with a drying agent (sodium sulfate), and ground on the bench top where they were set aside to dry. Once dried, the samples were poured into individual ASE cells and internal standards (13C12-Triclosan, d10-Pyrene, d5-Bifenthrin) were added tracking analyte recovery losses throughout the extraction process. The sediments were extracted on an ASE 200 (Accelerated Solvent Extraction) instrument. Once extracted, the samples were treated with copper for 24 h to remove any sulfur, and filtered through sodium sulfate to remove any residual water that may have remained in the extracts. Samples were then concentrated, solvent exchanged into hexane, and transferred into autosampler vials for instrument analysis.

Grab Samples

Grab Samples were collected at six different time points over the course of a month for each dosing study (t=0, t=24 h, t=7 d, t=14 d, t=21 d, t=28 d). Samples were collected in pre-cleaned, solvent rinsed, 1 L glass amber bottles and labeled appropriately. Water levels in the bottles were also marked for later volume measurements. The grab samples were immediately brought back to the laboratory where they were acidified with an 18% HCL solution with a pH of 2. 50 mL of methylene chloride was also added to each sample bottle as well. These steps were necessary to protect the integrity of each sample against degradation over time. Samples were then stored at +4 °C until extractions were performed on them at a later date.

A liquid / liquid extraction method was selected as the best option for extracting these samples while yielding higher recoveries for the analytes of interest. Each sample was poured into a 1 L

separation funnel. Carbon labeled and deuterated internal standards (13C12-Triclosan, d10-Pyrene, d5-Bifenthrin) were added to the grab samples at the beginning of the extraction process to track recovery losses over time. Methylene chloride (50 mL) was then added to each funnel. The separatory funnels were shaken and vented for 1 minute and returned to their original ring stands. A phase separation between the water fraction and methylene chloride fraction was observed after a few minutes with the water fraction settling on top, and the methylene chloride fraction settling on the bottom of the separatory funnel. The methylene chloride fraction was then collected into a pre-cleaned 500 mL bottles and set aside. This process was repeated two more times for a total of three extractions per sample. This ensured that all compounds were captured in the organic fraction. All three fractions were combined into one 500 mL bottle for each sample. Sample extracts were then filtered through sodium sulfate into a 200 mL TurboVap tube to remove any residual water. The extracts were concentrated, solvent exchanged into hexane, and transferred into autosampler vials for instrumental analysis.

CLAM HLB Samples

CLAM samples were deployed once each week in the mesocosm, 8 h at a time, for six different time points throughout the course of each dosing study. A detailed method and protocol pertaining to CLAM sample deployment and functionality are described in EPA method 3535a (Campisano et al., 2017). Each CLAM unit consisted of a battery powered pump, a (GF8) pre-filter, an HLB filter, and a counter for tracking volume throughput of water passing through the filters over time. Flow rates were determined using conversion tables provided by the manufacturer (Aqualytical Inc.) CLAM units were collected at each time point by trained laboratory personnel and the date, time, and counter number of each unit was recorded. The pre-filters and HLB filters were then separated from the units and placed into individual labeled Mylar bags and stored in a cooler for transport to the laboratory.

Detailed extraction protocols and methods for the CLAM samples were determined by the manufacturer (Aqualytical Inc. 2019). Samples were spiked with internal standards to track recovery losses of individual analytes throughout the extraction process. Pre-filters and HLB filters were conditioned and extracted using a combination of organic solvents pulled through the filters under vacuum using vacuum manifolds and collected into beakers. A liquid/liquid extraction was then performed on the samples to separate the methanol fraction from the dichloromethane fraction. The dichloromethane fraction was collected, and this procedure was repeated two more times for a total of three extractions per sample. Samples were then filtered through sodium sulfate to remove any residual water, concentrated, solvent exchanged into hexane, and transferred to autosampler vials for instrumental analysis.

POCIS Samples

POCIS samples were deployed at the beginning of each dosing study and were left in the mesocosm tanks throughout the duration of the study (28 d/dosing experiment). Samples consisted of a HLB sorbent, for capturing contaminates in the water column, encased on both sides by a flow-through filter membrane. Filter membranes were secured using stainless steel housing attached and anchored to a larger stainless steel unit to keep the samples stationary for the duration of the dosing experiment (Alvarez, 2010). Samples were then collected on t = 28 d by trained laboratory personnel. The stainless units were then disassembled, and the filter membranes were individually wrapped in foil, stored in coolers, and transported to the lab in preparation for extractions.

An SPE extraction method was selected for working with POCIS samples during the extraction process. Filter membranes were detached from their stainless steel housing, and pulled apart exposing the HLB sorbent. A pre-cleaned spatula was used to scrape the sorbent away from the filter membranes and into sterile 6-mL glass columns lined with 20 µm Teflon frits (Biotage, 2019). Columns were then installed onto a vacuum manifold where they were extracted with a combination of organic solvents to separate and capture the contaminants of interest from non-target contaminants. Extracts were collected into 50-mL TurboVap tubes (Biotage, 2019) and concentrated down to 0.5 mL where they were solvent exchanged into hexane and transferred to autosampler vials for instrumental analysis.

SB Samples (Silicone Bands)

Silicone Band samples are a relatively new technology that is a low cost inexpensive way to quantitate targeted contaminants in the environment. These bands were pre-conditioned in the laboratory prior to deployment in the mesocosm. Once deployed at t = 0 h, the silicone bands were left in the mesocosm tanks for the duration of each dosing study and collected at t = 28 d which were similar to the POCIS samples. Once collected, the bands were individually wrapped in foil, and placed in a cooler for transport to the laboratory for extraction purposes.

The extraction method followed methods reported by USGS (Swanson et al., 2018). Silicone Bands were taken out of their initial foil wraps and rinsed with DI water into a waste container to remove any bioaccumulation that may have occurred during the totality of the dosing study. They were then dried under a steady stream of nitrogen, placed in a beaker of ethyl acetate, which in turn was placed on a shaker table where they were extracted over a period of 4 to 5 hours being stirred and shaken vigorously. The beakers were then decanted into a 200 mL TurboVap tube (Biotage Inc.), and the process was repeated once more. Samples were concentrated, solvent exchanged into hexane, and transferred to autosampler vials for instrumental analysis.

Chemical Analysis

All extracted samples were analyzed using an Agilent 6890 Gas Chromatograph coupled with a 5973 Mass Selective Detector in EI (Electron Ionization) mode. Instrument parameters generally followed protocols detailed in CCR-043. Briefly, the inlet parameters included a Gerstel PTV Inlet (Programmable Temperature Vaporization Inlet) equipped with a Large Volume Injector (LVI) and a baffled quartz inlet liner. An inlet temperature ramp was used to focus all analytes onto the head of the column during sample acquisition. An Agilent J&W HLB 30m x 0.25um x 0.25mm capillary column operated in constant flow mode with a flow rate of 1.1mL/min was used with helium being the carrier gas of choice. All analytes were acquired in SIM mode (Selective Ion Monitoring) during mass spectrometer operation.

Data Analysis

The agreement between discrete and CLAM sample concentrations were compared by performing a concordance correlation (Lin, 1989 and Zar, 1999) in Microsoft Excel (version 2016). Briefly, the CLAM concentrations (Y-axis) were plotted against the discrete sample concentrations (x-axis) along with a line showing an idealized "perfect agreement" of the two datasets. Using the equation found in Zar (1999), the concordance correlation coefficient [r_c], which is a modification of the Pearson Correlation Coefficient [r], was calculated for each analyte. Theoretically, r_c can range from -1 to 1, but in all likelihood the values will be positive for almost all cases. An r_c=1 would indicate perfect agreement between the two methods. The absolute value of r_c will never be greater than r. CLAM data were also used to calculate loss constants using chemical degradation kinetics models (U.S. EPA, 2015). Chemical mass data from SB and POCIS samples are qualitatively reviewed in this report as specific coefficients and sampling rates are sought. Additional data is being collected in order to estimate a mesocosm system mass balance for each chemical.

Results

Comparison of CLAM and Discrete Water samples

Based on the fact that the CLAM units are capable of actively recording a measured volume, determining a TWA concentration is directly related to the mass of chemical extracted and measured at each time point and is most directly comparable to concentrations determined from discrete grab samples. For each chemical, plots of time versus chemical concentration resulted in first order loss curves at both Low and High dose treatments. First order degradation follows:

$$\text{(equation 2) } C_0 = C_t * e^{-kt}$$

where C_0 represents the initial concentration, C_t represents the concentration at a given time, t is time and -k is the rate of loss.

Figures 4, 5 and 6 display the decreasing concentrations for triclosan, pyrene and bifenthrin, respectively. Table 1 shows the rate (loss) constant and general fit for the exponential curve (R^2) for each chemical and by water and CLAM. The range of rate loss (k) for aqueous concentrations ranged from -0.13 to -0.278 (except for discrete sampling for bifenthrin where concentrations were found to be less than the minimum detectable concentrations). The average rate loss (k; omitting the bifenthrin NC data) was determined to be -0.202 with an average R^2 of 0.74. Based on these consistent results (by evaluating the % Relative Standard Deviation for k (24.5%) and R2 (11.7%), we determined the concordance between CLAM and discrete concentrations (Figure 7, 8 and 9). The concordance (r_c; or an estimate of the agreement between data plotted on the x and y axis) analysis was determined to be 0.967 for triclosan, 0.884 for bifenthrin and 0.841 for pyrene. Observationally, while these data sets show excellent concordance, these data are slightly biased toward the grab sampling data.

Comparison of Silicone Bands and POCIS

The remaining two PSD are more diffusive and integrative over 28 days. In order to estimate TWA concentrations using either SB or POCIS results, a rate constant must be calculated for each contaminant under the relative environmental conditions found during exposure. While there are general rate constants that are occasionally used for this conversion, using a standard rate constant in these closed and replicated systems would not change the relative relationships between the silicone bands and the POCIS. Therefore, we examined SB and POCIS data on total extracted mass from each sampling replicate. Realizing that the differences in Low versus High dose were 2x (for triclosan) and 4x (for bifenthrin and pyrene); neither diffusive integrating device (SB or POCIS) matched the expected increase in dose. Differences in dose for triclosan were minimal at best, with ratios of High/Low masses resulting in 0.8 and 0.9 for SB and POCIS respectively. Ratios describing the High/Low relationship for bifenthrin were better where results were 1.8 (SB) and 2.5 (POCIS). Ratios for pyrene were 2.7 (SB) and 1.7 (POCIS).

Sediment as a sink for contaminants

In addition to aqueous measurements of contaminants, sediment is considered as a sink for many organic pollutants. Building on the model of diffusive binding, relationships of sediment and water chemistry have been documented (i.e. Equilibrium Partitioning Theory, EqPT). DiToro et al. (1991) initially described EqPT in terms of estimating sediment quality criteria for nonionic organic chemicals and has been used extensively to estimate both aqueous and organismal chemical concentrations (Spacie, McCarty and Rand, 1995 as an introduction and Lee, et al. 1990; Landrum et al 1992 and DeWitt et al., 1992 as examples). Table 3 summarizes average

chemical concentrations for the three contaminants. No detectable concentrations for each of the contaminants was found prior to dosing. Within 8 hours (0.33days) signatures for each chemical was measurable in sediment samples and based on this sampling design, peaked on day 7. After day 7; sediment concentrations decreased. There was an approximate 2X fold increase in triclosan concentrations after 0.33 days and pyrene concentrations were observed at ~4x. Sediment concentrations for bifenthrin were roughly equal between "Low" and "High" dose.

Discussion

As the data is presented here, it is difficult to gain a holistic view of these sampling platforms but we are able to evaluate and highlight observations for each PDS. Grab samples are ideal for evaluating time specific concentrations but may miss situations where pulses of higher concentrations (such as a post rain event) may be present. Historically, discrete sampling is considered the standard for environmental water analysis. In this effort, the results of discrete sampling after dosing each mesocosm system generally confirmed the anticipated nominal dose. Average chemical concentrations were measured for triclosan, bifenthrin and pyrene were calculated from sampler collected 30 minutes after dosing (to allow for some mixing) and the respective measured average concentrations for the low dose were 100.4, 0.013, 1.5 µg/L respectively (relative to nominal concentrations of 100, 0.02 and 2.2 µg/L). The measured concentrations from discrete samples also affirmed the nominal concentrations for the high dose; where triclosan was measured at 349 µg/L (nominal ~200µg/L), bifenthrin was measured at 0.08 µg/L (nominal ~0.08 µg/L) and pyrene was measured at 15.3 µg/L (nominal ~8.8 µg/L).

The most directly comparable PSD platform used in this study was the CLAM. These HLB-based platforms that also measure pumped volume across the sampler matrix allows for a direct comparison with data collected from discrete samples since the variables of time and volume extracted are known. The concordance among all three tested chemicals in this study ranged from 0.841 (pyrene) to 0.967 (triclosan), indicating excellent agreement between sampling protocols (Figures 7, 8, and 9, respectively) although the concordance for bifenthrin may have been limited by the number of samples where the concentration was not detected. While this study only examined three chemicals, it should be noted that the agreement between grab and CLAM concentrations for these three chemicals generally decreases as a function of concentration. *It can be easily concluded that a CLAM unit may be appropriate for monitoring marine and estuarine water concentrations in areas where discrete sampling is difficult, from a field-logistics or sample transport perspective.*

Results from POCIS and SB deployments are less quantifiable in this experimental design. Valid estimation of water concentrations are nearly impossible without having direct laboratory assessments of sampling rate (R_s) and partition coefficients (K_{sw}) for triclosan, bifenthrin or pyrene under similar environmental conditions. It is therefore inappropriate to compare these

PSD results in this study to concentrations from discretely collected water samples. However, there are several useful observations to note. First, all three chemicals were detected on both the POCIS and SB. In this study, the log K_{ow} of chemicals ranged from 4.79 to 6.0, targeting more hydrophobic chemicals. POCIS (using HLB) sampling targets log K_{ow} values less than 5 but there are reports that the PES membrane that encases the HLB media in POCIS has an affinity for compounds with a log K_{ow} greater than 5 (Silvani et al., 2017). Additionally, observations of triclosan in both POCIS and SB did not result in increases in chemical mass between the low and high dose. It was expected that there would be a general increase in chemical mass with increasing dose and this trend was noticed for both bifenthrin (~ a 2-3 fold increase) and pyrene (~ a 1.5-2.5 fold increase) (Table 2). The observation with triclosan may be related to the relatively high target concentrations (100 and 200 µg/L) compared to the other chemicals (all nominal targets were less than 10 µg/L) and long deployment time (28 days). Triclosan may have overwhelmed the capacity of both SB and POCIS samplers. From this data it is not surprising that POCIS can be effectively used to determine the presence of chemicals in aqueous systems since these units have been used in aqueous monitoring studies for over a decade. (Alvarez et al., 2009). It is exciting, although maybe not surprising, that SBs were able to detect all three compounds and helps to justify using these extremely inexpensive units as a screen for a wide range of chemical contaminants. While there are models and reports where both POCIS and SB PSDs generate quantitative data, the need for NOAA to continue examining laboratory and mesocosm level calibration studies to determine R_s and K_{sw} coefficients is evident.

Data Availability

All of the raw chemistry data for this project is located in the Chemistry Information Management System, also known as ChemLIMS, which is an NCCOS administrative application, housed on the Microsoft Azure Cloud. The ChemLIMS database is only accessible to NCCOS personnel, and is maintained by the NCCOS Web and App support team. Photographs taken throughout the duration of the study are located in both the NCCOS shared "projects" drive, under the CCR swap folder as well as on the Google share drive. Access to both of these locations is restricted to NCCOS personnel.

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Figures and Tables

Figure 1: Practical relationships of passive air monitoring sampling (from Zabiegala et al., 2010) that builds the foundation for using PSDs in environmental matrices such as water and sediment.

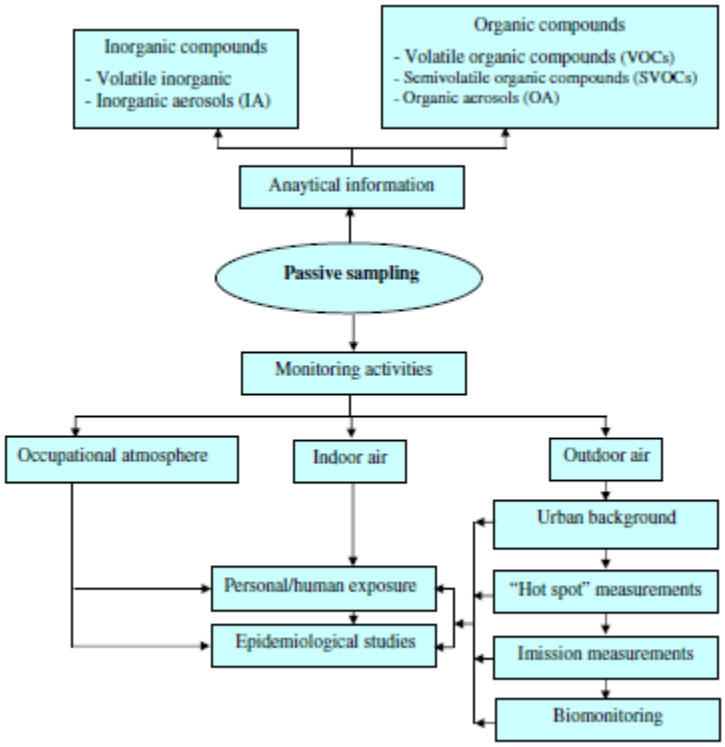


Figure 2: The general relationship that drives the mathematical model and equations supporting environmental monitoring of chemicals using PSDs (from Stuer-Lauridsen, 2005).

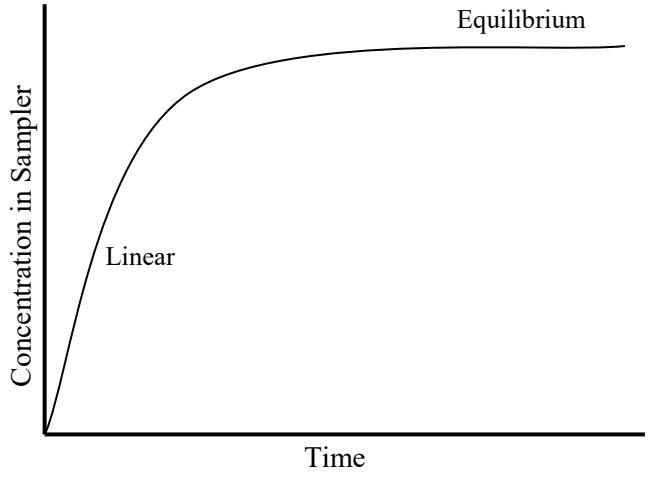
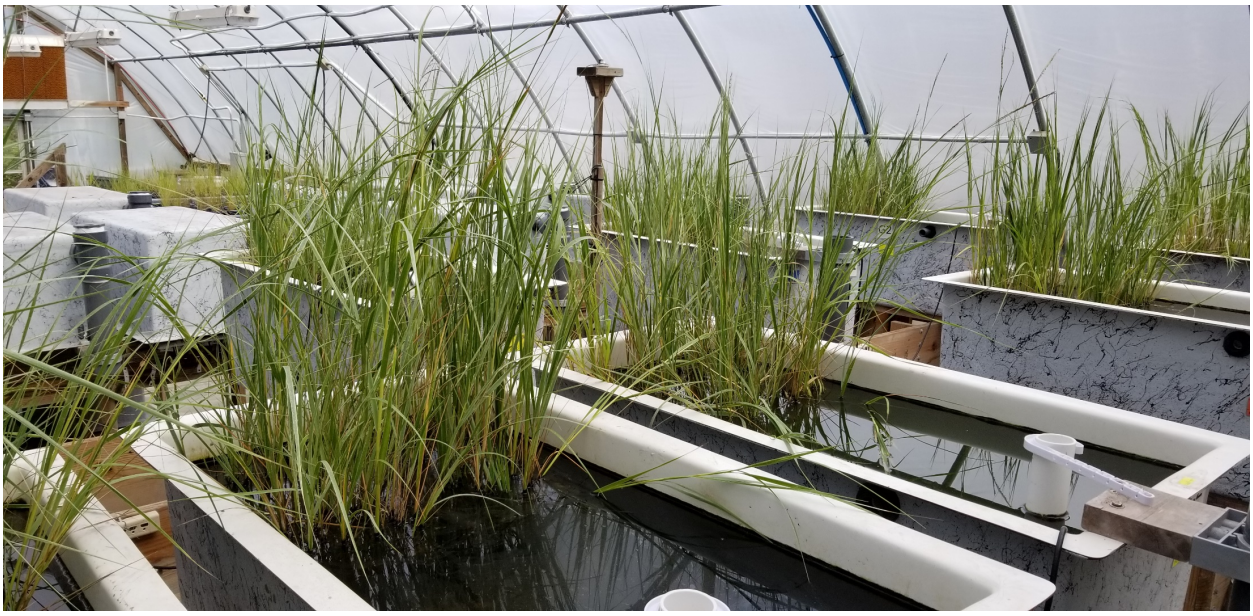


Figure 3a: Mesocosm facility and 3b: Experimental Design

a) 

b)

CLAM #1	POCIS #1	SB #1
CLAM #2	POCIS #2	SB #2
CLAM #3	POCIS #3	SB #3

(a discrete grab sample of water and sediment from all 9 mesocosm systems)

Table 1: Y intercept (C_0), rate constants (k) and R^2 fitting the exponential rate degradation equation (equation 2) for each chemical and dose (NC= not calculated; most of the data was below detection limits).

Chemical	**Dose**	**Platform**	**C_0 / Y-intercept**	**Rate Constant (k)**	**R^2**
Triclosan	*Low*	*Grab*	39.386	-0.263	0.88
	High	*Grab*	49.696	-0.278	0.72
	Low	*CLAM*	12.211	-0.213	0.88
	High	*CLAM*	11.55	-0.237	0.61
Bifenthrin	*Low*	*Grab*	NC	NC	NC
	High	*Grab*	NC	NC	NC
	Low	*CLAM*	0.0021	-0.13	0.70
	High	*CLAM*	0.0183	-0.15	0.78
Pyrene	*Low*	*Grab*	0.3417	-0.157	0.69
	High	*Grab*	2.3377	-0.215	0.74
	Low	*CLAM*	0.1405	-0.17	0.67
	High	*CLAM*	0.8656	-0.203	0.73

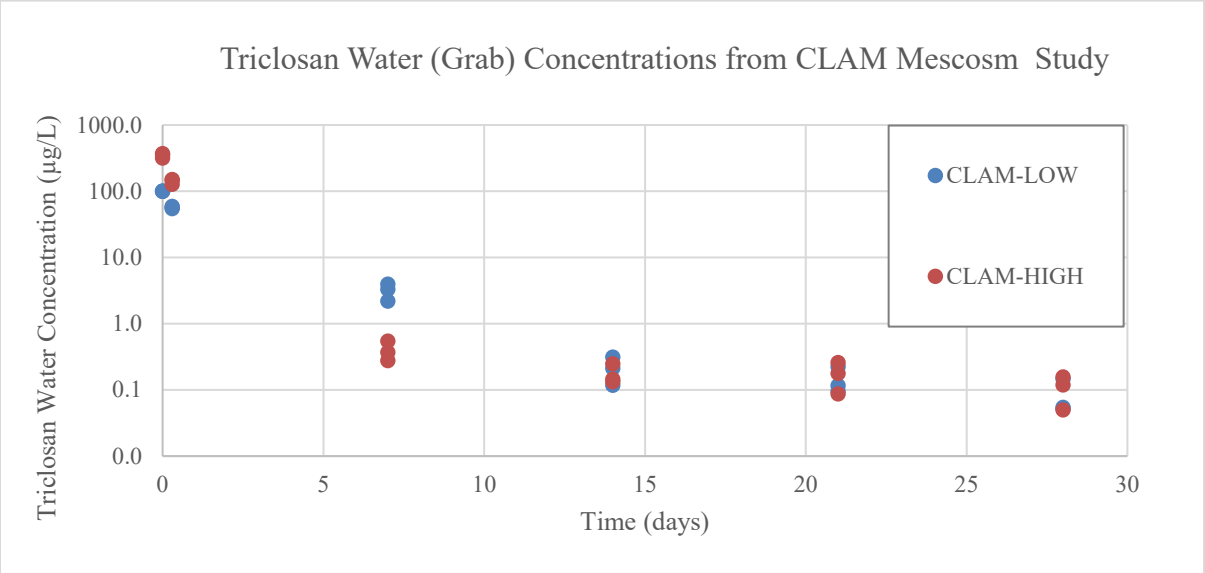
Table 2. Extracted masses of chemicals by both SB and POCIS samplers after 28 days in the mesocosm (AVG = average of three replicates; SD = standard deviation).

			SB		***POCIS***	
		Log K_{ow}	***AVG (ng)***	***SD***	***AVG (ng)***	***SD***
Low Dose	*Triclosan*	*4.79*	*670.9*	*22.5*	*34495.4*	*5704.8*
	Pyrene	*4.88*	*2266.5*	*631.8*	*312.2*	*247.8*
	Bifenthrin	*6.0*	*15.6*	*0.7*	*1.6*	*0.3*
High Dose	*Triclosan*	*4.79*	*553.0*	*83.2*	*32657.0*	*858.9*
	Pyrene	*4.88*	*6185.9*	*450.3*	*517.0*	*76.7*
	Bifenthrin	*6.0*	*28.5*	*5.4*	*4.0*	*0.5*

Table 3. Summary of sediment concentrations for "Low" and High" dose treatments. The high dose was 2x the low dose for bifenthrin and pyrene when the difference was 4x in initial nominal concentrations, whereas the triclosan high dose was ~2x the "low" dose (as nominally dosed).

Sediment concentration (ng/g dry)							
		Triclosan		**Bifenthrin**		**Pyrene**	
Time (d)	**Dose**	**Average**	**Standard Deviation**	**Average**	**Standard Deviation**	**Average**	**Standard Deviation**
0	*LOW*						
	HIGH						
0.33	*LOW*	381	34.9	0	0	21.2	1.23
	HIGH	858	28.9	0.194	0.173	79.5	5.04
7	*LOW*	278	70.9	0.135	0.405	16.8	5.17
	HIGH	376	132	0.138	0.136	44.0	9.83
14	*LOW*	155	1.32	0	0	14.7	4.60
	HIGH	261	90.7	0	0	28.1	7.61
21	*LOW*	92.9	3.03	0	0	12.8	2.81
	HIGH	171	60.3	0	0	23.7	5.44
28	*LOW*	61.2	12.2	0	0	11.3	0.872
	HIGH	138	39.1	0	0	20.0	2.43

Figure 4: Changes in Triclosan concentrations as measured in water and HLB media from the CLAM unit.



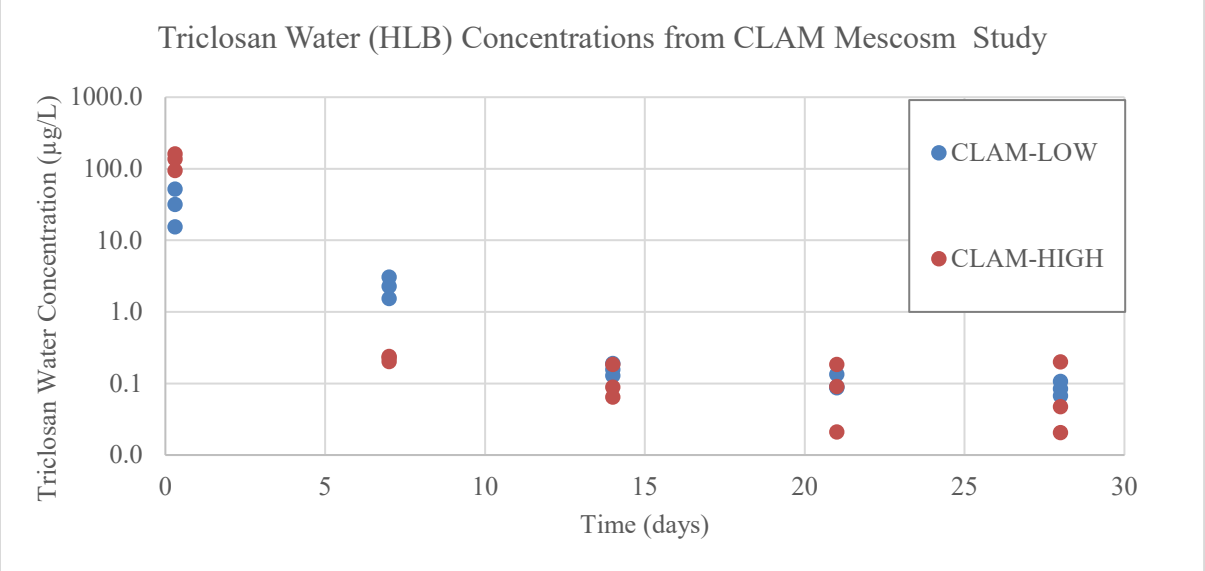
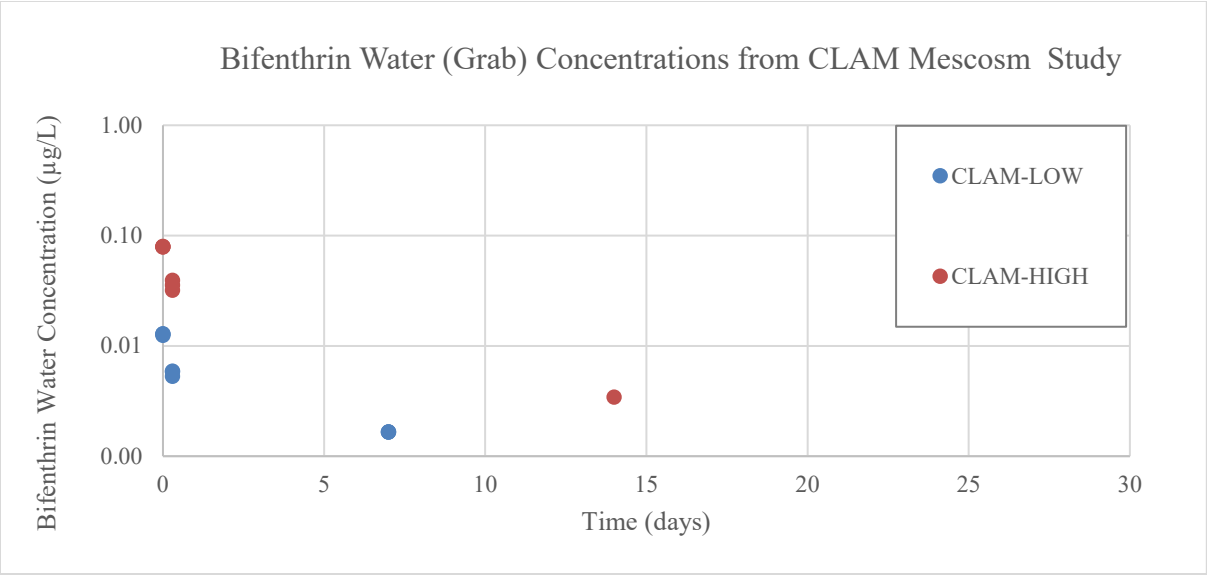


Figure 5: Changes in Bifenthrin concentrations as measured in water and HLB media from the CLAM unit.



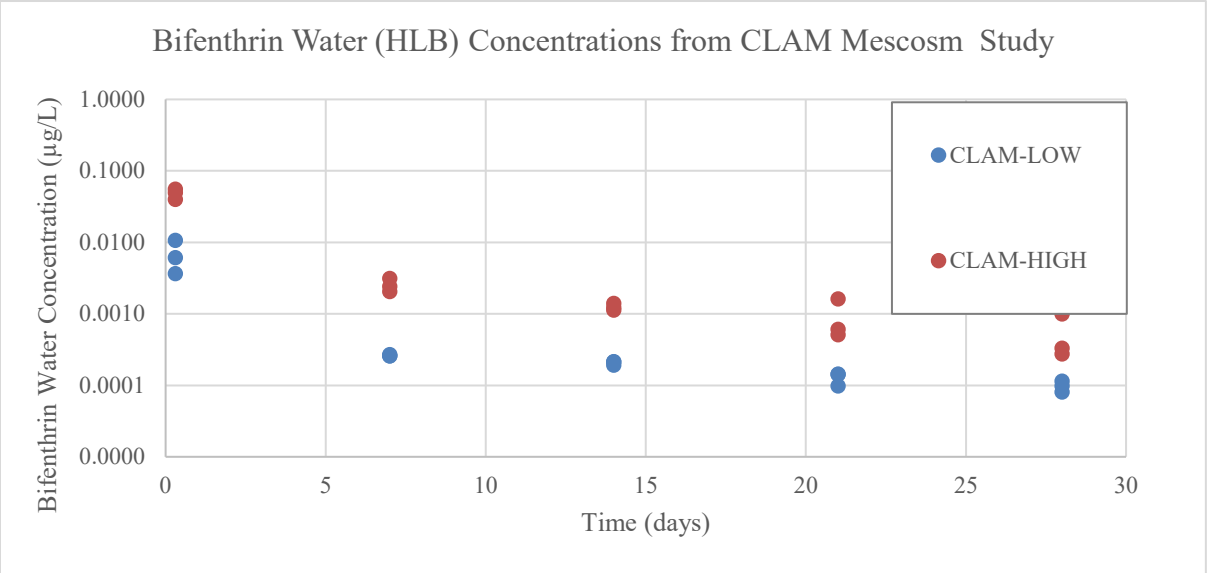
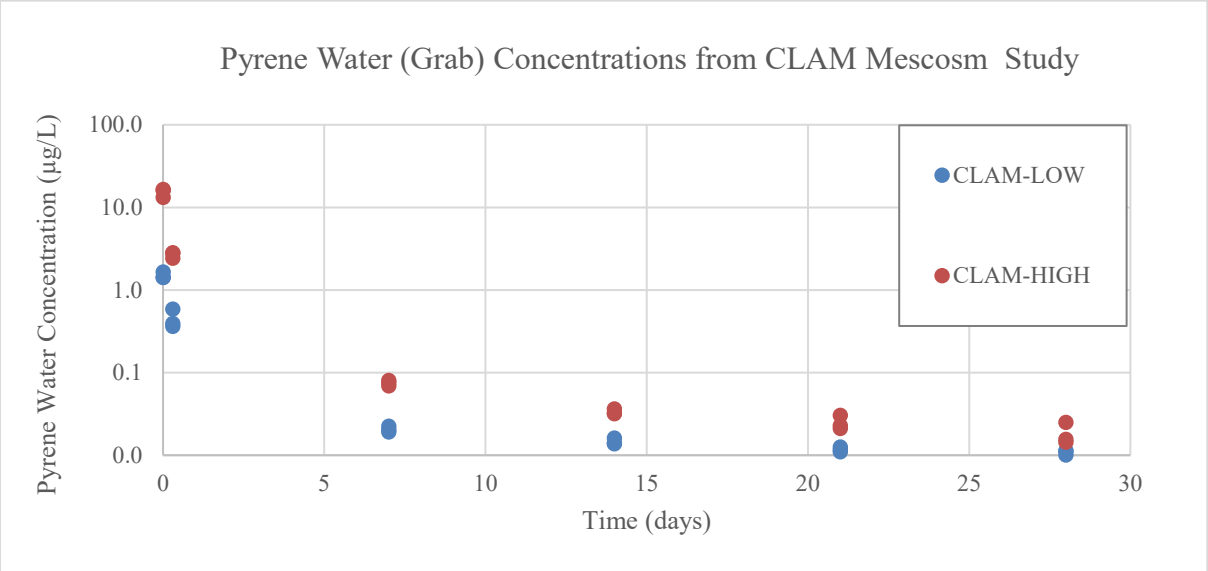


Figure 6: Changes in Pyrene concentrations as measured in water and HLB media from the CLAM unit.



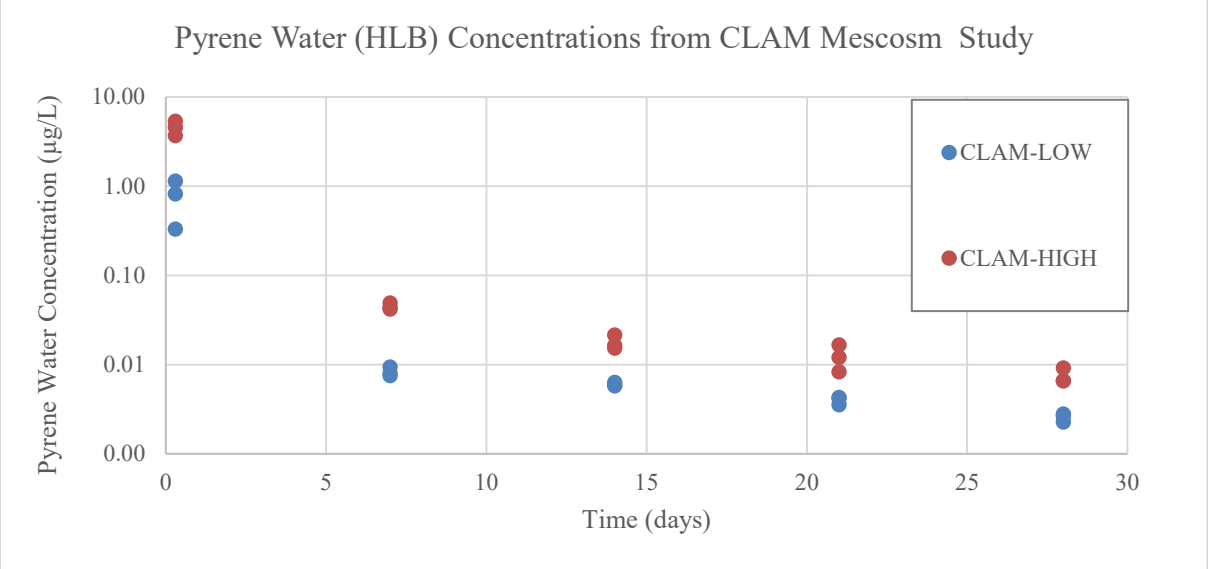


Figure 7. Concordance of triclosan concentrations between discrete and CLAM sampling.

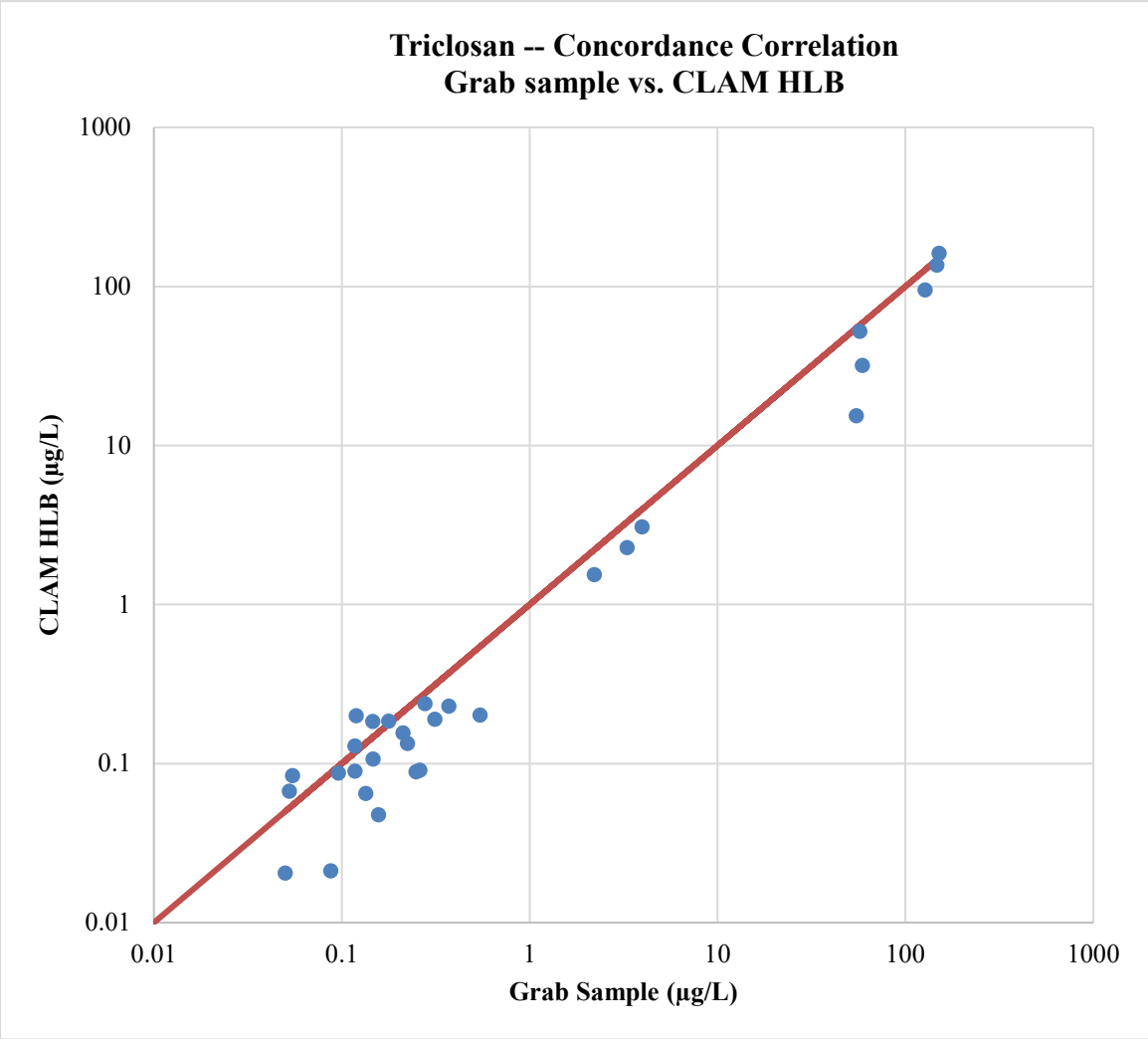


Figure 8. Concordance of bifenthrin concentrations between discrete and CLAM sampling.

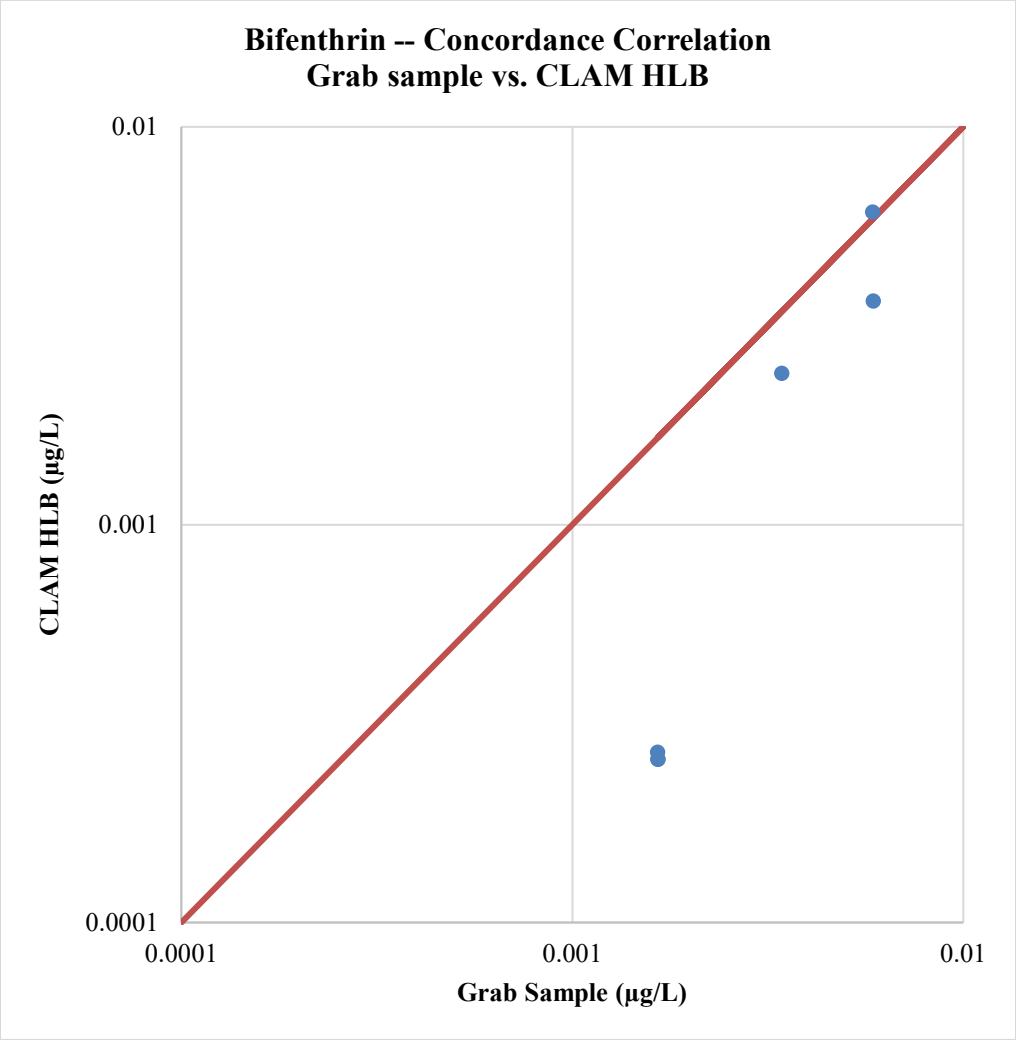
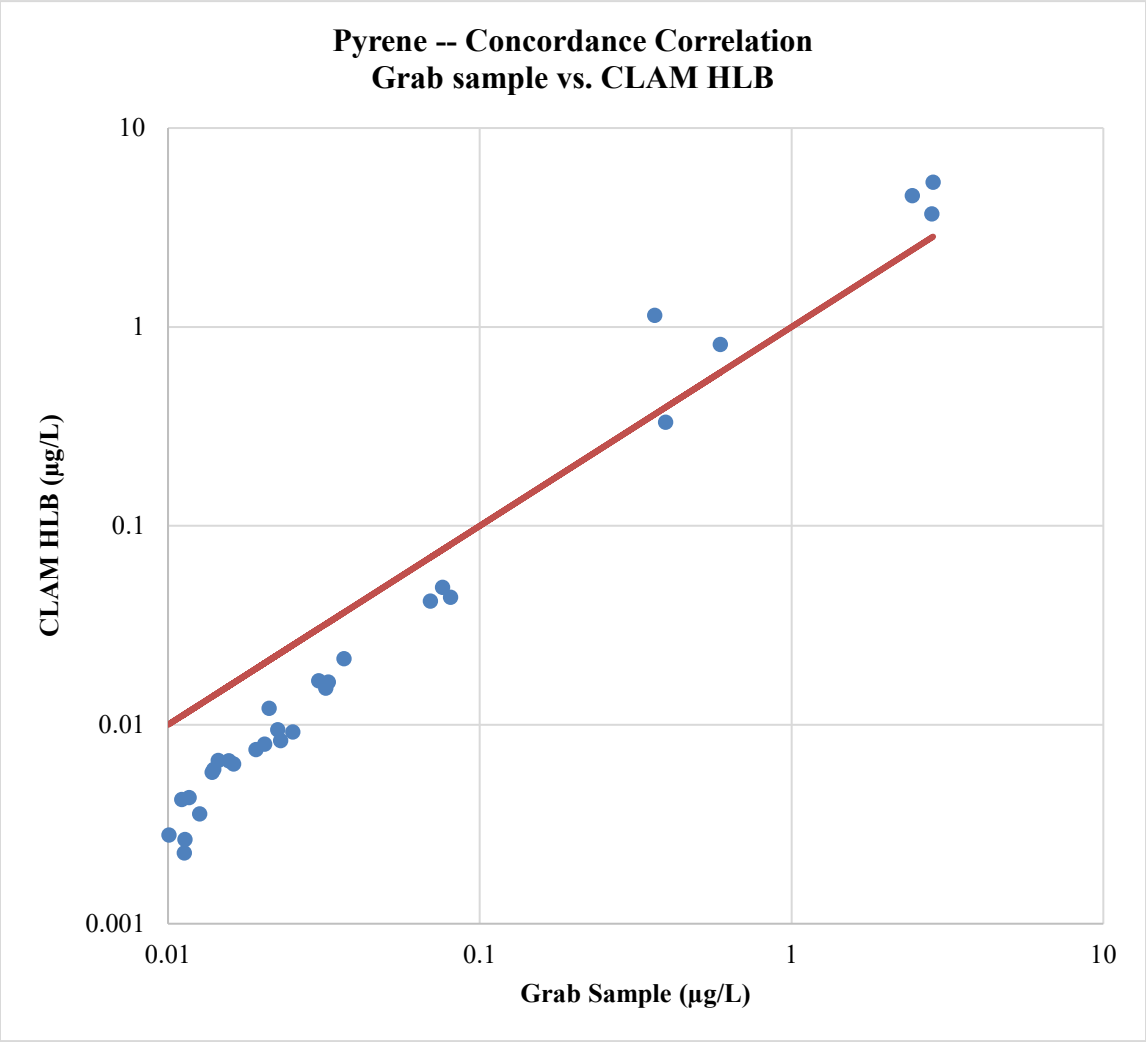


Figure 9. Concordance of pyrene concentrations between discrete and CLAM sampling.



Appendix 1. List of selected internal Ecotoxicology Branch standard operating procedures (SOP) used in this study for the chemical analysis of water and sediment samples. SOPs are located at within an internal shared directory and are available by request.

Task Category	SOP ID	SOP Title
Sample Handling and extraction	CCR-002	Organic glassware cleaning
	CCR-046	Cleaning of ASE Cells
	CCR-075	Preparation of Passive Sampler Devices for Deployment
	CCR-047	Sample receipt
	CCR-004	Dionex ASE 200 Extraction
	CCR-005	ASE extract filtering
	CCR-006	TurboVap II Workstation
	CCR-044	Combustion cleaning of sodium sulfate for ASE extraction
	CCR-010	Alumina SPE Clean-up
Instrumental Analysis	CCR-070	GC/MS Basic Maintenance
	CCR-043	Analysis of Persistent Organic Pollutants by GS/MS
	CCR-041	Integration of sample chromatograms
	CCR-042	Integration of procedural blanks for MDL calculation
Data Analysis	CCR-035	SOG Data guidelines v2
	CCR-061	AZURE ChemLIMS Database

Title: Long term monitoring data for environmental assessments: SC Estuarine and Coastal Assessment Program and California Bight - 2018 Channel Islands National Marine Sanctuary Regional Assessments

Edward F. Wirth[1], Emily Pisarski[2], Lou Ann Reed[1], Brian Shaddrix[2], and Dennis A. Apeti[2]

[1] NOAA/NOS/NCCOS; Stressor Detection and Impacts Division
[2] CSS Inc., under contract to NOAA/NOS/NCCOS; Stressor Detection and Impacts Division

Abstract

The Chemical Contaminants Research (CCR) group has a long history of providing chemistry data for trace metals and persistent organic pollutants (POPs) to regional monitoring programs such as the South Carolina Estuarine and Coastal Assessment Program (SCECAP) and the National Status and Trends Mussel Watch Program. SCECAP uses our chemistry data in conjunction with other types of data (e.g., water quality, biological indices) to assess the overall health of an area. The goal of this report is to take sediment quality guidelines, such as the effects range low (ERL) and effects range median (ERM), which are used in SCECAP assessments, and apply those tools to a set of data that we analyzed for the Southern California Coastal Water Research Project Bight 2018 assessment. In this study, we analyzed 15 sediment samples from the Channel Islands National Marine Sanctuary for trace metals, POPs, and contaminants of emerging concern (CECs) and compared the ERL/ERM results from 2018 to results from the 2008 Bight assessment to understand if and how conditions have changed over the last ten years. In 2018 samples, ten sites exceeded the ERL for total DDTs, eight sites exceeded the ERL for p,p'-DDE, and the ERL was exceeded for nickel, cadmium, and mercury at one site apiece. CECs were also detected at CINMS sites, but there are no ERL/ERM criteria available. When taking paired amphipod toxicity tests into account, all sites were designated as "not toxic" (Parks et al. 2020).

Introduction

The nation's extensive coastal zone supports an abundance of natural resources; it is enjoyed by residents and tourists alike. Tourism, coastal recreational fishing, and commercial fisheries all contribute to the economic value of this natural resource. These estuarine areas serve as nursery or primary habitat for fishery and ecotourism wildlife resources but are sensitive to anthropogenic impacts. In many coastal areas, the population has rapidly increased in recent years and is expected to continue to do so. With the increase in population density comes an expansion of infrastructure and an increase in recreational use of coastal waters. This may result in risk for impact to coastal habitats.

Thus, it is important to protect these coastal habitats from degradation. Monitoring and assessment programs like the Southern California Coastal Water Research Project (SCCWRP), the South Carolina Estuarine and Coastal Assessment Program (SCECAP), and the National Status and Trends (NS&T) program provide temporal data and can document changes to these areas. These programs provide a mechanism for detecting and addressing human impacts to our valued coastal resources.

SCECAP is a collaboration between the South Carolina Department of Natural Resources (SCDNR), South Carolina Department of Health and Environmental Control (SCDHEC), and the National Oceanic and Atmospheric Administration (NOAA). The SCECAP program has collected legacy contaminant data for South Carolina ecosystem assessment for each year since 1999. Water quality, sediment quality, and biological condition indices are compiled to calculate an integrated overall Habitat Quality Index. Stations with good, fair, or poor scores for the Habitat Quality Index are mapped. With 20 years of data available now, factors like coastal development, impervious cover, precipitation, drought, and severity of winter weather are being investigated to determine impact on estuarine environmental quality. SCECAP data is being used in a national effort led by the United States Environmental Protection Agency (USEPA) to

develop a national benthic index with species abundance tied directly to sediment contaminant data, which can be used to evaluate pollution sensitivity of various species.

The Southern California Coastal Water Research Project (SCCWRP) is a public research and development agency that develops and applies science to improve management of aquatic systems. Information on the status and trends of the sediment quality is critical to effective management of resources of the Channel Islands National Marine Sanctuary (CINMS). SCCWRP was founded in 1969 and has been developing strategies, tools and technologies for protecting and enhancing the ecological health of Southern California's coastal ocean and watersheds.

The NS&T program, which began in 1984, has three program areas: Benthic Surveillance, Mussel Watch, and Bioeffects. Broadly speaking, these programs assess sediment and water quality by measuring chemical contaminants in sediments and aquatic organisms (e.g., bivalves, benthic fish), as well as measuring any associated biological effects due to water/sediment quality (Gottholm et al. 1993). Historically, programs within NS&T have focused on legacy contaminants (e.g., trace metals, persistent organic pollutants (POPs)), however, advances in analytical chemistry and the development of new chemicals and their subsequent release into the environment, have led programs, such as Mussel Watch, to start including contaminants of emerging concern (CECs) as part of their analyses. Benthic assessments within NS&T have used sediment quality guidelines to better understand how measured sediment chemistry could induce potential adverse biological effects and identify potential areas of concern (Balthis et al. 2015; Balthis et al. 2018). Long and Morgan (1990) developed informal guidelines to use in sediment quality assessments which include an effects range low (ERL) and an effects range median (ERM) for nine trace metals, total polychlorinated biphenyls (PCBs), low molecular weight polycyclic aromatic hydrocarbons (LMW PAH), high molecular weight PAHs (HMW PAHs) and total PAHs, and two pesticides (Tables 1-2) (Long et al. 1995). The ERL and ERM are used to determine if the measured sediment concentrations would rarely be associated with adverse effects (<ERL), occasionally be associated with adverse effects (>ERL, <ERM), or frequently be associated with adverse effects (>ERM) (Long et al. 1995). While ERL and ERM values are based on single analytes, these values have been used to calculate mean ERM quotients (mERM-Q), which incorporate mixtures of these contaminants (Long et al. 1998), thus providing a more detailed analysis of measured sediment chemistry.

The Chemical Contaminants Research (CCR) group has a history of providing chemical data to all of these programs for both organic and inorganic analytes. Historically, our data contribution has centered around legacy contaminants, but as our client needs have shifted towards including CECs, we have increased our analytical capabilities to keep up with this demand.

The goal of this report is to demonstrate how the CCR group has an instrumental role in providing chemistry data to these long term monitoring programs and use sediment quality guideline tools (e.g., ERL, ERM, mERM-Q), to understand the health of an area. In this particular case study, we will focus on data that we provided to SCCWRP's Channel Island National Marine Sanctuary (CINMS) assessment that occurred in 2018. In this study, we analyzed fifteen sediment samples for a suite of trace metals, POPs, and CECs.

Methods

Experimental Design

Samples for this study were collected during SCCWRP's 2018 Southern California Bight Regional Monitoring Program. A subset of the samples collected, which are from the CINMS, were analyzed at the NOAA/NCCOS lab in Charleston, SC for trace metals, POPs, and CECs. Details on sample collection are

found in Parks et al. (2020). Figure 1 shows the locations of the fifteen samples collected from the CINMS.

Organic Chemical Analysis

A suite of POPs, which include polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), and organochlorine (OC) pesticides, and CECs, including alkylphenols (APs), alkylphenol ethoxylates (APEOs), fipronils, and pyrethroids were the organic compounds analyzed in this study (Table 3). The POP analytes of interest were extracted from sediment samples through Accelerated Solvent Extraction (ASE; ThermoFisher ASE 200). A ten gram aliquot of sample is dried with sodium sulfate, spiked with isotopically labeled internal standards (Table 4) and then extracted with 1:1 (v/v) acetone/dichloromethane. After extraction, samples are passed through additional sodium sulfate, and then cleaned up to remove matrix interferences by copper treatment, gel permeation chromatography (GPC; J2 Scientific) and alumina solid phase extraction.

Fipronil and pyrethroid compounds were extracted from ten gram aliquots spiked with a suite of isotopically labeled internal standards (Table 4) by ASE (100% dichloromethane), and were cleaned up using copper treatment, activated charcoal (Phenomenex CarboPrep) and alumina SPE.

APs and APEOs were extracted from sediments using ASE. Roughly two grams of sample was mixed with anhydrous sodium sulfate, spiked with isotopically labeled internal standards (Table 4), and extracted with 1:1 (v/v) acetone/hexane. Extracts were passed through additional anhydrous sodium sulfate and cleaned up using copper treatment and aminopropyl SPE (Supelco Supelclean LC-NH_2).

Sample extracts for these projects were analyzed using gas chromatography mass spectrometry (GC/MS; Agilent 6890/5973) or high performance liquid chromatography tandem mass spectrometry (LC-MS/MS; Agilent 1100/API 4000). Data for PCBs, PBDEs (except PBDE 209), DDT's, fipronils, and pyrethroids were acquired using a programmable temperature vaporizer (PTV) inlet, DB-XLB analytical column (Agilent; 30 m x 0.25 µm x 0.25 mm), and electron impact (EI) ionization and selected ion monitoring (SIM) modes. The remaining OC pesticides were run on a GC/MS containing a PTV, Rtx-5ms analytical column (Restek; 30 m x 0.25 µm x 0.25 mm), and negative chemical ionization (NCI) and SIM modes. Data for PBDE 209 was acquired using a split/splitless inlet, DB-5ms analytical column (Agilent; 15 m x 0.25 µm x 0.25 mm), and NCI and SIM modes. PAHs were acquired using a split/splitless inlet, DB-17ms analytical column (Agilent; 60 m x 0.25 µm x 0.25 mm), and EI and SIM modes. AP and APEOs were acquired using LC-MS/MS. A C18 analytical column (Waters XBridge; 2.5 µm, 2.1 x 50 mm), with a gradient mobile phase of either methanol and water (APs) or 10 mM ammonium acetate in methanol and 10 mM ammonium acetate in water (APEOs). Samples were run under negative electrospray ionization (ESI; APs) or positive ESI (APEOs). Instruments were calibrated with standards prior to running samples, and continuing verification standards (CCV) were run every 10-15 samples to ensure the validity of the calibration curves. All analytes had coefficients of determination (r^2 values) greater than or equal to 0.995. Data was analyzed using MSD Chemstation software (ver E.02.02.1431).

Inorganic Chemical Analysis

In preparation for inorganic elemental analysis, sediments are dried, ground and mixed well prior to microwave or hotblock digestion with ULTREXII ultrapure nitric acid in closed Teflon vessels. A Perkin Elmer (PE) Sciex ELAN DRCII Inductively Coupled Plasma Mass Spectrometer is used to measure isotopes of interest. A multiple element internal standard (^{45}Sc, ^{72}Ge, ^{103}Rh, ^{175}Lu) is added to each sample and calibration standard. Samples are diluted as necessary for bracketing by calibration curves.

For total Hg determination, sediments are not dried prior to analysis. Subsamples are accurately weighed into nickel boats for analysis with a Thermal Decomposition Amalgamation Atomic Absorption Spectrophotometer Direct Mercury Analyzer, the DMA-80.

Quality Assurance/Quality Control

A series of control solutions, certified standard reference materials, matrix spikes, reagent spikes and reagent blanks were run with each extraction. Standard laboratory data quality guidelines are applied to all CCR projects. Spiked analyte recoveries must be within 80-120% of the expected concentration for at least 80% of the analytes. Reagent blanks are used to calculate method detection limits (MDLs). Reagent blanks containing measurable quantities of POPs/CECs are subtracted from samples associated with that reagent blank. The MDLs for POPs/CECs were determined according to methods detailed in Ragland et al. (2013).

Results

Alkyl Phenols

There were no sediment concentrations for AP analytes measured above the MDL in any of the sampled sites. For the APEOs, NP1EO was detected in seven of the fifteen sampled locations. Concentrations ranged from 66.3-241.5 ng/g dw. Only one location had a concentration for NP2EO above the MDL, which was measured at 63.7 ng/g dw. As APs and APEOs are considered CECs, there are no sediment criteria for ERL/ERM that have been established yet.

DDTs

Sediment concentrations for DDT and their degradation products are found in Table 5. All sites had values below the MDL for 2,4'-DDD and 2,4'-DDT. Ten sites (i.e., 67%) had total DDT concentrations that exceeded the ERL (1.58 ng/g dry), but they did not exceed the ERM (46.1 ng/g dry) (Figure 2). Eight sediment samples (i.e., 53%) that exceeded the ERL for total DDTs also exceeded the ERL for p,p'-DDE (2.2 ng/g dry) (Figure 3). The average sediment concentration for sites that had total DDT (4,4'-DDMU not included) concentrations above the MDL was 3.94 ± 3.04 ng/g dw.

Metals

Of the 15 inorganic analytes (Table 6), there were published ERL and ERM values for nine of them: arsenic, cadmium, chromium, copper, lead, mercury, nickel, silver, and zinc. None of the sites had sediments exceeding the guidelines for arsenic, chromium, copper, lead, silver, or zinc. None of the sediments exceed the MDL for silver. Three sites, NOAA sample numbers 18-0710, 18-0711, and 18-0713 had concentrations of metals that exceeded the ERL for nickel, cadmium, and mercury, respectively (Figures 4-6).

PAHs

Sediment concentrations for PAHs that have ERL/ERM criteria were never exceeded at any of the CINMS sites. In fact, in most instances, PAHs that have ERL/ERM criteria were never measured in concentrations about the MDL. Total PAH50, which is the sum of 50 parent and alkylated PAHs, and a common way that PAH data is reported in the literature, was also calculated. PAH50 concentrations ranged from 3.4-116.9 ng/g dw (Table 7). The majority of PAHs that were most frequently detected were high molecular weight PAHs (i.e., PAHs with four or more rings).

PBDEs

PBDE 47 and 99 were the most frequently detected PBDEs, being detected in 86.7% and 73.3% of the sites, respectively (Table 8). Detectable concentrations of PBDE 47 ranged from 0.01-0.04 ng/g dw while detectable sediment concentrations of PBDE 99 ranged from 0.01-0.02 ng/g dw. Other detectable concentrations of PBDEs include PBDE 153, PBDE 183, and PBDE 209, however, these PBDEs were only detected at one (PBDE 153 and 183) and two (PBDE 209) sites. PBDE 17, PBDE 28, PBDE 66, PBDE 71, PBDE 85, PBDE 100, PBDE 138, PBDE 154, and PBDE 190 had no sites with sediment values exceeding the MDL. There are currently no ERL/ERM criteria for PBDE compounds.

PCBs

A suite of 100 PCB congeners, encompassing all ten homolog groups, were measured and summed for PCB analysis ($\sum PCB_{100}$; Table 9). Total PCB concentrations measured in sediments ranged from 0.07-2.17 ng/g dw. ERL criterion was not exceeded (ERL = 22.7 ng/g dw) for any of the sites sampled in this study. While there are 209 possible PCB congeners, which is what the ERL/ERM criteria is based upon, measuring all possible congeners is not necessary as environmental PCB contamination is almost always the result of a release of an Aroclor mixture, and some PCBs were never part of any Aroclor mixture, or their contribution to the Aroclor mixture is so low that they would not be measured above instrument detection limits (Battelle Memorial Institute et al. 2012). Studies have reported that measuring 80-120 PCB congeners yields an accurate representation of total PCB concentration (Battelle Memorial Institute et al. 2012). For example, a study in the Ashtabula River which looked at 117 PCB congeners was found to represent 97-98% of the total PCB concentration (Battelle Memorial Institute et al. 2012), thus it is appropriate for the CCR group to be able to make comparisons between the 100 PCBs we measure and the ERL/ERM values that incorporate all 209 congeners.

OC Pesticides

Of the nineteen measured OC pesticides, only three compounds were found to be above the MDL; those compounds were lindane, hexachlorobenzene, and γ-chlordane. Lindane was detected at seven of the fifteen sites (0.0027-0.0044 ng/g dw), hexachlorobenzene was detected at six of the fifteen sites (0.0063-0.048 ng/g dw), and γ-chlordane was detected at only one site (0.011 ng/g dw). There are no ERM/ERL criteria for any of the OC pesticides.

Fipronils and Pyrethroids

Of the eight pyrethroid compounds measured, there was only one single detection of an analyte in any of the fifteen stations; bifenthrin was detected at 2.24 ng/g dw. There were no instances of fipronil or its associated degradation products above the MDL at any of the sampled sites. There are currently no ERL/ERM criteria for fipronil or pyrethroid compounds.

Discussion

Analyzing sediment chemistry in the Southern California Bight began in 1994, and since then, has occurred in 1998, 2003, 2008, 2013, and 2018. The location of sampling sites change over the years, but they target a diverse range of locations, including heavily trafficked areas such as marinas, ports, and bays to protected areas like the Channel Islands National Marine Sanctuary (CINMS). One of the goals of their

assessment is to understand the magnitude and impact of chemical contaminants in the Bight, and if that varies among the habitats in the Bight (Schiff et al. 2011). Prior to the 2018 sampling in CINMS, the area was last sampled as part of SCCWRP's 2008 Bight assessment.

Organics Data

The average sediment concentration for total DDTs in the 2018 CINMS samples was 3.94 ± 3.04 ng/g dw, while in 2008, the average concentration was 2.98 ± 4.30 (SCCWRP 2015). While the average concentration for total DDTs has slightly increased over the past ten years, the means were not significantly different (t-test, p=0.0638, log transformed concentrations). However, it was found that there was an increase in the percentage of sites above the ERL for both p,p'-DDE and total DDTs between 2008 and 2018 sampling (Figure 7). DDT has a long half-life and is subject to atmospheric transport and deposition (EPA 2017). Despite DDT being banned in the United States in the 1970's, in the mid 1990's, DDTs were still being introduced into the Bight, albeit at a greatly reduced rate, by publicly owned treatment works, which are located offshore at depths of 60-100 m (Schiff et al. 2000). Historically, the highest concentrations of DDTs in the Bight have been observed in the continental shelf and slope, close to Los Angeles Harbor and Palos Verdes, where the outfalls are located (Schiff et al. 2011). Additionally, acid waste containing DDTs was dumped in the Santa Monica and San Pedro Basins during the 1960's (Schiff et al. 2000). Anoxic sediment conditions in the basins have prevented the degradation of DDTs, and the disruption and subsequent dispersion of these sediments over the years, is likely the cause of widespread DDT contamination in the Bight (Schiff et al. 2000), which would include the area of the CINMS.

The average PCB concentration in sediments from 2018 CINMS samples was 0.70 ± 0.64 ng/g dw. In 2008 CINMS, of the PCBs measured, only PCB206 was detected in one sample (0.51 ng/g dw). Differences in PCB concentrations between 2008 and 2018 are likely due to differences in method detection/reporting limits and number of congeners analyzed. In the Bight 2008 study, only 41 congeners were analyzed while in 2018, 100 congeners were analyzed. Additionally, MDLs in 2008 (average MDL = 0.55 ± 0.22 ng/g) were generally an order of magnitude greater than MDLs in 2018 samples (average MDL = 0.04 ± 0.04 ng/g). Regardless, concentrations of PCBs measured in 2018 are less than the ERL, and are representative of background levels of PCBs (Kennicutt 2017).

PAH sediment concentrations never exceeded the ERL in 2018, nor did they in 2008. PAH concentrations in 2008 were generally found to be below the MDL in CINMS sites. In fact, PAHs were only detected above the MDL at three sites during the 2008 assessment, for only two compounds, perylene and 2,6-dimethylnaphthalene. Perylene is commonly detected in marine sediments as it is often derived from plant origins, however, it does have anthropogenic origins as well (Venkatesan 1988). While PAHs were more frequently detected in 2018, as in the case with PCBs, MDLs in 2008 were generally an order of magnitude higher than MDLs used in 2018, which would allow for a greater frequency of detection (20.1 ± 7.1 versus 1.7 ± 4.8 ng/g dw, respectively).

Pyrethroids and PBDEs were analyzed in a subset of samples collected during the 2008 Bight assessment, however, the CINMS samples were not part of the subset, thus there is no historical data present for these two classes of compounds. As in the case with PCBs, for PBDEs, there are 209 possible congeners.

PBDEs are used as flame retardants in a wide variety of consumer products; their use in the United States has been voluntarily halted in 2004 for the commercial penta- and octaBDE formulations, but the decaDBE (PBDE 209) is still produced (NOS 2020). PBDE 47 and 99 were the two most frequently detected PBDEs found in this study. Both PBDE 47 and 99 are part of the commercially used pentaBDE formulation and are also two of the most prevalent PBDEs detected in humans, wildlife, and environmental matrices (Erratico et al. 2011). The concentrations of PBDE congeners in this study are similar to concentrations documented in sediments from the Canadian Arctic (Kelly et al. 2008). While it is thought that sediment concentrations of PBDEs serve as a pathway for biomagnification into bivalves and eventually marine mammals (Schiff et al. 2011), concentrations of this magnitude are expected to be negligible for biomagnification potential (Kelly et al. 2008).

Of the eight pyrethroid compounds analyzed in CINMS samples, only one pyrethroid, bifenthrin, was detected at one location. Pyrethroid pesticides, which are used in both urban and agricultural applications, are likely to be found in sediments receiving stormwater runoff, so it is not surprising that pyrethroids were generally not detected in the CINMS (Schiff et al. 2011).

For the APEO compounds NP1EO and NP2EO, this is the first time these contaminants have been part of the Bight assessment, but archived sediment and tissue samples from previous NS&T MW studies were reanalyzed for select CECs which included NP1EO and NP2EO (Maruya et al. 2015). Samples from Marina del Rey, San Francisco Bay, San Simeon (tissue only), and the Palos Verdes shelf representing urbanized, mixed development, and undeveloped watersheds, and discharged effluent, respectively, were extracted for CECs (Maruya et al. 2015). NP1EO and NP2EO were detected at the urbanized and mixed use watersheds, as well as at the discharged effluent site; concentrations ranged from 20-1000 ng/g dw, with the highest concentrations detected at urban and discharged effluent sites (Maruya et al. 2015). No sediments from the undeveloped site were measured, but tissue samples from there were analyzed, and NP1EO was detected in tissue samples from 1994-2009 (Maruya et al. 2015). In this study, we detected NP1EO in seven of the fifteen sites at 66.3-241.5 ng/g dw in the CINMS. APEOs are surfactants that have been in use since the 1970's, however they have been found to be largely non-toxic during acute toxicity exposures (Maruya et al. 2015). Their detection in the environment indicates that they are relatively stable over long periods of time, and more research is needed to understand what their persistence means and why they are detected in areas like CINMS.

For trace metals, in 2008, one site had cadmium and nickel sediment concentrations that exceeded the ERL and a second site exceeded the ERL for nickel. None of the 2008 sites had concentrations that exceeded the ERL values for the other inorganic analytes with ERL values: arsenic, chromium, copper, lead, mercury, and zinc (Figures 8-10). The percentage of sites with trace metal concentrations above the ERL has remained relatively consistent between 2008 and 2018 (3% and 7% respectively; Figure 11).

Overall, sediment concentrations for trace metals, POPs, and CECs were detected at low levels in the 2018 CINMS samples. While the ERL was exceeded for total DDT concentrations in ten of the fifteen stations, with three of those stations also having an exceedance of the ERL for one metal, this does not necessarily mean that detrimental effects to benthic organisms will be observed. As stated earlier, ERL/ERM guidelines are used in an informal setting, and are typically combined with other sediment metrics, such as sediment toxicity assays and total organic carbon to understand the overall sediment quality. In projects like SCECAP, sediment quality is combined with water quality and biological condition assessments to obtain an overall habitat quality index, which provides a more holistic approach

to an area. In short, chemical analyses are only one part of understanding the overall health of an area. As part of SCCWRP's assessment, sediment toxicity and benthic infauna are combined with sediment chemistry to understand the sediment quality as a whole, which is then combined with microbiology, ocean acidification, harmful algal blooms and trash indices for their total regional monitoring assessment (Parks et al. 2020).

While data from other aspects of the Bight 2018 assessment are still being analyzed, the sediment toxicity portion of this study was reported earlier this year. Sediment toxicity assays were run for all sites in which samples for chemical analysis were obtained. The sediment toxicity assays were standard ASTM 2010 10-day survival tests with amphipods (*Eohaustorius estuarius*) (Parks et al. 2020). In the case of the CINMS samples, fourteen of the fifteen stations were considered nontoxic, and one station was found to have low toxicity (Parks et al. 2020). Low toxicity levels means that the control normalized response was between 82-89% (Parks et al. 2020); the sample in question that had low toxicity levels was NOAA ID 18-0711, which exceeded the ERL for total DDTs, p,p'-DDE, and cadmium. While one station was deemed to have low toxicity, it is important to note that in SCCWRP's assessment, low toxicity is considered "not toxic" in their assessment, thus all CINMS samples were found to be not toxic (Parks et al. 2020).

The chemistry data that CCR provides to organizations such as SCECAP, SCCWRP, and NS&T MW is very important in order for these organizations to assess the overall health of an area. Data from these assessments are a useful tool for coastal managers to understand how our activities, both positive and negative, affect coastal environments. For example, legacy contaminants such as PCBs and DDTs are still detected and continue to cause environmental effects today, but their environmental levels continue to decline thanks to legislative actions that were put into effect decades ago. Conversely, as new chemicals enter the market, we need to be cognizant of their presence in the environment, their ability to persist and bioaccumulate, and their potential to induce environmental effects. Thus, it is important to continue to be able to monitor legacy contaminants while incorporating new tools to screen for CECs to inform coastal managers of the next potential threat. To adapt to changing needs in environmental contaminant analysis, CCR has made efforts to increase its analytical abilities by developing new methods to measure fipronils, pyrethroids, and AP/APEOs, which were measured for the CINMS samples, but we have also developed methods to screen for ~150 pharmaceutical and personal care products, organic sunscreen ultraviolet filters, munition compounds, and phthalate metabolites. This flexibility and adaptability in capabilities will allow us to continue to work with our existing collaborators while also allowing us to forge new relationships with other partners and institutions.

Data Availability

Chemistry data for this project is located in the Chemistry Information Management System (ChemLIMS), which is housed on the Microsoft Azure Cloud. The ChemLIMS database is maintained by NCCOS IT and only accessible to NCCOS personnel. Data files may be exported from ChemLIMS and shared with partners.

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Supporting Documents

CCR-002 Organic Glassware Cleaning Protocol
CCR-003 Dry Weight Determination
CCR-004 Dionex ASE 200 Extraction System
CCR-005 ASE Extract Filtering Protocol
CCR-006 TurboVap II Concentration Work Station
CCR-007 Protocol for the Operation of the J2 Scientific Gel Permeation Chromatography System
CCR-010 Alumina Column Cleanup for Organic Extracts
CCR-016 Extraction of Pyrethroids in Sediments
CCR024 SOP Drying and Grinding Sediment
CCR025 SOP Microwave Digestion of Sediments using Nitric Acid
CCR030 SOP Inorganic Instrumental Analysis
CCR031 SOP Mercury Analysis
CCR-041 Integration of sample chromatograms
CCR-042 Integration of procedural blanks for MDL calculation
CCR-043 Analysis of Persistent Organic Pollutants by GC-MS

CCR-044 Combustion Cleaning of Sodium Sulfate for ASE Extraction
CCR-046 Cleaning of ASE Extraction Cells
CCR-047 CCR Sample Receipt
CCR-059 Extraction of APs and APEOs from sediments
CCR-061 Azure ChemLIMS Database
CCR-073 Extraction of Fipronils and Pyrethroids from Sediments

SCECAP Reports
SCCWRP Data Portal
NS&T Reports

Tables

Table 1: ERL and ERM values for nine trace metals as reported in Long et al. (1995)

Trace Element	ERL (ppm dry wt)	ERM (ppm dry wt)
Arsenic	8.2	70
Cadmium	1.2	9.6
Chromium	81	370
Copper	34	270
Lead	46.7	218
Mercury	0.15	0.71
Nickel	20.9	51.6
Silver	1	3.7
Zinc	150	410

Table 2: ERL and ERM values for organic compounds as reported in Long et al. (1995)

Organic Compound	ERL (ppb dry wt)	ERM (ppb dry wt)
acenaphthene	16	500
acenaphthylene	44	640
anthracene	85.3	1100
fluorene	19	540
2-methylnaphthalene	70	670
naphthalene	160	2100
phenanthrene	240	1500
LMW PAH[1]	552	3160
benz[a]anthracene	261	1600
benzo[a]pyrene	430	1600
chrysene	384	2800
dibenzo[ah]anthracene	63.4	260
fluoranthene	600	5100
pyrene	665	2600
HMW PAH[2]	1700	9600
Total PAH[3]	4022	44792
p,p'-DDE	2.2	27
Total DDT[4]	1.58	46.1
Total PCBs[5]	22.7	180

1 LMW PAH is the sum of acenaphthene, acenaphthylene, anthracene, fluorene, 2-methylnaphthalene, naphthalene, and phenanthrene

2 HMW PAH is the sum of benz[a]anthracene, benzo[a]pyrene, chrysene, dibenzo[ah]anthracene, fluoranthene, and pyrene

3 Total PAH is the sum of all individual PAHs in the table

4 Total DDT is the sum of 2,4' DDT, 4,4' DDT, 2,4'-DDD, 2,4'-DDE, 4,4'-DDD, 4,4'-DDE

5 Total PCB is the sum of all 209 possible congeners

Table 3: List of all organic analytes measured in this study

	PCBs	**PAHs**	**OC Pesticides**	**PBDEs**
PCB 1	PCB 115	naphthalene	Aldrin	PBDE 17
PCB 2	PCB 118	biphenyl	Alpha-HCH	PBDE 28
PCB 3	PCB 119	acenaphthene	Beta-HCH	PBDE 47
PCB 5	PCB 126	acenaphthylene	Chlorpyrifos	PBDE 66
PCB 8	PCB 128	fluorene	Cis-chlordane	PBDE 71
PCB 9	PCB 130	dibenzofuran	Cis-nonachlor	PBDE 85
PCB 12	PCB 132/153/168	dibenzothiophene	Desulfinyl fipronil	PBDE 99
PCB 15	PCB 138	phenanthrene	Dieldrin	PBDE 100
PCB 18	PCB 141	anthracene	Endosulfan I	PBDE 138
PCB 20	PCB 146	fluoranthene	Endosulfan II	PBDE 153
PCB 26	PCB 149	pyrene	Endosulfan Sulfate	PBDE 154
PCB 28	PCB 151	benz[a]anthracene	Endrin	PBDE 183
PCB 29	PCB 154	benzo[b]naphtho[2,1-d]thiophene	Fipronil	PBDE 190
PCB 31	PCB 156	chrysene+triphenylene	Fipronil sulfide	PBDE 209
PCB 37	PCB 157	benzo[b]fluoranthene	Fipronil sulfone	
PCB 44	PCB 158	benzo[k]fluoranthene	Gamma-chlordane	**AP/APEO**
PCB 45	PCB 159	benzo[j]fluoranthene	lindane	4-nonylphenol
PCB 47	PCB 163	benzo[a]fluoranthene	Heptachlor	4-n-octylphenol
PCB 48	PCB 164	benzo[a]pyrene	Heptachlor epoxide	NP1EO
PCB 49	PCB 165	benzo[e]pyrene	Hexachlorobenzene	NP2EO
PCB 50	PCB 167	dibenzo[a,h]anthracene	Mirex	
PCB 52	PCB 169	indeno[1,2,3-c,d]pyrene	Oxychlordane	**Trace Metals**
PCB 56	PCB 170	benzo[g,h,i]perylene	Trans-nonachlor	Aluminum
PCB 60	PCB 172	C1-naphthalenes	bifenthrin	Antimony
PCB 61	PCB 174	C2-naphthalenes		Arsenic
PCB 63/76	PCB 177	C3-naphthalenes	**Pyrethroids**	Barium
PCB 66	PCB 180	C4-naphthalenes	cis-permethrin	Beryllium
PCB 69	PCB 183	C1-fluorenes	cyfluthrin	Cadmium
PCB 70	PCB 184	C2-fluorenes	Cypermethrin	Chromium
PCB 74	PCB 187	C3-fluorenes	Deltamethrin	Copper
PCB 77	PCB 188	C1-dibenzothiophenes	esfenvalerate	Iron
PCB 81	PCB 189	C2-dibenzothiophenes	Lambda cyhalothrin	Lead
PCB 82	PCB 190	C3-dibenzothiophenes	trans-permethrin	Mercury
PCB 87	PCB 193	C4-dibenzothiophenes		Nickel
PCB 88/95	PCB 194	C1-phenanthrenes/anthracenes	**DDTs**	Selenium
PCB 92/84/89	PCB 195	C2-phenanthrenes/anthracenes	2,4'-DDD	Silver
PCB 99	PCB 198	C3-phenanthrenes/anthracenes	2,4'-DDE	Zinc
PCB 101/90	PCB 200 / IUPAC 201	C4-phenanthrenes/anthracenes	2,4'-DDT	
PCB 103	PCB 201 / IUPAC 199	C1-fluoranthenes/pyrenes	4,4'-DDD	
PCB 104	PCB 202	C2-fluoranthenes/pyrenes	4,4'-DDE	
PCB 105	PCB 203/196	C3-fluoranthenes/pyrenes	4,4'-DDMU	
PCB 106	PCB 206	C4-fluoranthenes/pyrenes	4,4'-DDT	
PCB 107/123	PCB 207	C1-chrysenes/benzanthracenes		
PCB 108	PCB 208	C2-chrysenes/benzanthracenes		
PCB 110	PCB 209	C3-chrysenes/benzanthracenes		
PCB 114		C4-chrysenes/benzanthracenes		
		C1-naphthobenzothiophenes		
		C2-naphthobenzothiophenes		
		C3-naphthobenzothiophenes		
		C4-naphthobenzothiophenes		

Table 4: Isotopically labeled internal standards used for the analysis of POPs and CECs

PCBs	PAHs	Pesticides (OCs, Fipronils, Pyrethroids)	PBDEs	AP/APEOs
$^{13}C_6$ PCB 3	d_8 naphthalene	$^{13}C_{12}$ 2,4'-DDE	$^{13}C_{12}$ PBDE 28	d_{17} 4-n-octylphenol
$^{13}C_{12}$ PCB 15	d_{10} 1-methylnaphthalene	$^{13}C_{12}$ 4,4'-DDT	$^{13}C_{12}$ PBDE 47	d_4 4-n-nonylphenol
$^{13}C_{12}$ PCB 31	d_8 acenaphthylene	$^{13}C_{12}$ 4,4'-DDE	$^{13}C_{12}$ PBDE 77	$^{13}C_6$ NP2EO
$^{13}C_{12}$ PCB 52	d_{10} acenaphthene	$^{13}C_{10}$ mirex	$^{13}C_{12}$ PBDE 100	$^{13}C_6$ NP1EO
$^{13}C_{12}$ PCB 118	d_{10} fluorene	$^{13}C_{12}$ dieldrin	$^{13}C_{12}$ PBDE 126	
$^{13}C_{12}$ PCB 153	d_8 dibenzothiophene	$^{13}C_9$ endosulfan II	$^{13}C_{12}$ PBDE 153	
$^{13}C_{12}$ PCB 180	d_{10} phenanthrene	$^{13}C_{12}$ γ-chlordane	$^{13}C_{12}$ PBDE 183	
$^{13}C_{12}$ PCB 194	d_{10} anthracene	d_4 endo I	$^{13}C_{12}$ PBDE 190	
$^{13}C_{12}$ PCB 206	d_{10} fluoranthene	$^{13}C_{10}$ heptachlor epoxide	$^{13}C_{12}$ PBDE209	
$^{13}C_{12}$ PCB 209	d_{10} pyrene	d_{10} chlorpyrifos		
	d_{12} benz[a]anthracene	$^{13}C_6$ lindane		
	d_{12} chrysene	$^{13}C_{12}$ hexachlorobenzene		
	d_{12} benzo[b]fluoranthene	d_5 deltamethrin		
	d_{12} benzo[k]fluoranthene	d_6 cypermethrin		
	d_{12} benzo[e]pyrene	d_6 cyfluthrin		
	d_{12} benzo[a]pyrene	$^{13}C_6$ trans-permethrin		
	d_{12} perylene	$^{13}C_6$ cis-permethrin		
	d_{12} benzo[ghi]perylene	d_5 bifenthrin		
		d_5 cyhalothrin		
		$^{13}C_2$, $^{15}N_2$ fipronil sulfone		
		$^{13}C_2$, $^{15}N_2$ fipronil		

Table 5: Measured sediment concentrations for DDT and it's associated degradation products.

NOAA ID	2,4'-DDD	2,4'-DDE	2,4'-DDT	4,4'-DDD	4,4'-DDE	4,4'-DDMU	4,4'-DDT	Total DDT[1]	Total DDT[2]
18-0708	<MDL	0.35	<MDL	0.26	3.52	0.49	0.16	4.29	4.78
18-0709	<MDL	0.35	<MDL	0.21	3.53	1.18	0.22	4.31	5.49
18-0710	<MDL	0.39	<MDL	0.35	5.55	1.26	0.18	6.46	7.72
18-0711	<MDL	0.62	<MDL	0.52	6.17	1.47	0.36	7.67	9.14
18-0712	<MDL	0.46	<MDL	0.27	4.60	1.14	0.14	5.47	6.61
18-0713	<MDL	0.60	<MDL	0.34	5.79	1.66	0.14	6.87	8.53
18-0714	<MDL	0.74	<MDL	0.75	6.98	1.80	0.23	8.70	10.50
18-0715	<MDL	0.17	<MDL	0.11	1.44	0.46	0.07	1.79	2.24
18-0716	<MDL	<MDL	<MDL	<MDL	0.08	<MDL	<MDL	0.08	0.08
18-0717	<MDL	0.12	<MDL	<MDL	0.50	<MDL	<MDL	0.62	0.62
18-0718	<MDL	0.72	<MDL	0.49	6.46	0.59	0.07	7.73	8.33
18-0719	<MDL	0.16	<MDL	0.10	1.23	0.00	0.05	1.54	1.54
18-0720	<MDL	0.15	<MDL	0.08	1.10	0.26	0.04	1.37	1.63
18-0721	<MDL	<MDL	<MDL	<MDL	0.06	<MDL	0.04	0.10	0.10
18-0722	<MDL	0.25	<MDL	0.18	1.42	0.42	0.21	2.06	2.48

[1] 4,4'-DDMU is not included in the Total DDT calculation for ERL/ERM analysis

[2] 4,4'-DDMU is included in the Total DDT

Table 6: Sediment concentrations on trace elements in µg/g dw

	Ag	Al	As	Ba	Be	Cd	Cr	Cu	Fe	Hg	Ni	Pb	Sb	Se	Zn
18-0708	0.0	4159.7	2.4	34.4	0.1	0.6	16.1	6.0	7393.7	0.0	14.6	3.1	1.2	0.6	32.4
18-0709	0.0	3912.7	2.3	34.7	0.1	0.4	16.6	5.4	8056.1	0.0	15.0	2.8	0.0	0.6	31.7
18-0710	0.0	5304.3	2.4	78.5	0.2	0.5	21.8	7.7	11129.5	0.0	21.7	3.9	0.0	0.8	41.9
18-0711	0.0	3035.3	2.6	119.5	0.1	1.2	20.4	5.0	6672.7	0.0	13.9	3.5	1.3	0.6	29.9
18-0712	0.0	3727.1	2.4	42.6	0.2	0.8	16.8	5.7	7951.4	0.0	14.6	3.3	1.1	0.6	30.3
18-0713	0.0	4673.6	2.6	40.5	0.2	1.0	19.6	7.2	9460.6	0.2	15.6	4.1	0.0	0.7	35.2
18-0714	0.0	3620.9	2.8	129.2	0.2	1.2	22.7	5.9	9641.7	0.0	15.0	4.0	0.0	0.7	37.2
18-0715	0.0	2379.7	2.4	96.1	0.1	0.7	18.4	3.7	7510.2	0.0	12.3	3.3	0.0	0.5	33.4
18-0716	0.0	1270.4	3.7	16.9	0.0	0.2	6.7	2.4	3819.0	0.0	14.3	2.6	0.0	0.4	18.9
18-0717	0.0	1459.7	2.7	15.8	0.1	0.3	9.0	4.2	3971.9	0.0	15.2	3.2	0.0	0.5	14.1
18-0718	0.0	3655.7	2.0	226.5	0.3	0.8	23.7	4.2	10539.8	0.0	10.0	5.5	1.1	0.4	34.3
18-0719	0.0	3440.1	2.8	36.6	0.1	0.4	13.2	4.7	8156.2	0.0	15.3	3.0	0.0	0.6	33.5
18-0720	0.0	3844.7	2.0	57.7	0.1	0.3	12.9	3.6	7108.4	0.0	12.1	2.0	0.0	0.5	28.3
18-0721	0.0	3574.5	2.5	33.1	0.1	0.0	9.6	5.1	6659.4	0.0	7.0	2.3	0.0	0.0	25.9
18-0722	0.0	4120.9	1.8	23.4	0.1	0.3	12.7	3.8	5353.3	0.0	9.1	2.0	1.1	0.4	24.3

Table 7: PAH sediment concentrations in ng/g dw

NOAA ID	Total ERL/ERM PAH (ng/g dw)	Total PAH50 (ng/g dw)
18-0708	3.6	38.7
18-0709	2.9	34.1
18-0710	10.9	55.2
18-0711	1.2	33.6
18-0712	1.3	33.6
18-0713	5.0	37.3
18-0714	2.9	34.9
18-0715	0.6	8.9
18-0716	5.0	6.1
18-0717	6.0	14.4
18-0718	6.2	49.2
18-0719	2.5	20.4
18-0720	1.5	13.6
18-0720	<MDL	3.4
18-0722	4.4	116.9

Table 8: PDBE sediment concentrations in ng/g dw

	PBDE 66	PBDE 71	PBDE 85	PBDE 99	PBDE 100	PBDE 138	PBDE 153	PBDE 154	PBDE 183	PBDE 190	PBDE 209
18-0708	<MDL	<MDL	<MDL	0.011	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
18-0709	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.146
18-0710	<MDL	<MDL	<MDL	0.013	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
18-0711	<MDL	<MDL	<MDL	0.015	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
18-0712	<MDL	<MDL	<MDL	0.010	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
18-0713	<MDL	<MDL	<MDL	0.012	<MDL	<MDL	0.021	<MDL	0.138	<MDL	<MDL
18-0714	<MDL	<MDL	<MDL	0.015	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
18-0715	<MDL	<MDL	<MDL	0.010	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
18-0716	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
18-0717	<MDL	<MDL	<MDL	0.015	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
18-0718	<MDL	<MDL	<MDL	0.017	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.177
18-0719	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
18-0720	<MDL	<MDL	<MDL	0.007	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
18-0721	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
18-0722	<MDL	<MDL	<MDL	0.008	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL

Table 9: Measured sediment concentrations for 100 PCB congeners

NOAA ID	$\sum PCB_{100}$ ng/g dw
18-0708	0.39
18-0709	0.47
18-0710	0.61
18-0711	1.29
18-0712	0.74
18-0713	0.98
18-0714	1.63
18-0715	0.1
18-0716	0.04
18-0717	0.12
18-0718	2.17
18-0719	0.19
18-0720	0.42
18-0721	0.07
18-0722	1.21

FIGURES

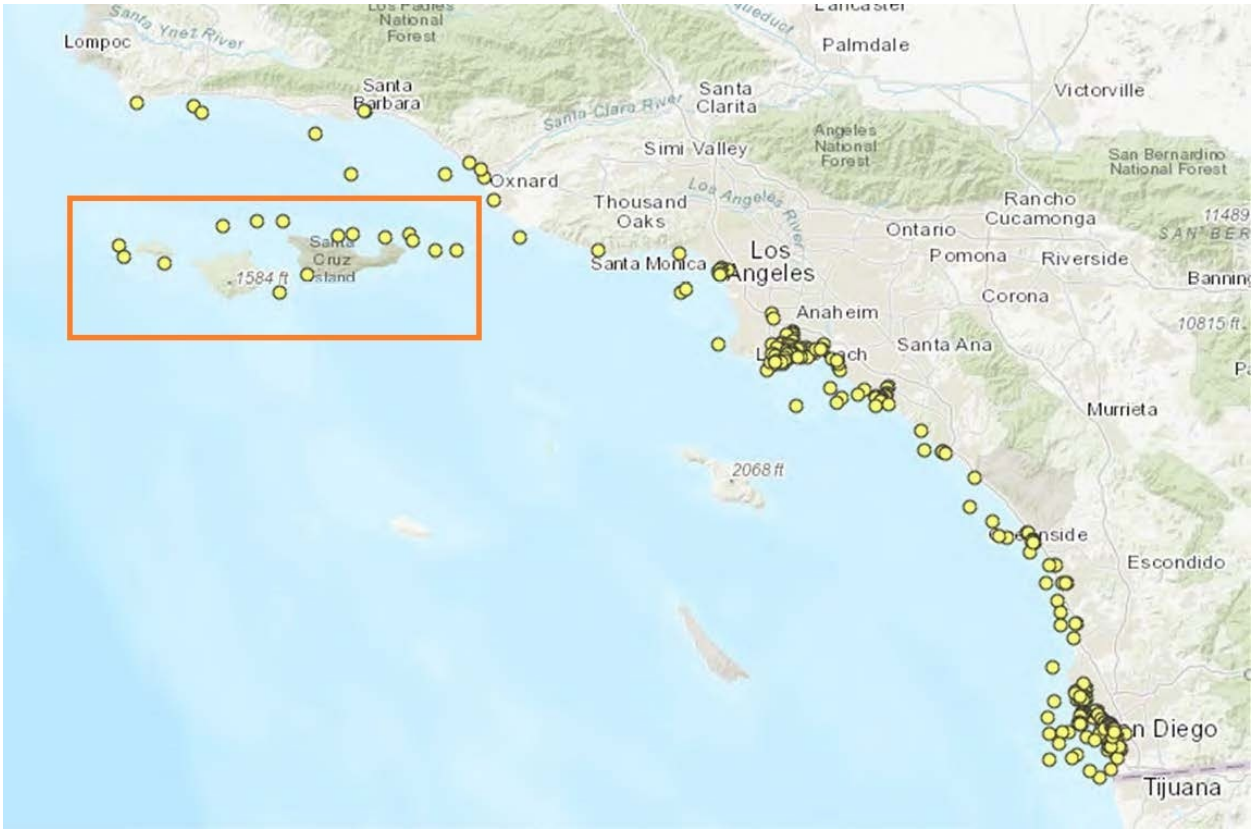

Figure 1: Sampling locations for the Bight 2018; the orange box denotes the sample locations from the Channel Islands National Marine Sanctuary, which were analyzed at NCCOS's Charleston Lab for trace metals, POPs, and CECs. Map figure was obtained from Parks et al. (2020).

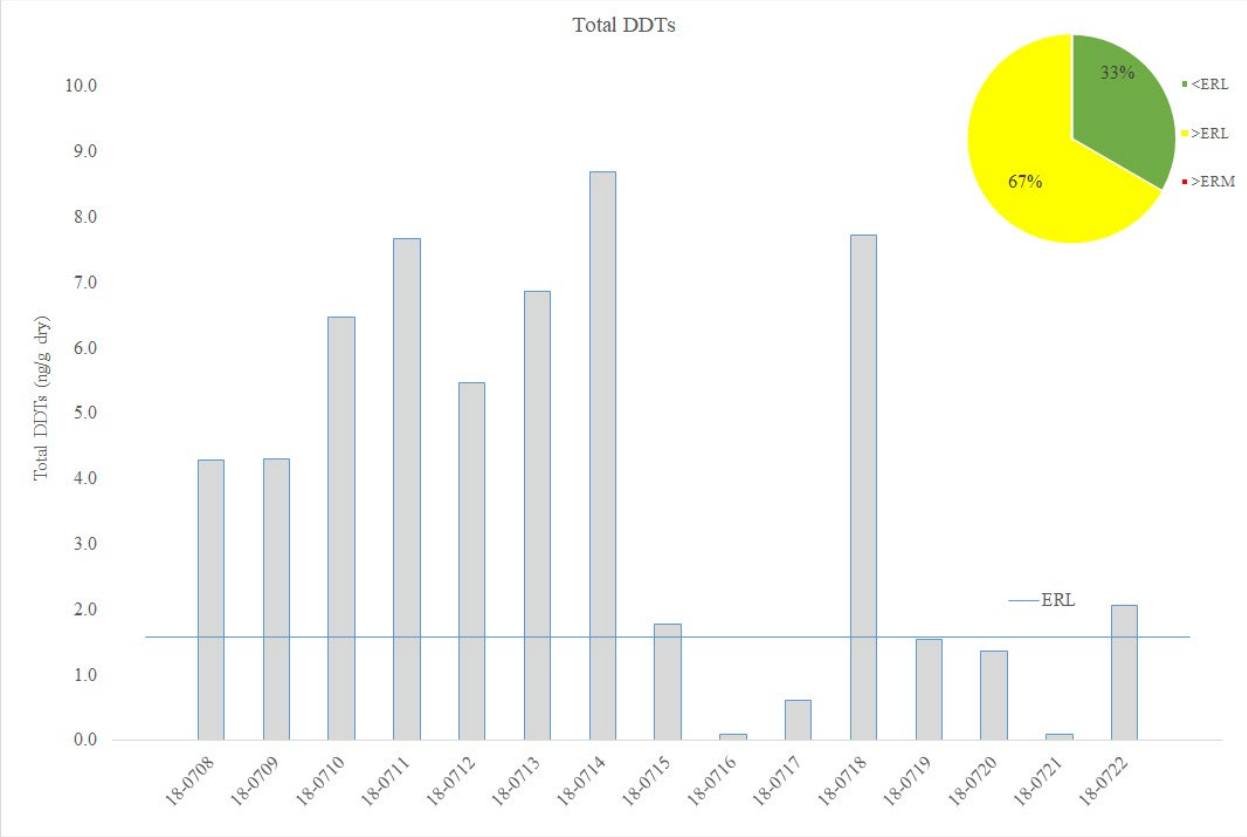

Figure 2: Total DDTs concentrations (in ng/g dw; 4,4'-DDMU not included) in Bight 2018 samples and percentages of samples <ERL, >ERL, and >ERM

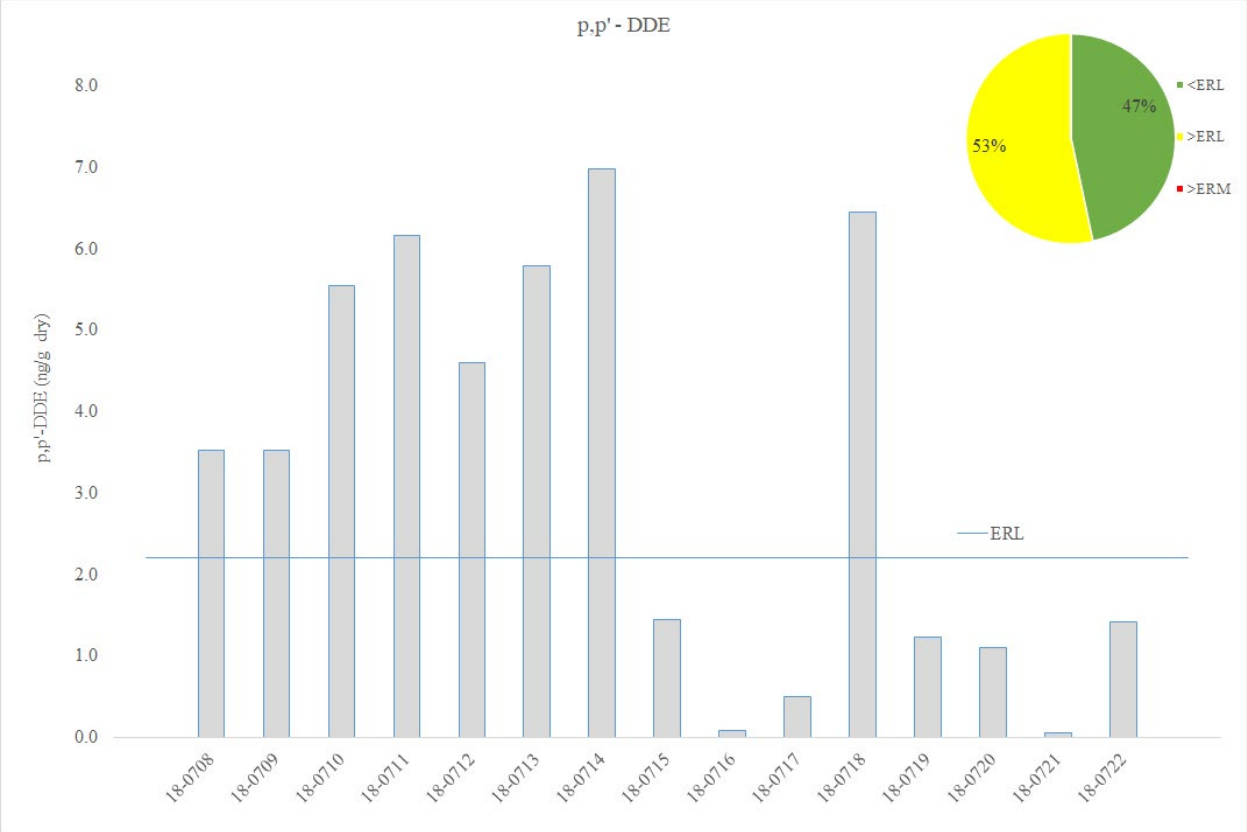

Figure 3: p,p'-DDE concentrations (in ng/g dw) in Bight 2018 samples and percentages of samples <ERL, >ERL, and >ERM

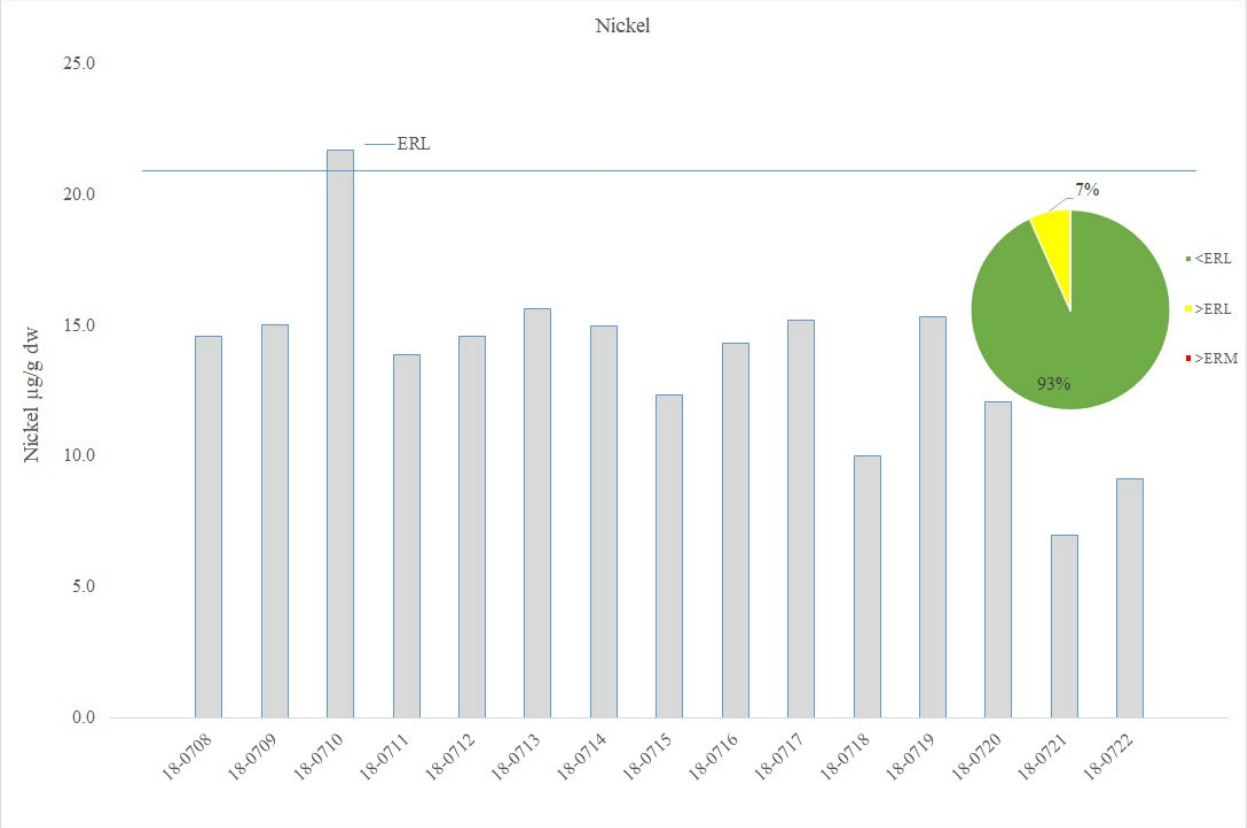

Figure 4: Nickel concentrations (in µg/g dw) in Bight 2018 samples and percentages of samples <ERL, >ERL, and >ERM

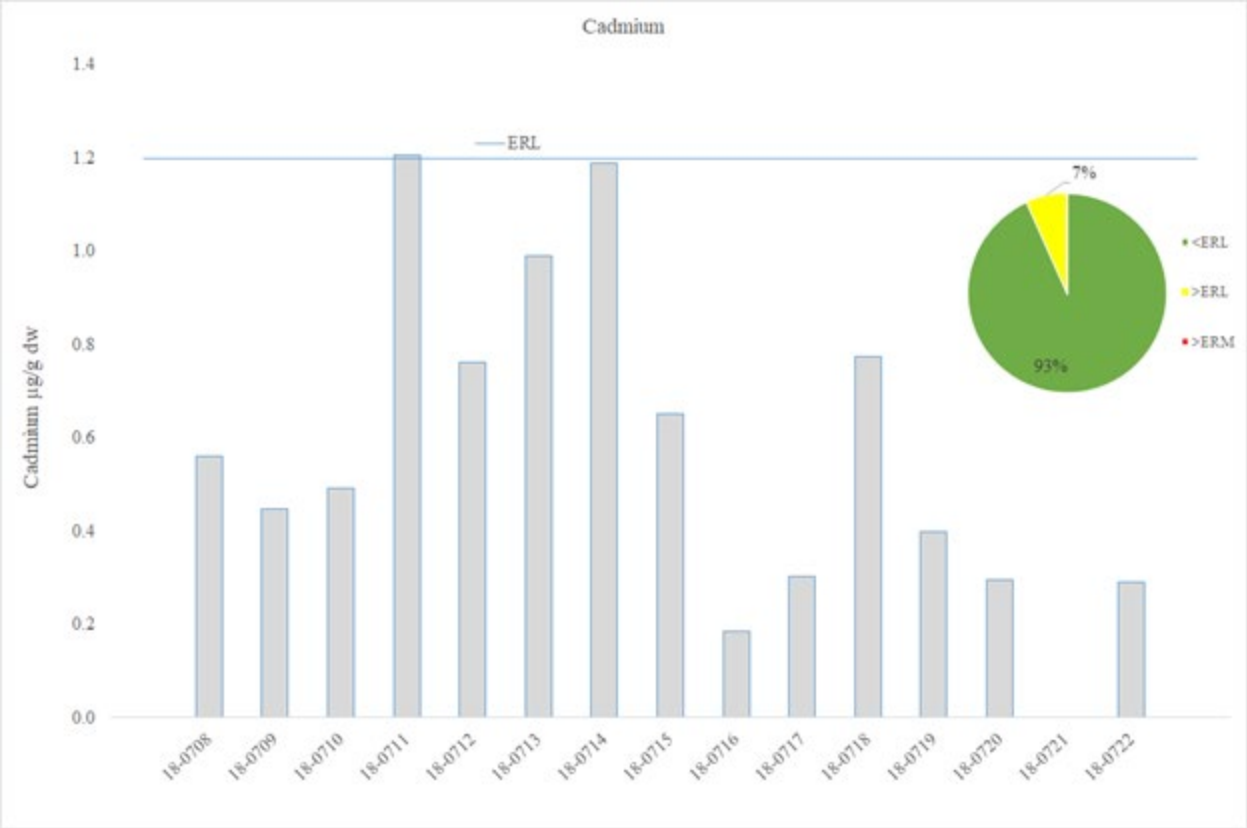

Figure 5: Cadmium concentrations (in µg/g dw) in Bight 2018 samples and percentages of samples <ERL, >ERL, and >ERM

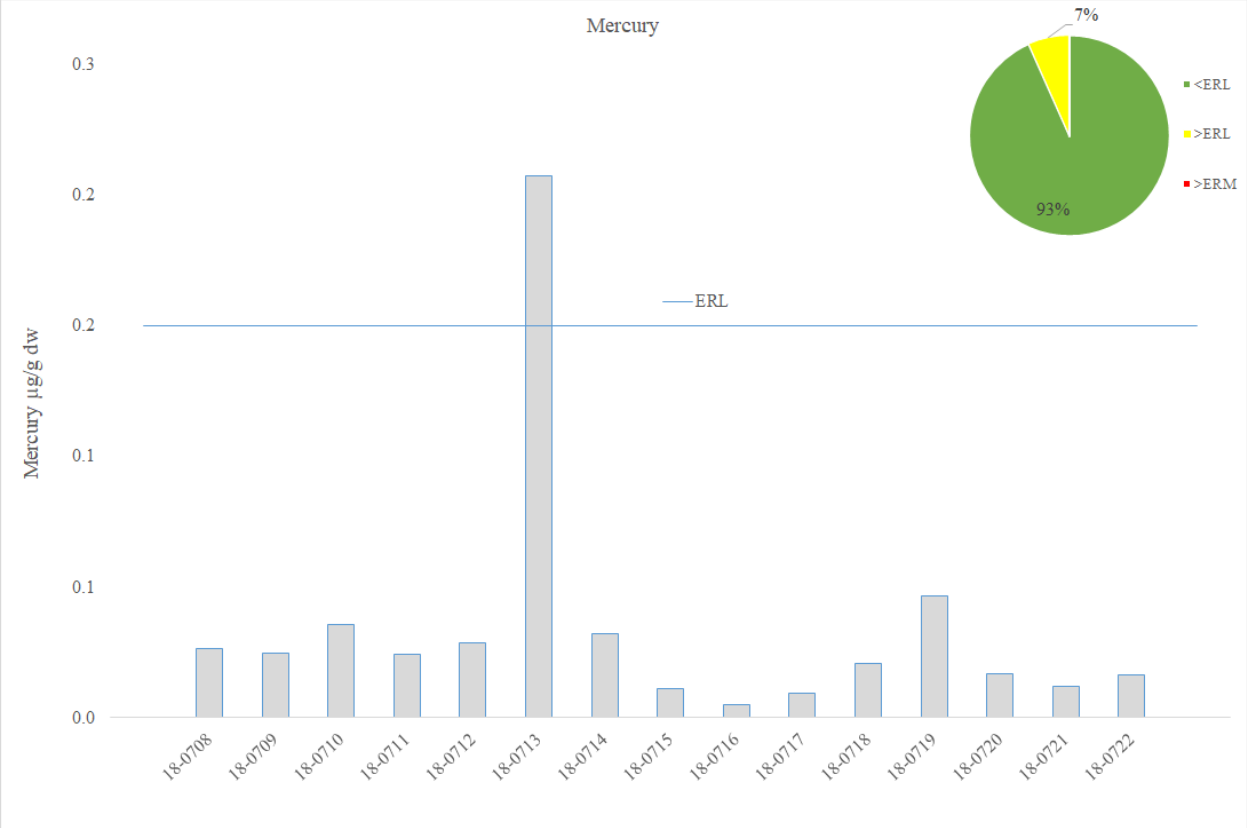

Figure 6: Mercury concentrations (in µg/g dw) in Bight 2018 samples and percentages of samples <ERL, >ERL, and >ERM

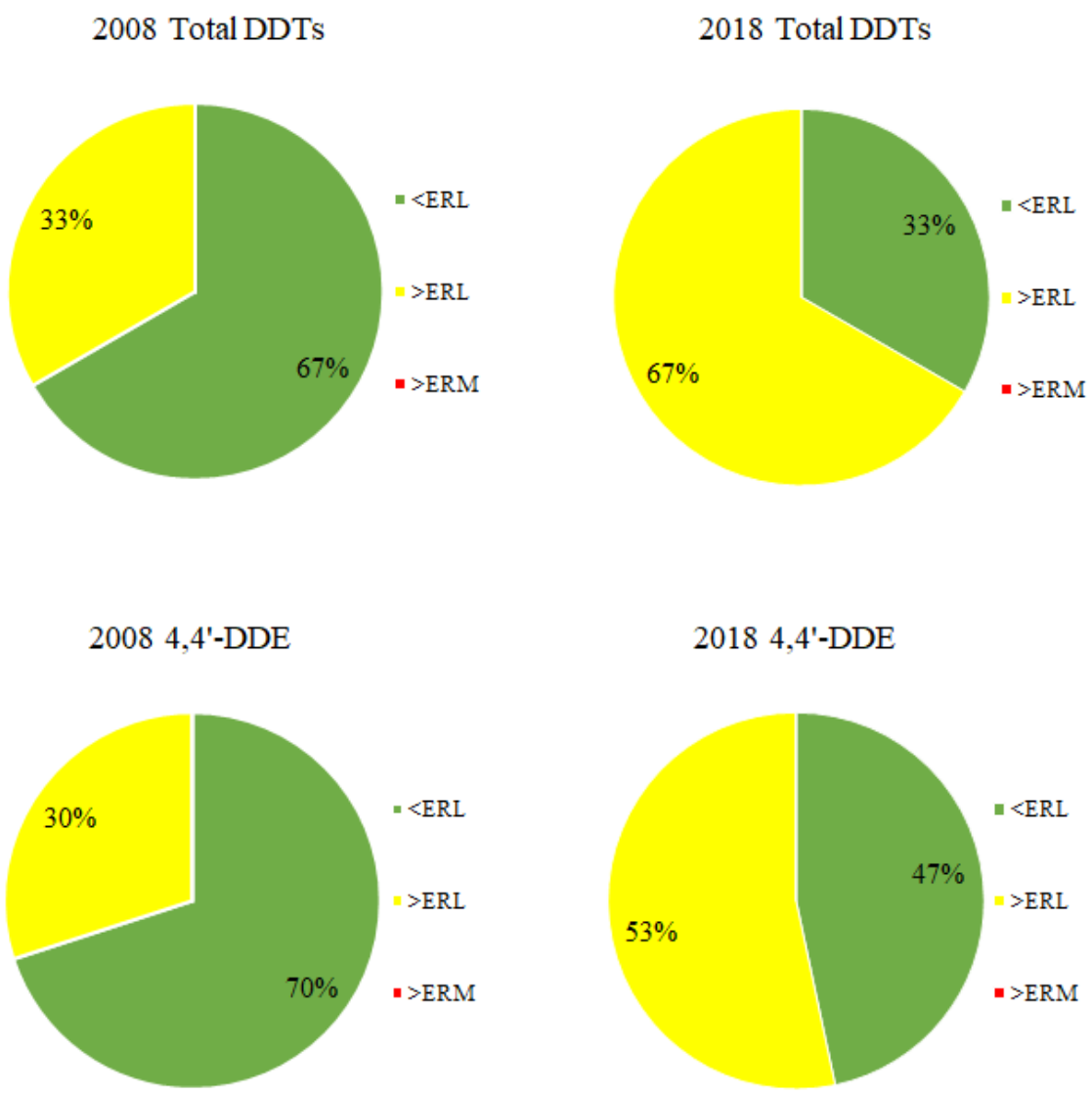

Figure 7: Percentage of samples <ERL and >ERL for 2008 and 2018 CINMS samples

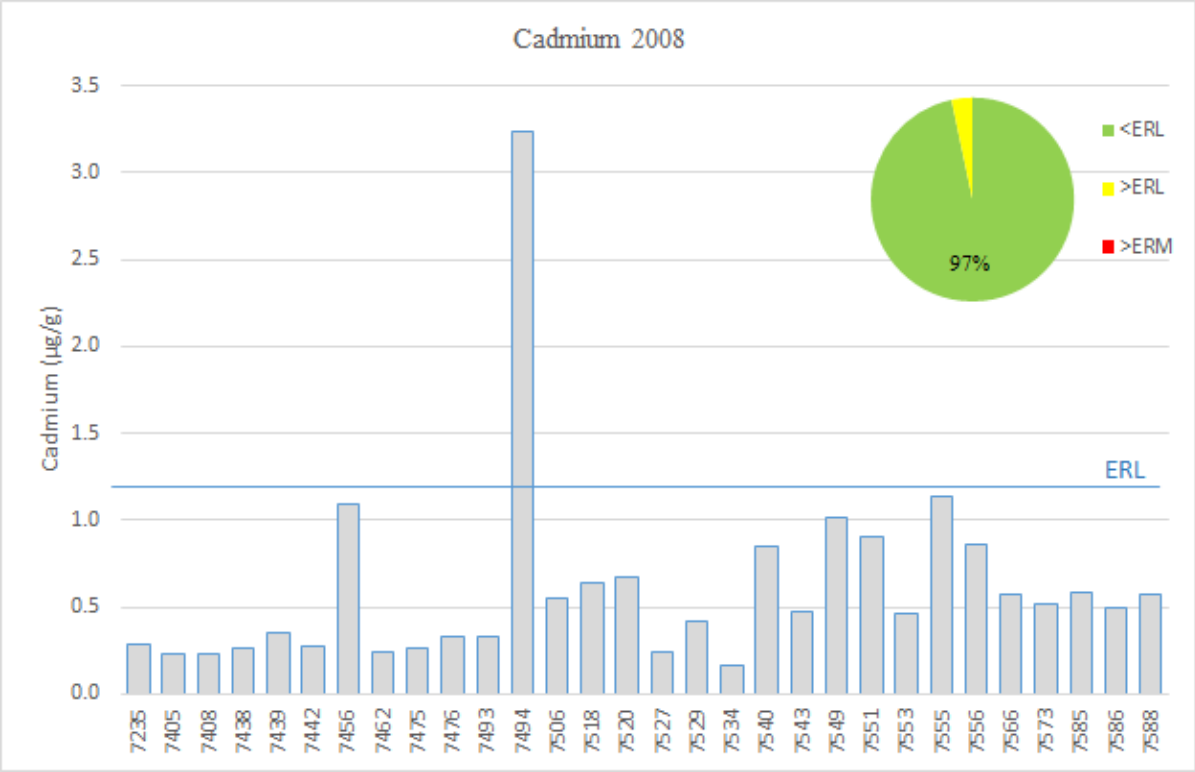

Figure 8: Cadmium concentrations (in µg/g dw) in Bight 2008 samples and percentages of samples <ERL, >ERL, and >ERM

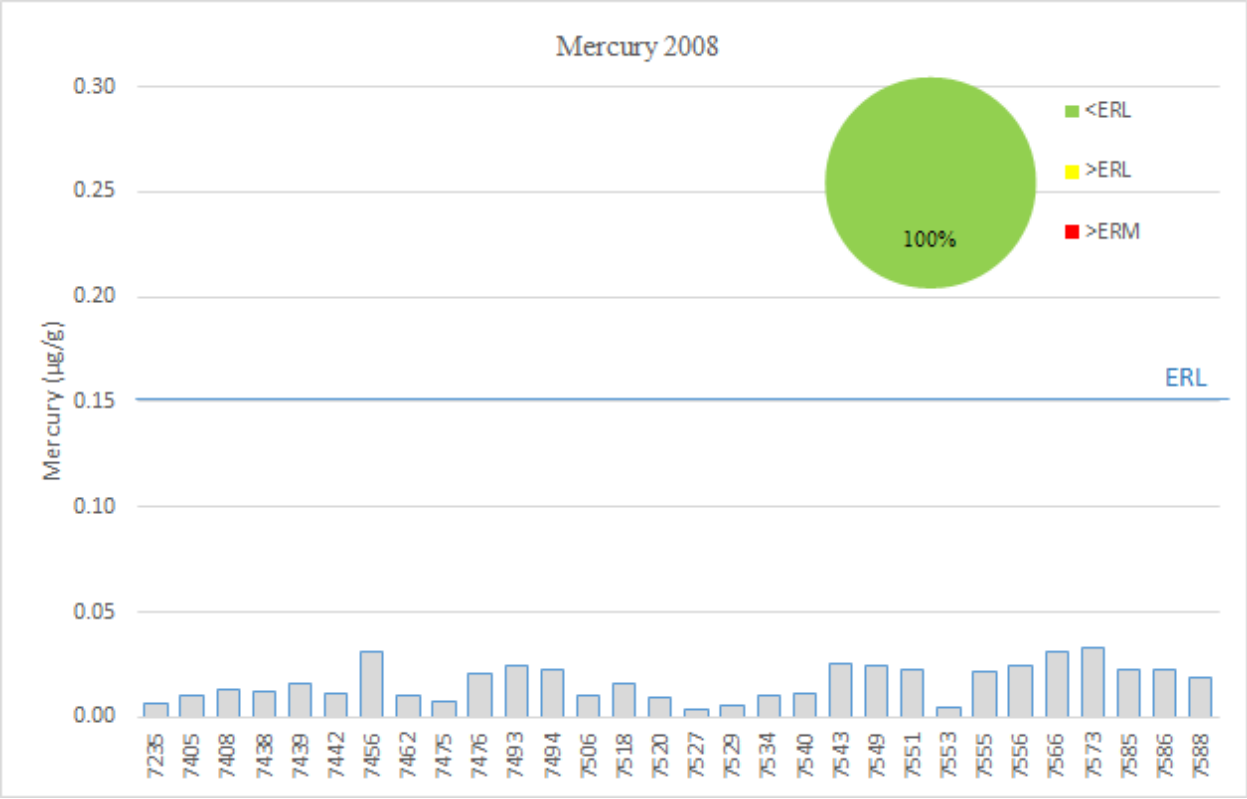

Figure 9: Mercury concentrations (in µg/g dw) in Bight 2008 samples and percentages of samples <ERL, >ERL, and >ERM

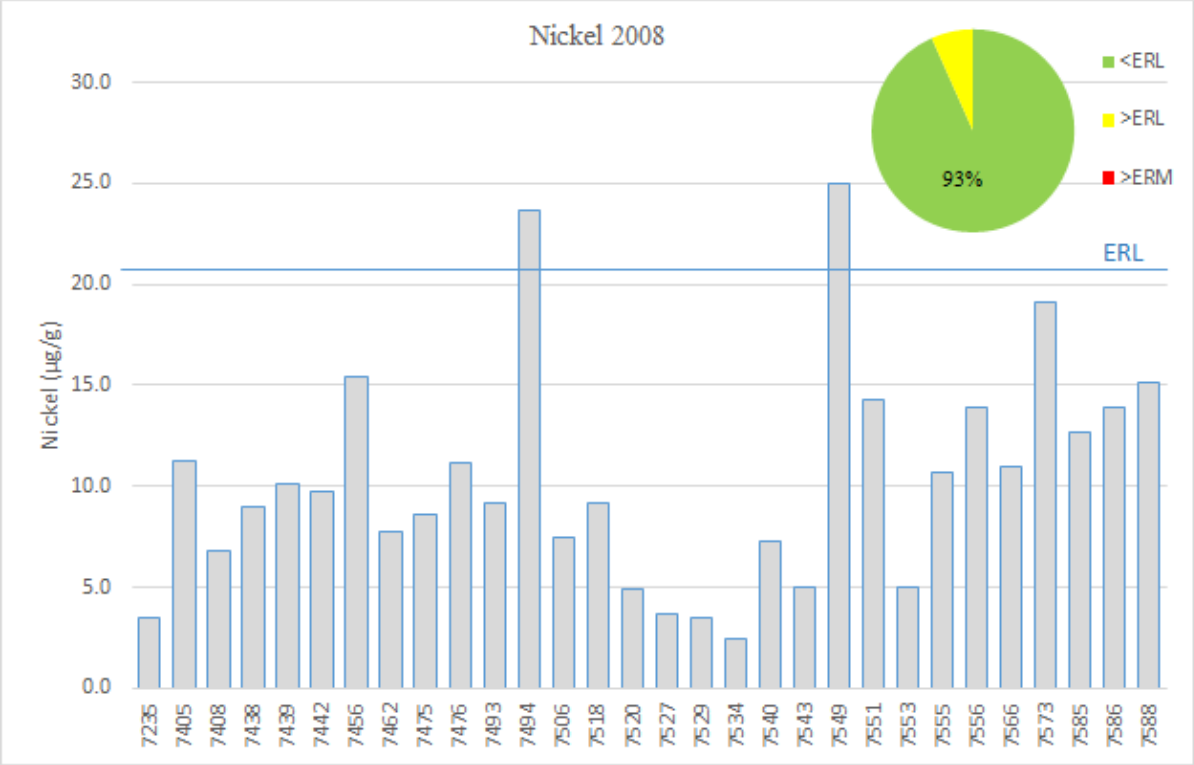

Figure 10: Nickel concentrations (in µg/g dw) in Bight 2008 samples and percentages of samples <ERL, >ERL, and >ERM

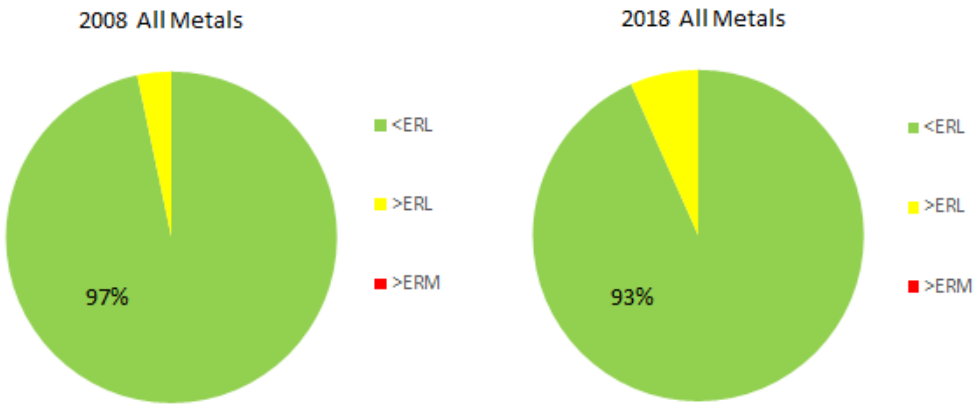

Figure 11: Percentage of samples <ERL and >ERL for 2008 and 2018 CINMS samples