



Bureau of Safety and Environmental Enforcement Oil Spill Preparedness Division

TRopical Oil Pollution Investigations in Coastal Systems [TROPICS]: Longitudinal Study in Support of Shoreline Spill Response Net Environmental Benefit Analysis (NEBA)

Final Report

February 2026



(Photo: RPI and Bermuda Biological Station for Research, 1984)

D Abigail Renegar, and Paul A Schuler

**US Department of the Interior
Bureau of Safety and Environmental Enforcement**

Oil Spill Preparedness Division

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Bureau of Safety and Environmental Enforcement**

Oil Spill Preparedness Division

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ABOUT THE COVER

Cover image is an aerial image of Dispersed site (Site D) taken during oiling in 1984. Image provided by Richard E. Dodge.

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EXECUTIVE SUMMARY

The TRopical Oil Pollution Investigations in Coastal Systems (TROPICS) study, initiated in 1984 on the Caribbean coast of Panama, sought to evaluate the comparative impacts of untreated crude oil versus chemically treated (dispersed) crude oil on nearshore tropical marine communities. From the initial two-year experiment and evaluation of effects, through a further 9 research visits between 1994 and 2016, the crude oil-only and dispersed crude oil treatments were found to have resulted in significantly different exposure conditions, environmental impacts, and recovery regimes at the respective sites. The trade-offs associated with exposure to floating or surface oil compared to water column exposure from chemically dispersed oil were demonstrated by the significant variation in mangrove, seagrass, and coral reef environmental response, depending upon whether the sites were exposed to dispersed oil or oil only. The results broadly established that the impacts to mangrove communities from non-dispersed crude oil included significant adult tree loss, with long-term implications for substrate stability. Dispersed oil resulted in less impact to the mangroves, but did result in significant short-term effects on coral cover and seagrass invertebrate communities, both of which recovered to pre-exposure levels within 10 years. Continuing oil contamination at the oil-only site remained an issue through the 29-year site visit, as did high erosion and sediment re-distribution that was not observed at the dispersed oil or reference sites.

TROPICS is one of the most comprehensive field experiments examining the acute and chronic impacts of oil and dispersed oil in nearshore tropical marine environments. Consequently, TROPICS has become the foundational and seminal field study which served as the historical antecedent for Net Environmental Benefit Analysis (NEBA), as well as the basis for follow-on Spill Impact Mitigation Analysis (SIMA) and Comparative Risk Analysis (CRA) for oil spill planning, preparation, and response. The primary objective of this project was to build upon the foundational 1984 longitudinal study by collecting new data to enhance understanding of the long-term environmental impacts of crude and dispersed oil on shorelines and nearshore ecosystems. This research directly supports the Net Environmental Benefit Analysis (NEBA) process, which is essential for optimizing oil spill response strategies, and was guided by an advisory committee of experts from academia, government, and industry was also formed to guide the project. Members of this JIP included academic institutions such as Nova Southeastern University, and Texas A&M University, and industry and government organizations including Clean Caribbean & Americas, Bureau of Safety & Environmental Enforcement, ExxonMobil, Oxy, American Petroleum Institute, Oil Spill Response Limited, and CEDRE.

The 2024 research expedition to the TROPICS locations replicated key biological assessment methodologies from earlier studies and introduced expanded chemical analyses of sediment samples. This study employed a comprehensive, multi-habitat ecological assessment methodology to evaluate long-term environmental impacts of oil and dispersed oil across mangrove, seagrass, and coral reef ecosystems, and evaluation of the persistence and potential migration of oil contamination across treatment islands, enabling more objective and data-driven comparisons over time and between sites. Mangroves, seagrass, and coral biological data were collected, using methods aligned with previous site visits to allow meaningful comparisons with

existing data; a rigorous sediment sampling and analytical protocol was implemented; and high-resolution remote sensing data were collected. This integrated approach provided robust, site-specific ecological data essential for evaluating the long-term fate of oil contamination and informing environmental response strategies.

After four decades of assessment at the TROPICS sites, the overall conclusion that dispersant use improved the long-term outcome for the mangrove forest remains clear. Oil exposure caused long-term structural changes in the mangrove forest, with severe initial mortality at the oil-only treatment site (Site O) and persistent adult tree losses over decades, despite strong seedling recruitment. After 40 years, adult tree numbers have declined significantly at Site O and the reference site (Site R) compared to the 10-year assessment, with the greatest loss at Site O (55.7% loss). Canopy density now exceeds pre-exposure levels at Site O and the dispersed oil treatment site (Site D), and is slightly below pre-exposure at Site R. Tree size has increased over time at Site D and to a lesser degree at Site R, but has decreased at Site O since the last assessment. Leaf morphometrics indicate that after 40 years malformed leaves were most frequent and leaf herbivory was significantly higher at Site D.

For seagrasses, after 40 years, plant density is similar to pre-exposure levels and no between-site difference was found. Blade area at Sites O and D was substantially higher than previous assessments. Sea urchin densities remain low across all sites, likely reflecting regional population declines rather than lingering effects of exposure to oil or dispersed oil. The coral reef was more significantly impacted by dispersed oil compared to oil only, however coral cover recovered to pre-exposure levels at both treatment sites at some point between 2-10 years after the exposures. After 40 years, coral cover at Sites O and D has declined compared to earlier assessments, while Site R remained stable; this decline may reflect regional Caribbean-wide coral losses rather than lingering effects from the experimental exposures, as no difference was observed between Site O and Site D. Overall, short-term coral impacts were severe but temporary, with long-term trends in coral cover likely related to broader ecological changes rather than lingering treatment effects.

Chemical analyses from sediment cores collected inside the sites highlighted the presence of hydrocarbons, alkanes, PAHs in all the samples whatever the site, depth, and tidal area. No petroleum biomarkers of the original test oil were detected in any of the core samples collected. However, a significant biogenic contribution was found in all samples; a diverse number of plant-synthesized hydrocarbons such as plant phytosterols (eg. stigmasterol, sitosterol, amyirin) and plant diterpenes (eg. rimuene, abietadiene) were found to be present. After four decades, sediments both within the original sites and immediately adjacent to the sites appear to be no longer contaminated with the original test oil and dispersed oil, although implications for long-term carbon storage in these environments after disturbances with variable recovery trajectories may be significant.

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1 Overview

The TRopical Oil Pollution Investigations in Coastal Systems (TROPICS) experiment, initiated in 1984 on small mangrove islands in Bahia Almirante on the Caribbean coast of Panama, has become one of the most comprehensive field experiments examining the long-term impacts of oil and dispersed oil exposures in nearshore tropical marine environments (Renegar et al. 2022). Over the initial 2.5-year study period, data collection occurred approximately quarterly both prior to and following the experimental exposure. Subsequent visits and analysis occurred 10, 11, 17, 18, 20, 25, 29, and 32 years after the exposure. As a result of these continuing research efforts to evaluate long-term effects at the sites over the years following the initial experiment, TROPICS is one of the most comprehensive field experiments examining the acute and chronic impacts of oil and dispersed oil in nearshore tropical marine environments. Consequently, TROPICS has become the foundational and seminal field study which served as the historical antecedent for Net Environmental Benefit Analysis (NEBA), as well as the basis for follow-on Spill Impact Mitigation Analysis (SIMA) and Comparative Risk Analysis (CRA) for oil spill planning, preparation, and response.

The conclusions of the previous TROPICS studies were that the use of dispersants reduced physical impacts and preserved the mangrove forest, sediments, and structural elevation of the environment; thus the relatively short-term loss of organisms was of less importance than preservation of the habitat structure itself. Mangroves were significantly affected at Site O, with high percentages of defoliation (43%) and death (17% mortality) of adult trees at 7 months post-exposure. This ultimately resulted in a significant decrease in canopy cover and an increase in the number of juvenile mangroves, although none of the experimentally planted juveniles survived to 4 months post-exposure. After 10 years, substantial additional mortality of mature trees (46%) had occurred, with a 9.1% reduction in total basal area of mangrove trees. A different pattern of exposure and effects was observed at the dispersed oil Site D; hydrocarbon concentrations in the water column were higher in the subtidal zone, but sediment concentrations were lower compared to the Site O in both the subtidal and intertidal areas, and oil sheens were not visibly released from the sediments after 10 years at Site D as was observed at Site O. No significant effects on adult mangroves were observed, with no significant defoliation or mortality, and neither short nor long-term effects on juvenile mangroves were apparent. For mangrove-associated invertebrates, minimal effects on mangrove oysters were observed at Site O and high tissue concentrations returned to preexposure levels after 1 year. Tree snail abundance was reduced by 51% immediately after the exposure but recovered to 94% of pre-exposure levels after 1 year. At Site D, the abundance of tree snails was reduced by 48% immediately after the exposure but recovered to 80% of pre-exposure levels after 7 months and 130% of preexposure levels after 1 year. Limited mortality was observed in mangrove oysters although tissue hydrocarbon levels were high (over 500 ppm) until approximately 1 year after the exposure.

Seagrasses withstood short direct contact with oil and dispersed oil without prolonged impact, but effects on associated fauna, particularly sea urchins, were severe. No significant effects on seagrass growth rate from exposure to either oil or dispersed oil were observed over time, although some impact on seagrass population dynamics were indicated by declining plant density at both Site D and Site O compared to Site R. Leaf morphometrics (blade area) indicated some long-term effects at Site O, as increases in blade area may be a response to stress (van

Tussenbroek, 1996). In contrast, significant effects were found in sea urchins from exposure to dispersed oil; abundance was severely reduced, with no surviving urchins immediately after the exposure at Site D, although the population recovered by 1-year post-exposure.

The key endpoints for coral assessment at the TROPICS study sites were coral % coverage and the mortality/growth rate of four coral species: *Orbicella annularis*, *Agaricia tenuifolia*, *Porites porites* (or *Porites furcata*), and *Acropora cervicornis*. Immediately following exposure, percent coral coverage declined abruptly and significantly, and continued to do so for an entire year (Ballou et al., 1987). Growth of *P. porites* (or *P. furcata*) and *A. tenuifolia* was significantly reduced at Site D. The short-term effects of dispersed oil on corals were clear, with coral coverage remaining significantly lower for at least two years following exposure, showing little indication of recovery. After 10 years of recovery, parameters at all sites were indistinguishable and no significant changes to coral coverage were found when comparing sites; short-term growth effects observed in some species, but not others, were not observed (Dodge et al., 1995).

Overall, both corals and seagrasses were found to be less impacted by floating oil but possible chronic effects from sediment contamination were observed. In contrast, oiling of mangrove environments, and subsequent leaching of entrapped oil from contaminated substrates, resulted in the loss of mature trees over time; this has significant implications for the complex balance between long-lived trees and short-lived inhabitants. Long-term recovery of the mangrove forest after oiling appeared to track the loss over time of detectable hydrocarbons in the substrate and maturation of colonizing juveniles. The dispersed oil exposure resulted in lower tree mortality and enhanced foliage recovery of surviving trees, and reduced sediment contamination.

During an oil spill response all aspects of both the direct impacts of spilled oil on the environment, as well as the indirect response-induced impacts must be considered. The United States has adopted the National Interagency Incident Management System (NIIMS) as the primary response management structure, and the tool to implement this that is most widely used is the Incident Command System (ICS) structure. Within the ICS, The Environmental Unit (EU) is charged with developing and recommending response options to the Federal On-Scene Coordinator (OSC), who oversees these incidents. In the development of these response options, the EU relies on a process known as NEBA, the Net Environmental Benefit Analysis process. IPIECA (2015) defines the NEBA as “a structured approach used by the response community and stakeholders during oil spill preparedness planning and response, to compare the short- and long-term environmental risks and benefits of potential response tools and develop a response strategy that will reduce the impact of an oil spill on the environment”.

1.1 Project Benefits

Benefits of this research center primarily on expanding the collective knowledge of the long-term impacts of oil and dispersed oil on tropical and nearshore tropical habitats. The opportunity for field research at the TROPICS sites represented a collaborative platform for federal, academic, and industry personnel to work together in a longitudinal assessment of the current state of the environment in and near the original 1984 test area which can then be used in decision-maker processes. All research partners, including BSEE, have benefited by having a much more comprehensive, non-theoretical, assessment of the potential long-term implications of oil, and dispersed oil impacts on tropical ecosystems, allowing a more comprehensive

assessment of spill response options detailed in Facility Response Plans (FRPs) associated with BSEE jurisdictional facilities operating in on the Outer Continental shelf (OCS). A key feature of the project was the inclusion of early career researchers and responders, fostering the development of future oil spill science professionals through hands-on, collaborative fieldwork. This project exemplifies a multi-sectoral approach to advancing oil spill science, combining historical context, rigorous fieldwork, and collaborative expertise to inform future environmental response planning. This JIP provided opportunities for multiple organizations representing BSEE, academic institutions, non-profit, and corporate entities to support and conduct simultaneous research during the site visit. This collaboration was crucial in promoting oil spill science, which expand understanding of the implications of long-term spilled oil and dispersed oil on shorelines and nearshore habitats and inform the Net Environmental Benefit Analysis (NEBA) process.

1.2 Project Location

TROPICS was designed to assess the ecological effects of fresh crude oil and chemically dispersed fresh crude oil at three experimental sites near Bocas Del Toro, Panama (Figure 1). The three experimental sites are located on small islands within Almirante Bay and were chosen for their similarity to each other and to represent typical tropical marine habitats; a complete initial characterization of each site is described in Ballou et al. (1987). The dispersed oil site (Site D) and the crude oil only site (Site O) are located approximately 0.5 km apart on Cayo Fresca, and the reference site (Site R) is located on Cayo Ramírez approximately 5 km to the east. Each of the three 30 m x 30 m treatment sites included a well-developed intertidal, overwash red mangrove (*Rhizophora mangle*) forest. Invertebrate fauna included mangrove oysters, sea urchins, starfish, shrimp, and tree snails (Ballou et al., 1987). The subtidal area of each experimental site consisted of shallow (≤ 1 m in depth) seagrass beds immediately seaward of the mangrove fringe, and a shallow, fringing coral reef oriented parallel to the mangroves. The reef crest was at a water depth of ≤ 1 m (Ballou et al., 1987).

Each site was fully enclosed within an oil spill containment boom (45 cm deep, anchored at 6 points) immediately prior to the exposures, and sites were separated by an open water containment boom (1 m deep and 100 m in length). Oil and dispersed oil were released into the treatment sites at the rate of 10 L/min through tubing distributed within each site (two release points were positioned in each of the coral, seagrass, and outer mangrove fringe areas). Site R was also enclosed within a containment boom, however no oil or dispersed crude oil was released. At Site O, 953 L (6 barrels) of Prudhoe Bay crude oil was released over two days; 4 barrels over a 4-hour period on one day, followed by 2 barrels over a 2-hour period on the next day. This dose was chosen to represent a moderate exposure to untreated oil and was based on estimates of field exposures that caused significant mangrove impacts, or the amount of oil that would strand from a 100 to 1,000 barrel spill. At Site D, 715 L of Prudhoe Bay crude oil pre-mixed with a commercial dispersant concentrate (20:1 oil to dispersant ratio) was released over 24 hours. The target dose (50 ppm, or a target exposure of 1200 ppm hours) was chosen to represent a high exposure of chemically dispersed oil, realistic if fresh oil (with an average slick thickness of 0.1 mm) was dispersed adjacent to a coral reef area in shallow nearshore waters, a worst-case scenario for dispersant use near coral reefs (Ballou et al., 1987).



Figure 1. Location of experimental sites within Almirante Bay, Panama, and (inset) views of dispersed oil site (Site D) and oil site (Site O) during exposure in 1984. Map data: Imagery ©2022 CNES/Airbus, Landsat/Copernicus, Maxar Technologies, US Geological Survey. (figure reproduced from Renegar et al. 2022).

1.3 Project partners and stakeholders

The project lead is Clean Caribbean & Americas (CCA), with CCA acting as the point of financial management. CCA has a 45-year history of managing projects efficiently and cost-effectively. CCA is a US 501(c)4 not-for-profit corporation. CCA has previously financed and participated in periodic TROPICS site assessments since 2001 (study years 17, 19, 25, 32). These site assessments and the resultant publications have contributed greatly to the body of knowledge regarding NEBA/SIMA and are highly cited and referenced in the science, regulatory, and operational communities.

For the 40-year site assessment, project partners and stakeholders include:

- Nova Southeastern University (Principal Investigator Abigail Renegar, Original Investigator Richard Dodge, Bernhard Riegl)
- Texas A&M University (Original Investigator Anthony Knap)
- Clean Caribbean & Americas (Paul Schuler)
- CEDRE (Ronan Jezequel, Ivan Calvez)
- Tom Sleeter (Original Investigator)
- Panama Environmental Protection Agency representative
- Nova Southeastern University graduate students/research assistants
- US oil spill regulators: BSEE, NOAA, EPA, USCG, ICCOPR

- Oil Spill Response Limited: Dispersant Core Group representative
- National Academy of Science, Engineering, and Medicine
- Canada Multi-Partner Research Initiative
- Oil Industry (Chevron, ExxonMobil, Shell, Oxy)

Project permitting was coordinated through the Smithsonian Tropical Research Institute, in Panama City and Bocas del Toro, Panama. Analytical chemistry and analysis of collected sediment cores were conducted at Texas A&M University in College Station, Texas and at CEDRE in Brest, France.

2 Objectives

The primary project objectives were to collect data that builds upon the 1984 longitudinal study, to expand our comprehensive knowledge of both crude oil and dispersed oil short- and long-term impacts on shorelines and the nearshore environment to better inform the NEBA process so critical to the optimum response. Between-site and temporal comparisons at the TROPICS sites were designed to provide non-subjective information for use in decision-making planning and processes, by evaluating the long-term fate and degradation of nontreated, versus chemically treated oil in the environment in support of NEBA. Further, the inclusion of early career researchers and responders on the research team provided next generation spill professionals an opportunity to participate in a multi-party collaborative study.

A total of nine site visits have been conducted since the conclusion of the initial experimental period in 1986, however site visit assessment metrics have varied considerably. The methodology for the 2024 visit replicated the biological assessment methods employed in the original and 10-year site visits, with expanded chemical characterization of site sediments to assess the long-term persistence and potential migration of oil contamination in sediments and substrates across the treatment island.

2.1 Project tasks

2.1.1 Task 1: Preparation for Assessments

Task 1 comprised the preparatory phase for development of the research team and planning for the field research trip.

- Fully assemble the members of the JIP:
 - Clean Caribbean & Americas
 - Bureau of Safety & Environmental Enforcement
 - ExxonMobil
 - Oxy
 - American Petroleum Institute
 - Oil Spill Response Limited
 - CEDRE
- In addition to the JIP, an advisory committee of industry professionals was established to support the project's direction. The invited members included:
 - Paul Schuler - Clean Caribbean & Americas

- Abigail Renegar – Nova Southeastern University
- Richard Dodge – Nova Southeastern University
- Anthony Knap – Texas A&M University
- Tom Sleeter - Bermuda Biological
- Ken Lee – Environment Canada
- Jacqui Michel – Research Planning, Inc.
- Steve Buschang - BSEE
- Adriana Bejarano – NOAA
- Boyd Mckew – University of Essex
- Brad Benggio - NOAA
- Ivan Calvez – CEDRE
- Gina Coelho – BSEE
- Robyn Conmy – Consultant
- Deborah French McCay - RPS Group
- Erich Gundlach – Mangrove Restoration
- Kelly McFarlin – ExxonMobil
- Victoria Broje – Shell
- Mike Drieu – Oxy
- Elizabeth Petras – USCG, ICCOPR
- Timothy Steffek - API
- Rob Holland – OSRL
- Lisa Symons – NOAA
- Nancy Kinner – UNH/CRRC
- Vanessa Principe – EPA
- Audrey Moore – OSRL
- Rhea Shears - OSRL
- Jake Smallbone – University of Essex
- Lee Britton – Memorial University
- Develop a ground/data collection plan, acquire equipment and field supplies.
 - A data collection plan with detailed methodology was presented to the members of the research team during the research team meeting on 8/28/24. A copy of this presentation was submitted with the 8/31/24 quarterly report. Supplies and equipment were acquired for field data collection, including transect tapes, quadrats, field books and slates, GPS units, and canopy densitometers. The Vibecore Mini coring device, along with core barrels were ordered directly from Specialty Devices, Inc. (the manufacturer) as this equipment was lease-only.
- Organize field teams and field activities. The field team for research conducted in Bocas del Toro included:
 - Abby Renegar
 - Steve Buschang
 - Bernhard Riegl
 - Tony Knap
 - Tom Sleeter
 - Michael Hernandez
 - Bryony Wood

- Oscar Garcia
- Junior Riegl
- Kyle Pisano
- Austin Blakeslee
- Ellen Skelton
- Cailey Dorman
- Secure all international permits and requirements (U.S. and Panama) for research and sampling (STRI).
 - The project was approved by STRI research and sample export permits were received from the Panamanian Ministry of the Environment (Mi Ambiente).
- Analyze and document biomarkers from the original oil that will be used to determine linkages:
 - The original oil used was Prudhoe Bay crude oil, for which biomarkers are well established; data on biomarkers for this oil were available at the Texas A&M GERG laboratory
- Travel arrangements to/in/from Panama for the field team:
 - The research team traveled to Bocas del Toro (BOC - Bocas del Toro Intl.) via Panama City, Panama (PTY - Tocumen Intl. and PAC-Marcos A. Gelabert Intl.), from various US and international locations, arriving on 12/08/24 or 12/09/24, with team members staying at two hotels for the duration of the research time in Bocas del Toro, and departing on 12/15/24.
- Conduct a virtual Preparation Assessment Team Meeting
 - The assessment team meeting was conducted on August 28th, 2024, at 1400 EST. In addition to the research team meeting, a project kickoff meeting with BSEE was conducted on 8/22/2024 at 1300 EST. A copy of both presentations was submitted with the 8/31/24 quarterly report.

2.1.2 Task 2: Field Assessments/Data Collection

Task 2 included the research phase of the project.

- Sample corals, seagrasses, and invertebrate communities.
 - 12/9/24: Research team members assembled in Bocas del Toro, Panama, to prepare for 5 days of field work on the TROPICS islands in Bahia Almirante.
 - 12/10/24: Day 1: All team members travelled to the Smithsonian Tropical Research Institute (STRI) Bocas del Toro Research Station for project check-in, Smithsonian facility/resource/safety orientation, and field trip registration (using STRI Field App) with Bocas Station Scientific Coordinator Plinio Gondola. After orientation, the team was introduced to STRI boat captains David and Caito who would be transporting the team to and from the TROPICS sites daily, then boarded the STRI vessels Pavona and Scarus and departed the STRI dock. Each TROPICS site (Reference, Oil, and Dispersed) was visited; team members assessed each site, located and flagged previously tagged trees, and flagged the approximate boundaries of each site. On Cayo Fresca, the island where the Oil and Dispersed Oil treatment sites are located, Oscar Garcia first calibrated, then conducted a multi-spectral survey/collected data utilizing his drone/UAS for the entire treatment island. The team then returned to Bocas, with most of the team disembarking at the Diver's Paradise

- Hotel dock; Abby Renegar and Michael Hernandez returned to STRI to check-in the STRI boats. The day's research trip was closed, and the following day's research trip plan was filed using the STRI Field App.
- 12/11/24: Day 2: Abby Renegar, Michael Hernandez, and Kyle Pisano travelled to STRI at 0830 to check-out the STRI boats, then picked up the rest of the research team at the Diver's Paradise Hotel dock at 0900. The team then traveled to the Reference site to begin Day 2 of data collection. Tom Sleeter, Ellen Skelton, and Cailey Dorman assessed the seagrass and coral reef environment and mangrove oyster populations. Bernhard Reigl, Bernhard Riegl Jr., Michael Hernandez, Bryony Wood, and Austin Blakeslee assessed mangrove forest parameters and tree snail populations. Oscar Garcia conducted multispectral and mapping drone surveys of the island. Abby Renegar, Steve Buschang, and Kyle Pisano collected 19 sediment cores; after core collection was complete, the Scarus with Tony Knap and the core collection team onboard returned to STRI to immediately extrude, divide, and place the core samples in cold storage. The remainder of the team returned to Bocas onboard Pavona after data collection was complete, with most of the team disembarking at the Diver's Paradise Hotel dock. Michael Hernandez and Austin Blakeslee returned to STRI onboard Pavona to complete check-in the STRI boats. After sediment core processing was complete, all team members returned to Bocas. The day's research trip was closed and the following day's research trip plan was filed using the STRI Field App.
 - 12/12/24: Day 3: Abby Renegar and Michael Hernandez travelled to STRI at 0830 to check-out the STRI boats, then picked up the rest of the research team at the Diver's Paradise Hotel dock at 0900. The team then traveled to the Dispersed Site to begin the second day of data collection. Tom Sleeter, Ellen Skelton, and Cailey Dorman assessed the seagrass and coral reef environment and mangrove oyster populations. Bernhard Reigl, Bernhard Riegl Jr., Michael Hernandez, Bryony Wood, and Austin Blakeslee assessed mangrove forest parameters and tree snail populations. Abby Renegar, Steve Buschang, and Kyle Pisano collected 30 sediment cores; after core collection was complete, the Pavona with the core collection team and Tony Knap onboard returned to STRI to immediately extrude, divide, and place the core samples in cold storage. Ellen Skelton and Cailey Dorman also assisted with sediment core processing. The remainder of the team returned to Bocas onboard Scarus after data collection was complete, with most of the team disembarking at the Diver's Paradise Hotel dock. Michael Hernandez and Austin Blakeslee returned to STRI onboard Scarus to complete check-in of the STRI boats. After sediment core processing was complete, all team members returned to Bocas. The day's research trip was closed, and the following day's research trip plan was filed using the STRI Field App.
 - 12/13/24: Day 4: Abby Renegar, Michael Hernandez, and Austin Blakeslee travelled to STRI at 0830 to check-out the STRI boats, then picked up the rest of the research team at the Diver's Paradise Hotel dock at 0900. The team then traveled to the Oil Site to begin Day 4 of data collection. Tom Sleeter, Ellen Skelton, and Cailey Dorman assessed the seagrass and coral reef

environment and mangrove oyster populations. Bernhard Riegl, Bernhard Riegl Jr., Michael Hernandez, Bryony Wood, and Austin Blakeslee assessed mangrove forest parameters and tree snail populations. Oscar Garcia onboard Scarus conducted repeat drone multispectral and mapping surveys of the Reference site island, then returned to Cayo Fresca and collected additional drone video of activities on the Oil and Dispersed oil island. Abby Renegar, Steve Buschang, and Kyle Pisano collected 31 sediment cores; after core collection was complete, the Pavona with the core collection team, Tony Knap, and Ellen Skelton onboard returned to STRI to immediately extrude, divide, and place the core samples in cold storage. The remainder of the team returned to Bocas onboard Scarus after data collection was complete, with most of the team disembarking at the Diver's Paradise Hotel dock. Michael Hernandez and Austin Blakeslee returned to STRI onboard Scarus to complete check-in of the STRI boats. After sediment core processing was complete, all team members returned to Bocas. The day's research trip was closed, and the following day's research trip plan was filed using the STRI Field App.

- 12/14/24: Day 5: Abby Renegar and Michael Hernandez travelled to STRI at 0830 to check-out STRI boat Pavona, then picked up Bernhard Riegl and Bryony Wood at the Diver's Paradise Hotel dock at 0900. The team then revisited each site to review and reassess the adult mangrove trees, identifying, tagging and measuring additional trees at each site. The team then returned to Bocas, and Abby Renegar and Michael Hernandez returned to STRI to check-in the STRI boat Pavona. Abby Renegar and Michael Hernandez then travelled to STRI, and met Kyle Pisano, Ellen Skelton, and Cailey Dorman to complete packing of research equipment and sediment cores. All personnel, equipment, and samples were then returned to the Diver's Paradise Hotel. The day's research trip was closed, concluding activities at STRI Bocas del Toro Station.
- 12/15/24: All team members travelled from Bocas del Toro to Panama City, then on to home destinations. Before returning to the US, team members Abby Renegar and Bernhard Riegl, after coordination with Felix Rodriguez, the Scientific Coordinator at STRI in Panama City, placed the sediment cores samples in cold storage in the STRI Tupper Building facility to await export approval. Samples were exported to the US by STRI personnel, and received at NSU on, after which they were sorted and forwarded to Texas A&M University and CEDRE.
- The Research Team achieved 100 percent completion of field Data Collection goals of Phase II for all JIP objectives.
- Texas A&M University and CEDRE will collect core samples of substrate for chemical analysis. These samples will attempt to identify persistent hydrocarbons associated with the 1984 release.
 - 80 core samples were collected and sent to Texas A7M University in College Station, TX and CEDRE in Brest, France for analysis

2.1.3 Task 3: Analysis of samples

- Analysis and interpretation of data.

- Data collected on mangroves (forest structure, mangrove tree morphometrics, mangrove forest invertebrates) seagrasses (plant density, leaf morphometrics, seagrass invertebrates) and the coral reef (% coverage of corals, plants, and other invertebrates), was analyzed and the results are contained herein. The visual and multispectral drone data analysis was completed by Oscar Garcia of Water Mapping LLC.
- Texas A&M University and CEDRE conducted a chemical analysis of sediment cores and identified petroleum hydrocarbon biomarkers.
 - The export permit for the core samples was received in January 2025, and samples were received at NSU in February. A total of 27 core samples were then sent to Ronan Jezequel at CEDRE, with the remaining 53 cores sent to Tony Knap at Texas A&M. These samples were analyzed and the reports compiled by CEDRE and Texas A&M were submitted to BSEE.

2.1.4 Task 4: Reporting

- Quarterly progress reports submitted to BSEE by CCA and NSU.
 - Quarterly reports have been submitted to BSEE by NSU and CCA on 08/30/24, 12/31/24, and 03/31/25.
- NSU prepared draft and final reports and submitted to BSEE for review and comment. The report examined the longitudinal effects observed associated with the oil and dispersed oil 1984 intentional release.
- A draft final technical report (objectives, methods, procedures, results, discussion, conclusions, and recommendations), and final report.
- Copies of all raw, and processed data conveyed to BSEE in digital format.
- Peer-reviewed, open-access publications (Marine Pollution Bulletin); 2) presentations at conferences and workshops (Interspill 2025, Clean Gulf 2025); and 3) outreach to the public, OSROs, and regulatory agencies (API Spill Advisory Group, IPECA Marine Spill Group).

3 Methods and procedures

For the 40-year site visit, assessments of biological compartments (mangroves, seagrasses, and corals) were conducted using the same methods applied during the original experiment (1984-1986) and the 10-year site visit in 1994. Boundaries at each site were reestablished by identifying tagged tree margins, then site edges were measured and marked with transect tapes. This site boundary re-identification was also conducted for site visits at 10 and 17 years, however published reports do not indicate that this was done at the 20, 25, or 29-year site visits.

At each TROPICS site, mangrove areas were surveyed for both structural and biological parameters. All propagules, seedlings, and mature trees were counted, tagged, and measured for height and diameter at breast height (DBH), following standardized forestry protocols. Nine sampling stations were established across three transects per site to measure canopy density and leaf area index (LAI). Microstructural traits such as leaf dimensions and aerial root lenticles were recorded for selected trees. Additionally, tree snail (*Littorina angulifera*) populations were quantified across six vertical strata within a 5-meter radius of each station. In seagrass areas, seagrass health and associated fauna were evaluated using quadrat and transect methods. Plant

density and blade morphometrics were measured within randomly selected quadrat cells. Sea urchin density was assessed both areally (within quadrats) and linearly (along transects). Mangrove oyster species counts were conducted on roots adjacent to seagrass beds. Coral reef community structure was characterized by the point plotless method with four 10-meter transects per site. Substrate types were classified at 10 cm intervals, identifying organisms to species or taxonomic group. Coverage percentages for corals, other organisms, plants and bare substrate were calculated to determine relative abundance and long-term ecological shifts.

To evaluate potential long-term hydrocarbon contamination at experimental sites, a rigorous sediment sampling and analytical protocol was implemented. A total of 80 sediment cores were collected using a GPS-indexed grid with 5-meter spacing, utilizing a Vibe-Core handheld coring device and precleaned aluminum barrels. Sampling targeted three tidal zones: high intertidal, mid-intertidal, and subtidal, with additional cores collected outside site boundaries to assess background contamination. Cores were extruded and segmented into three depth intervals (0–5 cm, 5–10 cm, and >10 cm), photographed, and preserved in cold storage for chemical analysis. Cores collected from within the site boundaries were analyzed at CEDRE in Brest France, and cores collected from outside site boundaries were analyzed at Texas A&M University GERG laboratory in College Station, Texas. This dual-laboratory approach ensured high-resolution chemical characterization of sediment samples, enabling robust assessment of hydrocarbon persistence and migration across the TROPICS islands.

To support a multidisciplinary study on oil spill impacts, high-resolution remote sensing data were collected using an Uncrewed Aerial System (UAS) equipped with a multispectral imaging system. The UAS platform—a quadcopter—was outfitted with the Altum-PT MicaSense sensor, capable of capturing synchronized imagery across seven spectral bands: Visual, Blue, Green, Red, RedEdge, Near Infrared, and Infrared. Imagery was acquired in NADIR mode (directly downward-facing) with a minimum 50% overlap between images to enable the construction of detailed orthomosaics. Additional oblique RGB imagery and video footage were collected to provide visual context and enhance spatial interpretation of the study sites. Over the course of multiple flight missions, more than 7,000 multispectral images were captured, covering all designated areas of interest.

3.1 Mangroves

All mangrove propagules/seedlings and mature trees were counted within each site. For each mature tree within the sites, the GPS coordinates were recorded and a numbered aluminum tree tag was affixed to the trunk at DBH. Trees were also re-tagged at the 10- and 17-year site visits, however published reports do not indicate that this was done at the 20, 25, or 29-year site visits. The height of each tree was measured (meters, surface of substrate to highest point of tree) using a graduated PVC pole. The trunk (bole) DBH was measured with a tree diameter tape at breast height (1.3 m above substrate), according to the following guidelines:

- If the tree had multiple boles and these boles forked or sprouted from a common base below breast height, each was measured as a separate bole.
- If the tree had multiple boles and they forked at or above breast height, the DBH was measured at 1.3 m or just below the swelling caused by the fork (if the fork was at or above breast height, it was considered as only one bole).

- If the bole had a fluted trunk or prop roots at breast height, the diameter was measured above them at a location where the fluting and/or prop roots did not bias the measurements.
- If the bole had swellings, branches, or abnormalities at breast height, the DBH was measured either above or below (whichever was closest to breast height) the irregularity where it stopped affecting normal form.

Tree height and DBH were measured during the original experiment (1984) and at the 10- and 17-year site visits, using the same methods described here. These two parameters were also assessed at the 29-year site visit, but collected only for 9 random tree plots with each site and therefore do not likely represent the complete within-site tree population.

For assessment of canopy density, LAI, and leaf morphometrics, three transects were established perpendicular to the water's edge in the mangrove forest at each site; a center transect, and two transects midway between the center transect and the edge of the study site. Three sample stations were established on each of these transects, for a total of 9 transect sampling stations at each site. At each sampling station, mangrove canopy density was measured three times at 1.3 m above the substrate using a spherical densiometer. At each sampling station, LAI was measured 5 times, by counting the number of leaves touching a PVC pole inserted up through the forest canopy.

Three trees on the center transect were selected for measurement of microstructural characteristics (leaf morphometrics). One tree was located near the water's edge, one in the center of the site, and one at the rear of the study site. The length and width of each mangrove leaf on three branches of each tree was measured with plastic calipers, and percentage of malformed leaves and leaf herbivory was visually evaluated.

Canopy density and LAI were also measured, using the same apparatus and using the same transect locations as described here, at the 17-18-year site visits; canopy density was also measured at the 29-year site visit, but it is unclear if similar transect and sampling station locations were used. Leaf herbivory at the 17-year visit was conducted on 100 randomly selected leaves at each site, in contrast to the method used in the original study, the 10-year visit and the 40-year site visit (this study).

At each of the 9 sampling stations within each site, the number of tree snails (*Littorina angulifera*) in each of 6 vertical compartments (from the sediment surface to the canopy: 0-1 cm, 1-24 cm, 25-60 cm, 61-100 cm, 101-170 cm, <170 cm) within a 5 m radius of each station was counted. Tree snail counts were also conducted at the 10- and 29-year site visits, but only the total number of snails at each sampling station was counted, without evaluation of vertical distribution in the mangrove canopy.

Data were tested for normality (Shapiro-Wilk, Komologorov-Smirnov) and homoscedasticity (Bartlett's, Brown-Forsythe), then parametric (One-way ANOVA) or non-parametric (Kruskal-Wallis) tests were used to assess significant differences between sites as appropriate.

3.2 Seagrasses

Long-term trends in seagrass plant density and blade morphometrics, as well as seagrass associated invertebrates, were evaluated. Four 0.5 m x 0.5 m PVC quadrats (divided into 25 internal 100 cm² cells) were haphazardly placed in the seagrass area. Sea urchin areal density (urchins/m²) was measured by counting all the sea urchins within each quadrat. Seagrass plant density (plants/m²) was measured by counting the number of seagrass plants in 16 randomly selected cells in each quadrat. Seagrass blade morphometrics were determined by measuring the length (inflexible margin of basal sheath to distal end of blade) and width of the inner (newest) undamaged central blades with plastic calipers for 10 randomly selected plants within each quadrat.

Plant density was also measured at the 10-, 18-, 25-, and 32-year site visits, using the same quadrat methodology described here, although specific quadrat locations varied as the original quadrat markers were no longer present. Sea urchin areal density was also assessed at the 32-year site visit using the same quadrats, but only total urchins were enumerated, without species discrimination.

Three 30 m tape transects were placed perpendicular to the shoreline at each site, and sea urchin linear density was measured by counting the number of urchins of each species at 2 m intervals along each transect. The number and species (*C. rhizophorae*, *I. alatus*, and *P. imbricata*) of mangrove oysters was counted on five randomly selected mangrove roots adjacent to the seagrass area at each site.

Sea urchin linear density was also assessed at the 10- and 29-year site visits, although the transect locations differed from the originally marked transect locations. Similarly, mangrove oyster abundance on subtidal mangrove prop roots were assessed at the 10- and 29-year site visits, although the specific root enumerated varied from the original experiment.

Data were tested for normality (Shapiro-Wilk, Komologorov-Smirnov) and homoscedasticity (Bartlett's, Brown-Forsythe), then parametric (One-way ANOVA) or non-parametric (Kruskal-Wallis) tests were used to assess significant differences between sites as appropriate.

3.3 Coral reefs

To assess long-term changes in the reef community structure, reef flora and fauna composition were characterized using the point plotless method: Four 10 m transects were deployed at each site, parallel to the reef crest: 2 transects were located on the crest at a depth of approximately 1 m, and 2 transects in slightly deeper water (approximately 1.3 m depth) seaward of the reef crest. The substrate directly beneath 100 points (located at 10 cm intervals on each transect line) was identified and classified as bare substrate, animal, or plant (Table 1). Corals and zoanthids were identified to species, and other animals to phylum or order as possible. Seagrass was identified to species, and algae classified as fleshy or calcareous. The mean % coverage for each category (total organisms, total corals, total animals, and total plants) was then calculated as a measure of abundance.

The reef community was assessed using the same point plotless methodology at the 10-, 18-, 25-, and 32-year site visits, although specific transect locations varied from the original experiment as the original transect markers were no longer identifiable.

Table 1. Substrate and organisms ID codes used for the point-plotless method.

Substrate Codes:	ID codes:
B=Bare	R=rubble S=sand
O=Other animals	Z=zoanthid S=sponge A=anemone U=urchin
P=Plant	C= calcareous algae F=fleshy algae S-seagrass
C=Coral	PF= <i>Porites furcata (porites)</i> PA= <i>Porites astreoides</i> AT= <i>Agaricia tenuifolia</i> MA= <i>Millepora alcicornis</i> SS= <i>Siderastrea siderea</i> DS= <i>Diploria strigosa</i> DC= <i>Diploria clivosa</i>

3.4 Sediment cores

To assess potential long-term hydrocarbon contamination in the sediments of the experimental sites, sediment core samples were collected at each site in a GPS indexed grid, with cores spaced 5 m apart. A total of 80 sediment cores were collected using a Vibe-Core handheld, battery powered coring device and 2-inch diameter, 34-inch long precleaned aluminum core barrels (from Specialty Devices, Inc.).

The GPS coordinates of each core were recorded, and after the core barrel was extracted from the sediment, excess water was drained and the cores were capped and placed in a cooler for transport to the Smithsonian Research Station in Bocas del Toro. On the same day as collection, cores were extruded on a clean foil surface and subdivided into three depths (0-5 cm, 5-10 cm, and >10 cm) from the sediment surface. Cores were photographed, and subsamples were wrapped in precleaned foil, placed in labelled Whirl-Pak sample bags, and transferred immediately into cold storage at STRI in Panama City, Panama, before they were shipped first via FedEx Priority International Overnight to Nova Southeastern University in Ft. Lauderdale, Florida. Cores collected inside the experimental sites were then shipped via FedEx Priority International Overnight to CEDRE in Brest, France for analysis, and cores collected outside the sites were shipped via FedEx Priority Overnight to Texas A&M GERG in College Station, Texas for analysis. Distribution of samples between the two laboratories was determined in collaborative meetings between the project PI and the laboratory directors. Sample analysis at

both laboratories included assessment of the presence of petroleum biomarkers from the original test oil and quantification of alkanes and PAHs.

3.4.1 Within sites: CEDRE

3.4.1.1 Extraction

Prior to analysis, samples were homogenized. Approximately 5 grams (wet weight) were sub-sampled to determine water content (40°C @60°C for 12h). Approximately 10 grams were sub-sampled for hydrocarbon analysis. An internal standard solution (deuterated alkane, d42-eicosane) was added prior to extraction using an EXTREVA ASE system (Accelerated Solvent Extraction). The final extract was concentrated to 200 µL under a gentle nitrogen stream prior to instrumental analysis by gas chromatography with flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC-MS).

3.4.1.2 Analysis

Quantification of hydrocarbons (C10–C40 range) was conducted by GC-FID using liquid injection. Calibration was performed using the BAM K010 diesel/mineral oil standard (BAM, Germany), in accordance with the NF EN ISO 9377-2 method. Analyses were carried out using an HP 7890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a pulsed splitless injector (splitless time: 1 min; purge flow: 54 mL/min). The injector temperature was set to 320 °C, and the detector interface was maintained at 300 °C. The GC oven temperature program was as follows: initial temperature of 45 °C, ramped at 10 °C/min to 320 °C, with a final hold of 12 minutes. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Separation was achieved on an HP-5ms capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies). The FID detector was operated at 300 °C.

The analysis of PAH and alkanes was performed by Gas Chromatography coupled to Mass Spectrometry (GC-MS). The GC was an HP 7890N (Hewlett-Packard, Palo Alto, CA, USA) equipped with a CIS-4 injector used in “splitless” mode (Splitless time: 1 min, flow 54 mL/min). The injector temperature was maintained at 300 °C. The interface temperature was 280°C. The GC temperature gradient was: from 42°C (1.1 min) to 320°C at 5.5°C/min (16 min). The carrier gas was Helium at a constant flow of 1 mL/min. The capillary column used was a HP-5 ms (HP, Palo Alto, USA): 30 m x 0.25 mm ID x 0.25 µm film thickness. The GC was coupled to an Agilent 5977B used in SIM mode (Electronic Impact: 70 eV, voltage: 2000 V). Compounds quantifications were done using Single Ion Monitoring mode with the most representative fragment of each compound at a minimum of 2 cycles/s.

3.4.2 Outside sites: Texas A&M GERG

3.4.2.1 Extraction

As the samples were very peaty, a simple sonication extraction with dichloromethane (DCM) followed by a sodium sulfate extraction column for moisture removal were not successful, and only phytosterols and elemental sulfur were found. Therefore, a more comprehensive extract clean-up technique using silica gel column chromatography was necessary to remove the polar compounds and activated copper to remove elemental sulfur.

Five hundred milligram subsamples were extracted in DCM using ultrasonication for 30 minutes and transferred to clean vials following centrifugation. This sonication step was repeated a total of three times. The resulting extract is considered the total lipid extract (TLE). The TLEs were then concentrated under a gentle nitrogen stream. These extracts were found to have interferences from elemental sulfur and plant biomolecules and required further cleanup using activated silica gel column chromatography. The silica gel columns (containing 1 gram of activated silica gel) were first washed with hexane followed by 1:1 hexane:DCM. The samples were reconstituted in 1:1 hexane:DCM and loaded onto the columns. The fraction containing less-polar hydrocarbons, including petroleum biomarkers, was eluted using 3 mL of 1:1 hexane:DCM. The second, more polar, fraction containing plant phytosterols was eluted using 3 mL of 1:1 DCM:methanol. The addition of activated copper was essential to remove elemental sulfur from the majority of extracts to remove matrix interference. This was added to the fraction 1 extract (1:1 hexane:DCM). The final extracts were concentrated to near dryness with a gentle stream of nitrogen and reconstituted in 400 μ L of DCM for analysis. NIST Standard Reference Material (SRM) 1944 (New York/New Jersey Waterway Sediment) was extracted for every twenty samples to verify method performance.

3.4.2.2 Analysis

Extracts were analyzed on an Agilent 7890 Gas Chromatograph (GC) coupled to a 5977B Single Quadrupole Mass Spectrometer (MS). Several NIST petroleum SRMs (2779 and 2722) were run with each analytical batch to verify petroleum biomarker peak positions. Extracts were injected in “splitless” mode using a 2 μ L injection volume and a splitless time of 1.5 minutes. The inlet was held at 300°C and the helium carrier gas was held constant at 2 mL/min. The capillary column used was an Agilent DB-1ms ultra inert column (60 m x 0.25 mm ID x 0.25 μ m film thickness). The GC oven was initially held at 35°C for 2 minutes, followed by a 4°C/min ramp to 300°C where it was held for 20 minutes. A post-run ramp to 320°C was held for 10 minutes before the next sample injection. The MS was operated in SCAN mode with a scan range of 40-450 m/z following an initial solvent delay of 10 minutes. The electron impact (EI) source was held at 250°C while the quadrupole was kept at 150°C. Total run time is 98.25 minutes. The biomarkers measured in this study are shown in the detailed report from GERG, along with their corresponding mass-to-charge ratio (m/z).

3.5 Remote sensing

With the objective of collecting high resolution remote sensing data over various targets related to a multidisciplinary study of oil spill impacts, WM utilized an Uncrewed Aerial System (UAS) rigged with a multispectral sensor. The UAS is a Quadcopter with capacity to payload a multispectral Altum-PT micasense system. This system has 7 independent sensors that collect synchronous imagery within the Visual, Blue, Green, Red, RedEdge, Near Infrared, and Infrared wavelengths, at a maximum altitude of 200 feet. The multispectral data was obtained in NADIR mode (Straight look down camera angle) with a minimum overlap of 50% between images to construct the Orthomosaics. An oblique view of the study sites was collected with additional RGB (visual) sensor, and synchronous multispectral imagery was collected over multiple flight missions to capture the areas of interest of the various study sites. The multispectral dataset collected during the field campaign consisted of more than 7000 individual images recorded during multiple flights throughout the field campaign. In addition to the multispectral dataset, extensive RGB visual photo stills and video were also collected with the UAS.

4 Results and discussion

4.1 Mangroves

4.1.1 Trees

The number of live trees and live seedlings at the sites has varied over time (Figure 2). Pre-exposure tree density was initially highest at Site O and lowest at Site D, but overall tree densities were within the normal range for similar Caribbean environments (Pool et al., 1977). Four months after the oil exposure, severe effects were observed in the adult mangroves at Site O, with significant mean defoliation (43.1%) throughout the entire site, concentrated in the area where the oil was observed to collect during the exposure; 18 trees were completely defoliated and dead. Low survival of natural seedlings and propagules was noted. An additional 7 trees were lost at Site O after 7 months (Ballou et al., 1987). At the next site visit, 10 years post-exposure, a substantial number of additional trees (44, for a total of 69) had been lost at Site O, concomitant with a significant increase in the number of seedlings and juveniles. The number of live adult trees was unchanged at Site R, and only 2 trees had been lost at Site D (Dodge et al., 1995). Live tree numbers decreased slightly at Site D and Site R, and increased slightly at Site O, at 17-18 and 20 years post exposure (Ward et al., 2003; Baca et al., 2005; Schuler and Baca, 2007). Live tree numbers increased at Site O and Site R after 25 years, and at Site D and Site O after 29 years. Substantial increases in the number of seedlings at all sites were seen at the 20, 25, and 29 year site visits, with the most substantial increase observed at Site O (DeMicco et al., 2011; Baca et al., 2014). After 40 years, the number of live trees within site boundaries decreased compared to tree numbers at the 10-year assessment at all sites, to 68 at Site R, 66 at Site O, and 53 at Site D. This represents an overall 34.3% loss of trees at Site R, a 55.7% loss at Site O, and a 25% loss at Site D since the beginning of the experiment; this loss is significant at Site R and Site O, but not at Site D (simple linear regression). Seedling numbers have continued to increase at all sites, particularly at Site R.

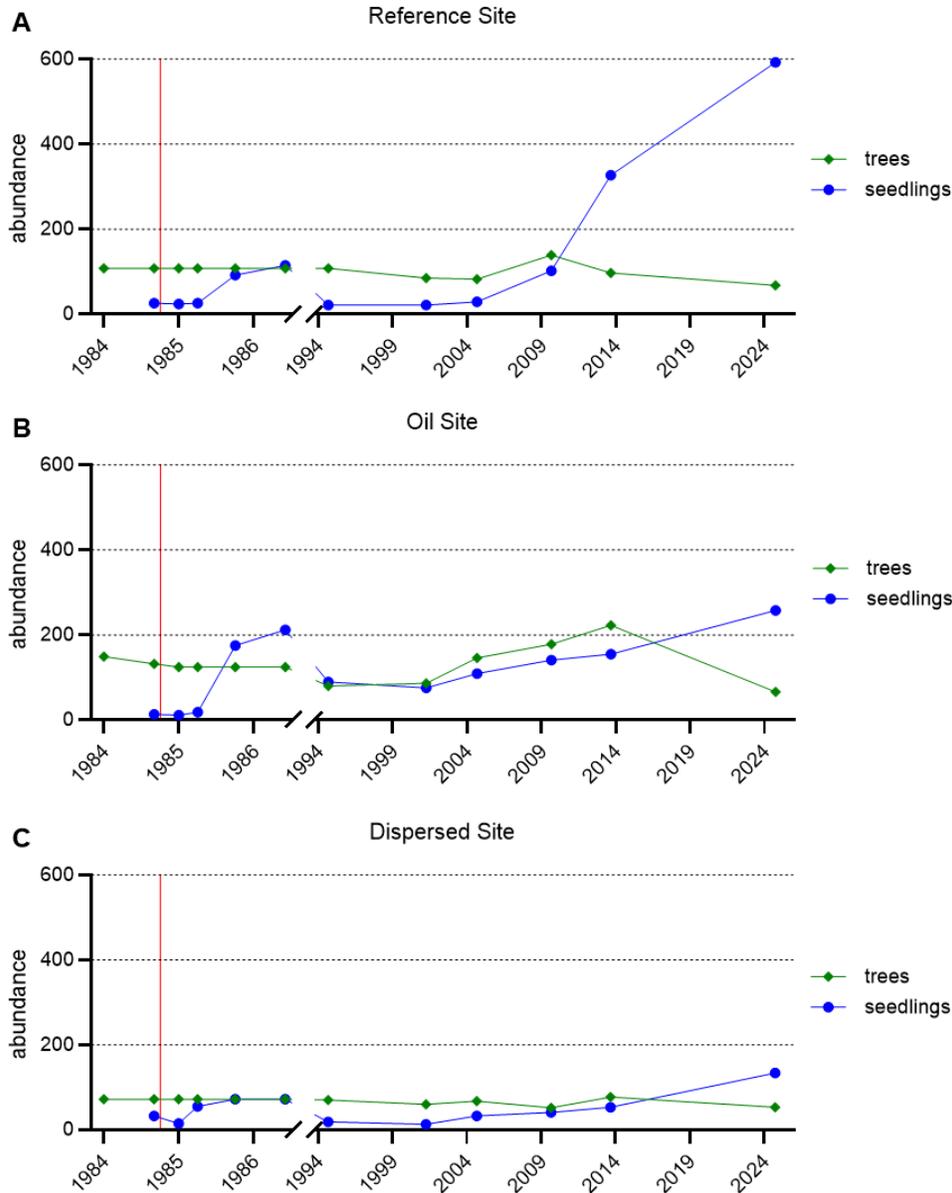


Figure 2. Live mangrove tree and mangrove seedling abundance over time at A) the Reference site, B) the Oil site, and C) the Dispersed oil site. Red vertical lines indicate the exposure date.

Mangrove canopy density (Figure 3) was reported over time as either as % open canopy (the % of overhead area not occupied by canopy, or percentage open canopy, as in Ballou et al., 1987) and as canopy density (100 - % open canopy, as in Dodge et al., 1995). The percentage of open canopy was initially higher at Site O compared to Site D and Site R; at 4 months post exposure, the % open canopy remained relatively constant at Site D and Site R, but increased substantially at Site O as a result of defoliation and tree loss. After 20 months, continuing defoliation at Site O was evident, with some defoliation observed in 78% of the surviving trees; the average estimated defoliation was 47.5%, and the % open canopy continued to increase. After 10 years, canopy density had decreased substantially at all sites, but Site R and Site D remained higher than Site O (Dodge et al., 1995). Canopy density had increased at all sites after 29 years but remained overall

lower than pre-exposure levels at all sites (Baca et al., 2014). Canopy density in 2024, after 40 years, is slightly greater than pre-exposure levels at Site O and Site D, and only slightly less than pre-exposure levels at Site R. Between site differences were significant, (Kruskal Wallis, $p=0.0027$) with canopy density at Site R significantly less than at Site D (Dunn's, $p=0.0018$); no significant difference was found between Site D and Site O (Dunn's, $\alpha=0.05$).

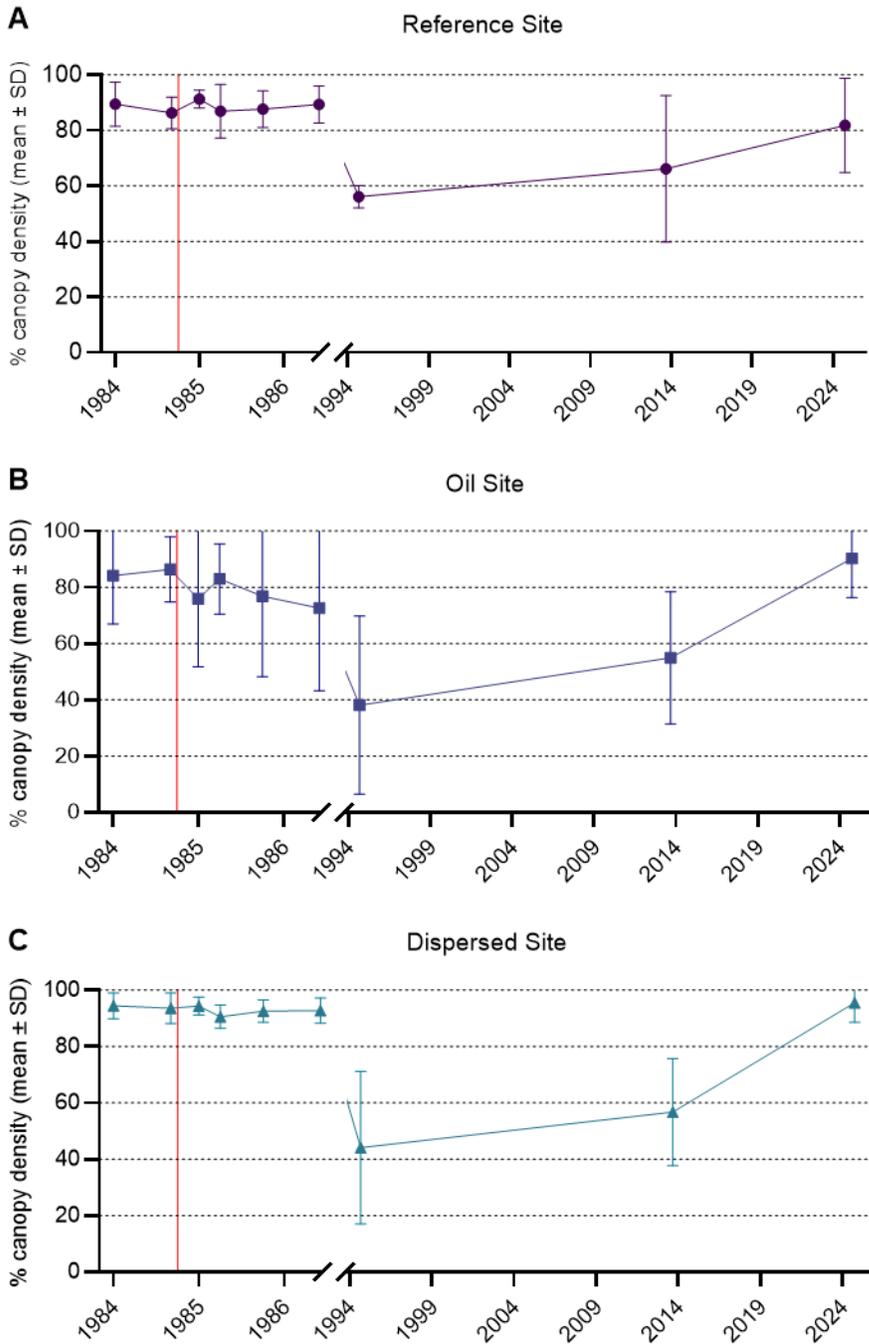


Figure 3. Mangrove canopy characteristics over time, as % canopy density (mean ± SD) (100 - % open canopy, as in Dodge et al., 1995) for A) the Reference site, B) the Oil site, and C) the Dispersed oil site. Red vertical lines indicate the exposure date.

Mangrove tree size (DBH) and canopy height (Figure 4) have been measured 4 times since the project began: pre-exposure, and 10-, 17-, and 40-years post exposure. Large within-site variability has limited the statistical significance of these parameters, but overall canopy height has remained relatively consistent over time, and overall tree DBH has increased over time at all sites. However, while mean tree size has increased over the 23 years since the last measurements at Site R (by 13.6%) and at Site D (by 14.5%), mean tree size has decreased at Site O (by -3%) during the last measurement interval. After 40 years, no significant difference in canopy height or LAI was found between sites (Kruskal Wallis, $p > 0.05$). However, mean DBH is significantly different between sites (Kruskal Wallis, $p < 0.0001$), with mean DBH significantly higher at Site D compared to Site O and Site R, with no difference between Site R and Site O; this is a departure from previous observations, where Site O DBH was significantly less than Site R after 10 years, and no difference between sites was found after 17 years.

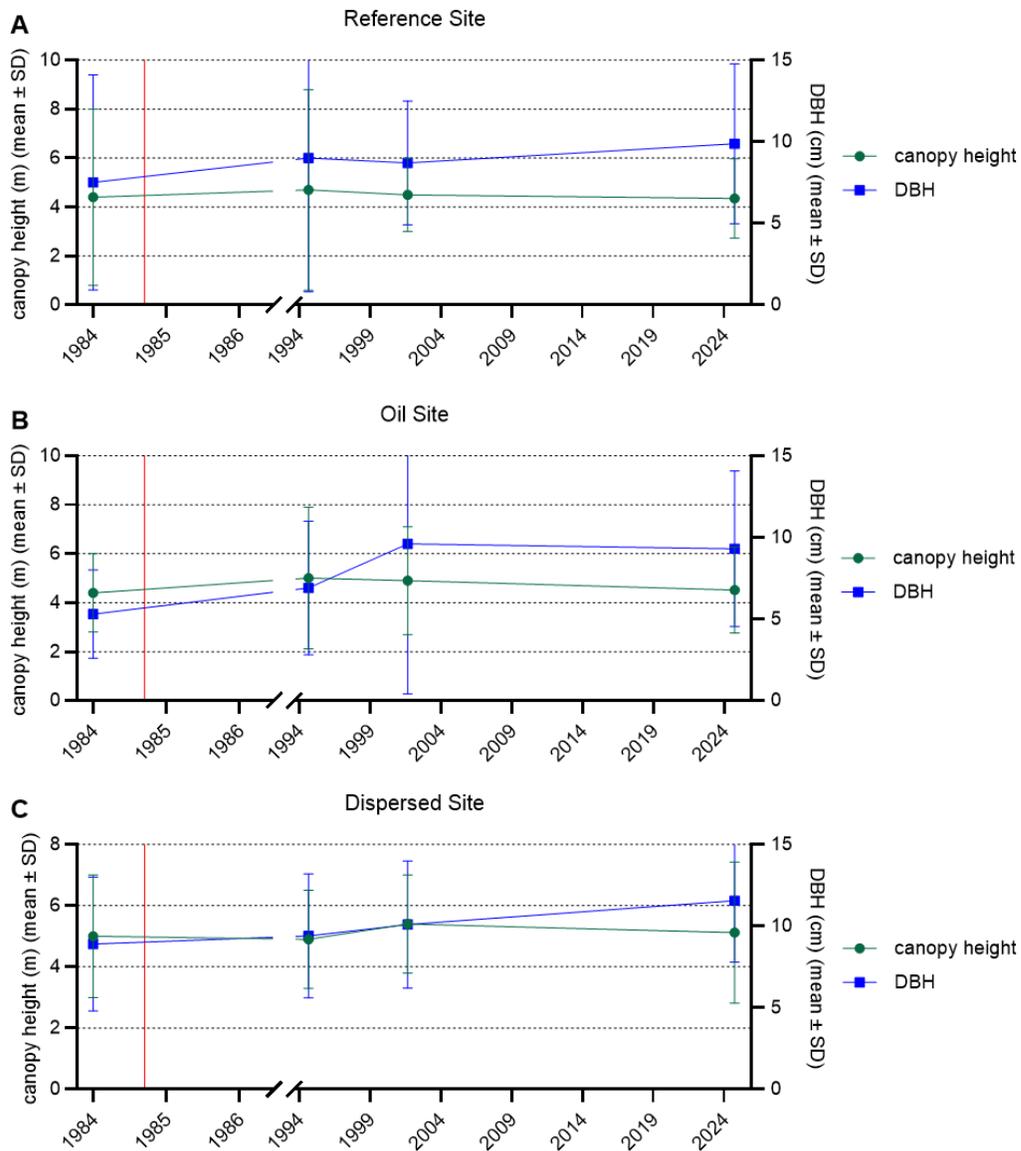


Figure 4. Mean (\pm SD) canopy height (m) and mean (\pm SD) diameter breast height (DBH) (cm) of the mangrove trees over time at A) the Reference site, B) the Oil site, and C) the Dispersed oil site. Red vertical lines indicate the exposure date.

Mangrove leaf morphometrics over time are shown in Figure 5. A significant decrease in leaf length compared to baseline measurements was observed at 7 months post exposure at Site D. After 12 months, significant decreases in all mangrove leaf parameters (leaf width, length, and length/width ratio) were observed at Site O; a significant decrease in leaf length compared to baseline was observed at Site D, and significant decreases in leaf width and length were observed at Site R. At 20 months post- exposure, significant decreases in leaf width and length were also observed at Site O, and a significant decrease in the length/width ratio of adult leaves was found at Site D. (Ballou et al., 1987). No statistically significant difference was found between leaf length or width among the sites after 10 years (Dodge et al., 1995). After 40 years, the L/W ratio and leaf length has increased at all sites, and the leaf width has decreased at all sites. No

significant difference between sites was found for leaf width, but was found between sites for leaf length and L/W ratio (Kruskal Wallis, $p < 0.05$), with leaf length greater at Site D compared to Site O (Dunn's, $p = 0.0126$), and L/W ratio was significantly greater at Site D compared to Site R (Dunn's, $p = 0.0024$). A highly significant difference between sites was found for leaf herbivory, with % herbivory greater at Site D (15.1%) compared to both Site R (9.5 %) and Site O (6.2%).

The percentage of malformed leaves was 30% at Site D, 21.1% at Site R, and 16.7% at Site O; leaf malformation was evaluated during the original experiment only, but the results were not reported in Ballou et al. (1989). Leaf herbivory was previously assessed at the 10- and 17-year site visits; no significant difference between sites was noted at the 10-year visit, but herbivory was significantly higher at Site D compared to Site O and Site R at the 17-year site visit, the same pattern seen after 40 years.

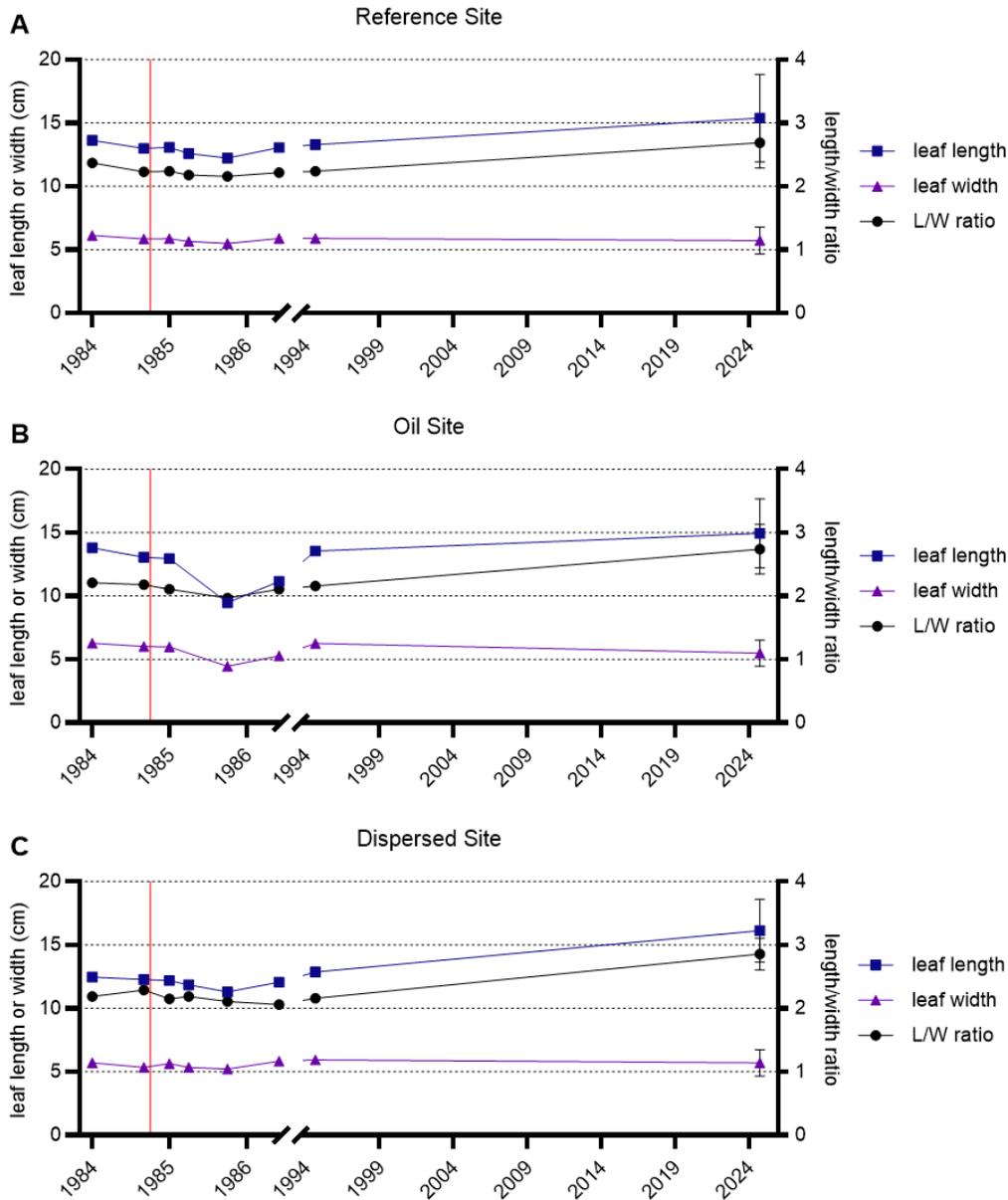


Figure 5. Mean (\pm SD, where available) mangrove leaf width (cm), mean (\pm SD, where available) mangrove leaf length (cm), and mean (\pm SD, where available) leaf length/width ratio over time at A) the Reference site, B) the Oil site, and C) the Dispersed oil site. Red vertical lines indicate the exposure date.

Acute effects from oil exposure in mangroves have included defoliation and mortality of adult trees and loss of seedlings and juveniles, followed by secondary and residual effects frequently including the continuing loss of mature trees (Burns et al., 1993; Mackey and Hodgkinson, 1996; Proffitt, 1997; Hensel et al., 2010; Lewis et al., 2011; Santos et al., 2011; Duke and Burns, 1999; Michel and Rutherford, 2014; Duke, 2016). The crude oil-only treatment at Site O resulted in heavy oil contamination of mangroves and their intertidal substrate and sediments, with significant defoliation and death of adult trees in the short term. After 10 years, substantial additional mortality of mature trees (46%) had occurred. At Site D, no significant effects on

adult mangroves were observed, with no significant defoliation or mortality, and neither short nor long-term effects on juvenile mangroves were apparent. After 40 years, adult tree numbers have declined significantly at Site O and Site R compared to the 10-year assessment, with the greatest loss at Site O (55.7% loss). The number of adult trees at all three sites has declined over time based on the 40 year counts; it is unclear why substantial increases in tree counts were reported from site visits between 10 and 29 years post-exposure, but it is possible that either single trees with multiple boles were inadvertently counted as individual trees, or that juvenile trees with a DBH of <2.5 cm were included in the tree count (as has been done for the current dataset, based on STRI mangrove assessment protocols). Seedling recruitment has steadily increased since 17 years post-exposure; the ongoing increase in seedling numbers observed since then would appear to be consistent with the 40-year tree counts, although the long-term survival rate of seedlings and juvenile trees may have been reduced by continuing substrate contamination at Site O.

Canopy density now exceeds pre-exposure levels at Site O and Site D, and is slightly below pre-exposure at Site R. Recovery of the mangrove community to pre-exposure levels of canopy cover (or greater) has now occurred, despite the decline in tree numbers at all three sites. Mangrove canopy density can increase despite a decrease in the total number of trees as a result of natural forest maturation, which can result in fewer trees which have larger, spreading crowns. Reduced resource competition from the loss of trees, particularly at Site O, may have been offset by growth inhibition in surviving adult trees from continuing substrate oil contamination, which delayed gap infilling and crown expansion. Once large trees are lost post-disturbance, recovery in terms of seedlings progressing to adulthood may not occur if they are ultimately out-competed for resources, and shaded out by the surviving larger adults.

Forest height has been relatively stable over time; no significant differences between sites in was found in 2024. Tree size (DBH) has increased over time at Site D and to a lesser degree at Site R but has decreased at Site O since the last measurement. Leaf morphometrics have shown variable between-site patterns over time, however after 40 years malformed leaves were most frequent at Site D (30%), followed by Site R (21.1%) and Site O (16.7%), and leaf herbivory was significantly higher at Site D (15.1%) compared to Site R (9.5%) and Site O (6.2%). The organism(s) primarily responsible for this leaf herbivory have not been identified at any TROPICS site visits, but are likely insects and crabs. Increased leaf herbivory is associated with increased stress and pollution, but is also commonly observed in mature trees at 5-20 % (Farnsworth and Ellison 1991, Duke 2002); therefore although herbivory is higher at Site D, it is with the typical range for this mangrove species, as is herbivory at Site O and Site R. Given the overall canopy density and canopy health after 40 years at all 3 sites, the significant difference in herbivory may be the result of natural variation in herbivory patterns and not related to the oil or dispersed oil site treatments.

4.1.2 Invertebrates

Significant initial impacts of the exposures on tree snails (Figure 5) (*L. angulifera*) were observed at both Site O and Site D; snail populations were reduced to 51% of pre-exposure abundance at Site D and to 50% of pre-exposure abundance at Site O immediately after the exposures. After 4 months, snail abundance had increased at both exposure sites, but remained

below pre-exposure levels (Ballou et al., 1987). After 7 months, tree snail populations increased to within 80% of pre-exposure abundance at both treatment sites, approaching pre-exposure population density. After 12 months, tree snail populations increased to above pre-exposure abundance at Site D, and to 94% of pre-exposure abundance at Site O. After 20 months, tree snail abundance decreased overall at all sites, with a general shift of the remaining organisms upward in the mangrove forest; analysis indicated that the treatments had a significant effect on snail populations over time, although a significant change in vertical distribution (a shift away from the forest floor) was only observed at Site O. At Site R, the tree snail distribution was observed to shift bidirectionally, towards both the canopy and the forest floor (Ballou et al., 1987).

Total tree snail abundance (only) was assessed at 10 and 29 years post exposure, with overall abundance increasing at all sites. A similar trend was observed in 2024, with large increases compared to the 29-year post-exposure assessment; overall the snail population was highest at Site O, and lowest at Site R, where the Site R population now is within the same range as previously observed, while Site O and Site D populations are substantially higher (Dodge et al., 1995; Baca et al., 2014).

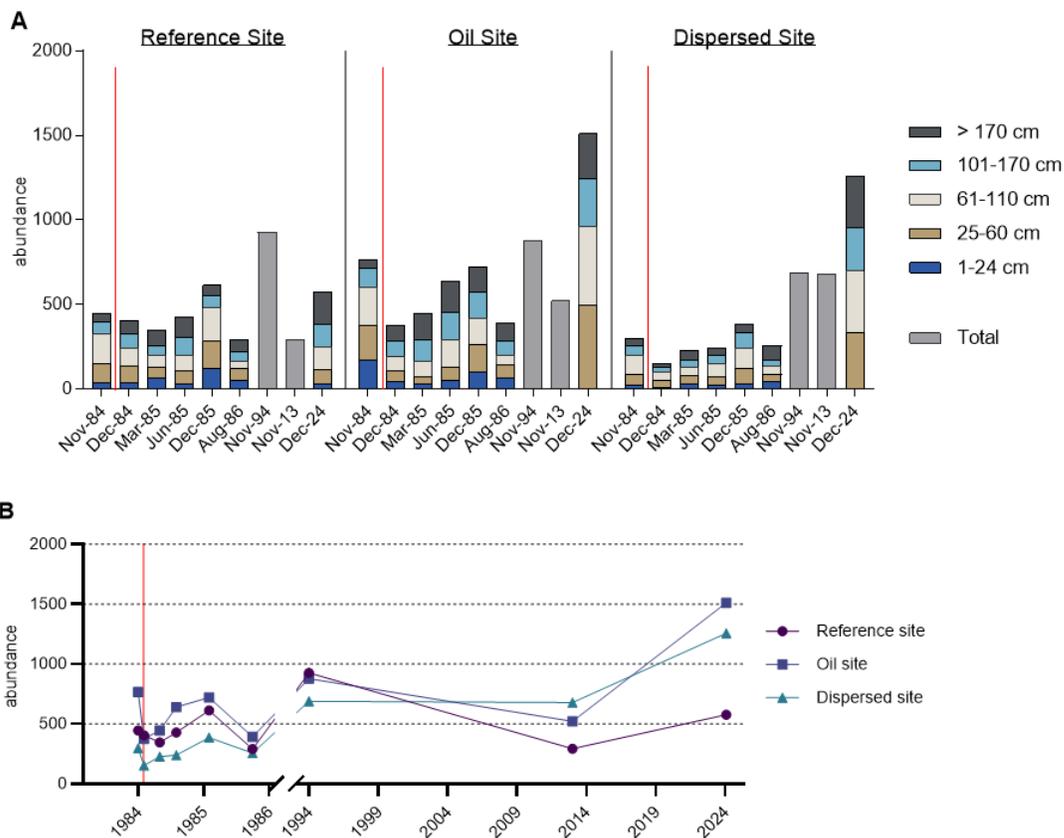


Figure 6. Mangrove tree snail abundance at each treatment site over time, shown as A) the number of snails in each elevation compartment above the substrate, and B) the total number of snails over time. Red vertical lines indicate the exposure date.

Mangrove oyster populations on selected mangrove roots over time is shown in Figure 7. Immediately after the exposures, and despite significant exposure to oil, *Isognomon alatus* and *P. imbricata* had 100% survival at both Site D and Site O, and *C. rhizophorae* had 96.9% survival at Site D and 87.5% at Site O. Survival at Site R was 100% for all oyster species. After 4 months, oyster survival was more variable but remained relatively high, with 100% survival of *C. rhizophorae* at Site R and 81.2% survival at Site O, with the lowest survival rate (60.9%) at Site D (Ballou et al., 1987). After 10 years, the population size of all three species increased from the 20-month levels, with no significant differences between Site O and Site R (Dodge et al., 1995). Oysters were reassessed after 29 years, The population size of all three oyster species increased at all sites compared to pre-exposure levels, with a significant increase at Site D (Baca et al., 2014). After 40 years, oyster populations at Site R and Site D have decreased to levels similar to the 10-year assessment, but have continued to increase at Site O. Since the 1994 assessment, an ongoing shift in population composition towards *P. imbricata* has been evident.

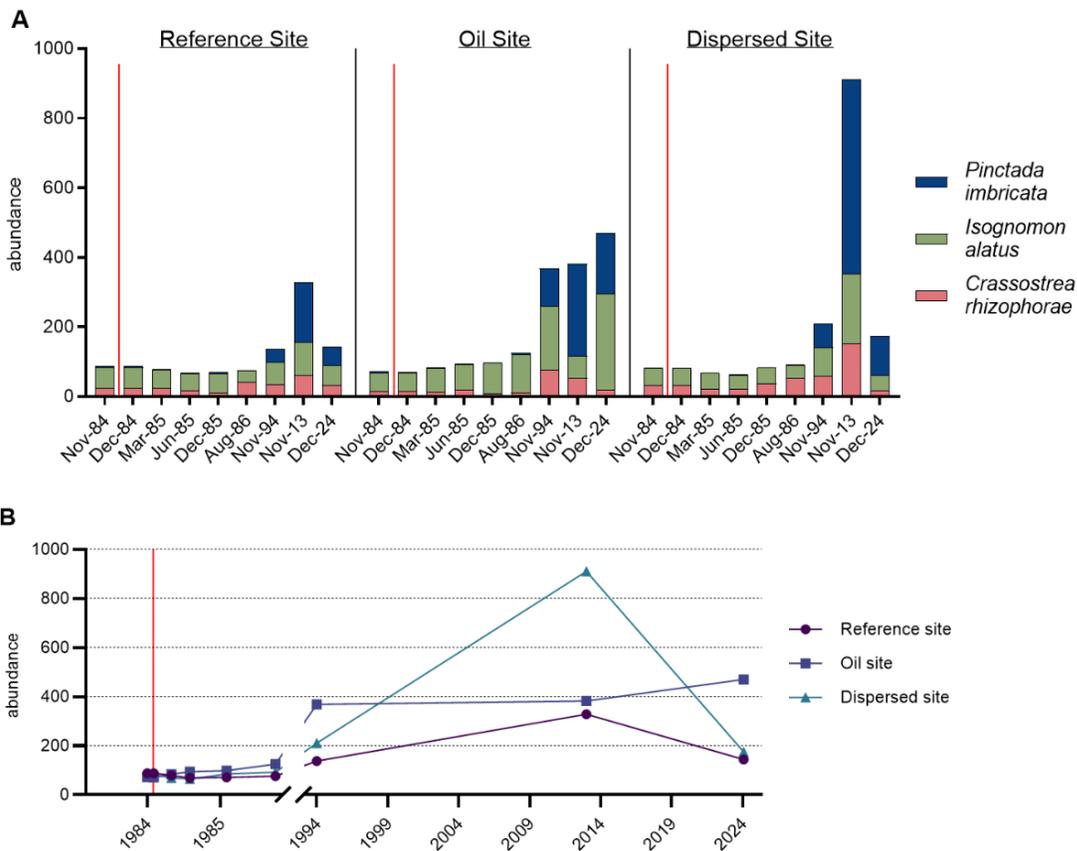


Figure 7. Mangrove oyster species abundance at each treatment site over time, shown as A) the number of each species of mangrove oyster (*Crassostrea rhizophorae*, *Isognomon alatus*, and *Pinctada imbricata*) which were counted on 5 permanently marked prop roots at each site (from November 1984 to August 1986, with different roots evaluated at subsequent assessment dates), and B) the total number of mangrove oysters over time. Red vertical lines indicate the exposure date.

Studies of oil spill impacts on mangrove invertebrate communities are limited and results remain unclear regarding if a greater impact results from dispersed vs whole oil (Lai, 1986; Duke et al.,

2000). At TROPICS Site O, tree snail abundance was reduced immediately after the exposure but recovered to near pre-exposure levels after 1 year. Minimal effects on mangrove oysters were observed (96.9% survival of *C. rhizophorae* 4 days post-exposure) despite high tissue concentrations (506 ppm) 4 days after the exposure had returned to preexposure levels after 1 year. At Site D, the abundance of tree snails was reduced by >50% immediately after the exposure but recovered to 130% of preexposure levels after 1 year. Limited mortality was observed in mangrove oysters (87.5% survival of *C. rhizophorae* 4 days post-exposure), although tissue hydrocarbon levels were high (over 500 ppm) until approximately 1 year after the exposure. Overall, no lingering impacts to tree snails or mangrove oysters after 10, 29 and 40 years are observed, with populations of both now at or above pre-exposure levels.

4.2 Seagrasses

4.2.1 Plants

Impacts on seagrass plant density (Figure 8) at the sites has been difficult to interpret, as significant differences between sites were found for both pre-exposure periods and all post-exposure periods. During the original experimental period, plant densities were initially reduced at Site D, but were significantly higher at other sampling points. At Site O, plant density declined gradually but significantly over time. Plant density at Site R did not demonstrate a specific trend (Ballou et al., 1987). At 10 years post-exposure, plant density was again variable between and within sites, however no significant difference in plant density between sites was found (Dodge et al., 1995). At 17-18, 25, and 32 years post-exposure, while overall variability was observed, plant density was consistently the lowest at Site O and highest at Site R (Ward, 2003; Ward et al., 2003; DeMicco et al., 2011; Renegar et al., 2017a). After 40 years, no significant difference in mean plant density was found between sites (One-Way ANOVA, $p=0.1206$, $F=2.701$). In comparison to earlier time points, plant density is relatively lower than previous measurements at a comparable time of year, including immediately before the exposures in December 1984.

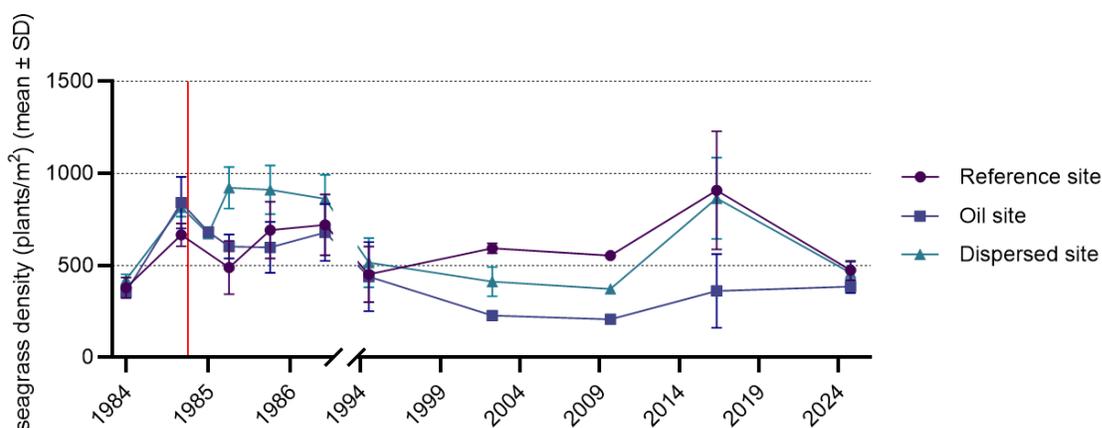


Figure 8. Seagrass plant density (plants/m²) (mean ± SD) of *Thalassia testudinum* over time at each treatment site. Plant density was not measured at Site R in March 1985. Red vertical line indicates the exposure date.

Seagrass blade area (Figure 9) was similar between sites during pre-exposure assessments but changed over time post-exposure. In the original 20-month experimental time period, at Site D, blade area generally increased over time but was not found to change significantly over time

when compared with pre-exposure values. Blade area at Site O generally decreased over time, but again these changes were not significant. Blade area did not vary significantly over time at Site R. (Ballou et al., 1987). No significant difference between sites was observed at 10 years post-exposure (Dodge et al., 1995), however blade area was significantly greater at Site O compared to Site D and Site R at 17-18 years post-exposure (Ward, 2003; Ward et al., 2003). After 40 years, a significant difference in blade area was found between sites (Kruskal Wallis, $p=0.043$). Post-hoc analysis did not identify significant differences between sites, but compared to previous time points, seagrass blade area was higher at Site O and Site D.

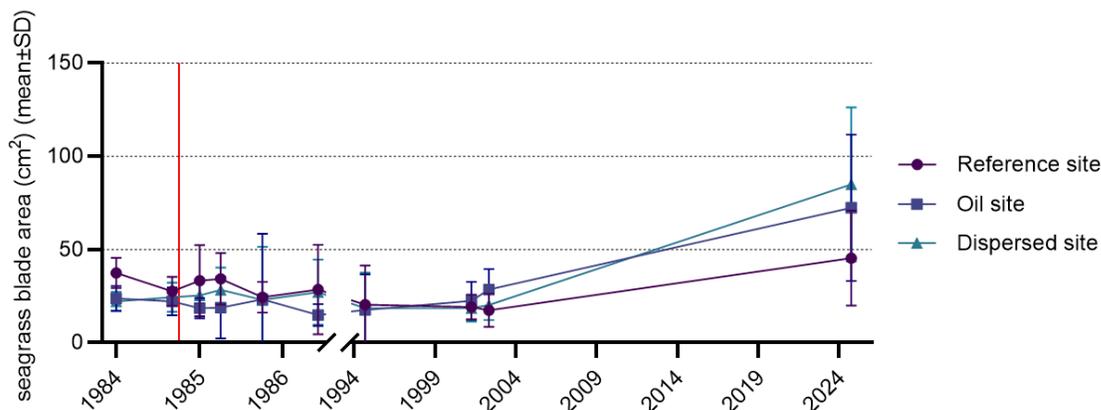


Figure 9. Seagrass blade area (cm²) (mean ± SD) of *Thalassia testudinum* over time at each treatment site. Red vertical line indicates the exposure date.

Previous studies of oil and dispersed oil impacts to seagrasses have observed complete mortality, leaf exfoliation, metabolic impairment, loss of pigment, decreased photosynthetic efficiency, and overall chronic and sublethal stress (Thorhaug and Marcus, 1987; Jackson et al., 1989; Ralph and Burchett, 1998; Sandulli et al., 1998; Peirano et al., 2005; Scarlett et al., 2005; Kenworthy et al., 2017).

No mortality or overall effects on seagrass growth rate (which could result from metabolic impairment, loss of pigment, or decreased photosynthetic efficiency) from the TROPICS exposures were observed based on the lack of consistently significant differences in growth rates between experimental sites and within sites over time. Leaf blade area, however, indicated some long-term effects, as increases in blade area may be a response to stress (van Tussenbroek, 1996). Blade area showed minimal short-term changes following oil exposure, with no significant differences between sites during the initial 20-month period and at 10 years post-exposure. However, by 17–18 years, blade area was significantly greater at Site O, and after 40 years, blade area is now substantially higher at Site D and Site O, and plant density is similar to pre-exposure levels at all three sites. Seagrass plant density trends have been variable, with initial reductions at Site D and gradual declines at Site O during the initial experimental period; no significant differences remained after 40 years, despite overall lower densities compared to earlier time points. Declining plant density, observed during previous assessments at both Site D and Site O, suggest that long-term exposure of tropical nearshore environments to crude oil, and subsequent chronic sediment contamination and structural habitat alterations, may result in greater disturbance to subtidal seagrasses than exposure to dispersed oil (Ward et al., 2003).

4.2.2 Invertebrates

Sea urchin density over time at the experimental sites is shown in Figure 10. The impacts of the dispersed oil exposure on sea urchin density were marked, with significant declines and almost no live urchins observed in the seagrass beds at Site D through 4 months post exposure (Ballou et al., 1987). Recovery in urchin populations was observed 12-20 months after the exposure at Site D (Ballou et al., 1987), and no significant differences in urchin density between sites was found at the 32-year site visit (Renegar et al., 2017a). After 40 years, no significant difference in mean total areal urchin density was found between sites (One-Way ANOVA, $p=0.6224$, $F=0.500$). Overall urchin density was lower than at many previous assessment timepoints, even at Site R.

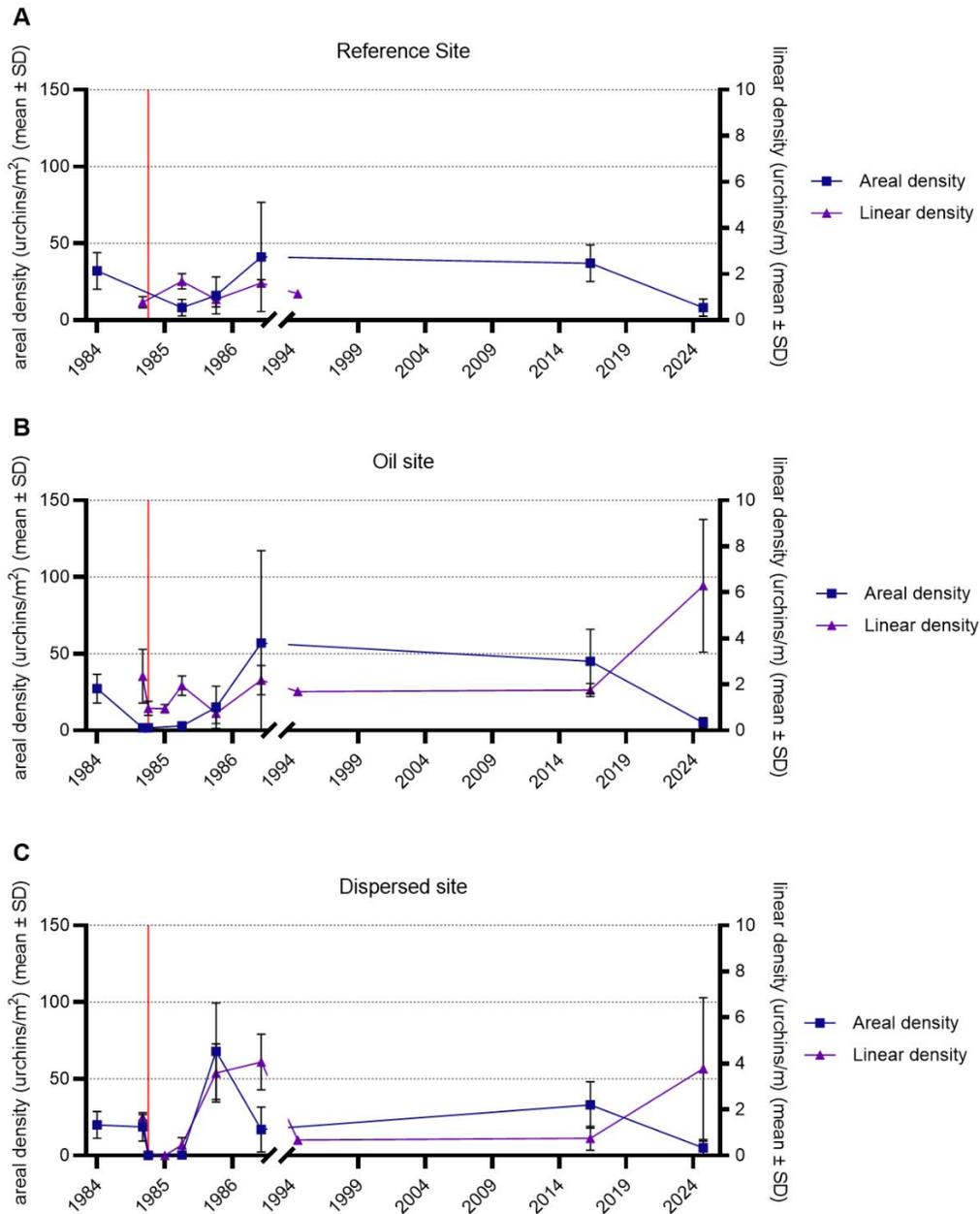


Figure 10. Total areal urchin density (urchins/m²) and total linear urchin density (urchins/m) (mean ± SD) of both *Echinometra lacunter* and *Lytechinus variegatus* over time at A) the Reference site, B) the Oil site, and C) the Dispersed oil site. Areal density was not measured at Site R in Nov-84 and Dec-84, and at any site in Mar-85 or Sep-94. Linear density was not measured at Site R in Dec-84, Mar-85, and Jul-16, and at any site in Mar-84. Red vertical lines indicate the exposure date.

Seagrass invertebrates, specifically sea urchins, were significantly affected by exposure to dispersed oil in TROPICS; urchin abundance was severely reduced, with no surviving urchins immediately after the exposure at Site D, although the population recovered by 1-year post-exposure. A similar, but less severe decline at Site O is consistent with observations in several other field studies where oiling of nearshore habitats resulted in significant declines in urchin populations. (Peterson et al., 1996; Moore et al., 1997; Edwards and White, 1999; Barillé-Boyer

et al., 2004). After 40 years, urchin densities are low across all sites, likely reflecting regional population declines from environmental factors such as heat stress rather than lingering effects of exposure to oil or dispersed oil (Collin and Chan, 2016). The high variability in urchin abundance over time highlights the importance of long-term continuous data in understanding the difference between natural variability and oil exposure impacts (Alvarado, 2008).

4.3 Coral reefs

Percent coverage of corals, other fauna (including sponges, zoanthids, and anemones), plants (including algae and seagrass), and bare substrate (sand or rubble) over time at each treatment site is shown in Figure 11. Pre-exposure surveys found significant differences in total plant and total organism % coverage between all sites, but no significant differences between sites for total animal or total coral % coverage. Algal % coverage was similar between the sites, although plant % coverage was higher at Site R due to greater seagrass coverage. Immediately after the exposures, coral bleaching and dead sponges were observed in shallow areas at Site D; % coverage of total organisms, animals, and corals declined immediately after the exposure, and total algae and total plants increased slightly. At Site O and Site R, no significant reductions in % coverage of total corals, total animals, total plants or total organisms were found. From 4 to 20 months post exposure, trends in % coverage at Site O were short-term significant reductions in total plants and total organisms, concomitant with non-significant decreases in total corals and total animals. Total plant % coverage remained significantly less than pre-exposure observations through the end of the original experimental period; a non-significant decline in total coral % coverage was also observed, alongside a significant increase in total animal % coverage which partly resulted from an increase in the zoanthid population. At Site D, all organisms % coverage categories decreased, and bare substrate increased at 4- and 7-months post exposure. A complete loss of calcareous algae was observed after 7 months. At 12- and 20-months post exposure, significant decreases in coral and total organism % coverage (compared to pre-exposure) were observed along with increased % bare substrate. At Site R, no significant differences in total coral, animal, plant, or organism % coverage were found through the 12-month assessment; after 20 months, total animal % coverage was significantly less compared to pre-exposure levels, and total plant % coverage was significantly greater. Overall, regression analysis indicated that % coral coverage declined significantly over time at both Site D and Site O; a concomitant statistical difference was also indicated by ANOVA at Site D, but not at Site O. A consistent decline in assessed parameters over time occurred at Site D, with some evidence of recovery between 12- and 20-months post-exposure (Ballou et al., 1987).

After 10 years, total organism % coverage was not statistically different between the three exposure sites, and coral and total animal % coverage at Site D were not significantly different from pre-exposure values, or from Site R. No significant impact of the treatments to total plant % coverage was found, and no significant effects on coral growth rate were found for any of the species assessed (Dodge et al., 1995). No significant between site- differences in % coral cover were found between sites at the 18 year assessment, and no significant differences in % coverage of bare substrate, scleractinian coral, plants, soft corals, or other organisms (like sponges) was found between sites after 32 years (Ward, 2003; Ward et al., 2003; Renegar et al., 2017a).

After 40 years, no significant between-site differences in % coverage were found for coral, plant, total organisms, or bare substrate (Kruskal-Wallis, $p > 0.05$). Percent cover of other organisms

(sponges, zoanthids, etc.) was found to be significantly higher at Site R compared to Site O and Site D; this was primarily driven by a large zoanthid population at Site R, while more sponges were present at Site O and Site D. In comparison to previous time points, coral cover in particular is overall within the same range as previous assessments at Site R. This is not the case at Site O and Site D, where marked declines in coral cover have occurred, with a community composition shift to plants (particularly seagrass) at the treatment sites.

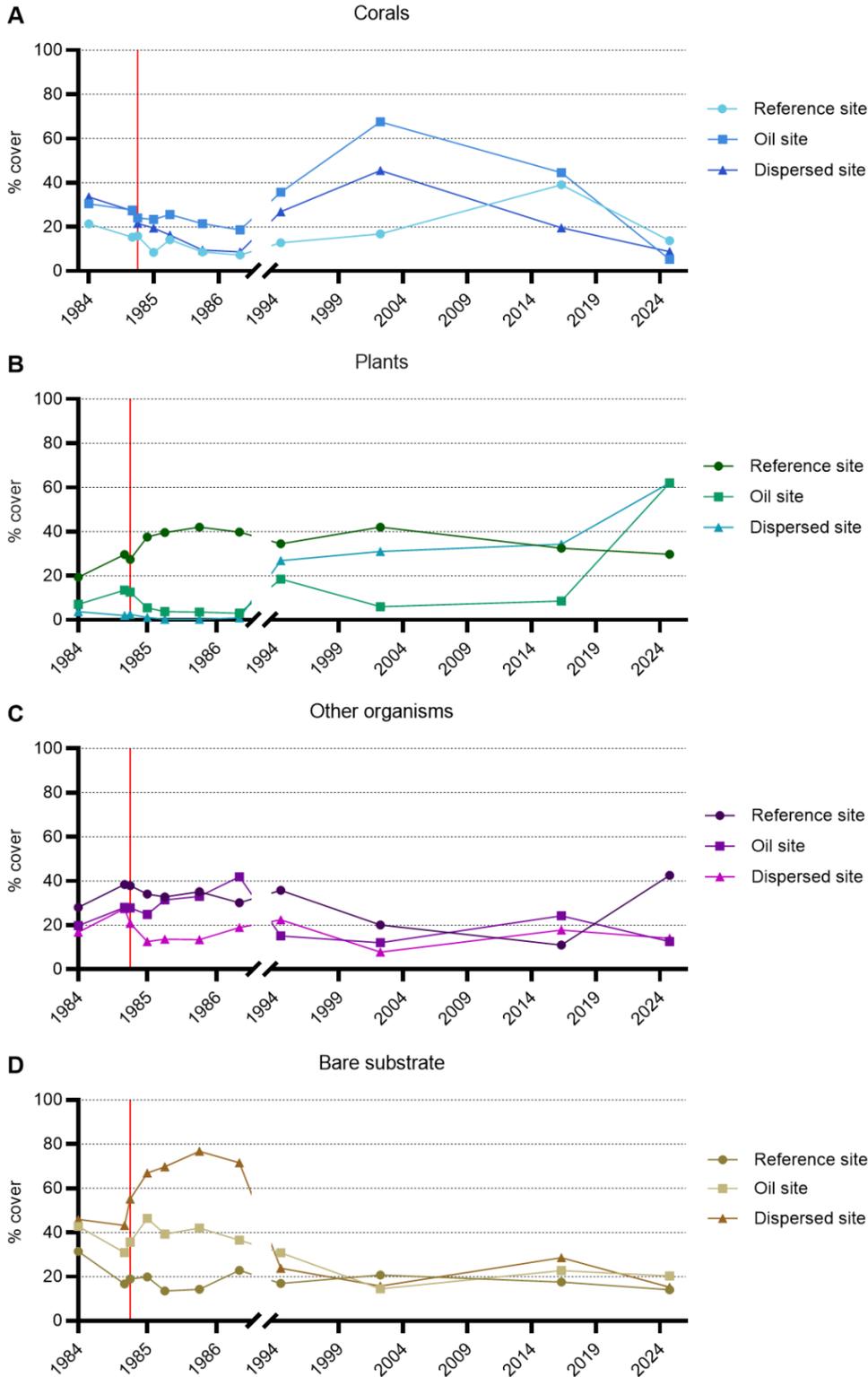


Figure 11. Percent coverage of A) corals, B) plants (including algae and seagrass), C) other fauna (including sponges, zoanthids, and anemones), and bare substrate (sand or rubble) over time at each treatment site. Red vertical lines indicate the exposure date.

The key endpoints for coral assessment at the TROPICS study sites were coral % coverage and the mortality/growth rate of four coral species. Immediately following exposure, Growth of *P. porites* (or *P. furcata*) and *A. tenuifolia* (which dominated the reef community) was significantly reduced by exposure to dispersed oil. Coral and benthic community percent coverage showed significant short-term impacts at Site D following dispersed oil exposure, including coral bleaching, sponge mortality, and declines in coral and total organism coverage, while Sites O and R exhibited minimal immediate changes (Ballou et al., 1987). Coral cover at Sites D and O remained significantly lower for up to two years, with reduced growth rates in dominant species, but by 10 years post-exposure, coverage and growth were comparable across all sites. Short-term growth effects observed in some species, but not others, were no longer evident (Dodge et al., 1995). Long-term assessments (18–32 years) revealed no significant differences in coral, plant, or organism coverage between sites, though community composition shifted toward plants and non-coral fauna at Site D and Site O.

After 40 years, coral cover, which was similar to (or greater than) pre-exposure levels at all three sites at the 32-year site visit is now greatly reduced at Site D and Site O, but it remains unclear if this reflects a spatial shift in the reef on the treatment island (with subsequent effects on transect locations) or observed regional and Caribbean-wide coral declines. Coral cover at all three TROPICS sites (5.25-13.75%) is lower than recorded for other shallow reef areas in the region (23-27%) (Seeman et al., 2013), although the regional data set was collected in 2010-2011, which is before the onset of Stony Coral Tissue Loss Disease (SCTLD) in 2014 and recent intense coral bleaching events in 2015 and 2023-2024. Although the primary hard coral at the TROPICS sites, *P. furcata*, generally shows strong recovery after bleaching and has a relatively higher tolerance to turbidity and eutrophication compared to other hard coral species (Seeman et al., 2013), the shallowest reefs in Almirante Bay may not have had sufficient time to recover from the 2024 bleaching event and this was reflected in the 40-year TROPICS reef transect data.

4.4 Sediment cores

Surface sediments and sediment cores have been collected from the treatment sites at multiple time points since the conclusion of the original experiment. At 10 years post-exposure, oil was still present in the surface sediments at Site O, as sheen was released by walking in the substrate; this was not observed at Site D. Sediment cores collected from the mangrove area and a few seagrass sediment areas found variable hydrocarbon concentrations in core samples, both within and between sites. Hydrocarbons were still present in the sediments at Site D, but at lower concentrations compared to 20 months post-exposure. Some core samples indicated relatively high concentrations at 10-12 cm depth in the sediments, with slightly higher levels at Site O compared to Site D. (Dodge et al., 1995).

Sheen was again observed at 17-18 years post exposure in the mangroves at Site O (Ward et al., 2003). Surface sediment samples collected 20 years post-exposure found hydrocarbon concentrations that were very similar to preexposure levels at both exposure sites (Baca et al., 2005). At 25 years post-exposure, surface sediment samples collected from three central locations in the mangrove area at each site showed a continuing decline in TPH, a stabilization in PAH concentration, and a marked increase in the concentration of naphthalenes at Site O (DeMicco et al., 2011). Surface sediment PAH concentrations were undetectable at the lowest

detection level 29 years post-exposure (Baca et al., 2014). Sediment cores were again collected at each site 32 years post-exposure, and although PAHs were detected, it was concluded that this was likely from small boat traffic as biomarker analysis did not indicate the presence of the original oil or degradation products of oil used in the experiment (Renegar et al., 2017a).

The sampling strategy and sample locations in this 2024 study replicated the within site sampling plan in the original experiment and 10-year site visit, with the substantial addition of sediment core samples collected outside of the experimental site boundaries. Sediment core locations relative to site boundaries are shown in Figure 12. Within each site, 9 cores were collected from 3 areas (high intertidal, mid-intertidal, and subtidal areas), with 3 replicate cores at 1 sampling point in each area. An additional 10-20 cores were collected at each site outside the site boundaries (10 cores at reference site, 20 cores at oil and dispersed oil sites), primarily inland and east (downwind) of the sites. The corer at times went about 60 cm into the substrate but due to compression the cores were between 15 and 32 cm in length. Each of the three sites was sampled on consecutive days with only one site visited per day.



Figure 12. Sediment core locations at Site R (Reference), Site O (Oil), and Site D (Dispersed). Cores marked red were collected within site boundaries and analyzed by CEDRE; cores marked blue were collected outside site boundaries and analyzed by Texas A&M GERG; and cores marked purple were collected outside site boundaries and are stored at Texas A&M GERG (not analyzed).

4.4.1 Within sites

A total of 27 cores were analyzed at CEDRE; nine cores (3 triplicates) were sampled at each of the 3 TROPICS sites, collected within site boundaries. The total length of the sediment cores

ranged from 17 cm for the smallest (R6) up to 32 cm for the longest (D36 and D45). The sediment was composed predominantly of water (approximately 80%), vegetal fibers and root fragments. Prior to extraction, the core sections were manually homogenized, and the largest debris were removed.

The total recoverable hydrocarbon concentrations (TRH) for each individual core collected at each site are shown in Figure 13. TRH can be used as a nonspecific quantitative screening tool to determine the quantity of organic compounds in an environmental sample, including petroleum hydrocarbons. The highest TRH concentrations were observed at Site D, and the lowest overall TRH concentrations were found at Site O, but these between site differences were not significant. At Site D, the TRH concentrations were highest in the high intertidal area with the highest concentrations in the 0-5 cm and 5-10 cm sediment layers. The same trend was observed for the other tidal areas (mid-intertidal and subtidal) even though the TRH concentrations were lower. TRH concentrations were next highest at Site R, with a similar distribution of total hydrocarbons through core depth in the high- and mid-intertidal areas. At Site O, TRH was higher in the two first sediment layers (0- 5 and 5-10 cm) of the high intertidal area compared to deeper sediments, but concentrations were similar throughout core depth in the mid intertidal area, and the unusual pattern of higher hydrocarbon concentrations in shallow and deep core depth, with low concentrations in the 5-10 cm core depth in the subtidal area. At Site R, no significant influence of depth was found on TRH distributions. Overall, the TRH concentrations as a measure of organic carbon content indicate organic carbon storage is highest in mangrove sediments inside the Dispersed and Reference site, and lowest at the Oil site.

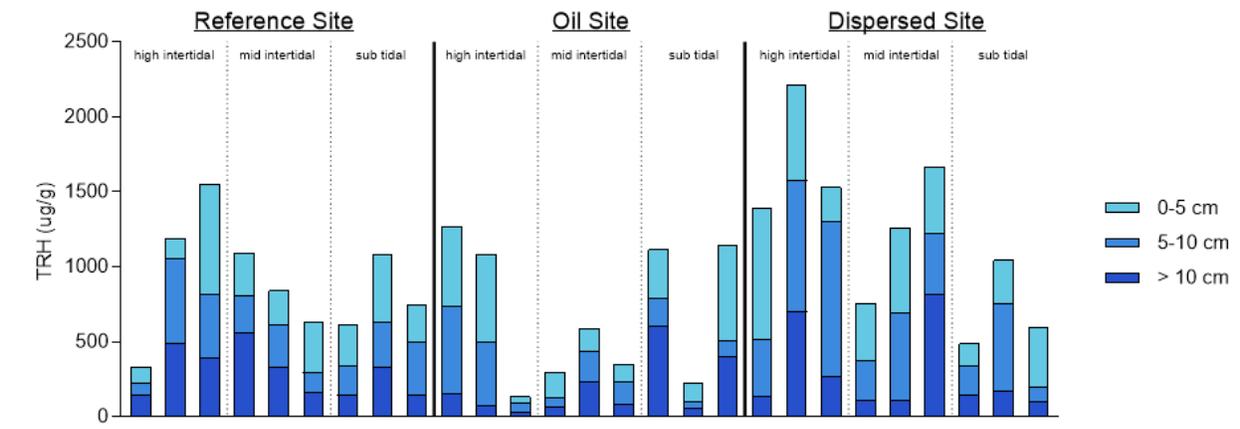


Figure 13. Distribution of total recoverable hydrocarbon concentrations (µg/g) in 3 individual sediment cores (core depth= 0-5cm, 5-10 cm, and >10 cm) for each tidal level (high intertidal, mid intertidal and subtidal) inside the 3 experimental sites (Reference, Oil and Dispersed sites).

Concentrations of alkanes and their distributions in sediment cores relative to core depth are shown in Figure 14. Alkane concentrations (Figure 14A) were the highest overall in the high- and mid-intertidal areas, in the shallowest 2 sediment layers at Site R. Overall alkane concentrations were the highest at Site R, and similar at Site O and Site D, but these differences were not significant. Total PAH concentrations (Figure 14B) were overall highest at Site O and lowest at Site D, especially for the high- and mid- intertidal zone at Site O, but the between site differences were not significant.

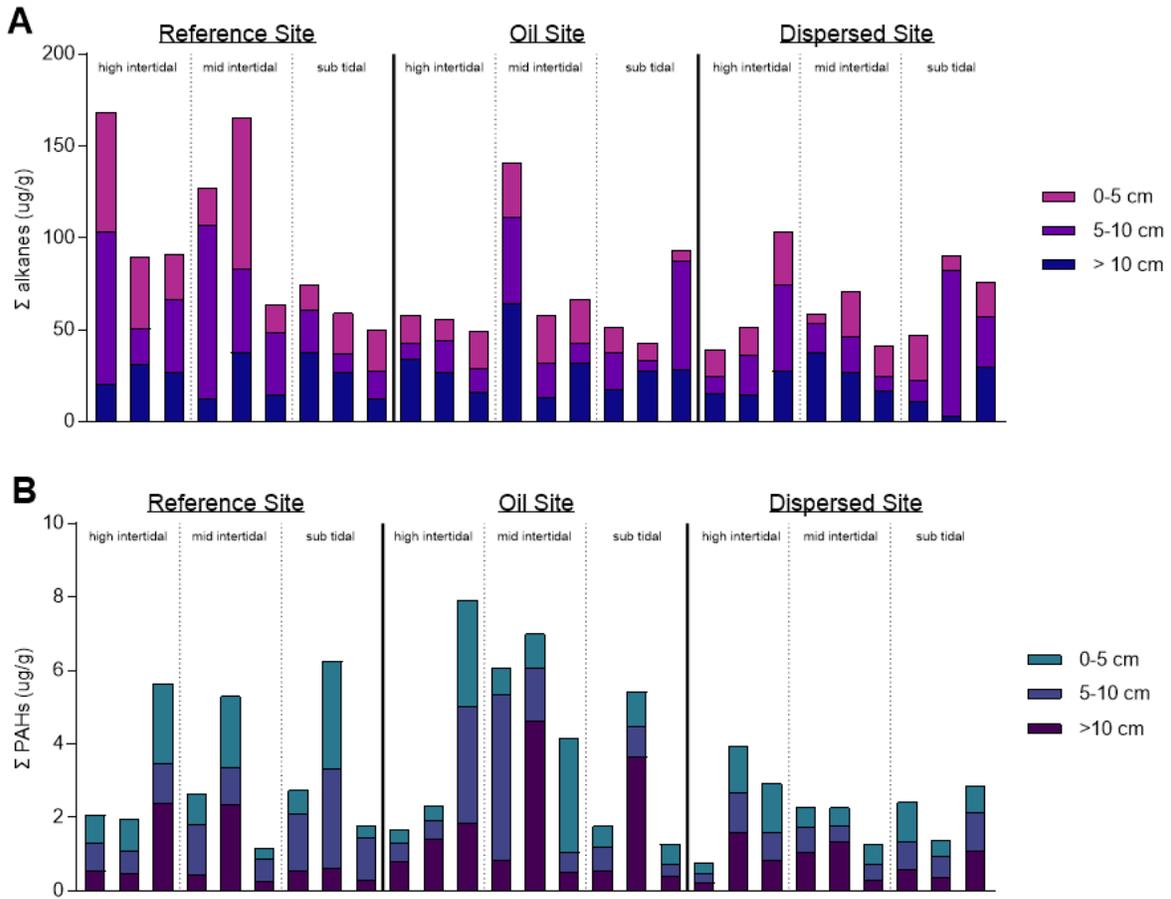


Figure 14. Distribution of A) total alkane concentrations ($\mu\text{g/g}$) and B) total PAH concentrations in individual sediment cores (core depth= 0-5cm, 5-10 cm, and >10 cm) for each tidal level (high intertidal, mid intertidal and subtidal) of the 3 experimental sites (Reference, Oil and Dispersed sites).

The PAH distributions in samples with the highest PAH concentrations [Site O, core O68 (5-10 cm); Site D, core D37 (>10 cm); Site R, core R10 (0-5 cm)] are shown in Figure 15. The cores from Site O and Site D are characterized by a PAH distribution similar to Site R, with a predominance of fluoranthene / pyrene family and naphthalene family. These two families represent respectively 45% and 28% of total PAHs. As noticed at the previous sampling time (Renegar et al., 2017), some PAHs signal detected in sediment samples can be related to small boat traffic in the area close to the site. For the 2024 sampling, this was also observed for core

O68 at Site O, with a presence of other PAHs such as C1-, C2 and C3- Phenanthrene / anthracene, dibenzothiophene and chrysenes.

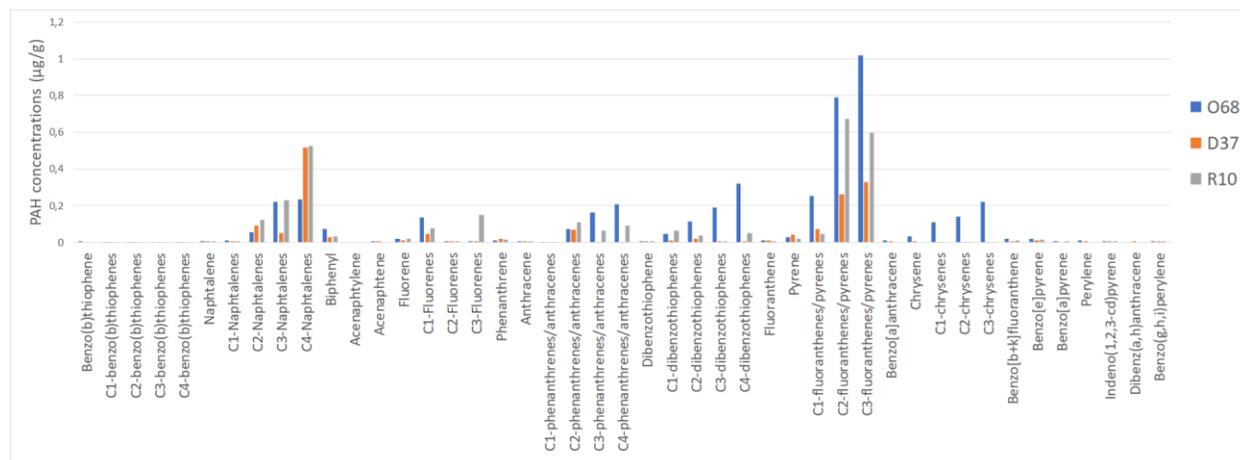


Figure 15. PAH distributions for the most concentrated extracts in the 3 sites [Oil site, core O68 (5-10 cm); Dispersed site, core D37 (> 10 cm); Reference site, core R10 (0-5 cm)].

In order to assess the presence of crude oil in the different sediment samples, chromatograms of two groups of geochemical biomarkers typical of petrogenic hydrocarbons (triaromatic steroids ($m/z=231$) and hopanes ($m/z=191$)) were investigated. The triaromatic steroids group is composed of polycyclic aromatic compounds from the aromatic fractions of oils and rock extracts. They are highly resistant to biodegradation due to their high molecular weight and benzene ring structure, their distribution and composition characteristics are preserved even for highly degraded crude oils. These compounds generally give valuable information about source of organic matter input (Wang et al., 2008). Triaromatic steroids can be found naturally in petrogenic oil, and are characterized by their high resistance to natural degradation processes such as dissolution, evaporation, biodegradation, photooxidation. Figure 16 presents examples of chromatograms and the GC/MS patterns obtained for the 3 samples, which appeared very similar between the 3 sites. No compounds from the triaromatic steroid or hopane families were identified; the most abundant compounds that were identified were taraxerol and stigmaterol, two chemical compounds naturally occurring in plants.

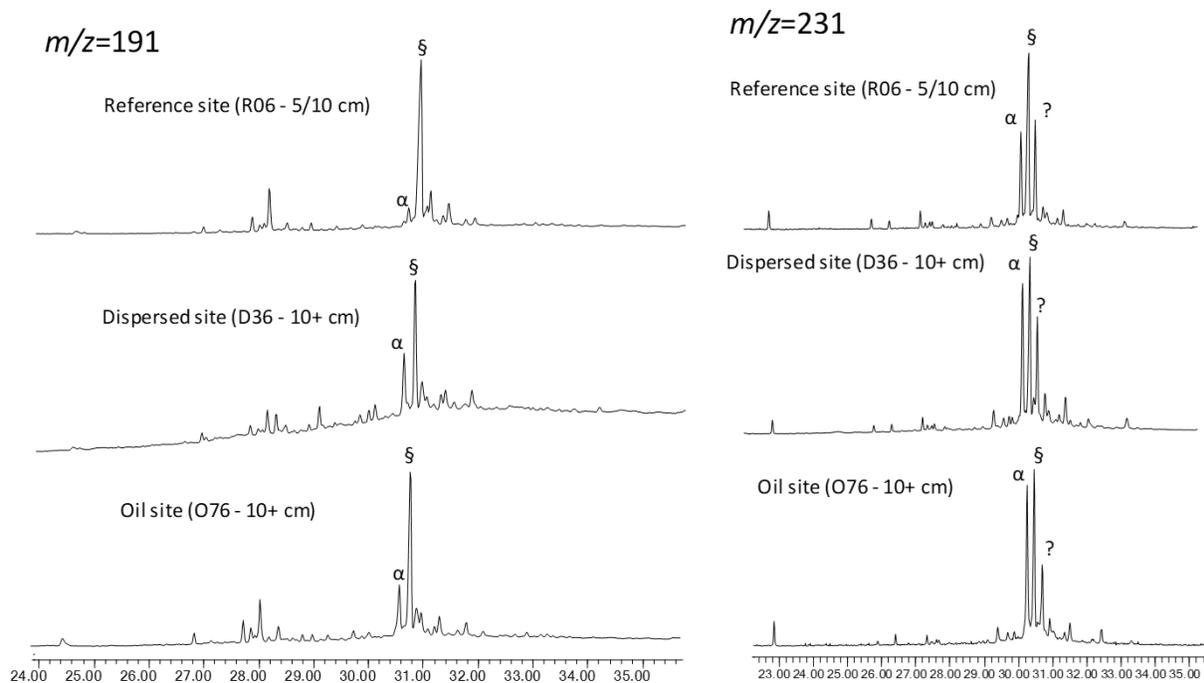


Figure 16. GCMS chromatograms of triaromatic steroids ($m/z=231$) and hopanes group ($m/z=191$) of cores collected in Site R (R06, 5-10 cm), Site D (D36, >10 cm) and Site O (O76, >10 cm) (α : stigmasterol, \S : taraxerol).

In order to identify alkanes and PAHs detected in the sediment samples, the perylene index was calculated. The presence of perylene in the environmental samples can be related to oil spill but also to anoxic sediments with high biological productivity (Venkatesan et al., 1988; Stout and Wang, 2018). The perylene index, defined as the concentration of perylene divided by the total of penta-cyclic PAH isomers has been developed to distinguish biogenic perylene from pyrogenic perylene (Venkatesan et al., 1988). A perylene index greater than 10% indicates biogenic inputs, whereas an index of <10% indicates pyrogenic origin of the compounds. Among the 81 samples analyzed during this project, the perylene index exceeded 10% for 72 samples. For the remaining 9 samples, the PAHs concentrations were too low to allow a perylene calculation.

4.4.2 Outside sites

Samples were received at the Geochemical and Environmental Research Group (GERG) on April 29, 2025. The cores arrived with blue ice packs and were immediately stored at 4°C. Individual core samples were subsequently split vertically using a razor blade to obtain an archived half and a working half. Both were stored frozen (-20°C). A total of 36 cores were analyzed at Texas A&M GERG with 104 individual sample analysis of core sections. The cores consisted of biological material of fiber and roots (mangrove debris) rather than classic sediment. The working half was freeze-dried and homogenized by hand. Moisture content was calculated by recording the wet weight and post-drying weight of the core section. The mean moisture content for all samples was 85%. A total of 36 cores were analyzed at GERG; 10 cores from Site R (29 sample intervals), 13 cores from Site D (37 sample intervals), and 13 cores from Site O (38 sample intervals).

Concentrations of alkanes and their distributions in sediment cores relative to core depth are shown in Figure 17. Alkane concentrations (Figure 17A) at Site R and Site O were relatively similar, although they were slightly higher at Site O. This is similar to the pattern observed in cores collected inside the sites, except alkanes were highest at the Reference site. Alkane concentrations were lowest at Site D, except for one core (D49, 5-10 cm core depth, collected approximately 15 m from the NE rear corner of the site) where alkane concentrations were approximately 3 times greater than the highest concentration observed at the Oil site (core O57) in the same core depth.

Total PAH concentrations (Figure 17B) were overall highest at Site O and lowest at Site D, which was the same pattern observed in the cores collected inside of the sites. As for alkanes, one core at Site D (D40, >10 cm core depth, collected approximately 10 m from the NE rear corner of the site) where PAH concentrations were approximately 12 times greater than the highest concentration observed at the Oil site (core O70) in the same core depth.

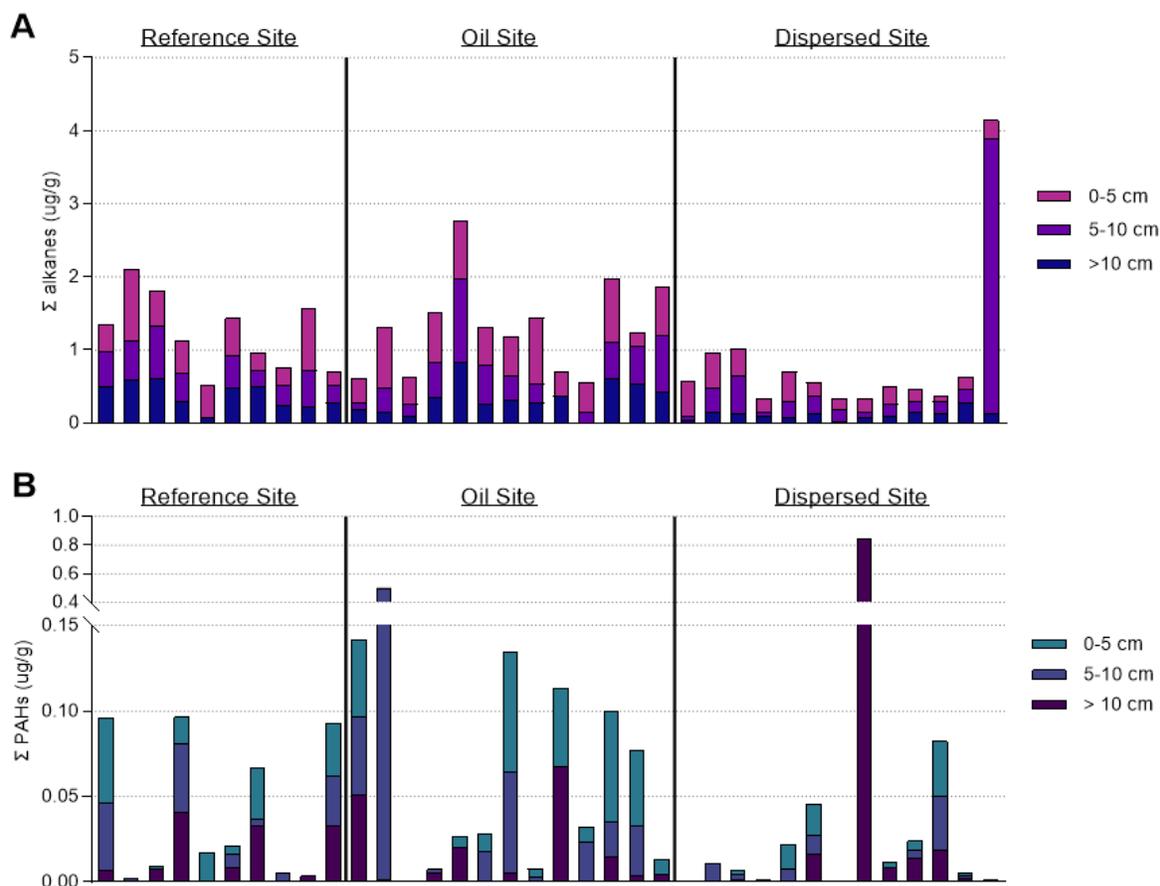


Figure 17. Distribution of A) total alkane concentrations ($\mu\text{g/g}$) and B) total PAH concentrations ($\mu\text{g/g}$) in individual sediment cores (core depth= 0-5cm, 5-10 cm, and >10 cm) at the 3 experimental sites (Reference, Oil and Dispersed sites).

The TLE analyzed for these core samples contained significant amounts of elemental sulfur and plant biomolecules (Figure 18). Each of these constituents swamped the signal of any petroleum biomarkers that may be present. The abundant plant biomolecules and elemental sulfur are consistent with the coastal mangrove ecosystem.

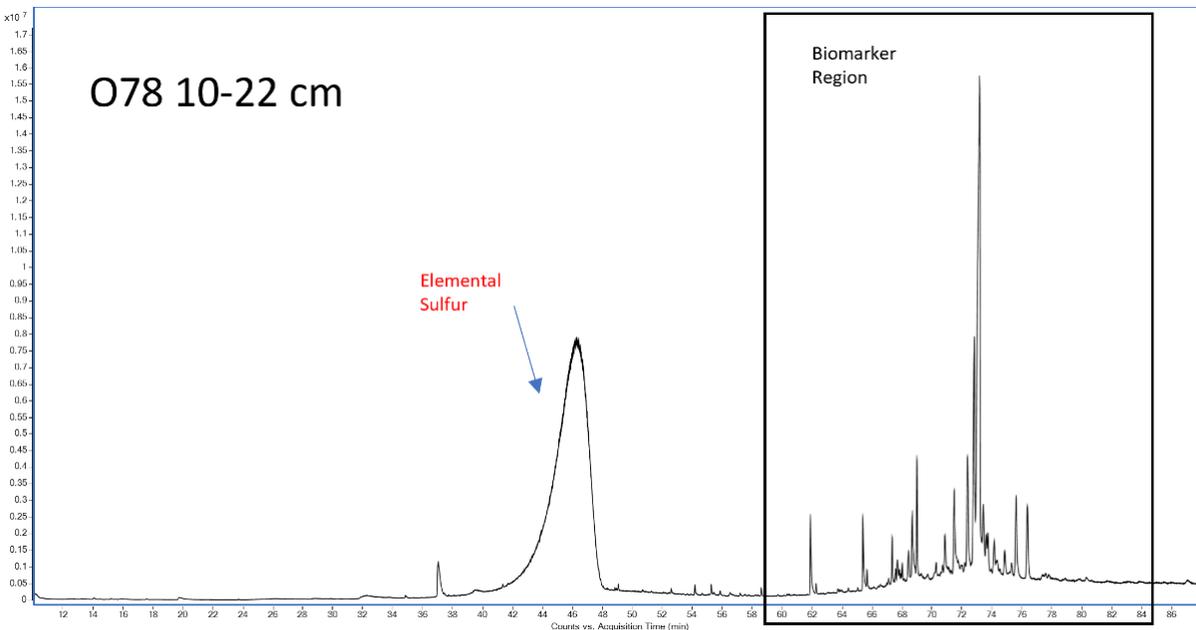


Figure 18. Total lipid extract chromatogram for core O78 (> 10 cm interval). Highlighted are the interferences from elemental sulfur and plant phytosterols in the biomarker region of the chromatogram.

Potential identities for these plant biomolecules based on mass spectral library matching include sitosterol, stigmasterol, amyirin, and several plant diterpenes. Therefore, additional silica gel column chromatography was conducted with added activated copper as described in the methods to remove these matrix interferences. Following further extract cleanup, the elemental sulfur and interfering plant sterols were removed. However, the extracts lack the hump for the unresolved complex mixture (UCM) commonly observed in petroleum-impacted samples (Figure 19).

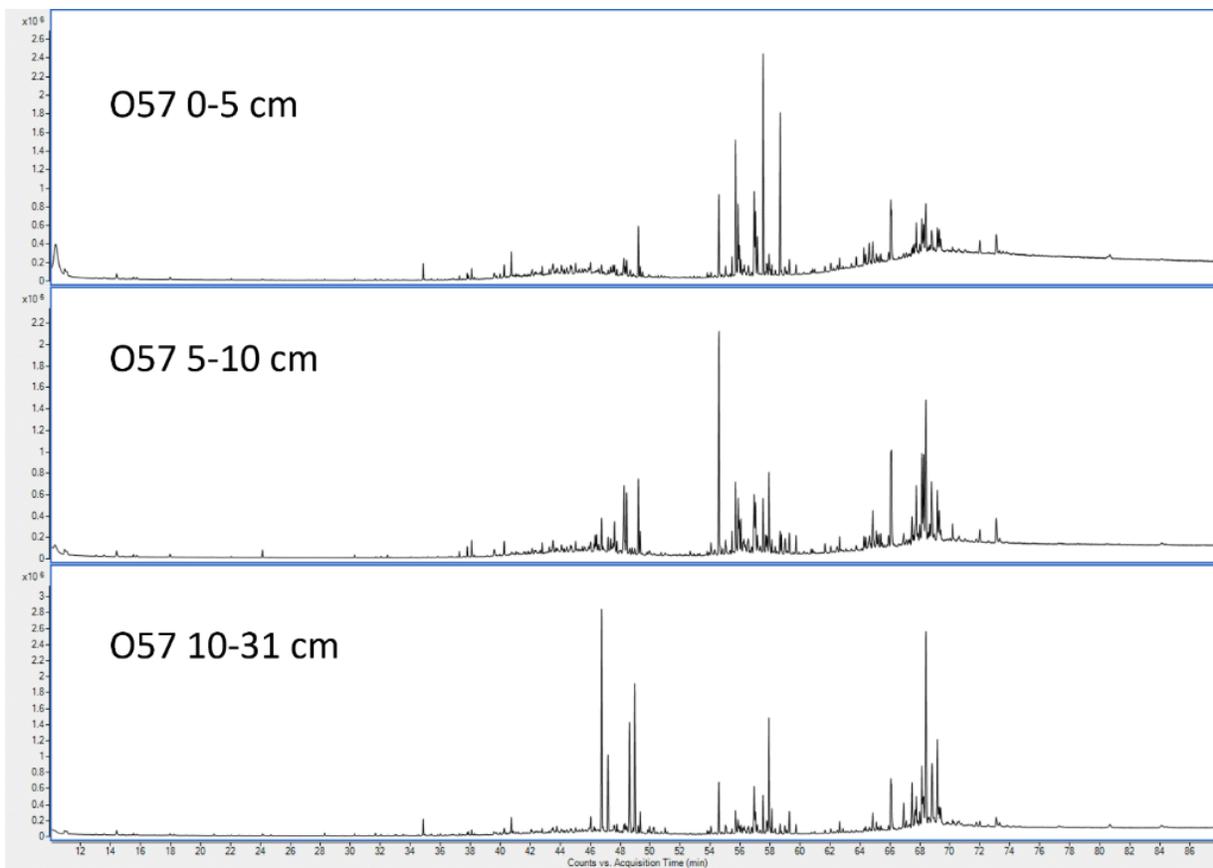


Figure 19. Total ion chromatographs for core O57 following silica gel chromatography and activated copper treatment.

The same procedure was used for the NIST 1944 sediment SRM to verify the recovery of the UCM and petroleum biomarkers following these additional cleanup steps (Figure 20 and 21).

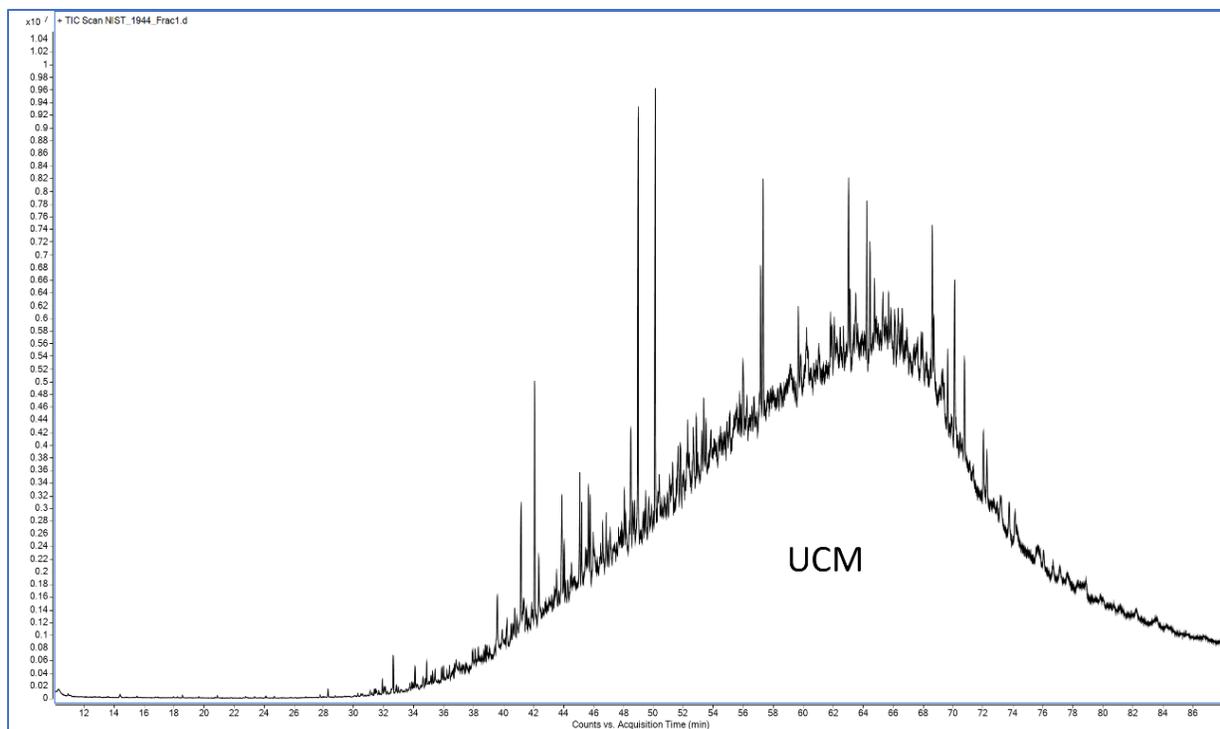


Figure 20. Total ion chromatogram of NIST 1944 sediment following silica gel chromatography and activated copper treatment.

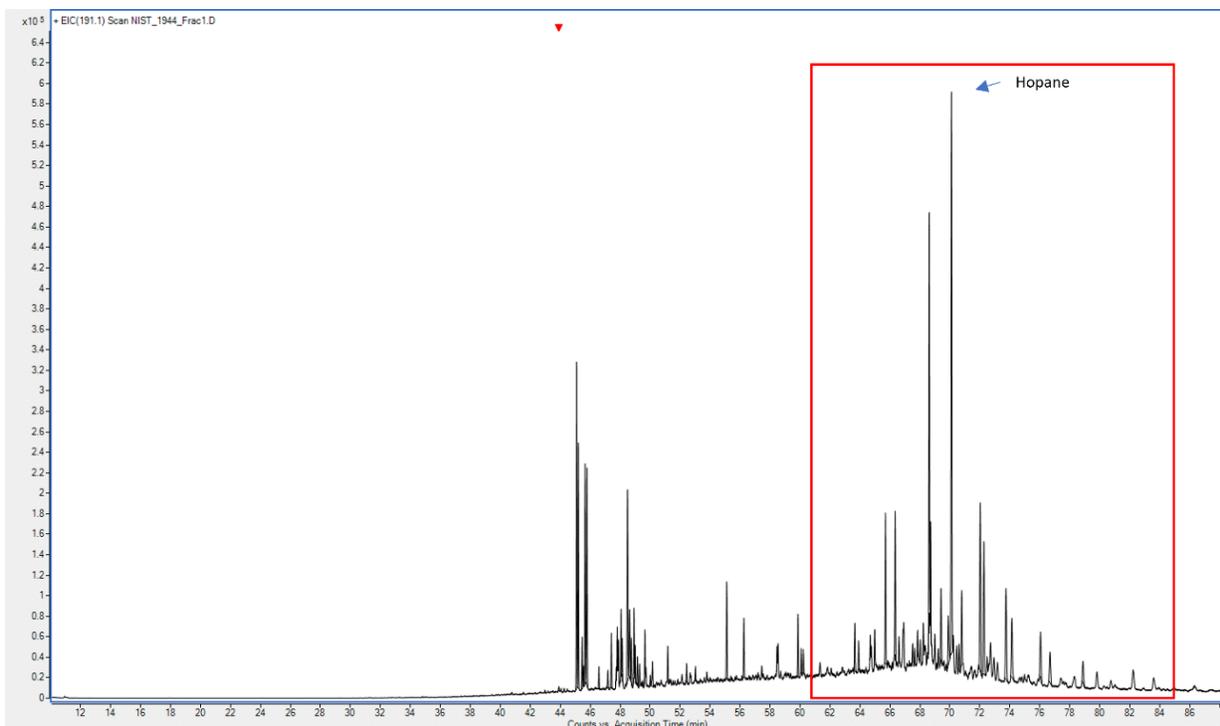


Figure 21. Extracted ion chromatogram (EIC) of m/z 191.1 for NIST 1944 sediment. Petroleum biomarkers were recovered following silica gel column chromatography.

Regardless of site (Reference, Dispersed, Oiled) and core, there were no quantifiable amounts of petroleum biomarkers present in these mangrove soils. Figure 22 shows the extracted ion chromatographs (EIC) for m/z 191.1. This mass is used to look for terpanes including hopane, which is commonly the most abundant biomarker in petroleum samples. The small peaks present in the chromatograms do not match the retention times and spectra for any petroleum biomarkers and are likely plant biomolecules based on the NIST library.

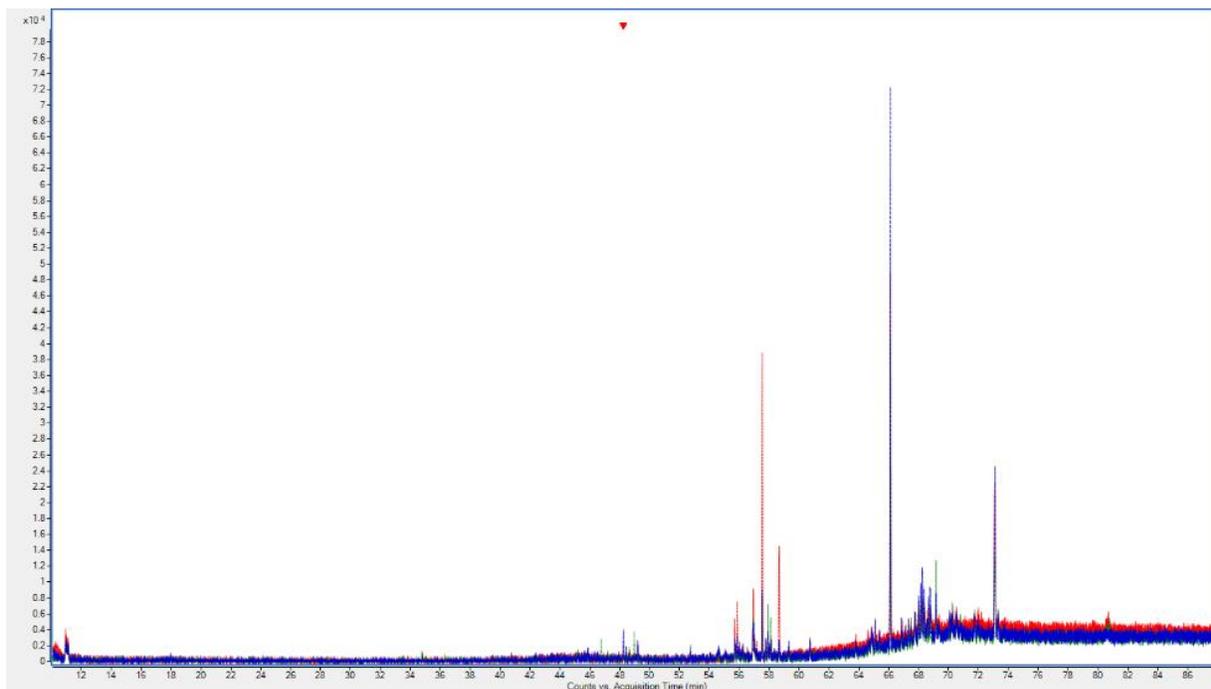


Figure 22. Extracted ion chromatograms (EIC) of m/z 191.1 for O57 (Interval 0-5 cm shown in blue; interval 5-10 cm shown in red, and interval 10-31 cm shown in green).

An overlay of the NIST 1944 sediment, which contains abundant petroleum biomarkers, is shown as a reference in Figure 23.

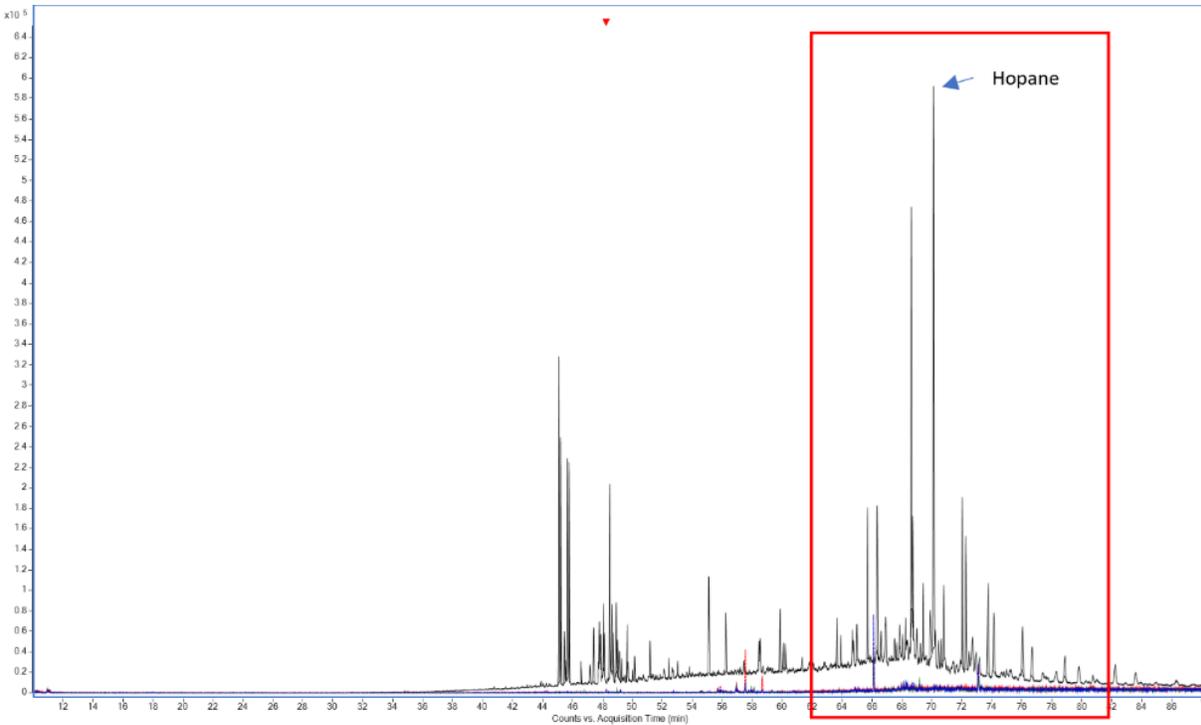


Figure 23. Extracted ion chromatograms (EIC) from Figure 22 with corresponding EIC from NIST 1944 sediment overlay.

Finally Figure 24 shows biomarkers (Terpanes) from Biomarkers for Alaska North Slope oil (Wang et al., 2006) which were used in the original oiling 40 years ago. If residues of the original test oil were still present, terpanes would have been found.

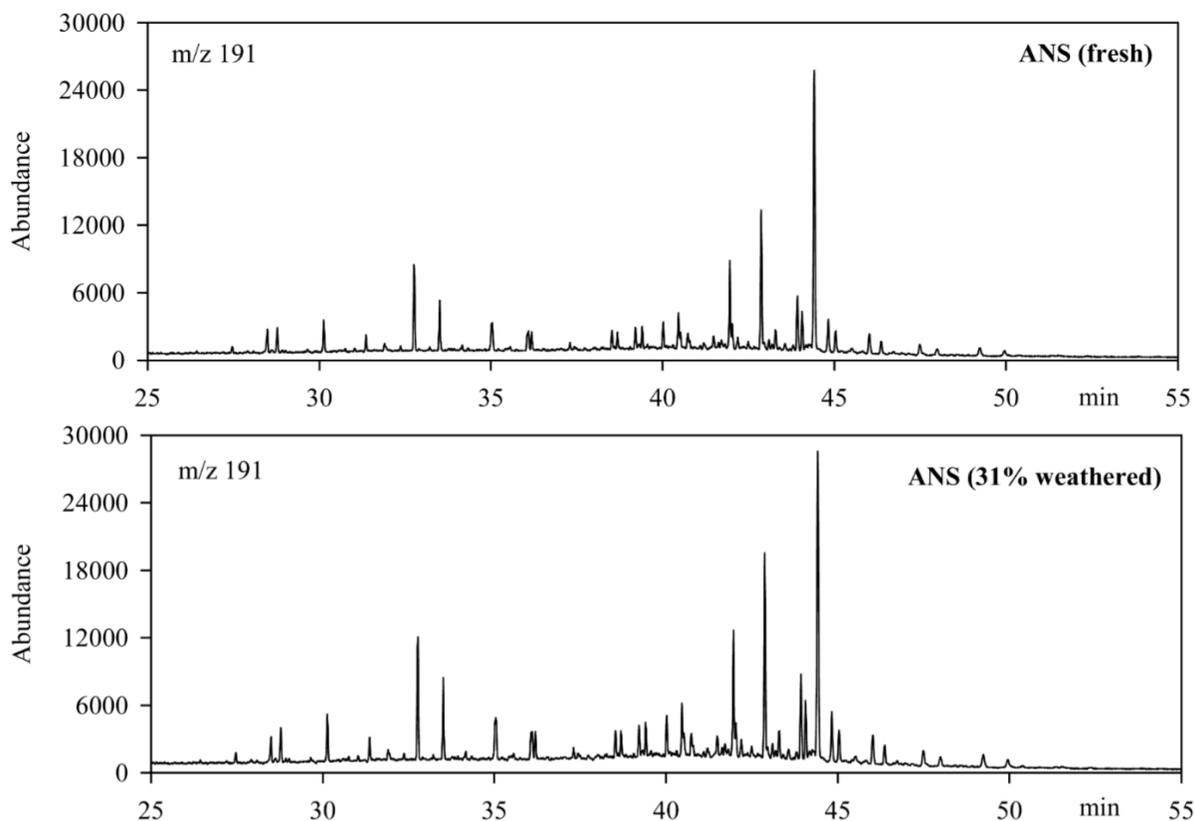


Figure 24. Alaska North Slope Terpanes (Wang et. al., 2006).

Oil on shorelines can become entrained in intertidal sediments and persist for years to decades after initial contamination, as the high complexity of prop roots on mangrove dominated coastlines facilitates oil entrapment and ingress into deep sediments, where anoxic conditions may reduce the efficiency of biological degradation (Burns et al., 2000; Owens et al., 2008). Subsequent leaching and resuspension of oil retained in sediments results in chronic hydrocarbon contamination of subtidal waters and sub-lethal effects which may continue for decades (Levings et al., 1994; Lee and Page, 1997; Burns et al., 1999). This was also observed at the TROPICS experimental sites; in sediment cores collected at 20 months post exposure, substantial oil penetration and apparent entrapment was found in some of the high and mid intertidal surface sediments, but not in any subtidal sediments, at Site D. Sediment hydrocarbon concentrations were overall higher at Site O compared to Site D, indicating less effect of tidal flushing and more entrapment in the sediments and substrate of Site O. However, a greater degree of oil penetration into subsurface sediments occurred at Site D compared to Site O, particularly in the mid intertidal mangrove forest; this could possibly be attributed to tidal impacts and burrowing organisms (Dodge et al., 1995). After 10 years, sediment hydrocarbon concentrations had decreased substantially at both treatment sites, although pockets of oil were still present at the oil-only site (sheen was released by walking on the substrate) and had appeared to migrate down through the sediments via dead prop roots. Little to no sediments were present in the mangrove area at Site O, possibly from the loss of mangroves which would have stabilized the substrate; subsidence from decomposition of mangrove roots may also have been a factor. Although no

qualitative difference in sediments between sites was noted after 40 years, the lower organic carbon content in the sediments at the Oil site may reflect previous observations of sediment loss.

Petroleum consists of thousands of individual compounds, producing a complex and unique distribution of peaks in a gas chromatography chromatogram, or “fingerprint” for each specific oil. However, this fingerprint is highly susceptible to both biotic and abiotic weathering processes. For example, PAHs are highly vulnerable to photo-oxidation, and evaporation steroids are both more indicative of the original petroleum source and are more stable during these weathering processes. This has led to their use of oil fingerprinting in oil spill forensics, such as those following the Deepwater Horizon oil spill in 2010. Diagnostic ratios (DRs), or ratios of petroleum biomarkers, are used to fingerprint petroleum samples for oil source matching, and analyze oil source similarity/dissimilarity. Although petroleum biomarkers were easily detected in Standard Reference Material, there were no petroleum biomarkers detected in any of the core samples collected; thus, after four decades, sediments on the treatment island (both inside and outside test site boundaries) appear to be no longer contaminated with oil and dispersed oil. A detailed overview of chemical compounds indicated significant biogenic contribution, including diverse plant -synthesized hydrocarbons such as plant phytosterols (eg. Stigmasterol, sitosterol, amyirin) and plant diterpenes (eg. Rimuene, abietadiene).

4.5 Remote sensing

Multispectral data was processed using Water Mapping custom software for orthomosaic projection. Orthomosaic maps were created from each of the UAS flights over the two study sites (Figure 25). These maps are ultra-high resolution of approximately 2cm per pixel. Orthomosaics were produced as geotiffs compatible with any GIS platforms for geospatial statistical analysis.

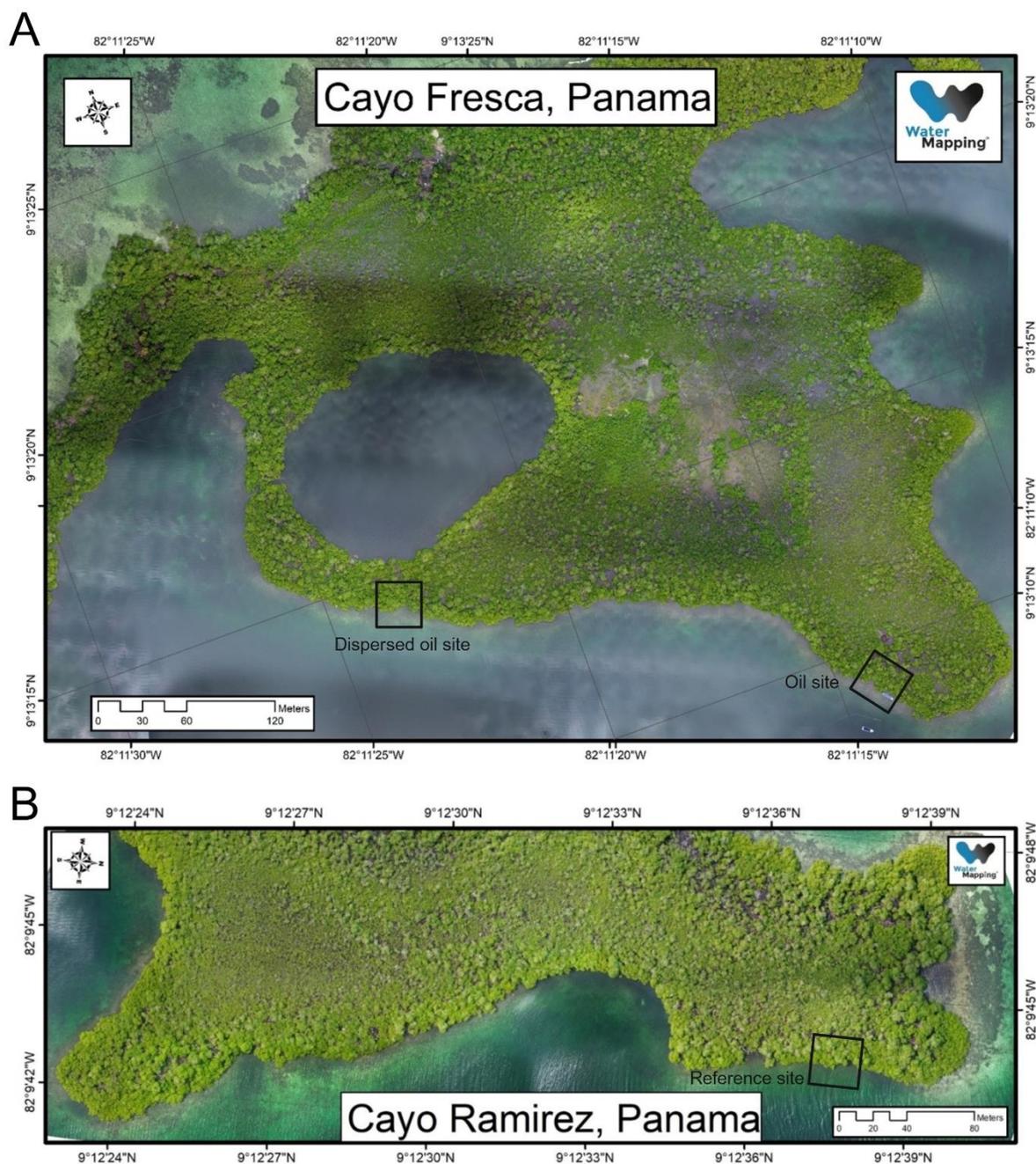


Figure 25. A) High resolution (2cm x pixel) orthomosaic map of the Dispersed oil site and the Oil site on Cayo Fresca from UAS data collected on December 10, 2024. B) High resolution (2.5 cm x pixel) orthomosaic map of the Reference site on Cayo Ramirez collected with multispectral imagery on December 11-12, 2024. Sites are outlined in black.

Multispectral imagery from each independent band was processed as orthomosaics to generate NDVI georeferenced maps. This processing requires the conversion from digital pixel values to reflectance values. The ‘Reflectance’ value describes how much light is reflected from a surface or optical element. It is equal to the ratio of reflected power and incident power when light is

shot onto a surface. The Transmittance describes how much light is transmitted from a surface or optical element. It is equal to the ratio of transmitted power and incident power when light is shot onto a surface. In order to conduct this conversion (from pixel to reflectance values) a reflectance calibration panel is used prior and after to each flight with the multispectral sensor.

For each flight, the calibration panel is used to set raw values on each of the multispectral bands prior and after the flights. NDVI stands for Normalized Difference Vegetation Index. To calculate this index, independent multispectral band orthomosaics with converted reflectance values are used to perform an arithmetic calculations among bands and generate the NDVI index map. As plants become healthier, the intensity of reflectance increases in the NIR and decreases in the Red, which is the physical basis for most vegetation indices. NDVI values can be a maximum value of 1, with lower values indicating lower plant vigor. Therefore, 0.5 typically indicates low vigor whereas 0.9 indicates very high vigor. NDVI is also effective for distinguishing vegetation from soil. NDVI is recommended when looking for differences in above-ground biomass in time or across space. NDVI is most effective at portraying variation in canopy density during early and mid-development stages but tends to lose sensitivity at high levels of canopy density. With reflectance value calculated on each of the independent multispectral bands, orthomosaic maps of NDVI indexes were generated for the three study sites (Figures 26-28).

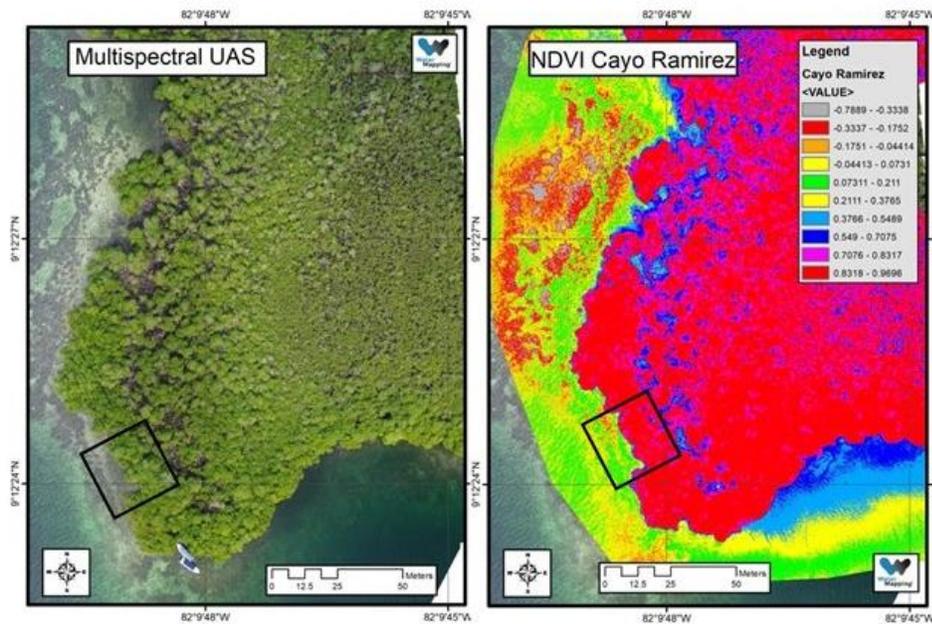


Figure 26. High resolution orthomosaic NDVI map of the Reference site on Cayo Ramirez, collected with multispectral imagery on December 11-12, 2024. Site is outlined in black.

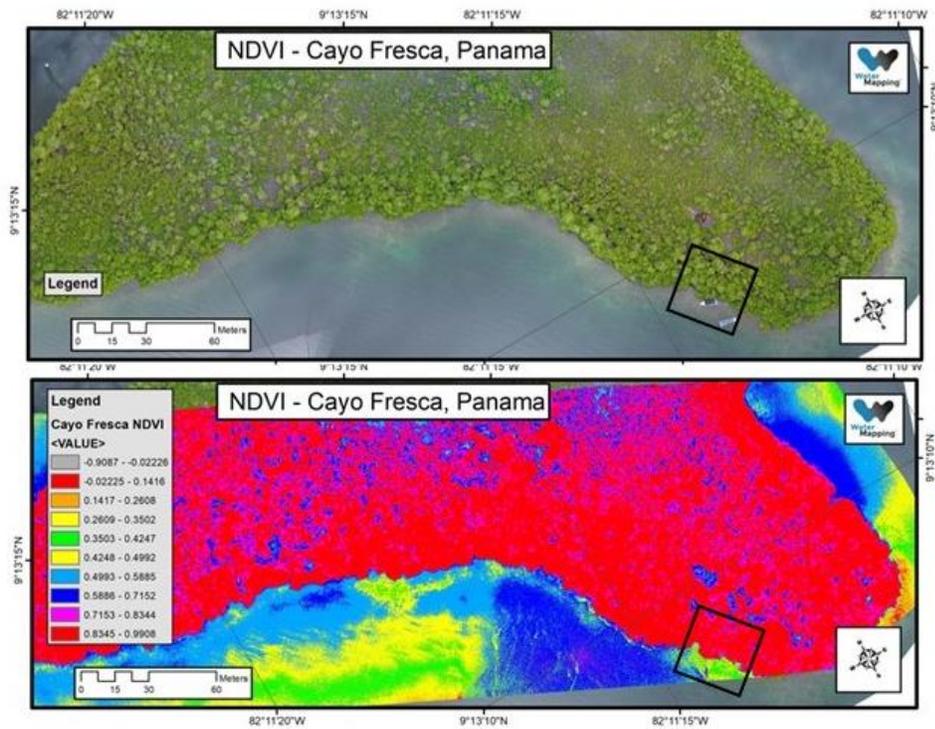


Figure 27. High resolution orthomosaic NDVI map of the Oil site on Cayo Fresca, collected with multispectral imagery on December 11-12, 2024. Site is outlined in black.

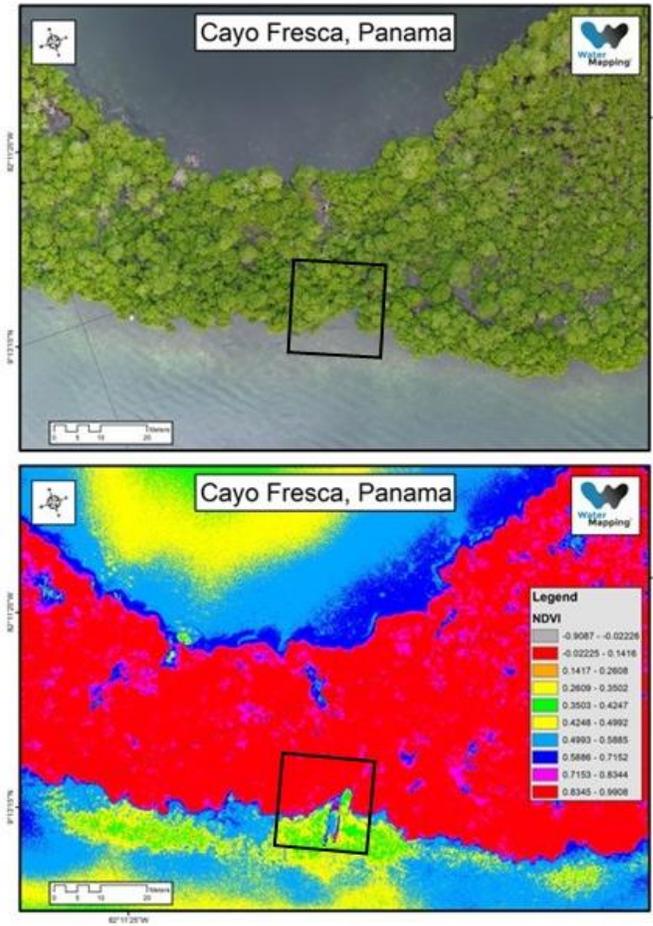


Figure 28. High resolution orthomosaic NDVI map of the Dispersed site on Cayo Fresca, collected with multispectral imagery on December 11-12, 2024. Site is outlined in black.

More than 7000 multispectral images collected over the Cayo Fresca & Ramirez islands were used to produce NDVI orthomosaic maps of the study sites. To produce these maps, a standard remote sensing methodology was followed to calibrate and convert digital pixel values to reflectance values on the multispectral imagery. With this procedure, calibrated NDVI values were obtained over the islands during the December 2024 field campaign, and the high NDVI values (near 1) across all sites indicated that the mangrove vegetation was very healthy. The NDVI dataset for the two study islands can be used to compare the spatial extent, density, and health of mangroves over time to similar data collected during future site visits. It may also be used as baseline data for assessment of progressive environmental stress in this mangrove species, and across the Almirante Bay region as a whole.

5 Conclusions and recommendations

5.1 Environmental impacts of oil vs dispersed oil

The key conclusion drawn from continuing assessment of the TROPICS sites over 4 decades of study is that the use of dispersants reduces impacts to the mangrove forest, specifically preserving adult mangroves and therefore the structural integrity of the shoreline environment. Oil exposure has caused long-term structural changes in the mangrove forest at the TROPICS sites, with severe initial mortality at the Oil site and persistent adult tree losses over decades, despite strong seedling recruitment. While canopy density has largely recovered, and even surpassed pre-exposure levels at some sites, structural metrics reveal uneven growth patterns, with the Oil site showing reduced tree size despite canopy recovery, possibly related to chronic growth limitation from oil contamination of sediments and substrates. The use of dispersant reduced initial impacts to the mangroves at the Dispersed site by reducing tree mortality and defoliation, and limiting long-term contamination of coastal sediments. Although biomarkers of the original oil were not detected inside or outside the sites after 4 decades, lingering impacts on the mangrove forest at the Oil site resulting from the loss of adult trees and their functional role as key environmental engineers in this coastal habitat are still apparent.

TROPICS demonstrated that both corals and seagrasses were less vulnerable to floating oil but may experience chronic effects from long-term sediment contamination or structural instability. The coral reef was more significantly impacted by dispersed oil compared to oil only, and % cover recovered to pre-exposure levels at both treatment sites at some point between 2-10 years after the exposures. Short-term coral impacts were significant, with long-term trends after 4 decades likely dominated by broader ecological changes, such as ocean warming, rather than lingering effects which can be associated with the experimental exposures. The subtidal seagrasses assessed at the TROPICS sites were the least affected, exhibiting a high degree of resilience and tolerating exposure to both oil and dispersed oil, although impacts to associated invertebrate fauna were significant. Indeed, invertebrates associated with all three environmental compartments (mangrove associated invertebrates tree snails and mangrove oysters, seagrass associated sea urchins, and coral reef associated echinoderms, soft corals and sponges) demonstrated high initial sensitivity with short-term declines followed by recovery with no evidence of lingering impacts after 10 years, and long-term population changes consistent with the broader ecological landscape.

Overall, 4 decades of post-exposure monitoring at the TROPICS sites has demonstrated that intertidal mangrove and subtidal seagrass and coral reef communities exhibited significantly different impact and recovery regimes (summarized in Figure 29), which illustrate the effects of untreated floating oil compared to water column exposure from chemically dispersed oil. Impacts of non-dispersed oil include significant loss of adult mangrove trees, with long-term effects related to the retention of hydrocarbons in the substrate; in contrast, the impacts of floating oil to seagrass and coral are generally regarded as less significant. Dispersed oil resulted in less impact to the mangroves by reducing tree mortality and limiting sediment contamination, had significant effects on seagrass invertebrate communities and coral reef organisms. The use of dispersants reduced physical impacts and preserved the mangrove forest, sediments and

structural elevation of the environment, with the relatively short-term loss of organisms balanced by the benefits of preservation of the habitat itself.

Observed recovery time to pre-exposure conditions

		Untreated oil	
Untreated oil	 <p>mangroves</p>	Tree abundance: 69 adult trees lost after 10 years, 55.7% decrease in adult trees over time	
		Canopy density: recovery after 40 years	
		Leaf morphometrics: 2-10 years for recovery	
		Seedling abundance: 25 years for recovery	
		Mangrove oyster abundance: 2-10 years for recovery	
		Mangrove oyster tissue hydrocarbon levels: returned to normal after 1 year	
	Tree snail abundance: 1-2 years for recovery		
	 <p>seagrass</p>	Plant density: variable, no clear impacts, has decreased over time	
		Blade area: 1 year for recovery	
Sea urchin abundance: 1 year for recovery			
 <p>coral reef</p>	Coral cover: 2-10 years for recovery		
	Coral growth rate: 2-10 years for recovery		
		Dispersed oil	
Dispersed oil	 <p>mangrove</p>	Tree abundance: 2 adult trees lost after 10 years, 25% decrease in adult trees over time	
		Canopy density: recovery after 40 years	
		Leaf morphometrics: 2-10 years for recovery	
		Seedling abundance: 20 years for recovery	
		Mangrove oyster abundance: 2-10 years for recovery	
		Mangrove oyster tissue hydrocarbon levels: returned to normal after 1 year	
	Tree snail abundance: 1 year for recovery		
	 <p>seagrass</p>	Plant density: variable, no clear impacts, has decreased over time	
		Blade area: no clear impacts	
Sea urchin abundance: 1 year for recovery			
 <p>coral reef</p>	Coral cover: 2-10 years for recovery		
	Coral growth rate: 2-10 years for recovery		

Figure 29. Time for recovery to pre-exposure conditions for the different habitat and organism health metrics after exposure to untreated oil or dispersed oil at the TROPICS experimental sites.

5.2 Recommendations for future TROPICS site visits and research

Significant challenges remain in understanding the relative contribution of impacts, after four decades, from the experimental exposures versus subsequent disturbances (anthropogenic or natural) observed changes in habitat structure and organism composition at the TROPICS sites. Further, directly applying the knowledge gained from TROPICS to spill response planning scenarios is challenging due to the complex exposure conditions and highly individual nature of actual spills. Specifically, variability in environmental stress factors can be significant factors influencing both initial impacts and recovery from disturbance, and failure to consider these variables can result in misinterpretation of outcomes for spill response planning exercises. However, the conclusion that dispersant use improved outcomes for the mangrove forest at TROPICS is clear, as are the potential for chronic, long-term effects of oil contamination on long-lived organisms such as red mangroves (life span of >150 years). For this reason, future research visits to the TROPICS sites should include continued monitoring of tree-specific mangrove growth rate with DBH and canopy height measurements (not currently possible with the historic data set), and assessment of canopy density. The collection and analysis of trunk cores from large trees at each site which are known to have been present during the original

experiment could be used to explore the relationship between the ecological disturbance caused by oil or dispersed oil exposure and tree ring formation, anatomical characteristics and wood properties of this mangrove species (Moya et al., 2024). This could be combined with additional sediment core collections and remote sensing data to estimate above- and below-ground biomass for the assessment of changes in high-density carbon storage in mangrove environments after a significant disturbance such as an oil spill. Future site visits should also include expanded assessment of the coral reef within the sites, to examine if the observed decrease in coral cover at the TROPICS sites is spatially driven by within-site transect location or is consistently observed at adjacent reef sites within the Almirante Bay region. Transect locations should be permanently marked if possible, to evaluate changes in the reef community at the same location over time.

TROPICS is one of the most comprehensive field experiments examining the long-term impacts of oil and dispersed oil exposures in nearshore tropical marine environments, and the length of the study has significantly contributed to knowledge of the effects of chronic oil contamination in the environment. The importance of such controlled field studies to oil spill science is in the real-world data they provide on the environmental impact of oil that cannot be fully simulated in laboratory settings. This information ultimately forms the basis for spill response technologies (such as clean-up and remediation methods) and decision-making. Field studies which integrate multi-disciplinary approaches to elucidate the persistence of petroleum hydrocarbons in sediments and the enduring impacts on ecosystem health demonstrate the differing recovery trajectories which can be discerned when monitoring and research efforts encompass multiple, differentially impacted environmental compartments and include organisms with a range of expected sensitivities and recovery times. This, combined with longitudinal observations, is essential to understand ecosystem-level effects which guide the NEBA/SIMA process. Future field experiments should therefore continue to include extended temporal monitoring of multiple key ecosystem components which are likely to reflect chronic effects, allowing the recognition of long-term spill impacts within the context of broader environmental change and shifting baselines.

6 References

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7 Appendixes

7.1 Appendix A: Technical Summary

REPORT TITLE: TRopical Oil Pollution Investigations in Coastal Systems [TROPICS]:
Longitudinal study in support of shoreline spill response Net Environmental Benefit Analysis
(NEBA)

CONTRACT NUMBER(S): 140E0124C0005

FISCAL YEARS(S) OF PROJECT FUNDING: FY2024-2025

CUMULATIVE PROJECT COST: \$150,000

COMPLETION DATE OF REPORT: 26 February 2026

BSEE COR(S): Kevin A. Cabaniss

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KEY WORDS: TROPICS field experiment, coral reefs, seagrasses, mangroves, oil spill,
dispersed oil

* The affiliation of the Principal Investigators(s) may be different than that listed for Project
Manager(s).

7.2 Appendix B: Sediment Core Analysis Report: CEDRE

Clean Caribbean & Americas

CHEMICAL ANALYSES OF SEDIMENT SAMPLES COLLECTED IN THE FRAMEWORK OF TROPICS PROJECT

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<p>Contract Reference: proposal Cedre P24071</p>		
<p>CHEMICAL ANALYSES OF SEDIMENT SAMPLES COLLECTED IN THE FRAMEWORK OF TROPICS PROJECT</p>		
<p>Drafted by: Ronan Jézéquel Research department</p>	<p>Proofread by: Stéphane Le Floch Research Department manager</p>	<p>Checked by: Arnaud Guéna Deputy Director, Production Manager</p>
<p>Key words: TROPICS, tropical, oil spill, weathering</p>		
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SUMMARY

The aim of the TROPICS project (Tropical Oil Pollution Investigations in Coastal Systems) is to assess the effect of an oil exposure and use of chemical dispersant on a tropical environment characterized by mangrove, coral reef and seagrass. For the 40th anniversary of the TROPICS project, Cedre was asked to take part in an on-site mission and to carry out the chemical analysis of the sediment samples taken during this mission.

A total of 27 sediment cores were collected within the 3 experimental sites: Dispersed site, Oil site and Reference site. Each core was divided into 3 sections in order to evaluate the distributions of hydrocarbons in sediment column. After solvent extraction, oil extracts were analyzed by GC-FID for TRH quantification (Total Recoverable Hydrocarbons) and GC-MS for *n*-alkanes, PAHs and biomarkers distributions.

Regarding the TRH quantification, hydrocarbons were detected in all the samples whatever the experimental sites, the tidal areas and the depths. It was not possible to establish a significative difference between the Reference site and the 2 other sites (Oil site and Dispersed site).

Detailed analyses of oil extracts highlighted that *n*-alkanes and PAHs were present in all the sediment samples. Regarding their distributions, as observed for TRH, it was not possible to establish a significative difference between the 3 experimental sites. Chromatograms highlighted that the oil extracts were characterized by the predominance of fatty acid and fatty alcohols, 2 groups of chemical compounds typically present in natural environment. Concerning the PAHs, same trends were observed for this chemical groups: PAHs were detected in all the samples and their distributions did not differ significantly between the Reference site and the 2 other experimental sites.

Geochemical biomarkers distributions were investigated in order to evaluate the presence of persistent petroleum biomarkers in the sediment cores. For both families of chemical compounds (triaromatics steroids, hopane), no traces of petroleum biomarkers were observed 40 years after the release of oil.

Considering all the results, it was not possible to confirm the presence of oil released 40 years ago within the TROPICS experimental sites.

1 CONTEXT

TROPICS (Tropical Oil Pollution Investigations in Coastal Systems) project - originally funded by API - began in 1984 with the main objective of assessing the effect of dispersant oil treatment of oil slick on a tropical environment (coral reef, seagrass, mangrove). Initially scheduled to last 2.5 years, the experimental site was monitored annually for the first 10 years, then more sporadically. The last visit was in 2016. For the 40th anniversary, Cedre was asked to take part in an on-site mission and to carry out the chemical analysis of the sediment samples taken during this mission.

2 OBJECTIVE AND CONTENT OF THE STUDY

The objective of this study is:

- to assess the spatial extension of the pollution through the TRH (Total Recoverable Hydrocarbons)¹ quantification in the 3 tidal areas (high-, mid-intertidal and subtidal areas);
- to characterize the oil extracts through the distributions of the different chemical families (alkanes, PAHs and petroleum biomarkers);
- to compare the TRH content and distributions of alkanes, PAHs, and biomarkers between the Reference site and the 2 other sites (Oil Site and Dispersed site).

3 SAMPLE DESCRIPTION

3.1 Sampling location

Nine cores (3 triplicates) were sampled in each of the 3 TROPICS sites (oil - O, dispersed – D and reference - R). Figure 30, Figure 31, Figure 32 and Figure 33 present the sampling locations for the 2024 sampling round.

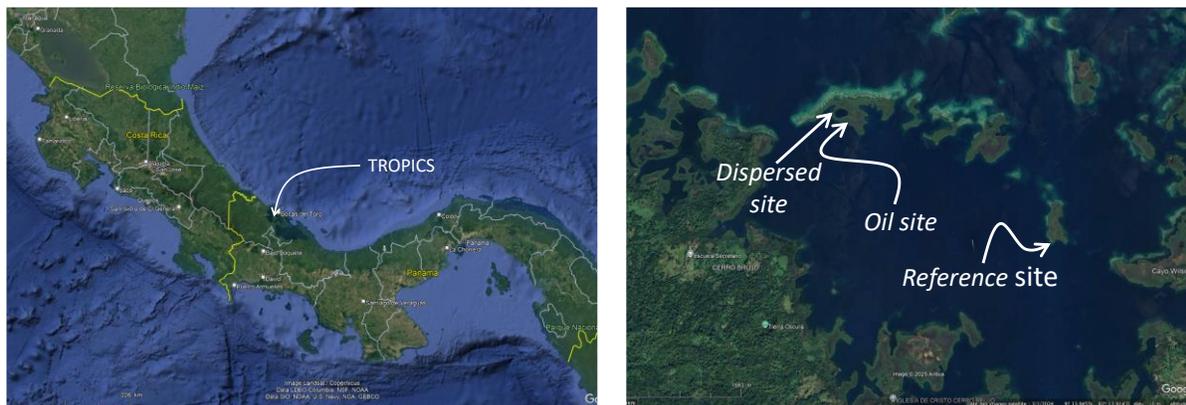


Figure 30. Experimental site location.

¹ TRH (Total Recoverable Hydrocarbons) analysis can be used as a nonspecific quantitative screening tool to determine the quantity of organic compounds in environmental sample (water, sediment organic tissue), including petroleum hydrocarbons.

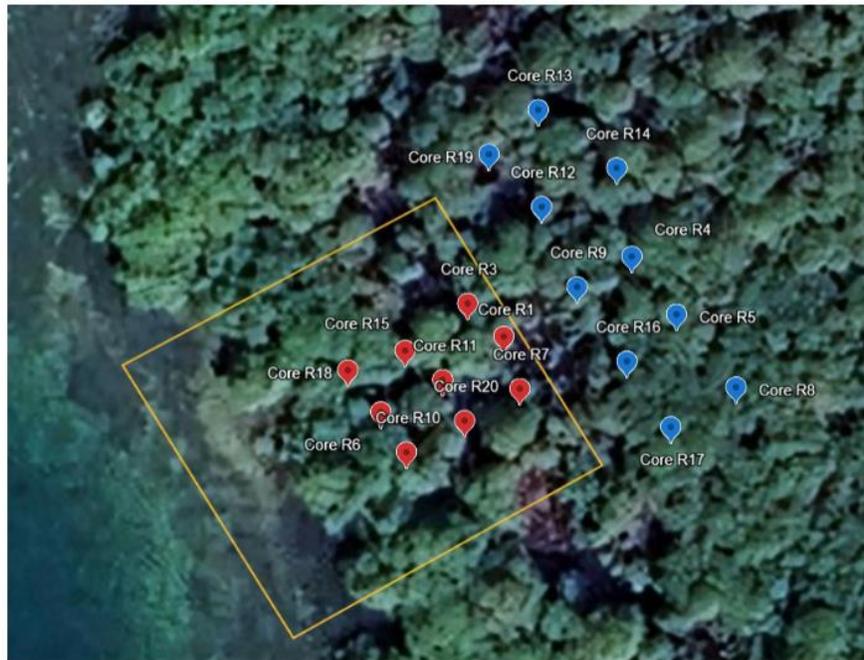


Figure 31. Picture of 2024 sample location within Reference site (R) (yellow square corresponds to the experimental area) (source: Nova Southeastern University).

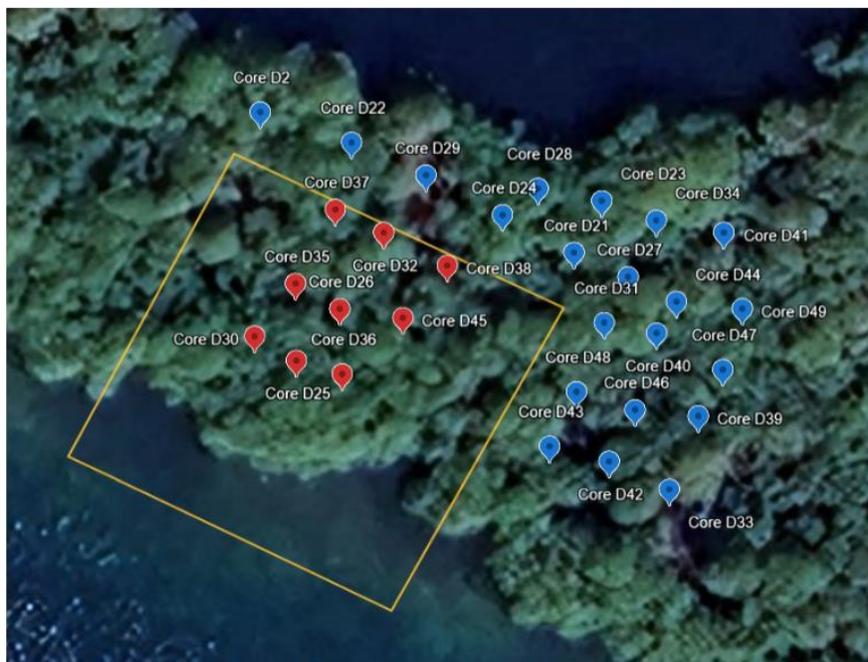


Figure 32. Picture of 2024 sample location within Dispersed site (D) (yellow square corresponds to the experimental area) (source: Nova Southeastern University).

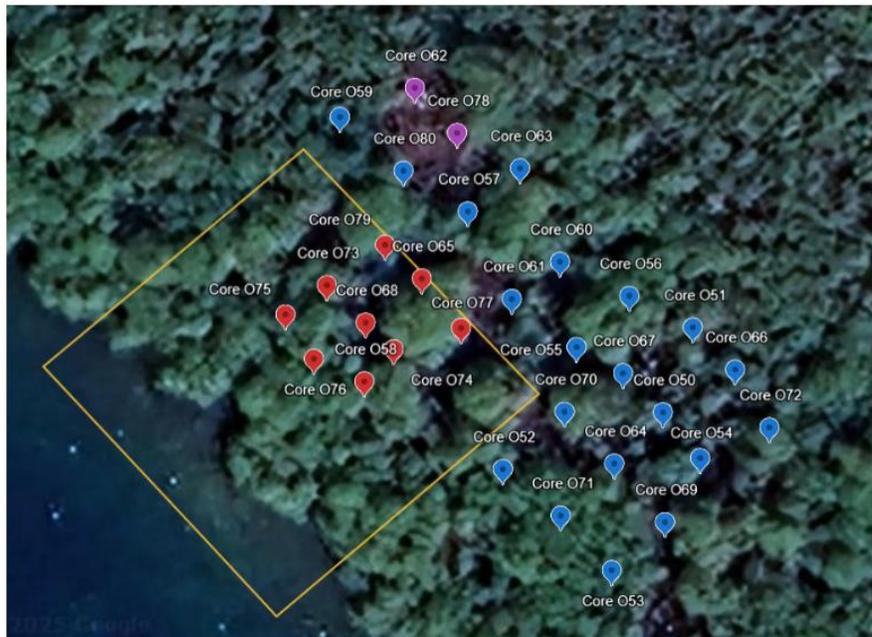


Figure 33. Picture of 2024 sample location within Oil site (O) (yellow square corresponds to the experimental area) (source: Nova Southeastern University).

3.2 Cores descriptions

Each core sample were divided into three sections: 0 – 5 cm / 5 – 10 cm / 10 + cm. The total length of the sediment cores differs from 17 cm for the smallest (R6) up to 32 cm for the longest (D36 and D45).

The sediment is composed predominantly of water (approximately 80%), vegetal fibers and root fragments. Prior to the extraction step, the core sections were manually homogenized, and the largest debris were removed (Figure 34).



Figure 34. Example of core samples (O74) divided into three sections.

4 **PROTOCOL OF ANALYSES**

4.1 **Oil extraction**

Prior to analysis, samples were homogenized. Approximately 5 grams (wet weight) were sub-sampled to determine water content (40°C @60°C for 12h). Approximately 10 grams were sub-sampled for hydrocarbon analysis. An internal standard solution (deuterated alkane and deuterated PAHs) was added prior to extraction using an EXTREVA ASE system (Accelerated Solvent Extraction). Table 2 presents the extraction conditions.

Table 2. Extraction conditions of sediment samples using the EXTREVA ASE system.

System Pressure	1500 psi
Oven Temperature	100°C
Sample Size	≈ 10 g
Oven Heatup Time	5 min
Static Time	5 min
Solvent:	Dichloromethane/acetone (80:20), (v/v)
Flush Volume	50% of extraction cell volume
Solvent flow rate	1,2 mL/min
Purge time	45 s
Nitrogen Purge	1 MPa (150 psi) for 60 s

The final extract was concentrated to 200 μL under a gentle nitrogen stream prior to instrumental analysis by gas chromatography with flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC-MS).

4.2 TRH quantification

Quantification of TRH was conducted by GC-FID using liquid injection. Calibration was performed using the BAM K010 diesel/mineral oil standard (BAM, Germany), in accordance with the NF EN ISO 9377-2 method. Analyses were carried out using an HP 7890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a pulsed splitless injector (splitless time: 1 min; purge flow: 54 mL/min). The injector temperature was set to 320 °C, and the detector interface was maintained at 300 °C.

The GC oven temperature program was as follows: initial temperature of 45 °C, ramped at 10 °C/min to 320 °C, with a final hold of 12 minutes. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Separation was achieved on an HP-5ms capillary column (30 m \times 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies). The FID detector was operated at 300 °C.

4.3 n-alkanes / PAH quantification

The analysis of PAH and alkanes was performed by Gas Chromatography coupled to Mass Spectrometry (GC/MS). The GC was an HP 7890N (Hewlett-Packard, Palo Alto, CA, USA) equipped with a CIS-4 injector used in “splitless” mode (Splitless time: 1 min, flow 54 mL/min). The injector temperature was maintained at 300 °C. The interface temperature was 280°C. The GC temperature gradient was: from 42°C (1.1 min) to 320°C at 5.5°C/min (16 min). The carrier gas was Helium at a constant flow of 1 mL/min. The capillary column used was a HP-5 ms (HP, Palo Alto, USA): 30 m \times 0.25 mm ID \times 0.25 μm film thickness. The GC was coupled to an Agilent 5977B used in SIM mode (Electronic Impact: 70 eV, voltage: 2000 V). Compounds quantifications were done using Single Ion Monitoring mode with the most representative fragment of each compound at a minimum of 2 cycles/s.

5 RESULTS

5.1 Evolution of TRH concentrations

Figure 35 presents the TRH concentrations (mean \pm SD) for each site studied. In this figure, the results of the 3 tidal areas and 3 depths are combined. For the dispersed site, the TRH concentrations reach $405 \pm 129 \mu\text{g/g}$. This concentration is lower for the Reference site ($299 \pm 99 \mu\text{g/g}$) and the Oil site ($230 \pm 113 \mu\text{g/g}$). However, due to the standard deviation is not possible to establish that TRH concentrations in sediment cores are significantly different between these 3 sites.

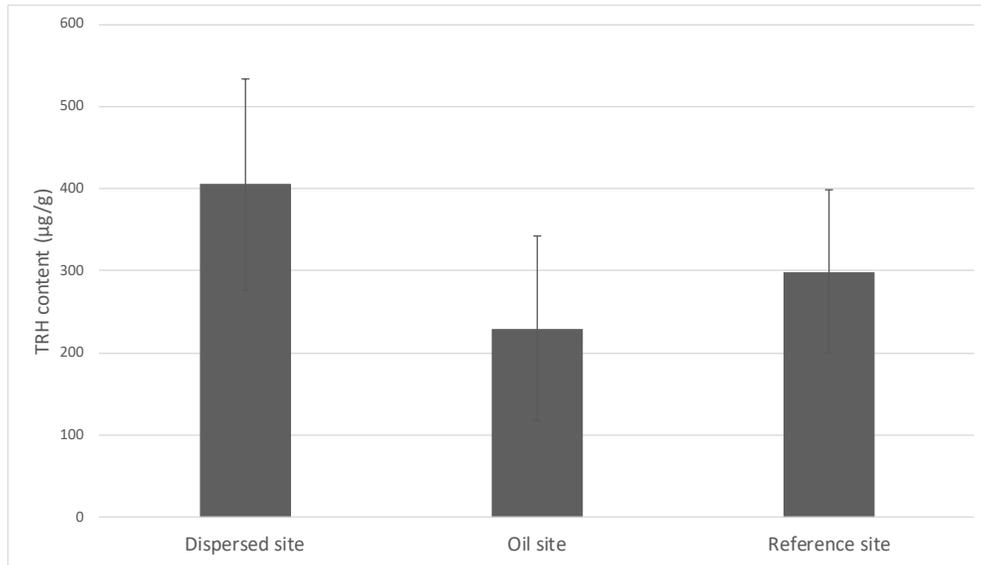


Figure 35. Mean (of TRH concentrations in the 3 layers (0-5cm / 5 - 10 cm / 10 cm+) of the sediment cores ($n=3$) of the 3 experimental areas (Dispersed, Oil site and Reference site).

Considering the layers and tidal level individually (Figure 36), some differences can be observed between the three sites. For the Dispersed site, TRH concentrations are higher in the High intertidal area compared to the other tidal areas. Moreover, TRH concentrations appear higher for the two first sediment layers (577 ± 328 ppm and 766 ± 339 ppm respectively for the 0-5cm and 5 - 10 cm) of the High intertidal area compared to deeper sediment (368 ± 298 ppm). The same trends are observed for the other tidal areas (mid-intertidal and subtidal) even if the TRH concentrations are lower. However, due to the standard deviation value, these trends are not significant.

Regarding the Oil site, TRH concentrations are higher in the two first sediment layers (0-5 and 5-10 cm) of the High intertidal area compared to deeper sediment. For the other tidal area, TRH are detected in the 3 sediment layers. Regarding the Reference site, TRH are detected in the 3 tidal areas without any significant influence of depth over the TRH distributions.

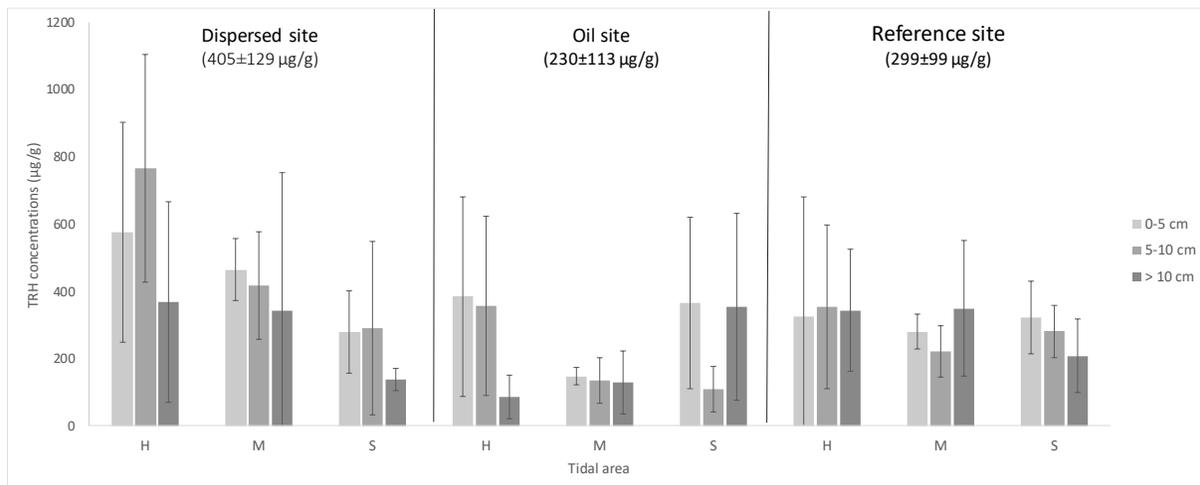


Figure 36. Distribution of TRH concentrations ($\mu\text{g/g}$) (mean \pm SD) in sediment cores ($n=3$) (depth = 0-5cm / 5 - 10 cm / 10 cm+) for each tidal level (H: high intertidal, M: mid intertidal and S: subtidal) of the 3 experimental areas (Dispersed site, Oil site and Reference site).

5.2 Evolution of alkanes and PAHs

Alkanes concentrations

Regarding alkanes concentrations in the 3 sites, Figure 37 presents the total concentrations of alkanes and their distributions in sediments core relatively to the depth of the sample (0-5 cm / 5-10 cm / 10 cm +) and the tidal area. The sums of alkanes are in the same range between the oil site ($23\pm 8 \mu\text{g/g}$) and the dispersed site ($21\pm 8 \mu\text{g/g}$). For the reference site, the sum of alkanes is higher ($33\pm 14 \mu\text{g/g}$) but the differences between the other sites are not significative.

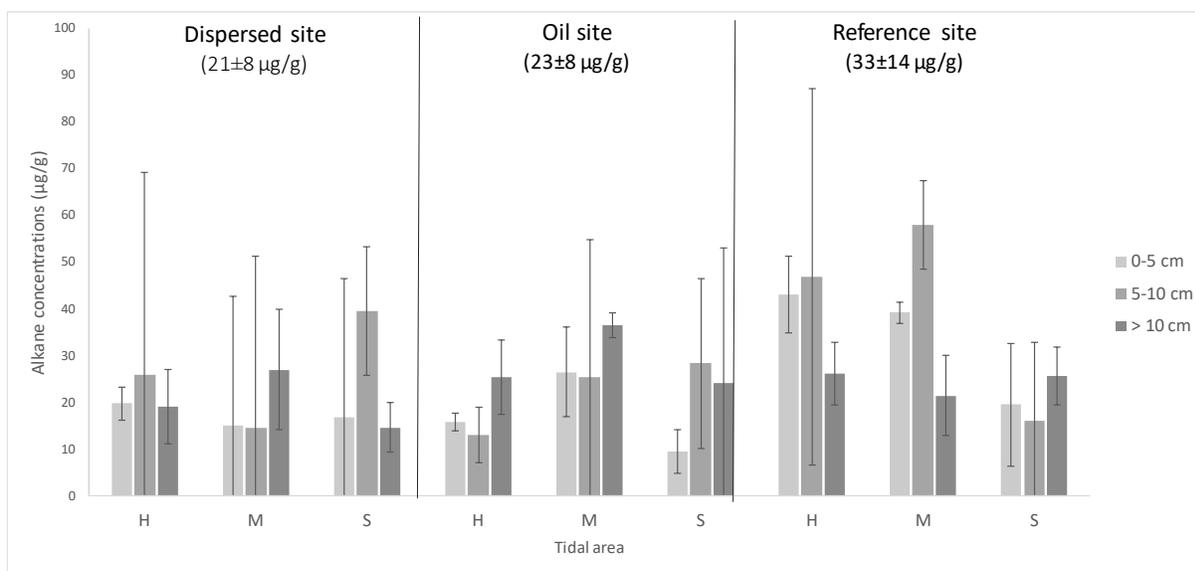


Figure 37. Total alkanes concentrations ($\mu\text{g/g}$) (mean \pm SD) in sediment cores ($n=3$) (depth = 0-5cm / 5 - 10 cm / 10 cm+) for each tidal level (H: high intertidal, M: mid intertidal and S: subtidal) of the studied areas (Dispersed site, Oil site and Reference site).

Examples of chromatograms used for alkanes quantification ($m/z=57$) are presented in Figure 38. These chromatograms highlight:

- A similarity of chemical compounds patterns between the oil or dispersed sites and the reference site;
- Even if alkanes are detected, there are in very low concentrations in comparison to other compounds such as fatty acids or fatty alcohols which are predominant in the extracts. These group of compounds are important groups of biogenic marker compounds as there are found in terrestrial organic matter and in all living organisms (Yunker et al, 1995, Wang et., 2002).

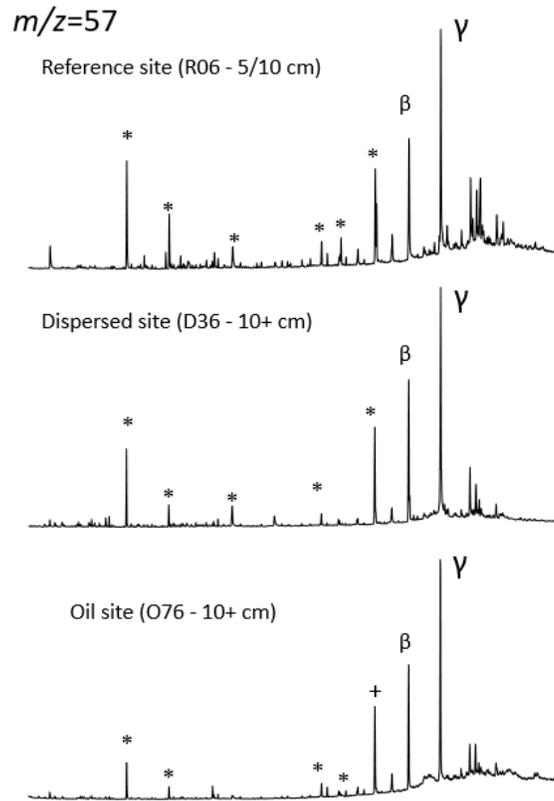


Figure 38. Examples of chromatograms (SIM mode - $m/z=57$) of sediment samples collected in the Reference site, Dispersed site and Oil Site (*: fatty acid / fatty alcohol, β : 1-eicosanol, γ : 1-triacontanol).

PAHs concentrations

Total PAHs concentrations are presented in Figure 39. Although PAHs concentrations in sediment cores appear to be higher at the Oil site ($1,4 \pm 0,5 \mu\text{g/g}$) especially for the High- and Mid- intertidal zone, the difference with the 2 other sites is not statistically significant ($0,7 \pm 0,2 \mu\text{g/g}$ for the Dispersed site and $1,1 \pm 0,4 \mu\text{g/g}$ for the Reference site).

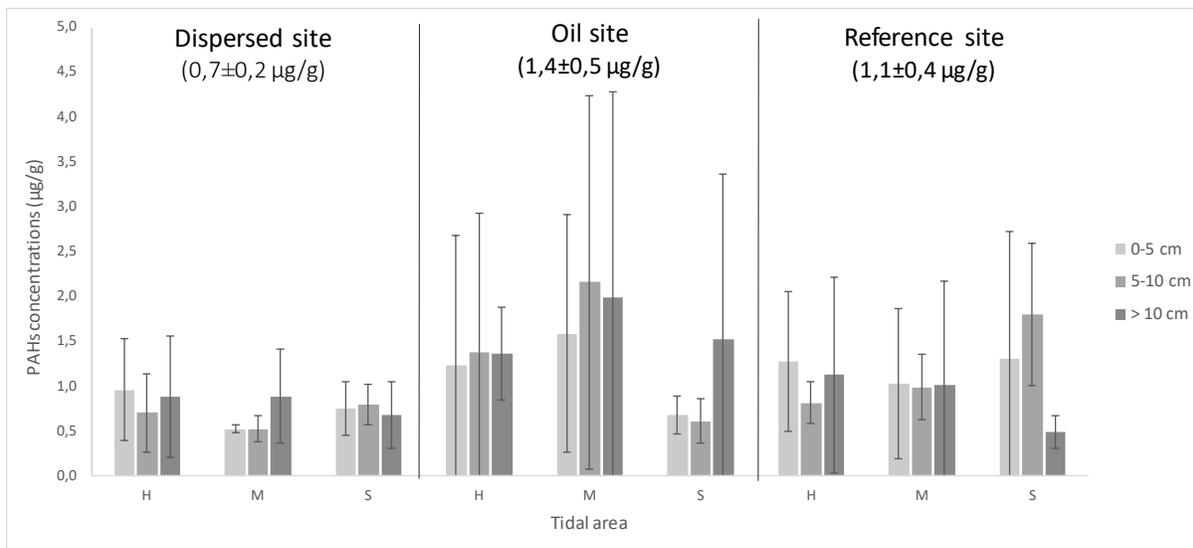


Figure 39. Total PAHs concentrations ($\mu\text{g/g}$) (mean \pm SD) in sediment cores ($n=3$) (depth = 0-5cm / 5 – 10 cm / 10 cm+) for each tidal level (H: high intertidal, M: mid intertidal and S: subtidal) of the experimental sites (Dispersed site, Oil site and Reference site).

Regarding the PAHs distributions, Figure 40 presents for each site the PAHs distributions of the sample with the highest PAH concentrations (Oil site, sample #68 – 5-10 cm; Dispersed site, Sample #37 10 + cm; Reference site, sample #10 – 0-5 cm). Samples of Oil site and Dispersed site are characterized by a PAHs distribution similar to Reference site with a predominance of Fluoranthene / pyrene family and Naphtalene family. These two families represent respectively 45% and 28% of total PAHs. As noticed during previous sampling round (Renegar et al., 2017), some PAHs signal detected in sediment samples can be related to small boat traffic in the area close to the site. For the 2024 sampling round, this was also observed for the sample #68 of the Oil site with a presence of other PAHs such as C1-, C2 and C3- Phenanthrene / anthracene, dibenzothiophene and chrysenes family.

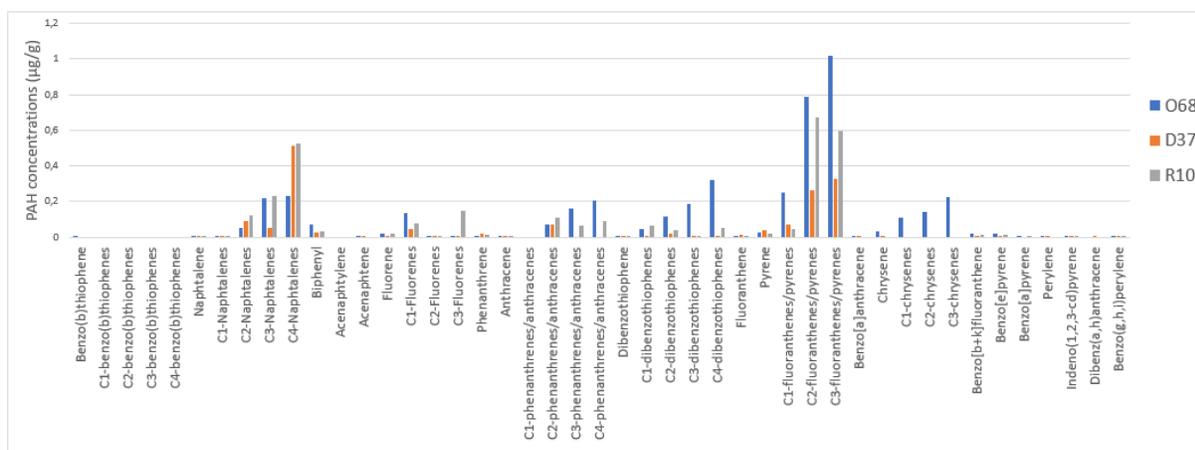


Figure 40. PAHs distributions for the most concentrated extracts in the 3 sites (Oil site, sample #68 - 5-10 cm; Dispersed site, Sample #37 - 10 + cm; Reference site, sample #10 - 0-5 cm).

Hydrocarbons identification

In order to assess the presence of crude oil in the different sediment samples, chromatograms of two groups of geochemical biomarkers typical of petrogenic hydrocarbons (triaromatic steroids ($m/z=231$) and hopane family ($m/z=191$)) were investigated. Triaromatic steroids group is composed of polycyclic aromatic compounds from the aromatic fractions of oils and rock extracts. There are highly resistant to biodegradation due to their high molecular weight and benzene ring structure, their distribution and composition characteristics are preserved even for highly degraded crude oils. These compounds generally give valuable information about source of organic matter input (Wang et al., 2008; Ando et al., 2017). With regard to the hopane family, as triaromatic steroid, these compounds can be found naturally in petrogenic oil. They are characterized by their high resistance to natural degradation processes such as dissolution, evaporation, biodegradation, photooxidation.

Figure 41 presents examples of chromatograms for each of the 3 sites. The GC/MS patterns obtained for the 3 samples appeared very similar between the 3 sites. For all samples, it was not possible to identify compounds from the triaromatic steroid or hopane families. The most abundant compounds that were identified were taraxerol and stigmasterol, two chemical compounds naturally occurring in plants.

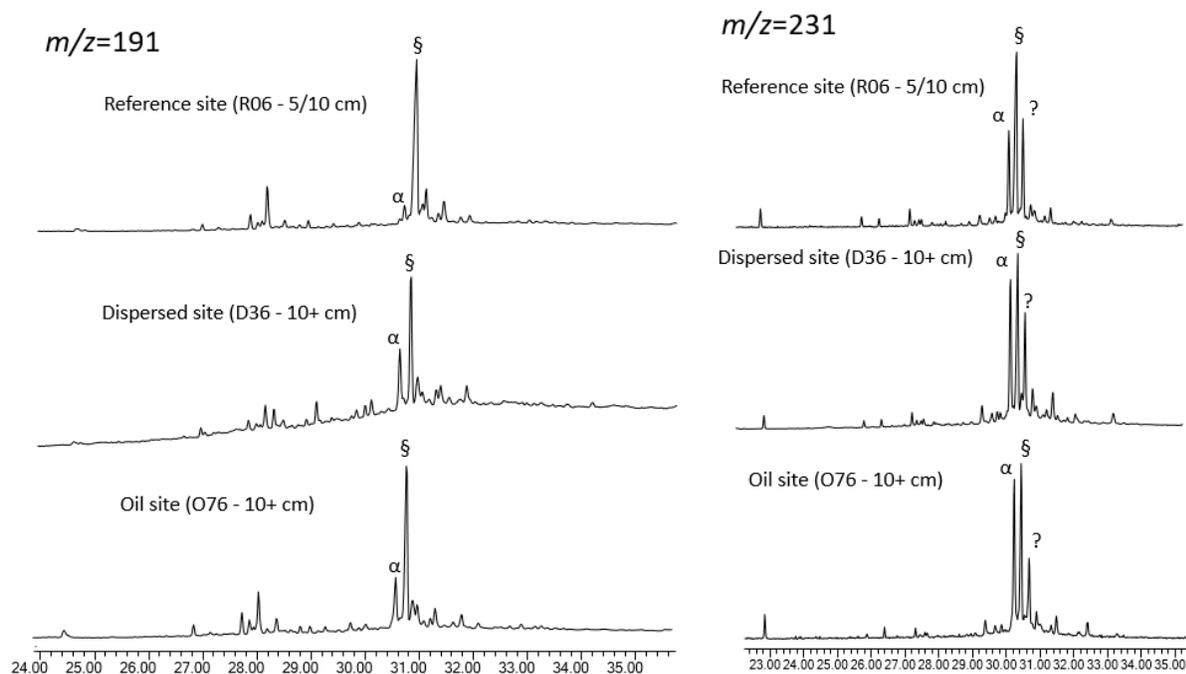


Figure 41. GCMS chromatograms of triaromatic steroids ($m/z=231$) and hopanes group ($m/z=191$) of samples collected in Reference site (R06 -5/10 cm), Dispersed site (D36 – 10+ cm) and Oil site (O76- 10+ cm) (α : stigmasterol, ξ : taraxerol).

In order to conclude on the identification of alkanes and PAHs detected in the sediment samples, perylene index was calculated. The presence of perylene in the environmental samples can be related to oil spill but also to anoxic sediments with high biological productivity (Venkatesan et al., 1988; Stout et Wang, 2018). The perylene index, defined as the concentration of perylene divided by the total of penta-cyclic PAH isomers has been developed to distinguish biogenic perylene from pyrogenic perylene (Venkatesan et al., 1988). A perylene index greater than 10% indicate the biogenic inputs whereas those <10% indicate pyrogenic origin of the compounds. Among the 81 samples analyzed during this project, the perylene index exceed 10% for 72 samples. For the remaining 9 samples, the PAHs concentrations were too low to allow a perylene calculation.

6 CONCLUSIONS

The aim of the TROPICS project was to evaluate the impact of a dispersant treatment of an oil slick drifting in a tropical environment (coral reef, mangrove, seagrass). The sediment cores samples collected in 2024 were analysed in order to assess the persistence of oil in the sediment 40 years after the oil release.

Results of chemical analyses highlighted the presence of hydrocarbons, alkanes, PAHs in all the samples whatever the site (Oil site, Dispersed site or Reference site), the depth and the tidal area (high-, mid- intertidal or subtidal). However, as the concentrations and the nature of the chemical compounds detected did not differ significantly between the Oil Site, the Dispersed site and the Reference site (i.e., without oil), it was not possible to conclude that oil released 40 years ago was still present in the sediment (from 0 to 30 cm depth). Moreover, detailed overview of chemical compounds indicated a significant biogenic contribution in all the cores samples.

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7.3 Appendix C: Sediment Core Analysis Report: Texas A&M



TEXAS A&M UNIVERSITY

Geochemical & Environmental
Research Group

Biomarker Analyses of Panama Mangrove Soils for TROPICS 40 Project

July 31, 2025

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Summary

The aim of the Tropics Project (Tropical Oil Pollution Investigations in Coastal Systems) was to assess the short and long-term effects of an oil exposure and use of chemical dispersants on a tropical mangrove, coral reef and seagrasses. The 40 year assessment was carried out by some members of the original team. This report is on the chemical measurements of Mangrove sediments. The chemistry task was divided between Texas A&M's GERG (Geochemical and Environmental Research Group and CEDRE (Brest, France). The tasks between the two groups was divided by CEDRE analyzing sediment cores inside the 3 sites and GERG a series of samples outside the sites as during the oiling 40 years before oil was detected outside the original site and as these areas had never been sampled before there was a view that these sites should be studied.

A total of 36 cores were analyzed at GERG with 104 individual sample analysis of core sections. Although Petroleum Biomarkers were easily detected in Standard Reference Material, there were no Petroleum Biomarkers detected in any of the core samples collected. The results of aliphatic and aromatic hydrocarbons were mostly below detection. What is present is a diverse plant - synthesized hydrocarbons such as plant phytosterols (eg. Stigmasterol, sitosterol, amyirin) and plant diterpenes (eg. Rimuene, abietadiene). If oil had contaminated these sites we would expect to have detected petroleum biomarkers.

Background and Motivation

Petroleum consists of thousands of individual compounds, producing a complex distribution of peaks in a gas chromatography chromatogram. This chromatogram is often called the fingerprint of the oil. However, this fingerprint is highly susceptible to weathering processes, both biotic and abiotic. For example, PAHs are highly vulnerable to photo-oxidation and evaporation (Figure 1).

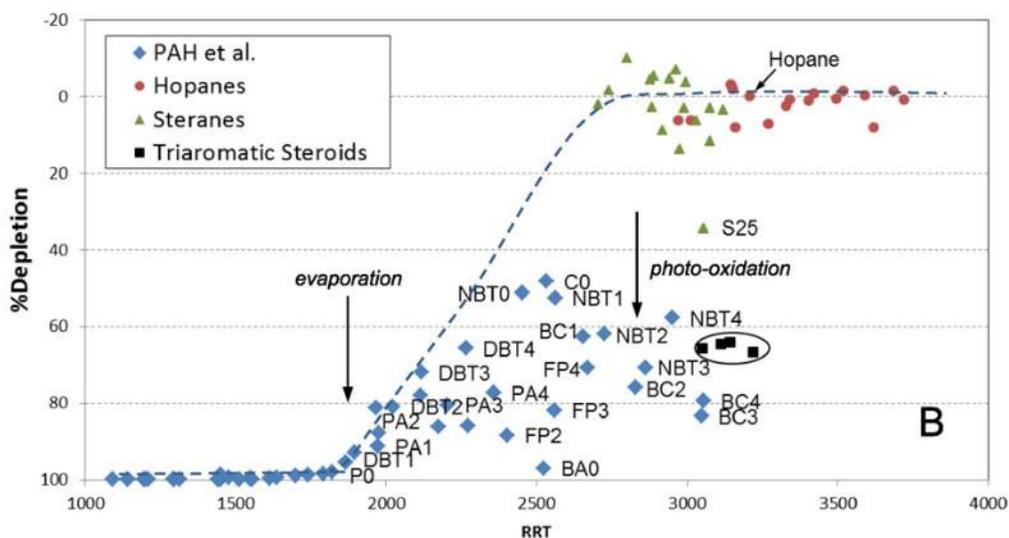


Figure 1: Percent depletion of PAHs and petroleum biomarkers for stranded Macondo oils (Deepwater Horizon oil spill). From Stout et al. (2016).

Petroleum biomarkers, which consist of terpanes, steranes, and triaromatic steroids, are both more indicative of the original petroleum source and are more stable during these weathering

processes. This has led to their use in oil spill forensics, such as those following the Deepwater Horizon oil spill in 2010.

Diagnostic ratios (DRs), or ratios of petroleum biomarkers, are used to fingerprint petroleum samples for oil source matching. Therefore, it was our objective to extract and measure petroleum biomarkers in the soil cores from oiled, dispersed, and reference sites to investigate the presence of crude oil at these locations. We then utilized diagnostic ratios to analyze oil source similarity/dissimilarity.

Methods Sampling

Samples were collected in all three sites (oil treatment, oil and chemical dispersant and reference) with a vibecorer in clean aluminum 2 inch barrels. The corer at times went about 60 cm into the sites but due to compression the cores were between 15 and 32 cm. The cores were extruded in the laboratory after each days sampling. Each of the three sites was sampled on consecutive days with only one site visited per day. The cores consisted of biological material of fiber and roots (mangrove debris) rather than classic sediment. The cores were sectioned into the top 5 cm, 510 cm, then 10 cm to bottom of core. The cores were wrapped in pre-combusted aluminum foil, place in a plastic Ziploc bad and stored in a cooler. They were then shipped to each laboratory CEDRE and GERG. The samples were delayed in Panama until the end of April 2025, but there was no evidence that this delay caused any issue to the samples.

Figures 2 , 3 and 4 indicate where samples were taken and what samples were analyzed by GERG. For the reference site there were 10 cores were analyzed (29 sample intervals). For the dispersed site 13 cores were analyzed (37 sample intervals). For the oil only site 13 cores were analyzed (38 sample intervals).



Figure 2: Reference site.

SAMPLES ANALYZED Reference Site

R4: top, middle, bottom	R5: top, middle, bottom
R8: top middle, bottom	R9: top, middle, bottom
R12: top, bottom	R13: top, middle, bottom
R14: top, middle, bottom	R16: top, middle, bottom
R17: top, middle, bottom	R19: top, middle, bottom



Figure 3: Dispersed Site

SAMPLES ANALYZED Dispersed Site

D2: top, middle, bottom	D21: top, middle, bottom
D22: top, middle, bottom	D23: top, middle, bottom
D28: top, middle, bottom	D29: top, middle, bottom
D33: middle, bottom	D40: top, middle, bottom
D42: top, middle, bottom	D46: middle, bottom
D47: top, middle, bottom	D48: top, middle, bottom
D49: top, middle, bottom	

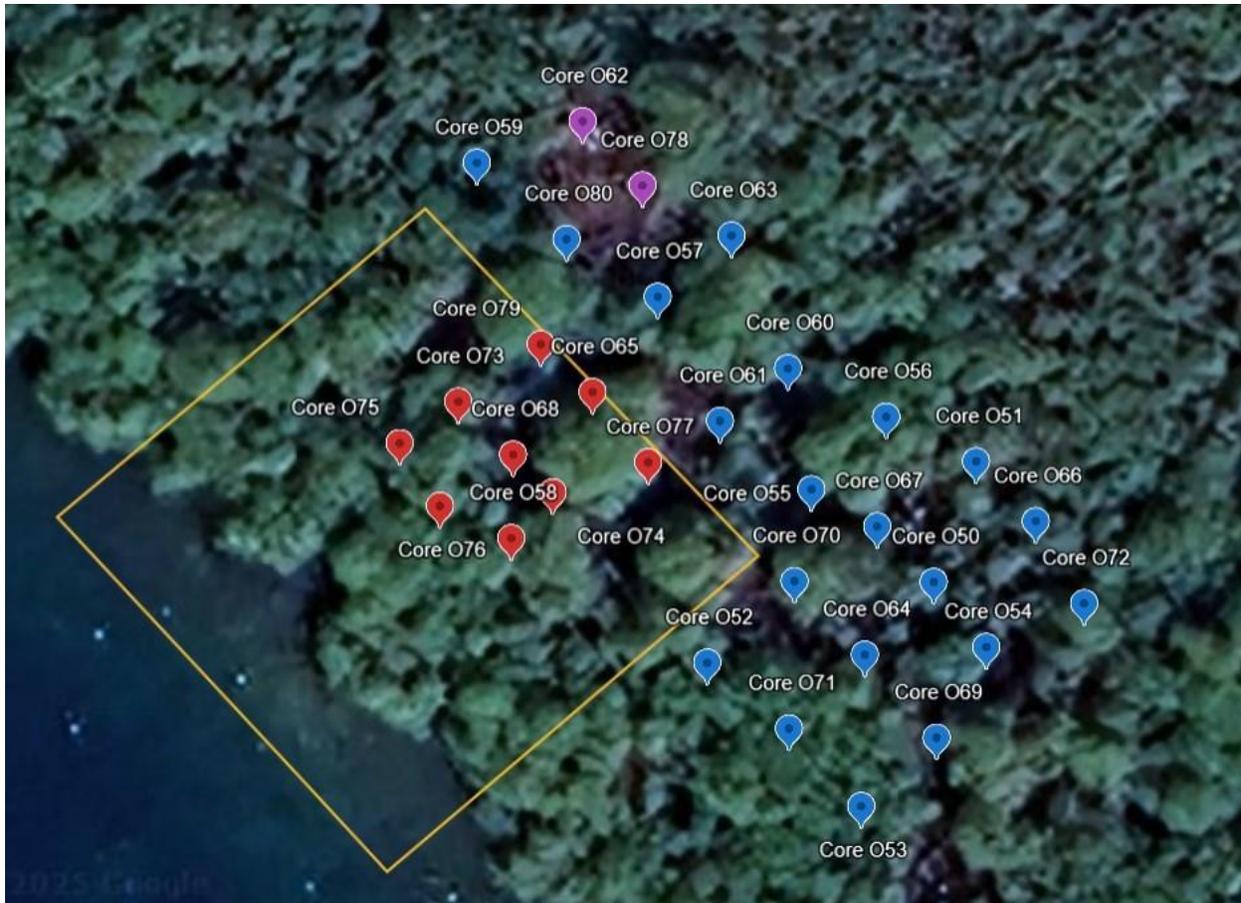


Figure 4: Oil Only Site

SAMPLES ANALYZED Oil Site

O50: top, middle, bottom	O52: top, middle, bottom
O55: top, middle, bottom	O56: top, middle, bottom
O57: top, middle, bottom	O59: top, middle, bottom
O62: top, middle, bottom	O67: top, middle, bottom
O70: top, middle, bottom	O71: top, middle
O72: top, middle, bottom	O78: top, middle, bottom
O80: top, middle, bottom	

Sample Handling

Sample Receiving: Samples were received at the Geochemical and Environmental Research Group (GERG) on April 29, 2025. The cores arrived with blue ice packs and were immediately stored at 4°C. Individual core samples were subsequently split vertically using a razor blade to obtain an archived half and a working half. Both were stored frozen (-20°C).

Sample Preparation: The working half was freeze-dried and homogenized by hand. Moisture content was calculated by recording the wet weight and post-drying weight of the core section. The mean moisture content for all samples was 85% (see Appendix).

Extraction

As these samples were very peaty, a simple sonication extraction with Dichloromethane (DCM) followed by a sodium sulfate extraction column for moisture removal did not work and we only saw phytosterols and elemental sulfur. Therefore, we had to use a more comprehensive extract clean-up technique using silica gel column chromatography to remove the polar compounds and activated copper to remove elemental sulfur.

Five hundred milligram subsamples were extracted in DCM using ultrasonication for 30 minutes and transferred to clean vials following centrifugation. This sonication step was repeated a total of three times. The resulting extract is considered the total lipid extract (TLE).

The TLEs were then concentrated under a gentle nitrogen stream. These extracts were found to have interferences from elemental sulfur and plant biomolecules (see Results section below) and required further cleanup using activated silica gel column chromatography. The silica gel columns (containing 1 gram of activated silica gel) were first washed with hexane followed by 1:1 hexane:DCM. The samples were reconstituted in 1:1 hexane:DCM and loaded onto the columns. The fraction containing less-polar hydrocarbons, including petroleum biomarkers, was eluted using 3 mL of 1:1 hexane:DCM. The second, more polar, fraction containing plant phytosterols was eluted using 3 mL of 1:1 DCM:methanol.

The addition of activated copper was essential to remove elemental sulfur from the majority of extracts to remove matrix interference. This was added to the fraction 1 extract (1:1 hexane:DCM).

The final extracts were concentrated to near dryness with a gentle stream of nitrogen and reconstituted in 400 μ L of DCM for analysis.

NIST Standard Reference Material (SRM) 1944 (New York/New Jersey Waterway Sediment) was extracted for every twenty samples to verify method performance.

Analysis

Extracts were analyzed on an Agilent 7890 Gas Chromatograph (GC) coupled to a 5977B Single Quadrupole Mass Spectrometer (MS). Several NIST petroleum SRMs (2779 and 2722) were run with each analytical batch to verify petroleum biomarker peak positions.

Extracts were injected in “splitless” mode using a 2 μ L injection volume and a splitless time of 1.5 minutes. The inlet was held at 300°C and the helium carrier gas was held constant at 2 mL/min. The capillary column used was an Agilent DB-1ms ultra inert column (60 m x 0.25 mm ID x 0.25 μ m film thickness). The GC oven was initially held at 35°C for 2 minutes, followed by a 4°C/min ramp to 300°C where it was held for 20 minutes. A post-run ramp to 320°C was held for 10 minutes before the next sample injection. The MS was operated in SCAN mode with a scan range of 40-450 m/z following an initial solvent delay of 10 minutes. The electron impact (EI) source was held at 250°C while the quadrupole was kept at 150°C. Total run time is 98.25 minutes.

The biomarkers measured in this study are shown in Table 1, along with their corresponding mass-to-charge ratio (m/z).

Table 1: Petroleum Biomarkers Measured in this method

Biomarker Class	Biomarker	Peak IDs	m/z
Terpanes	C24 Tetracyclic Terpane	T6a	191.1
	C26 Tricyclic Terpane-22S	T6b	191.1

	C26 Tricyclic Terpane-22R	T6c	191.1
	C28 Tricyclic Terpane-22S	T7	191.1
	C28 Tricyclic Terpane-22R	T8	191.1
	C29 Tricyclic Terpane-22S	T9	191.1
	C29 Tricyclic Terpane-22R	T10	191.1
	18a-22,29,30-Trisnorneohopane-Ts	T11	191.1
	17a(H)-22,29,30-Trisnorhopane-Tm	T12	191.1
	17a/b,21b/a 28,30-Bisnorhopane	T14a	191.1
	30-Norhopane	T15	191.1
	18a(H)-30-Norneohopane-C29Ts	T16	191.1
	17a(H)-Diahopane	X	191.1
	18a(H)&18b(H)-Oleananes	T18	191.1
	Hopane	T19	191.1
	Moretane	T20	191.1
	30-Homohopane-22S	T21	191.1
	30-Homohopane-22R	T22	191.1
	30,31-Bishomohopane-22S	T26	191.1
	30,31-Bishomohopane-22R	T27	191.1
	Tetrakishomohopane-22S	T32	191.1
	Tetrakishomohopane-22R	T33	191.1
Steranes	14a(H),17a(H)-20S-Cholestane + 13b(H),17a(H)-20S-Ethylcholestane	S12_S13	217.1
	14a(H),17a(H)-20R-Cholestane + 13b(H),17a(H)-20R-Ethylcholestane	S17_S18	217.1
	14b(H),17b(H)-20R-Cholestane	S14	218.1
	14b(H),17b(H)-20S-Cholestane	S15	218.1
	14b,17b-20R-Methylcholestane	S22	218.1
	14b,17b-20S-Methylcholestane	S23	218.1
	14a(H),17a(H)-20S-Ethylcholestane	S25	217.1
	14a(H),17a(H)-20R-Ethylcholestane	S28	217.1
	14b(H),17b(H)-20R-Ethylcholestane	S26	218.1
	14b(H),17b(H)-20S-Ethylcholestane	S27	218.1
	Triaromatic Steroids	C26,20R- +C27,20S- triaromatic steroid	RC26 SC27
C27,20R-triaromatic steroid		RC27	231.1
C28,20R-triaromatic steroid		RC28	231.1
C28,20S-triaromatic steroid		SC28	231.1

The quantified biomarker diagnostic ratios (DRs) are shown in Table 2.

Table 2: Diagnostic Ratios to be measured in this study. From Stout et al. (2016)

Ratio ID	Biomarker Ratio
DR05	T11/(T11+T12)
DR06	T16/(T16+T15)
DR07	X/(X+T15)
DR08	X/(X+T19)
DR09	T21/(T21+T22)
DR10	T26/(T26+T27)
DR11	T6a/(T6a+T19)
DR12	T6a/(T6a+T6b+T6c)
DR13	(T7+T8+T9+T10)/(T7+T8+T9+T10+T19)
DR14	T14a/(T14a+T19)
DR15	T15/(T15+T19)

DR16	T18/(T18+T19)
DR17	T20/(T20+T19)
DR18	(T32+T33)/(T32+T33+T19)
DR19	S25/(S25+S28)
DR20	(S26+S27)/(S26+S27+S25+S28)
DR21	(S12_S13+S17_S18+S25+S28+S22+S23+S26+S27)/ (S12_S13+S17_S18+S25+S28+S22+S23+S26+S27+T11+T12+T15+T19)
DR25	(S26+S27)/(S14+S15+S22+S23+S26+S27)
DR26	(RC26_SC27)/(RC26_SC27+RC27+RC28)
DR27	SC28/(SC28+RC28)
DR28	SC28/(SC28+RC27)
DR29	(RC26_SC27+RC27+RC28)/(RC26_SC27+RC27+RC28+T19)

Results

Matrix interferences

The TLE analyzed for these core samples contained significant amounts of elemental sulfur and plant biomolecules (Figure 5). Each of these constituents swamped the signal of any petroleum biomarkers that may be present. The abundant plant biomolecules and elemental sulfur are consistent with the coastal mangrove ecosystem.

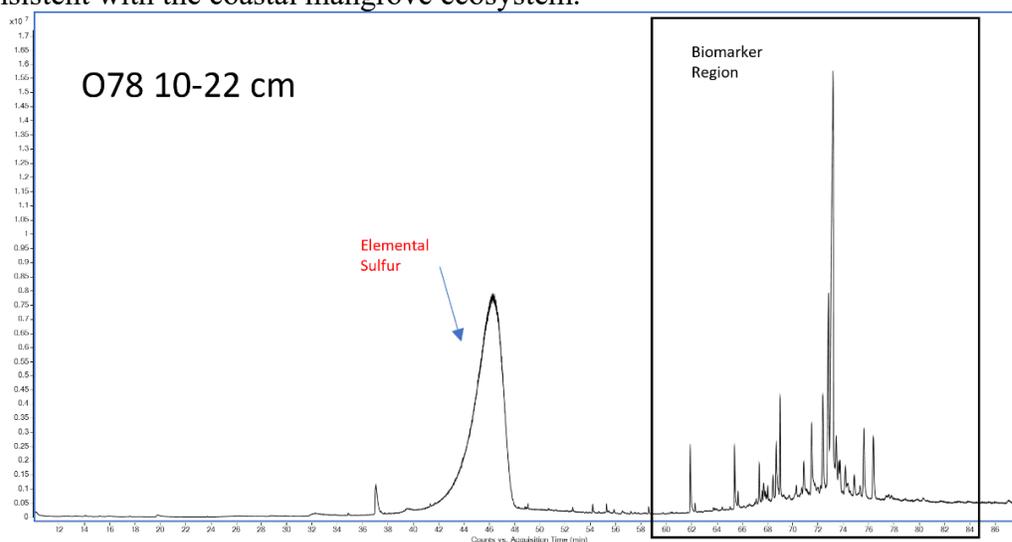


Figure 5: Total lipid extract chromatogram for core sample O78 (10-22 cm interval). Highlighted are the interferences from elemental sulfur and plant phytosterols in the biomarker region of the chromatogram.

Potential identities for these plant biomolecules based on mass spectral library matching include sitosterol, stigmasterol, amyirin, and several plant diterpenes. Therefore, we performed additional silica gel column chromatography and added activated copper as described in the methods to remove these matrix interferences. Following further extract cleanup, the elemental sulfur and interfering plant sterols have been removed. However, the extracts lack the hump for the unresolved complex mixture (UCM) commonly observed in petroleum-impacted samples (Figure 6).

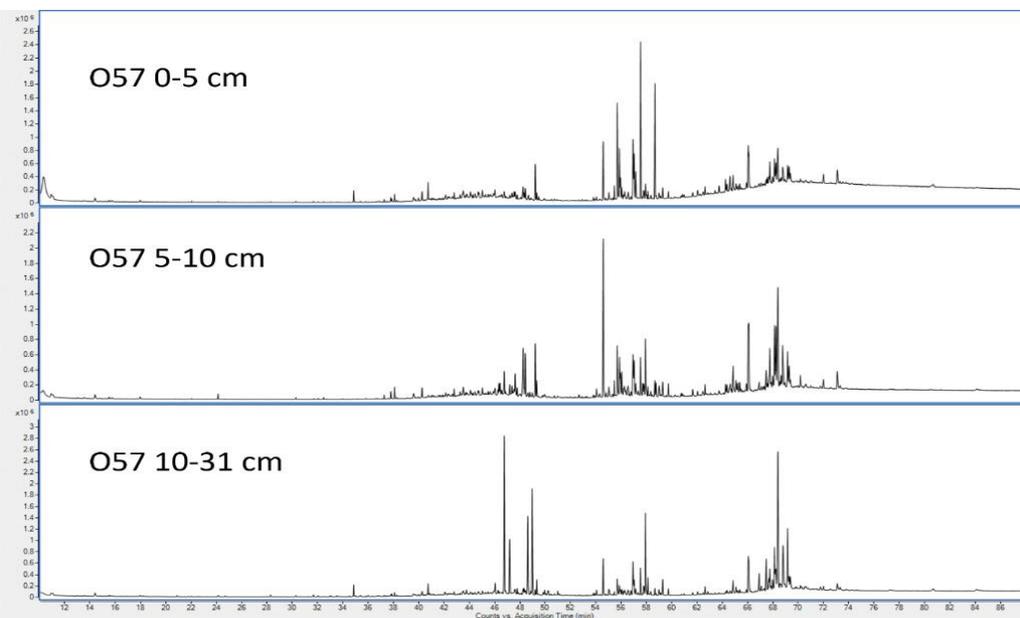


Figure 6: Total ion chromatographs for core O57 following silica gel chromatography and activated copper treatment.

The same procedure was used for the NIST 1944 sediment SRM to verify the recovery of the UCM and petroleum biomarkers following these additional cleanup steps (Figure 7 and 8).

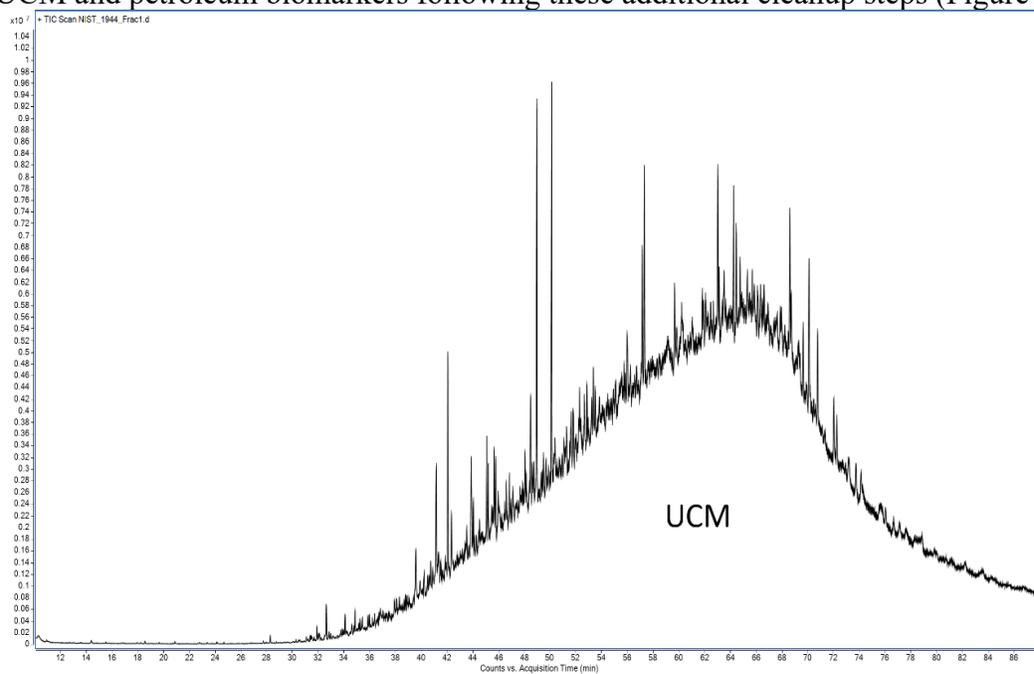


Figure 7: Total ion chromatogram of NIST 1944 sediment following silica gel chromatography and activated copper treatment.

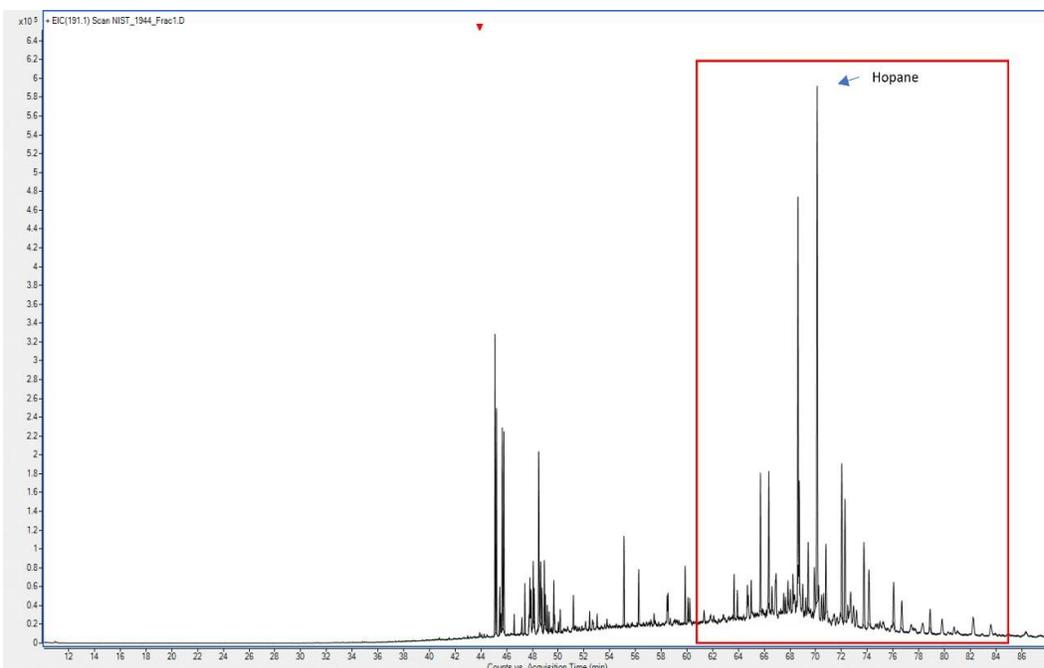


Figure 8: Extracted ion chromatogram (EIC) of m/z 191.1 for NIST 1944 sediment. Petroleum biomarkers were recovered following silica gel column chromatography.

Missing Petroleum Biomarkers in Mangrove Soils

Regardless of site (Reference, Dispersed, Oiled) and core, there were no quantifiable amounts of petroleum biomarkers present in these mangrove soils. Figure 9 shows the extracted ion chromatograms (EIC) for m/z 191.1. This mass is used to look for terpanes including hopane, which is commonly the most abundant biomarker in petroleum samples. The small peaks present in the chromatograms do not match the retention times and spectra for any petroleum biomarkers and are likely plant biomolecules based on the NIST library.

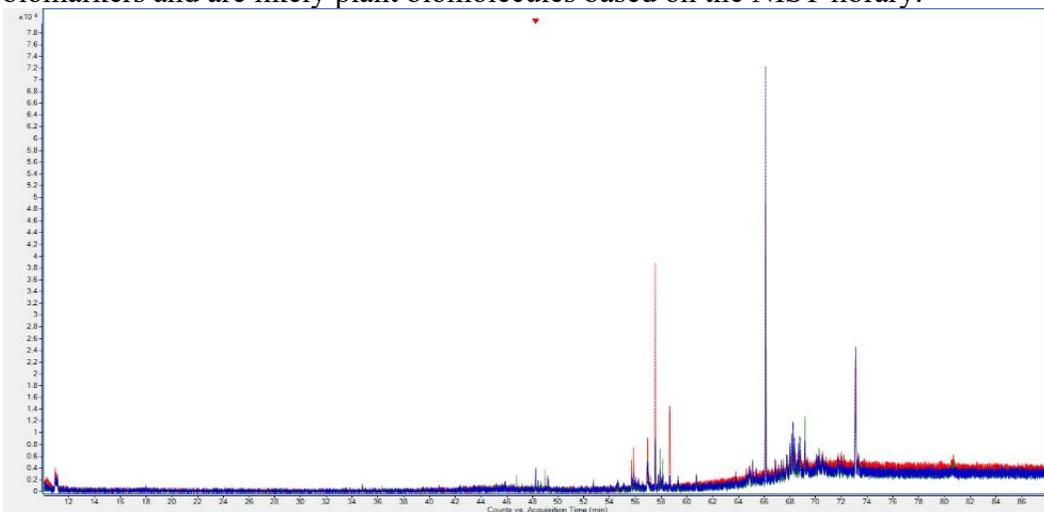


Figure 9: Extracted ion chromatograms (EIC) of m/z 191.1 for O57 (Interval 0-5 cm shown in blue; interval 5-10 cm shown in red, and interval 10-31 cm shown in green).

An overlay of the NIST 1944 sediment, which contains abundant petroleum biomarkers, is shown as a reference in Figure 10.

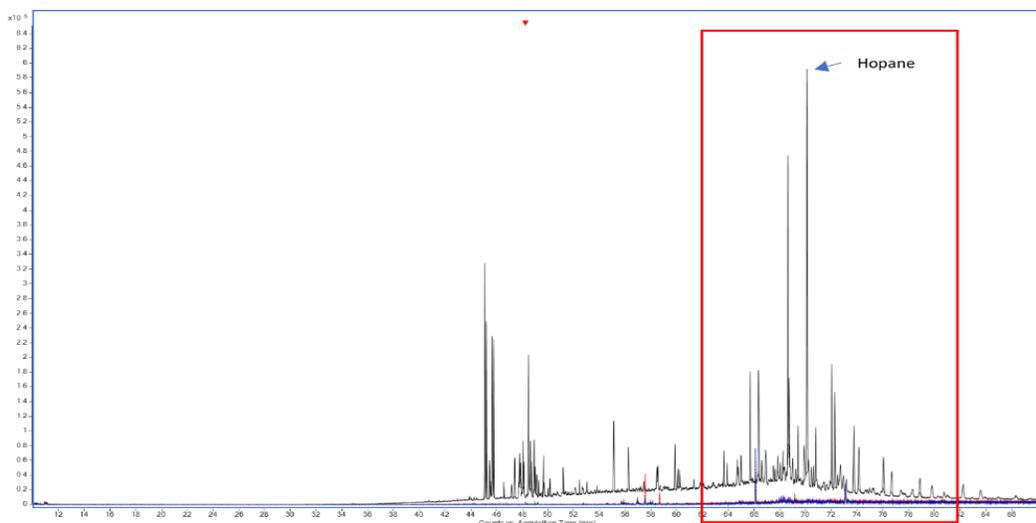


Figure 10: Extracted ion chromatograms (EIC) from Figure 9 overlaid with corresponding EIC from NIST 1944 sediment.

Finally Figure 11 shows biomarkers (Terpanes) from Biomarkers for Alaska North Slope oil (Wang et al. 2006) which we used in the original oiling 40 years ago. We would expect to have seen chromatographs similar to these if there was oil present.

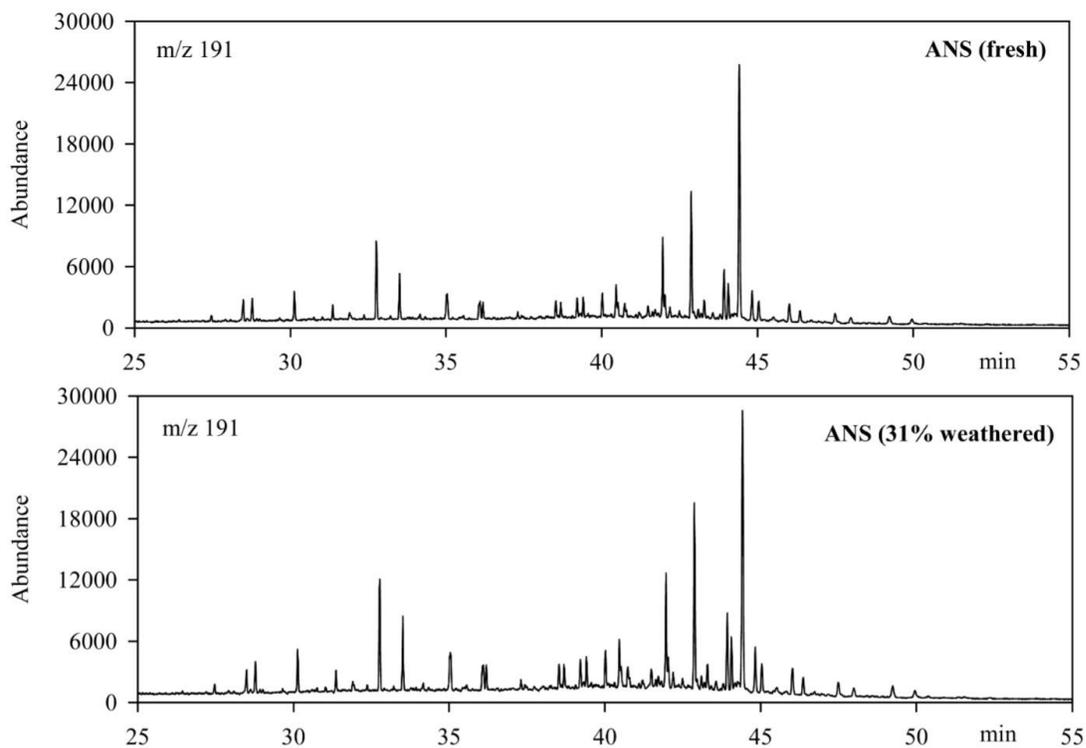


Figure 11: Alaska North Slope Terpanes (Wang et. al., 2006)

Appendix A: Moisture Content

Core ID	Interval	Length (cm)	Moisture Content (%)
D02	A	0-5	86.6
D02	B	5-10	86.9
D02	C	10-32	85.1
D21	A	0-5	86
D21	B	5-10	85.5
D21	C	10-28	81.5
D22	A	0-5	85.6
D22	B	5-10	85.6
D22	C	10-28	82.6
D23	A	0-5	88
D23	B	5-10	85.2
D23	C	10-35	85.7
D24	A	0-5	88.3
D24	B	5-10	87.2
D24	C	10-30	85.9
D27	A	0-5	84.6
D27	B	5-10	85.6
D27	C	10-39	85.8
D28	A	0-5	86.9
D28	B	5-10	85
D28	C	10-37	86.6
D29	A	0-5	86.8
D29	B	5-10	83.1
D29	C	10-29	83.6
D31	A	0-5	85.4
D31	B	5-10	83.6
D31	C	10-26	82.3
D33	A	0-5	84.5
D33	B	5-10	83.6
D33	C	10-20	81.5
D34	A	0-5	85.2
D34	B	5-10	85.1
D34	C	10-29.5	84.4
D39	A	0-5	84.6
D39	B	5-10	83.3
D39	C	10-24	82.7
D40	A	0-5	83.6
D40	B	5-10	84.4
D40	C	10-19	83.2
D41	A	0-5	85.1
D41	B	5-10	82.5
D41	C	10-30	81.6
D42	C	0-5	83.4
D42	B	5-10	85.3
D42	C	10-19.5	83.5

D43	A	0-5	86.3
D43	B	5-10	86.8
D43	C	10-22	85.4
D44	A	0-5	86.3
D44	B	5-10	83.2
D44	C	10-22	81.3
D46	A	0-5	84.2
D46	B	5-10	85.4
D46	C	10-20	84.2
D47	A	0-5	85.1
D47	B	5-10	84.1
D47	C	10-22.5	82.6
D48	A	0-5	84.8
D48	B	5-10	85.1
D48	C	10-23	84.1
D49	A	0-5	85
D49	B	5-10	84.4
D49	C	10-24	81.1
O50	A	0-5	83.2
O50	B	5-10	80.9
O50	C	10-18	78.6
O51	A	0-5	85
O51	B	5-10	83.3
O51	C	10-24	82.7
O52	A	0-5	83.4
O52	B	5-10	83.3
O52	C	10-22	82.1
O53	A	0-5	86.9
O53	B	5-10	85.8
O53	C	10-36	84.6
O54	A	0-5	83.8
O54	B	5-10	81.8
O54	C	10-19	82.3
O55	A	0-5	84.6
O55	B	5-10	82
O55	C	10-21.5	81.5
O56	A	0-5	83.3
O56	B	5-10	81.9
O56	C	10-22	81.3
O57	A	0-5	79.8
O57	B	5-10	80.7
O57	C	10-31	81.5
O59		0-5	83.6
O59		5-10	83.3
O59		10-23	80.6
O60		0-5	83.1
O60		5-10	82.5
O60		10-22	81.1

O61			84.4
O61			83.2
O61	C	10-22	80.7
O62	A	0-5	82.4
O62	B	5-10	81.9
O62	C	10-23	79.8
O63	A	0-5	79
O63	B	5-10	72.2
O63	C	10-16	75.6
O64	A	0-5	87.2
O64	B	5-10	83.2
O64	C	10-27	82.5
O66	A	0-5	82.8
O66	B	5-10	82
O66	C	10-21	78.7
O67	A	0-5	82.6
O67	B	5-10	82.7
O67	C	10-18	77.7
O69	A	0-5	84.5
O69	B	5-10	82.8
O69	C	10-15	78.1
O70	A	0-5	87.5
O70	B	5-10	
O70	C	10-23	80.9
O71	A	0-5	85.9
O71	B	5-10	86.6
O71	C	10-26	83.6
O72	A	0-5	80.8
O72	B	5-10	82
O72	C	10-22	79.5
O80	A	0-5	84.8
O80	B	5-10	89.3
O80	C	10-27	81.7
R04	A	0-5	83.8
R04	B	5-10	81.7
R04	C	10-17.5	80.1
R05	A	0-5	85.4
R05	B	5-10	84
R05	C	10-21.5	83.1
R08	A	0-5	85.2
R08	B	5-10	83.4
R08	C	10-19	80.9
R09		0-5	82
R09		5-10	79.8
R09		10-13	78.3
R12		0-5	83.1
R12		5-10	82.2
R12		10-16	83

R14			85
R14			83.2
R14	C	10-17	81.2
R16	A	0-5	85
R16	B	5-10	82.8
R16	C	10-16.5	83.3
R17	A	0-5	83.3
R17	B	5-10	84.1
R17	C	10-21	83.4
R19	A	0-5	83.6
R19	B	5-10	83.2
R19	C	10-16	81.1

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Appendix C: n-Alkane Results

Core	Interval	n-C9	n-C10	n-C11	n-C12	n-C13	n-C14	n-C15	n-C16	n-C17	Pristane	n-C18
D02	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12.07	<LOQ	<LOQ	25.29
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D02	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D21	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11.36	12.20	23.00	<LOQ	<LOQ	<LOQ
D21	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.87	<LOQ	18.09	<LOQ	<LOQ	<LOQ
D21	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D22	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12.88	<LOQ	<LOQ	13.08
D22	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	8.72	7.55	23.80	<LOQ	<LOQ	17.92
D22	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.08	<LOQ	<LOQ	7.91
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D23	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.74	<LOQ	<LOQ	<LOQ
D28	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	8.44	23.04	<LOQ	<LOQ	<LOQ
D28	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.41	<LOQ	8.14	<LOQ	<LOQ	<LOQ
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D33	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.57	<LOQ	<LOQ	<LOQ
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O50	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	16.42	<LOQ	<LOQ	<LOQ

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052	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.76	<LOQ	18.36	<LOQ	<LOQ	8.67
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056	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7.08	<LOQ	27.47	<LOQ	<LOQ	20.97
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057	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7.22	<LOQ	72.71	10.84	<LOQ	<LOQ
057	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7.65	<LOQ	28.98	<LOQ	<LOQ	<LOQ
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078	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	17.58	<LOQ	<LOQ	12.70
080	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	14.13	<LOQ	<LOQ	20.11
080	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	13.99	6.07	31.11	<LOQ	<LOQ	22.79
080	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	20.19	<LOQ	<LOQ	23.28
R04	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.05	<LOQ	12.57	<LOQ	<LOQ	<LOQ
R04	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.83	<LOQ	19.72	<LOQ	<LOQ	<LOQ
R04	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.18	<LOQ	19.99	<LOQ	<LOQ	9.95
R05	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.39	5.83	28.40	12.81	<LOQ	14.39
R05	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11.28	<LOQ	28.34	<LOQ	<LOQ	18.19
R05	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	23.51	<LOQ	35.01	<LOQ	<LOQ	14.09
R08	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	14.29	<LOQ	28.76	<LOQ	<LOQ	15.56
R08	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.43	<LOQ	20.56	<LOQ	<LOQ	14.43
R08	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	16.36	<LOQ	44.85	<LOQ	<LOQ	17.12
R09	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	9.51	4.13	20.16	<LOQ	<LOQ	<LOQ
R09	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	15.99	<LOQ	32.25	<LOQ	<LOQ	11.5

R09	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.81	<LOQ	18.99	<LOQ	<LOQ	10.56
R12	A	<LOQ	<LOQ	<LOQ	3.26	<LOQ	19.66	<LOQ	30.34	<LOQ	<LOQ	14.42
R12	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.20	<LOQ	20.60	<LOQ	<LOQ	15.64
R13	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.41	<LOQ	<LOQ	<LOQ
R13	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	10.89	<LOQ	<LOQ	11.81
R13	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11.83	<LOQ	<LOQ	<LOQ
R14	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.03	<LOQ	12.41	<LOQ	<LOQ	9.52
R14	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.56	<LOQ	19.44	<LOQ	<LOQ	8.07
R14	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.57	<LOQ	29.82	<LOQ	<LOQ	12.13
R16	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.70	<LOQ	20.29	<LOQ	<LOQ	10.84
R16	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	13.93	<LOQ	25.93	<LOQ	<LOQ	10.07
R16	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	13.87	<LOQ	21.70	<LOQ	<LOQ	10.05
R17	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.60	<LOQ	16.97	7.81	<LOQ	11.07
R17	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	16.51	3.99	29.13	<LOQ	<LOQ	13.69
R17	C	<LOQ	<LOQ	3.34	<LOQ	<LOQ	5.19	<LOQ	17.11	<LOQ	<LOQ	9.88
R19	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.81	3.68	18.82	<LOQ	<LOQ	7.98
R19	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	8.46	<LOQ	29.67	<LOQ	<LOQ	10.93
R19	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12.05	<LOQ	33.34	<LOQ	<LOQ	14.1

Core	Interval	Phytane	n-C19	n-C20	n-C21	n-C22	n-C23	n-C24	n-C25	n-C26
D02	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	14.80	<LOQ
D02	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D02	C	<LOQ	5.58	<LOQ						
D21	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	10.56	<LOQ
D21	B	<LOQ	53.46	<LOQ						
D21	C	<LOQ	20.82	<LOQ						
D22	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	8.36	<LOQ
D22	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7.59	<LOQ	<LOQ
D22	C	<LOQ	8.16	<LOQ						
D23	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D23	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D23	C	<LOQ	9.25	<LOQ						
D28	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D28	B	<LOQ	29.70	<LOQ						
D28	C	<LOQ	10.04	<LOQ						
D29	A	<LOQ	29.32	<LOQ	<LOQ	<LOQ	<LOQ	7.12	10.81	<LOQ
D29	B	<LOQ	28.24	14.05	<LOQ	<LOQ	<LOQ	17.50	<LOQ	<LOQ
D29	C	<LOQ	26.95	<LOQ						
D33	A	<LOQ	31.26	<LOQ						
D33	B	<LOQ	45.93	<LOQ						
D33	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D40	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D40	B	<LOQ	13.37	<LOQ						
D40	C	<LOQ	25.09	<LOQ	7.53	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D42	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	18.24	<LOQ

D42	B	<LOQ	36.38	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D42	C	<LOQ	20.65	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D46	A	<LOQ	40.69	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D46	B	<LOQ	44.66	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D46	C	<LOQ	33.73	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D47	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D47	B	<LOQ	7.88	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D47	C	<LOQ	12.35	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D48	A	<LOQ	11.39	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.87	<LOQ
D48	B	<LOQ	12.34	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D48	C	<LOQ	25.04	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D49	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	10.17	<LOQ
D49	B	<LOQ	19.66	324.49	<LOQ	489.03	<LOQ	592.6	<LOQ	588.78
D49	C	<LOQ	29.5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O50	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	13.48	<LOQ
O50	B	<LOQ	13.08	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.48	<LOQ
O50	C	<LOQ	16.42	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.48	<LOQ
O52	A	<LOQ	32.14	13.16	10.66	<LOQ	9.64	<LOQ	19.82	8.14
O52	B	<LOQ	30.18	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O52	C	<LOQ	14.28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O55	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11.28	<LOQ
O55	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O55	C	<LOQ	10.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O56	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	22.36	<LOQ	<LOQ
O56	B	<LOQ	16.92	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O56	C	<LOQ	34.09	<LOQ	12.9	<LOQ	<LOQ	<LOQ	7.82	<LOQ
O57	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	13.47	25.95	22.09
O57	B	<LOQ	28.18	15.04	16.07	<LOQ	14.81	10.64	32.03	18.26
O57	C	<LOQ	80.31	13.94	<LOQ	<LOQ	18.99	<LOQ	39.13	<LOQ
O59	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	10.79	<LOQ	<LOQ
O59	B	<LOQ	45.05	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	14.49	<LOQ
O59	C	<LOQ	19.71	<LOQ	<LOQ	<LOQ	8.92	<LOQ	21.82	<LOQ
O62	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11.09	<LOQ
O62	B	<LOQ	10.77	<LOQ	<LOQ	<LOQ	7.57	<LOQ	24.95	<LOQ
O62	C	<LOQ	18.68	<LOQ	<LOQ	<LOQ	15.2	<LOQ	37.59	<LOQ
O67	A	<LOQ	20.41	<LOQ	<LOQ	<LOQ	7.85	7.26	23.59	6.79
O67	B	<LOQ	22.25	<LOQ	<LOQ	<LOQ	6.82	<LOQ	13.05	<LOQ
O67	C	<LOQ	21.77	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.22	<LOQ
O70	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7.03	<LOQ
O70	C	<LOQ	18.34	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O71	A	<LOQ	51.38	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	10.95	<LOQ
O71	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O72	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	20.45	<LOQ
O72	B	<LOQ	41.03	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O72	C	<LOQ	9.28	<LOQ	<LOQ	<LOQ	11.95	<LOQ	37.62	<LOQ

O78	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O78	B	<LOQ	45.24	10.05	<LOQ	<LOQ	20.37	7.23	58.08	5.16
O78	C	<LOQ	13.27	6.44	6.9	<LOQ	23.22	8.05	91.24	5.08
O80	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11.91	7.45
O80	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12.23	<LOQ
O80	C	<LOQ	38.75	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11.99	<LOQ
R04	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11.38	<LOQ
R04	B	<LOQ	14.51	5.36	<LOQ	<LOQ	<LOQ	<LOQ	7.66	<LOQ
R04	C	<LOQ	14.9	<LOQ	10.29	<LOQ	<LOQ	<LOQ	5.88	<LOQ
R05	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	13.25	12.11	42.78	15.29
R05	B	<LOQ	26.48	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	13.38	<LOQ
R05	C	<LOQ	41.1	<LOQ	7.99	<LOQ	<LOQ	<LOQ	8.9	<LOQ
R08	A	<LOQ	30.29	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	16.48	<LOQ
R08	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12.98	17.61	7.97
R08	C	<LOQ	43.95	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	9.83	<LOQ
R09	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.59	6.44	10.2	<LOQ
R09	B	<LOQ	11.19	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.41	<LOQ
R09	C	<LOQ	24.39	<LOQ	12.34	<LOQ	<LOQ	<LOQ	4.04	<LOQ
R12	A	<LOQ	51.71	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12.41	<LOQ
R12	C	<LOQ	49.71	8.64	17.3	<LOQ	<LOQ	<LOQ	5.52	3.91
R13	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11.24	5.5
R13	B	<LOQ	15.65	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R13	C	<LOQ	25.58	<LOQ	15.64	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R14	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6.18	<LOQ
R14	B	<LOQ	15.16	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R14	C	<LOQ	19.33	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.16	<LOQ
R16	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	8.93	<LOQ
R16	B	<LOQ	14.51	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.3	<LOQ
R16	C	<LOQ	29.01	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R17	A	<LOQ	7.32	6.28	<LOQ	<LOQ	10.64	18.79	24.87	10.91
R17	B	<LOQ	<LOQ	7.47	<LOQ	<LOQ	5.89	6.03	12.95	4.9
R17	C	<LOQ	21.81	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.3	4.02
R19	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R19	B	<LOQ	20.53	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R19	C	<LOQ	23.39	<LOQ	9.67	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Core	Interval	n-C27	n-C28	n-C29	n-C30	n-C31	n-C32	n-C33	n-C34	n-C36
D02	A	37.08	29.95	252.26	39.54	76.74	<LOQ	<LOQ	<LOQ	<LOQ
D02	B	<LOQ	<LOQ	48.70	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D02	C	<LOQ	<LOQ	38.38	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D21	A	31.59	21.86	271.85	27.99	66.06	<LOQ	<LOQ	<LOQ	<LOQ
D21	B	<LOQ	18.87	198.50	<LOQ	40.66	<LOQ	<LOQ	<LOQ	<LOQ
D21	C	11.60	<LOQ	116.34	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D22	A	27.48	23.72	227.02	<LOQ	64.16	<LOQ	<LOQ	<LOQ	<LOQ

D22	B	28.60	25.51	280.39	34.81	77.66	<LOQ	<LOQ	<LOQ	<LOQ
D22	C	8.15	<LOQ	70.72	<LOQ	24.05	<LOQ	<LOQ	<LOQ	<LOQ
D23	A	17.04	<LOQ	149.47	<LOQ	26.84	<LOQ	<LOQ	<LOQ	<LOQ
D23	B	<LOQ	<LOQ	56.30	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D23	C	<LOQ	<LOQ	69.89	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D28	A	22.70	16.31	247.98	30.39	64.80	<LOQ	<LOQ	<LOQ	<LOQ
D28	B	21.15	<LOQ	127.56	<LOQ	28.56	<LOQ	<LOQ	<LOQ	<LOQ
D28	C	<LOQ	<LOQ	59.67	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D29	A	16.95	<LOQ	101.91	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D29	B	13.76	<LOQ	96.60	<LOQ	24.92	<LOQ	<LOQ	<LOQ	<LOQ
D29	C	14.09	<LOQ	86.55	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D33	A	<LOQ	<LOQ	103.48	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D33	B	<LOQ	<LOQ	117.02	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D33	C	<LOQ	<LOQ	25.16	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D40	A	<LOQ	<LOQ	146.39	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D40	B	<LOQ	<LOQ	52.99	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D40	C	<LOQ	<LOQ	42.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D42	A	36.36	<LOQ	143.67	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D42	B	13.39	<LOQ	103.42	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D42	C	<LOQ	<LOQ	76.16	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D46	A	<LOQ	<LOQ	124.81	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D46	B	<LOQ	<LOQ	99.23	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D46	C	<LOQ	<LOQ	91.42	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D47	A	4.85	<LOQ	67.62	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D47	B	10.11	<LOQ	95.9	<LOQ	19.01	<LOQ	<LOQ	<LOQ	<LOQ
D47	C	<LOQ	<LOQ	66.73	<LOQ	20.74	<LOQ	<LOQ	<LOQ	<LOQ
D48	A	13.57	<LOQ	86.33	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D48	B	14.06	<LOQ	118.07	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D48	C	13.33	8.66	152.53	15.13	48.05	<LOQ	<LOQ	<LOQ	<LOQ
D49	A	33.74	<LOQ	197.79	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D49	B	<LOQ	581.09	153.95	408.56	<LOQ	254.06	<LOQ	185.53	<LOQ
D49	C	<LOQ	<LOQ	94.51	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O50	A	24.38	12.4	211.24	<LOQ	53.11	<LOQ	<LOQ	<LOQ	<LOQ
O50	B	7.47	<LOQ	47.85	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O50	C	11.59	<LOQ	108.85	<LOQ	32.83	<LOQ	<LOQ	<LOQ	<LOQ
O52	A	60.86	27.85	353.05	30.87	119.83	<LOQ	<LOQ	<LOQ	<LOQ
O52	B	19.25	9.9	144.36	<LOQ	53.07	<LOQ	<LOQ	<LOQ	<LOQ
O52	C	9.5	<LOQ	72.53	<LOQ	28.74	<LOQ	<LOQ	<LOQ	<LOQ
O55	A	<LOQ	<LOQ	237.81	<LOQ	47.7	<LOQ	<LOQ	<LOQ	<LOQ
O55	B	12.07	<LOQ	122.48	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O55	C	<LOQ	<LOQ	51.67	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O56	A	36.61	23.56	386.52	38.52	101.86	<LOQ	<LOQ	<LOQ	<LOQ
O56	B	29.23	13.93	275.13	<LOQ	114.51	<LOQ	<LOQ	<LOQ	<LOQ
O56	C	20.51	<LOQ	183.39	<LOQ	42.14	<LOQ	<LOQ	<LOQ	<LOQ
O57	A	60.47	55.86	353.7	55.47	147.11	<LOQ	<LOQ	<LOQ	<LOQ

O57	B	68.6	53.96	501.9	79.24	203.32	<LOQ	<LOQ	<LOQ	<LOQ
O57	C	76.48	28.31	371.83	46.98	123.5	<LOQ	<LOQ	<LOQ	<LOQ
O59	A	35.71	22.94	330.38	26.39	57.32	<LOQ	<LOQ	<LOQ	<LOQ
O59	B	35.65	17.57	293.26	<LOQ	64.76	<LOQ	<LOQ	<LOQ	<LOQ
O59	C	34.95	<LOQ	107.59	<LOQ	21.98	<LOQ	<LOQ	<LOQ	<LOQ
O62	A	31.74	20.04	267.32	28.56	109.54	<LOQ	<LOQ	<LOQ	<LOQ
O62	B	33.76	<LOQ	94.64	<LOQ	27.05	<LOQ	<LOQ	<LOQ	<LOQ
O62	C	34.62	<LOQ	126.14	<LOQ	37.92	<LOQ	<LOQ	<LOQ	<LOQ
O67	A	78.43	30.04	489.25	39.59	150.65	<LOQ	<LOQ	<LOQ	<LOQ
O67	B	16.67	<LOQ	125.13	<LOQ	37.76	<LOQ	<LOQ	<LOQ	<LOQ
O67	C	15.19	<LOQ	152.19	<LOQ	46.1	<LOQ	<LOQ	<LOQ	<LOQ
O70	A	23.25	16.86	182.67	18.54	43.99	<LOQ	<LOQ	<LOQ	<LOQ
O70	C	17.48	7.96	188.48	<LOQ	51.14	<LOQ	<LOQ	<LOQ	<LOQ
O71	A	32.46	14.07	194.26	<LOQ	61.96	<LOQ	<LOQ	<LOQ	<LOQ
O71	B	8.34	<LOQ	72.13	<LOQ	19.71	<LOQ	<LOQ	<LOQ	<LOQ
O72	A	66.88	48.21	481.96	66.18	146.41	<LOQ	<LOQ	<LOQ	<LOQ
O72	B	33.83	24.18	242.69	33.43	92.1	<LOQ	<LOQ	<LOQ	<LOQ
O72	C	55.35	14.92	238.36	21.47	146.61	<LOQ	42.23	<LOQ	<LOQ
O78	A	10.38	<LOQ	105.66	11.48	46.39	<LOQ	<LOQ	<LOQ	<LOQ
O78	B	70.82	10.2	174.23	13.93	53.37	<LOQ	<LOQ	<LOQ	<LOQ
O78	C	86.76	8.7	184.69	13.42	56.15	<LOQ	<LOQ	<LOQ	<LOQ
O80	A	41.95	28.49	391.65	34.67	114.2	<LOQ	<LOQ	<LOQ	<LOQ
O80	B	39.36	23.71	449.9	45.67	129.13	<LOQ	<LOQ	<LOQ	<LOQ
O80	C	30.16	10.86	217.08	<LOQ	66.03	<LOQ	<LOQ	<LOQ	<LOQ
R04	A	32.6	21.73	187.74	27.13	65.99	<LOQ	<LOQ	<LOQ	<LOQ
R04	B	26.82	21.08	261.15	32.86	95.32	<LOQ	<LOQ	<LOQ	<LOQ
R04	C	22.87	21.3	252.46	38.56	91.17	<LOQ	<LOQ	<LOQ	<LOQ
R05	A	75.26	48.89	475.08	60.51	156.97	<LOQ	<LOQ	<LOQ	<LOQ
R05	B	33.33	24.44	260.42	28.48	100.39	<LOQ	<LOQ	<LOQ	<LOQ
R05	C	32.88	22.78	276.66	29.36	90.21	<LOQ	<LOQ	<LOQ	<LOQ
R08	A	32.77	18.94	236.27	20.81	67.79	<LOQ	<LOQ	<LOQ	<LOQ
R08	B	50.73	35.49	436.92	42.48	84.79	<LOQ	<LOQ	<LOQ	<LOQ
R08	C	30.47	24.5	287.32	30.54	96.86	<LOQ	<LOQ	<LOQ	<LOQ
R09	A	33.91	18.06	256.05	25.63	64.05	<LOQ	<LOQ	<LOQ	<LOQ
R09	B	22.25	14.19	191.68	20.28	60.33	<LOQ	<LOQ	<LOQ	<LOQ
R09	C	14.86	11.39	141.09	12.03	36.3	<LOQ	<LOQ	<LOQ	<LOQ
R12	A	29.22	16.61	195.28	20.56	62.58	<LOQ	<LOQ	<LOQ	<LOQ
R12	C	26.44	19.39	317.56	34.77	82.6	<LOQ	<LOQ	<LOQ	<LOQ
R13	A	34.63	22.06	293.56	32.31	104.92	<LOQ	<LOQ	<LOQ	<LOQ
R13	B	21.64	16.55	249.85	25.43	90.89	<LOQ	<LOQ	<LOQ	<LOQ
R13	C	19.52	16.34	267.31	28.64	93.64	<LOQ	<LOQ	<LOQ	<LOQ
R14	A	15.37	8.5	137.54	<LOQ	42.48	<LOQ	<LOQ	<LOQ	<LOQ
R14	B	10.96	8.35	129.01	<LOQ	28.83	<LOQ	<LOQ	<LOQ	<LOQ
R14	C	19.26	17.82	285.55	30.41	68.2	<LOQ	<LOQ	<LOQ	<LOQ
R16	A	21.25	7.98	150.08	<LOQ	30.47	<LOQ	<LOQ	<LOQ	<LOQ

R16	B	17.93	9.98	145.04	<LOQ	32.58	<LOQ	<LOQ	<LOQ	<LOQ
R16	C	10.82	6.55	122.66	<LOQ	22.1	<LOQ	<LOQ	<LOQ	<LOQ
R17	A	71.42	32.51	488.52	49.01	90.23	<LOQ	<LOQ	<LOQ	<LOQ
R17	B	33.97	12.06	267.61	21.28	57.02	<LOQ	<LOQ	<LOQ	<LOQ
R17	C	12.68	8.24	116.83	<LOQ	21.12	<LOQ	<LOQ	<LOQ	<LOQ
R19	A	9.91	7.64	111.11	<LOQ	33.59	<LOQ	<LOQ	<LOQ	<LOQ
R19	B	10.58	<LOQ	119.01	<LOQ	31.13	<LOQ	<LOQ	<LOQ	<LOQ
R19	C	10.27	6.62	131.1	8.34	27.48	<LOQ	<LOQ	<LOQ	<LOQ

Appendix D: PAH Results

Core	Interval	C1-	C2-	C3-	C4-	Acenap			C1-	C2-	C3-	C4-			
		Naphtalene	Naphtalenes	Naphtalenes	Naphtalenes	Biphenyl	hthylene	Acenaphthene	Fluorenes	Fluorenes	Fluorenes	Phenanthrene	dibenzothiophenes		
D02	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
D02	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
D02	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
D21	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
D21	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	10.96	<LOQ	
D21	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
D22	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.66	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.39	<LOQ	
D22	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.99	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.05	<LOQ	
D22	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.79	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.85	<LOQ	
D23	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
D23	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
D23	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.16	<LOQ	<LOQ	<LOQ	<LOQ	
D28	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	14.23	<LOQ	
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D29	A	<LOQ	<LOQ	<LOQ	<LOQ	4.27	<LOQ	<LOQ	<LOQ	1.25	<LOQ	<LOQ	<LOQ	12.5	<LOQ
D29	B	2.62	3.38	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.22	<LOQ
D29	C	<LOQ	2.1	<LOQ	<LOQ	<LOQ	1.09	<LOQ	<LOQ	1.32	<LOQ	<LOQ	<LOQ	11.61	<LOQ
D33	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D33	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.77	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D33	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D40	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D40	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D40	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	13.18	0.42	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
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D46	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.36	<LOQ
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D47	B	<LOQ	31.67	<LOQ	<LOQ	<LOQ	0.33	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D47	C	<LOQ	17.96	<LOQ	<LOQ	<LOQ	0.46	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D48	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.61	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.15	<LOQ
D48	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.51	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.18	<LOQ
D48	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.28	<LOQ
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D49	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.07	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O50	A	<LOQ	43.92	<LOQ	<LOQ	<LOQ	0.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.84	<LOQ

050	B	<LOQ	46.13	<LOQ	<LOQ	<LOQ	0.29	<LOQ							
050	C	<LOQ	50.33	<LOQ	<LOQ	<LOQ	0.37	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.8	<LOQ
052	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.51	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.18	<LOQ
052	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.75	2.35	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.44	<LOQ
052	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.24	<LOQ
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055	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
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062	C	<LOQ	3.87	<LOQ	<LOQ	<LOQ	0.44	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.73	<LOQ
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072	A	<LOQ	60	<LOQ	1.17	<LOQ									
072	B	<LOQ	17.44	<LOQ	3.33	<LOQ									
072	C	<LOQ	9.91	<LOQ	0.98	<LOQ									
078	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.78	<LOQ
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078	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.34	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.21	<LOQ
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080	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.15	<LOQ
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R04	B	<LOQ	38.37	<LOQ	<LOQ	<LOQ	0.79	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.81	<LOQ
R04	C	<LOQ	6.72	<LOQ	<LOQ	<LOQ	0.92	<LOQ	<LOQ						
R05	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R05	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.79	<LOQ
R05	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R08	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.96	<LOQ
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R08	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7.58	<LOQ

R09	A	<LOQ	15.24	<LOQ	<LOQ	<LOQ	0.36	<LOQ						
R09	B	0.43	39.02	<LOQ	<LOQ	<LOQ	1.12	<LOQ						
R09	C	<LOQ	39.61	<LOQ	<LOQ	<LOQ	0.69	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.62	<LOQ
R12	A	1.61	3.29	<LOQ	1.38	<LOQ	1.56	<LOQ	<LOQ	1.46	<LOQ	<LOQ	9.62	<LOQ
R12	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.31	<LOQ						
R13	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.83	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.36	<LOQ
R13	B	0.39	<LOQ	<LOQ	<LOQ	<LOQ	0.8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7.04	<LOQ
R13	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.03	<LOQ
R14	A	<LOQ	27.58	<LOQ	<LOQ	<LOQ	0.8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.68	<LOQ
R14	B	<LOQ	3.11	<LOQ	<LOQ	<LOQ	0.47	<LOQ						
R14	C	<LOQ	31.53	<LOQ	<LOQ	<LOQ	0.69	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.89	<LOQ
R16	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R16	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R16	C	<LOQ	0.58	<LOQ										
R17	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R17	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R17	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.16	<LOQ						
R19	A	<LOQ	29.92	<LOQ	<LOQ	<LOQ	0.73	<LOQ						
R19	B	<LOQ	29.59	<LOQ	<LOQ	<LOQ	0.35	<LOQ						
R19	C	<LOQ	33.2	<LOQ	<LOQ	<LOQ	0.38	<LOQ						

Core	Interval	C1- C2- C3- C4- fluorant fluorant fluorant fluorant henes/p hen/p hen/p hen/p				Benzo[a]	C1- chrysen	C2- chrysen	C3- chrysen	C4- chrysen	Benzo[b]	Benzo[k]
		Pyrene	yrenes	yrenes	yrenes	yrenes						
D02	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D02	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D02	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D21	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D21	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D21	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D22	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D22	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D22	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D23	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D23	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D23	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D28	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D28	B	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D28	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
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D33	C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

O56 A	<LOQ						
O56 B	1.16	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O56 C	<LOQ						
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O57 B	<LOQ						
O57 C	7.9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O59 A	<LOQ						
O59 B	1.41	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O59 C	<LOQ						
O62 A		2.31	1.77	<LOQ	<LOQ	<LOQ	<LOQ
O62 B	<LOQ						
O62 C	<LOQ						
O67 A	0.64	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O67 B	<LOQ						
O67 C	<LOQ						
O70 A	<LOQ						
O70 C		3.89	1.86	<LOQ	<LOQ	<LOQ	<LOQ
O71 A	<LOQ						
O71 B	<LOQ						
O72 A	1.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O72 B	<LOQ						
O72 C	<LOQ						
O78 A		3.46	3.01	3.39	<LOQ	<LOQ	<LOQ
O78 B		2.71	2.31	2.41	<LOQ	<LOQ	<LOQ
O78 C	0.69	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
O80 A	<LOQ						
O80 B	<LOQ						
O80 C	<LOQ						
R04 A	<LOQ						
R04 B	<LOQ						
R04 C	<LOQ						
R05 A	<LOQ						
R05 B	<LOQ						
R05 C	<LOQ						
R08 A	<LOQ						
R08 B	<LOQ						
R08 C	<LOQ						
R09 A	<LOQ						
R09 B	<LOQ						
R09 C	<LOQ						
R12 A	<LOQ						
R12 C	<LOQ						
R13 A	0.75	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
R13 B	<LOQ						
R13 C	<LOQ						

4.25

2.19

R14 A	<LOQ						
R14 B	<LOQ						
R14 C	<LOQ						
R16 A	<LOQ						
R16 B	<LOQ						
R16 C	<LOQ						
R17 A	<LOQ						
R17 B	<LOQ						
R17 C	<LOQ						
R19 A	<LOQ						
R19 B	<LOQ						
R19 C	<LOQ						

8 Abbreviations and Acronyms

CEDRE	Centre of Documentation, Research and Experimentation on Accidental Water Pollution
UAS	Uncrewed Aerial System
BSEE	Bureau of Safety and Environmental Enforcement
NOAA	National Oceanographic and Atmospheric Administration
EPA	Environmental Protection Agency
USCG	United States Coast Guard
ICCOPR	Interagency Coordinating Committee on Oil Pollution Research
NSU	Nova Southeastern University
NEBA	Net Environmental Benefit Analysis
CCA	Clean Caribbean and Americas
CRA	Comparative Risk Analysis
TROPICS	TRopical Oil Pollution Investigations in Coastal Systems
SIMA	Spill Impact Mitigation Analysis
IPIECA	International Petroleum Industry Environmental Conservation Association
FOSC	Federal On-Scene Coordinator
EU	Environmental Unit
ICS	Incident Command System
NIIMS	National Interagency Incident Management System
JIP	Joint Industry project
OCS	Outer Continental shelf
STRI	Smithsonian Tropical Research Institute
FRPs	Facility Response Plans
API	American Petroleum Institute
OSRL	Oil Spill Response Limited
DBH	Diameter breast height
LAI	Leaf area index
NADIR	Straight look down camera angle
PVC	Polyvinyl chloride
PAH	Polycyclic aromatic hydrocarbons
RGB	Red-green-blue (visual mode)
TLE	Total lipid extract
GC-FID	gas chromatography with flame ionization detection
GC-MS	gas chromatography–mass spectrometry
DCM	dichloromethane
SCTLD	Stony Coral Tissue Loss Disease
GERG	Geochemical and Environmental Research Group



Department of the Interior (DOI)

The Department of the Interior protects and manages the Nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors the Nation's trust responsibilities or special commitments to American Indians, Alaska Natives, and affiliated island communities.



Bureau of Safety and Environmental Enforcement (BSEE)

The mission of the Bureau of Safety and Environmental Enforcement works to promote safety, protect the environment, and conserve resources offshore through vigorous regulatory oversight and enforcement.

BSEE Oil Spill Preparedness Program

BSEE administers a robust Oil Spill Preparedness Program through its Oil Spill Preparedness Division (OSPD) to ensure owners and operators of offshore facilities are ready to mitigate and respond to substantial threats of actual oil spills that may result from their activities. The Program draws its mandate and purpose from the Federal Water Pollution Control Act of October 18, 1972, as amended, and the Oil Pollution Act of 1990 (October 18, 1991). It is framed by the regulations in 30 CFR Part 254 – *Oil Spill Response Requirements for Facilities Located Seaward of the Coastline*, and 40 CFR Part 300 – *National Oil and Hazardous Substances Pollution Contingency Plan*. Acknowledging these authorities and their associated responsibilities, BSEE established the program with three primary and interdependent roles:

- Preparedness Verification,
- Oil Spill Response Research, and
- Management of Ohmsett - the National Oil Spill Response Research and Renewable Energy Test Facility.

The research conducted for this Program aims to improve oil spill response and preparedness by advancing the state of the science and the technologies needed for these emergencies. The research supports the Bureau's needs while ensuring the highest level of scientific integrity by adhering to BSEE's peer review protocols. The proposal, selection, research, review, collaboration, production, and dissemination of OSPD's technical reports and studies follows the appropriate requirements and guidance such as the Federal Acquisition Regulation and the Department of Interior's policies on scientific and scholarly conduct.