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TITLE

TROPICS FIELD STUDY (PANAMA), 32-YEAR SITE VISIT: OBSERVATIONS AND CONCLUSIONS FOR NEAR SHORE DISPERSANT USE NEBA AND TRADEOFFS

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ABSTRACT (#2017-141)

The Tropical Oil Pollution Investigations in Coastal Systems (TROPICS) experiment initiated in 1984 on the Caribbean coast of Panama has become one of the most comprehensive field experiments examining effects of oil exposure to a combination of tropical marine reef, seagrass, and mangrove communities. The experimental dosage was chosen to simulate a severe but realistic spill scenario so that results could be useful in decisions about the extent to which dispersants reduced or exacerbated the effects of an oil spill on tropical environments of mangroves, seagrasses, and corals. Research has been conducted in the area prior to and 30+

years following exposure to evaluate long term effects. In July 2016, an international research team revisited the TROPICS field sites. In previous data collection visits, visual observations and core samples of the mangrove substrate at the non-treated (Oil only) site revealed the presence of oil. This "trapped" oil also apparently resulted in lower recovery rates for mangroves in that site. Of particular interest in the 2016 revisit was to determine the presence/non-presence of oil in core samples via new petroleum biomarker triple quadrupole mass spectrometry technology. Additionally, data collection and observations of the extent, diversity, and health of the shallow coral reefs, seagrass, and mangroves were conducted at the three sites. The focus was on the initial disruption and recovery of the study ecosystem over 32 years from the original dosing with crude oil or dispersed crude oil. Analysis qualitatively compared the 2016 results to 1984 pre-spill and post-spill conditions of each site. This paper discusses the results of the 2016 TROPICS study site revisit and conclusions for oil spill preparedness and response, particularly as it applies to the trade-offs for the use of dispersants in near shore tropical marine ecosystems.

INTRODUCTION

In November 1984, a research team conducted an experiment which involved exposing two 30m X 30m tropical nearshore marine sites to crude oil and crude oil pre-mixed with Corexit 9527 dispersant (approximately 6 barrels per site over 24 and 48 hours). A nearby reference (non-treated) site was included in the observations. These three sites were representative of the tropical nearshore marine ecosystem of coral, seagrass, and mangrove habitats and associated fauna in Bahia de Almirante, Panama. This **TR**opical **O**il **P**ollution **I**nvestigations in **C**oastal **S**ystems (TROPICS) study was designed to evaluate the relative short-term and long-term impacts of undispersed crude oil (Oil only site) and dispersed crude oil (Dispersed oil site) compared to a third non-treated site (Reference site) (Fig. 1). After initial dosing in 1984, observations and analyses were conducted at 30 days, 3 months and other periods over 24 months, with 8 subsequent visits in 29 years, including a comprehensive study after 10 years (Dodge et al, 1995). These studies became one of the seminal works for the concept of Net Environmental Benefit Analysis (NEBA).

In July 2016, an international research team, including two of the original 1984 investigators, revisited the TROPICS field sites in order to examine the long-term impacts and recovery of the study ecosystem 32 years after the original experiment.

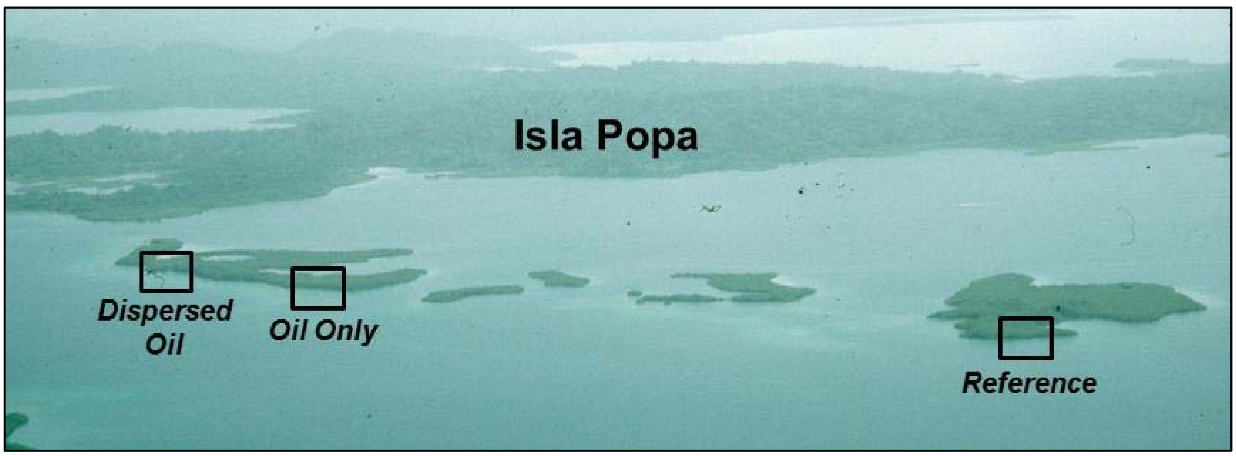

Figure 1. Oblique aerial view showing the relative location of experimental sites.

PRIOR RESULTS

Mangroves. Before and following exposure, mangrove leaf area, tree density, and propagule/seedling density were assessed at each site. Exposure effects were severe at the Oil only site, including increased mortality and defoliation compared to the Dispersed oil site. Defoliation at the Oil only site was observed in about 25% of the mangrove area by 4 months post-spill (Ballou et al. 1989). This continued to increase 7-, 12-, and 20- months after the spill to 45% defoliation of each tree in the Oil only site. Mortality immediately following exposure occurred in 18 trees, and increased to 25 trees (a 17% reduction) by June 1985. At 10 years post-exposure, there was a 9.1% reduction in total basal area of mangrove trees at the Oil only site, due to mortality (number of trees reduced by 46%, from 149 to 80 trees) (Dodge et al. 1995). There were no measurable long-term effects on mangroves at the Dispersed oil site.

Seagrass. Immediately following exposure, seagrass density declined at the Dispersed oil site but returned to pre-spill levels after 7 months. Seagrass density also declined at the Oil only site after 7 months, but returned to pre-treatment levels after 20 months. No significant effects on growth rate or blade area were found at either treatment site in 1985 (Ballou et al. 1987, Ballou et al. 1989). By 1994, after ten years of recovery, effects on seagrass components at both treatment sites included reduced plant densities. The initial short-term effects of reduced seagrass density at the Oil only site had diminished to non-significant levels (Dodge et al. 1995). Ward et al. (2003) revisited the study location in 2001/2002 and found that localized increases in seagrass leaf blade area and decreases in seagrass density had occurred. This was attributed to either competitive pressures or nutrient enhancement. Seagrass growth rate and blade area at the Dispersed oil site were similar to the 1994 Reference site in 2001 (Ward et al. 2003). Sea urchin populations were significantly negatively impacted immediately following exposure to both oil and dispersed oil treatments, but recovered to Reference site levels after 1 year (Ballou et al. 1987, Ballou et al. 1989).

Coral. Immediately following exposure, percent coral cover in the Dispersed oil site declined abruptly and this continued for a year (Ballou et al. 1987). Growth of *Porites porites* and *Agaricia tenuifolia* (which dominated the reef community) was significantly reduced at the Dispersed oil site. Corals at the Oil only site showed non-significant reduction in cover and growth of all species examined (Ballou et al. 1987, Ballou et al. 1989). Coral cover remained significantly lower at the Dispersed oil site for at least two years following exposure (Dodge et al. 1995), in contrast to non-significant changes in growth and coral cover at the Oil only site. By 10 years after the exposure in 1994, coral parameters at all sites were statistically indistinguishable with no significant differences in cover or growth between the Oil only, Dispersed oil, and Reference sites.

METHODS

The investigation in July 2016 examined the same site layout as in the previous experiment. Each site was a 30m x 30m square and included mangroves, shallow seagrass beds, and coral reefs. Water depth for the seagrass beds was less than 1 m; the coral reefs crested in 1 m and went to depths of 10 m.

Mangroves. After the perimeter of the site was identified, the mangrove area was divided into 9 equal sections. Each section was independently assessed by three two-person teams. Mature tree density was assessed via counts. Prop root density, canopy cover, and sapling to mature tree ratio were assessed using a semi-quantitative scoring metric. For prop root density and canopy cover, scores were assigned such that 1=none/thin, 2=medium and 3=dense/thick. For sapling to mature tree ratio, scores were assigned such that 1= majority sapling, 2=similar and 3=majority mature trees.

Seagrass. Four 0.25 m^2 PVC quadrats were haphazardly placed in the seagrass area at each site. Seagrass blade density and sea urchin density were determined by counts in each quadrat.

Reef. Reef organisms were assessed by percent occurrence of substrate or organism in 4 transects of 10 m at each site. Two transect lines were laid on and parallel to the reef crest in less than 1 m of water, and 2 were laid parallel to the first set seaward of the reef crest in l-2 m of water. Data was collected by divers swimming over the transect line and recording the identity of the substrate (organism type or bare) at 10 cm intervals. Organisms were categorized as hard coral (to species), soft coral, plant, or other.

Substrate chemical analyses. At each site, nine 10 cm x 24 cm Plexiglas core barrels were used for sampling, similar to past sampling periods. The substrate in the Oil only site was visibly more eroded, devoid of sediment, and largely composed of mangrove roots compared to other sites. The Oil only site mangroves still appeared significantly denuded compared to the trees at the Dispersed oil and Reference sites. The cores were taken by two persons pushing the core barrel into the substrate to obtain material. The longest core extracted was 18 cm, and most were between 12 and 18 cm. The cores were capped, placed in coolers, refrigerated in the laboratory, and shipped by air to Texas A&M University.

Polycyclic aromatic hydrocarbons (PAHs) and aliphatic hydrocarbon (ALHs) were measured in sediment samples based on established methods (Denoux, Gardinali, and Wade 1998, Qian, Sericano, and Wade 1998). In summary, macro-organic matter (e.g. roots, leaves) were manually separated from sediment and sediment samples were oven dried at 40 ºC. Homogenized samples were then mixed with anhydrous sodium sulfate and copper beads. Surrogate standards (e.g., d_8-naphthalene, d_{10}-acenaphthene, d_{10}-phenanthrene, d_{12}-chrysene, and d_{12}-perylene and d_{26}-n-C_{12}, d_{42}- n-C_{20}, d_{50} n-C_{24}, and d_{62} n-C_{30} for PAHs and ALHs, respectively) were added before the extraction. Extraction was done with methylene chloride using an automated solvent extractor (ASE). Then extracts were treated with acid washed copper beads to get rid of any sulfur. Any interfering compounds were removed by passing the extracts through alumina/silica gel. The volume of extracts was then reduced to (0.1-1 mL) using a hot water bath. Appropriate amounts of deuterated compounds as internal standards (e.g., d_{10}-Fluorene and d_{12}-Benzo(a)pyrene and d_{54}-n-C_{26} for PAHs and ALHs, respectively) were spiked before analysis. PAHs were quantitatively analyzed by gas chromatography with mass spectrometric detector (Agilent 6890N GC/5975C inert MSD) in the selected ion mode (SIM) using a 30 m x 0.25 mm i.d. (0.25 µm film thickness) DB-5 fused silica capillary column (J&W Scientific, Inc.). Alkanes were analyzed by GC-FID (Agilent) and data were processed through chrome-perfect software. The GC/MS and GC-FID both were calibrated by the injection of standards at five different concentrations. Analyte identification (for PAHs) was based on the retention time of the quantitation ion for each compound and a series of confirmation ions. Petroleum biomarker analysis was performed with an Agilent 7010 triple quadrupole GC Mass Spectrometer.

Statistical analyses. All data were tested for normality (Brown-Forsythe) and homoscedasticity (Komolgorov-Smirnov/Lilliefors) and transformed to meet these assumptions where applicable. Nonparametric methods were used if necessary. Tukey's Unequal N HSD (parametric) or Multiple Comparisons (nonparametric) was used for post-hoc analysis. All statistical tests were performed using STATISTICA 12.

Kruskal Wallis ANOVA on ranks (α=0.05) with untransformed data was used to compare mean mangrove prop root density, mangrove canopy cover, mangrove sapling to mature tree ratio, and mean reef transect % occurrence of substrate or organism between treatment sites. One-way ANOVA (α=0.05) on untransformed data was used to compare mean mangrove mature tree density, mean seagrass plant density, and mean sea urchin density between sites.

RESULTS AND DISCUSSION

The typical appearance of the mangroves, seagrass, and coral reef habitats for the Oil only site and the Dispersed oil sites is shown in Fig. 2.

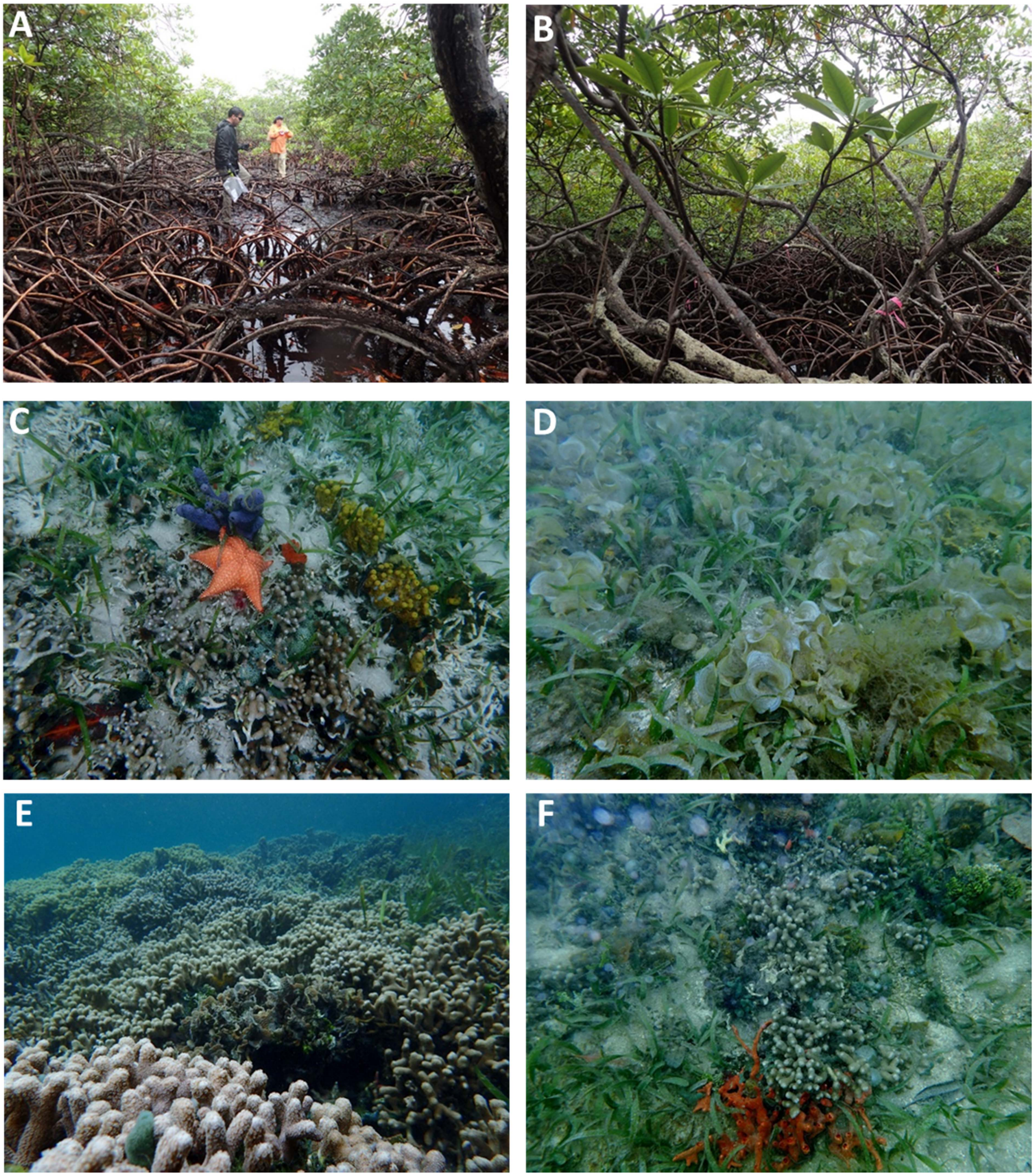

Figure 2. Typical appearance of mangrove, seagrass, and coral reef habitats at Oil only (first column) and Dispersed oil sites (second column); A) mangroves at Oil only site, B) mangroves at Dispersed oil site, C) seagrass at Oil only site, D) seagrass at Dispersed oil site, E) coral reef at Oil only site, and F) coral reef at Dispersed oil site.

Mangroves. No significant differences between sites were found for mature tree density or sapling to mature tree ratio score ($p>0.05$) (Fig. 3A and Fig. 3B). Significant differences between sites were found for prop root density (p=0.04) (Fig. 3C) and canopy cover (p=0.04) (Fig. 3D). Post-hoc analysis indicated that prop root density was significantly higher at the Dispersed oil site than at the Oil only site (p=0.04), and canopy cover was significantly higher at the Dispersed oil site than at the Oil only site (p=0.03).

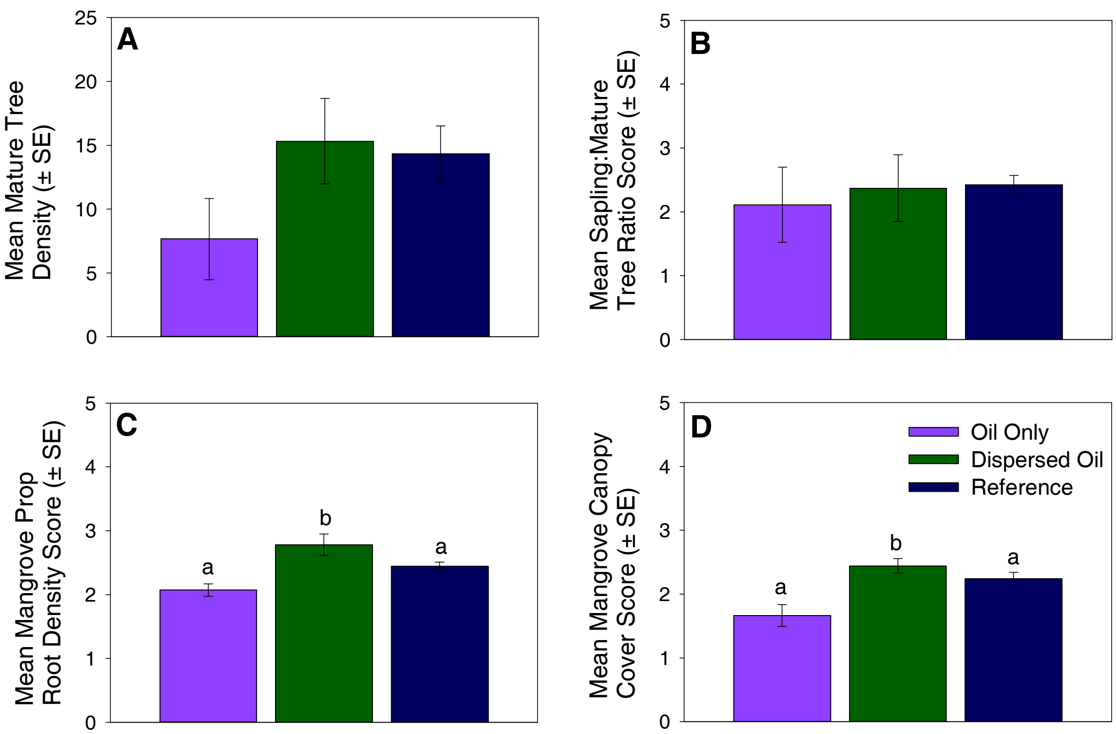

Figure 3. A) Mean mangrove mature tree density, B) mean mangrove sapling:mature tree ratio score, C) mean mangrove prop root density score, and D) mean mangrove canopy cover score between treatment sites (± SE). Significant differences between sites are denoted by different letters above each column.

Seagrass. Significant differences between sites were found for seagrass blade density (p=0.02) (Fig. 4A). Post-hoc analysis indicated that the Oil only site had significantly lower mean blade density (362 blades m^2) compared to the Dispersed oil (865 blades m^2) (p=0.05) or Reference sites (908 blades m^2) (p=0.03). No significant difference in urchin density (Fig. 4B) was found between sites (p=0.87).

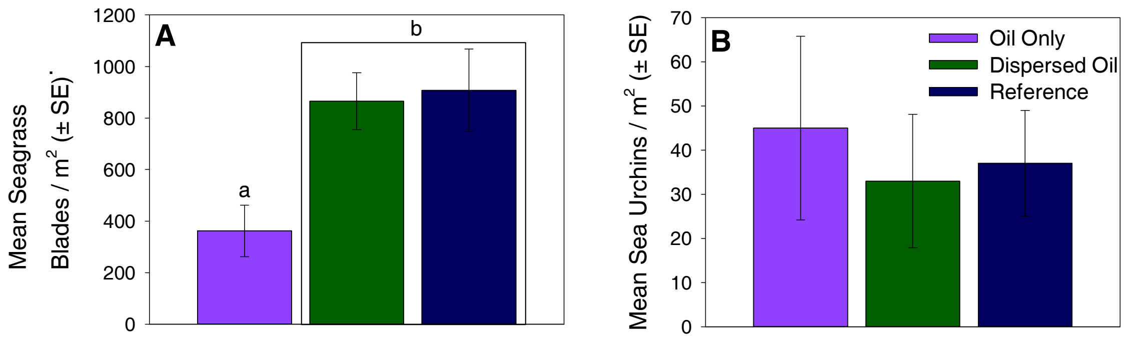

Figure 4. A) Mean seagrass blade density and B) mean sea urchin density at treatment sites (± SE). Significant differences between sites are denoted by different letters above each column.

Reef. No significant difference in percent occurrence of bare substrate (p=0.10), hard coral (p=0.12), plants (p=0.18), soft corals (p=0.36), or other organisms like sponges (p=0.10) was found between sites (Fig. 5) for transects on the reef crest or seaward of the reef crest.

Hydrocarbon and biomarker. Although PAH's were detected, there was no indication of the original oil or degradation products of that oil. Some PAH and alkane pattern (Fig. 6 and Fig. 7) were indicative of small, more recent oilings, most probably from the large amount of small boat traffic. Some of the PAH signal was due primarily to air contaminants and not petroleum. The petroleum biomarker (Fig. 8) analysis was not indicative of any crude oil input.

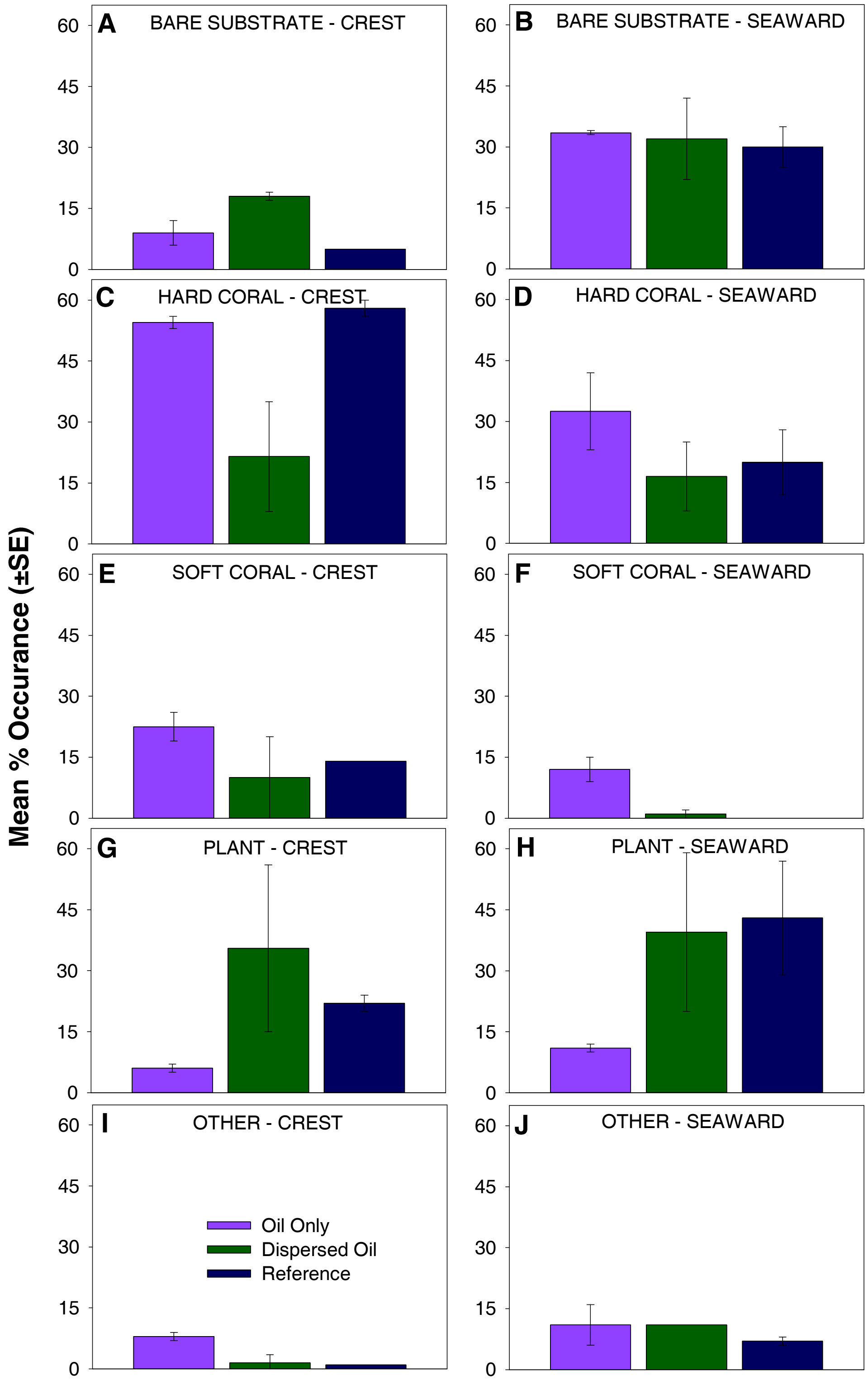

Figure 5. Mean (±SE) percent occurrence between sites of A) bare substrate on reef crest transects, B) bare substrate on seaward transects, C) hard coral on reef crest transects, D) hard coral on seaward transects, E) soft corals on reef crest transects, F) soft corals on seaward transects, G) plants on reef crest transects, H) plants on seaward transects, I) other organisms on reef crest transects, and J) other organisms on seaward transects.

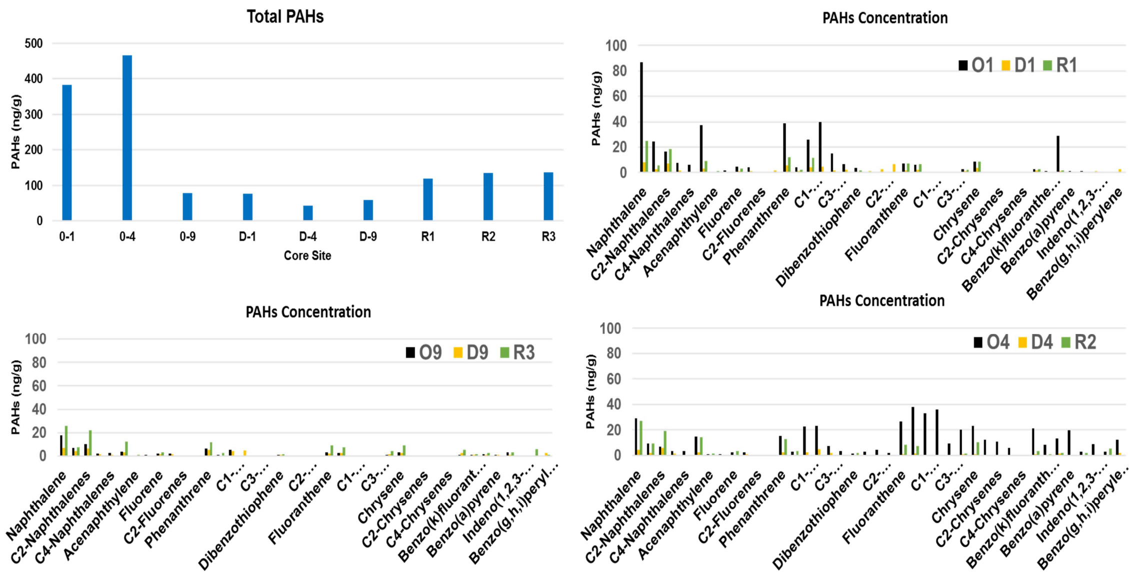

Figure 6. Total PAHs (ng/g) concentration in all the cores (left top corner) and comparison of PAHs concentrations at Oil only (O), Dispersed oil (D), and Reference (R) sites.

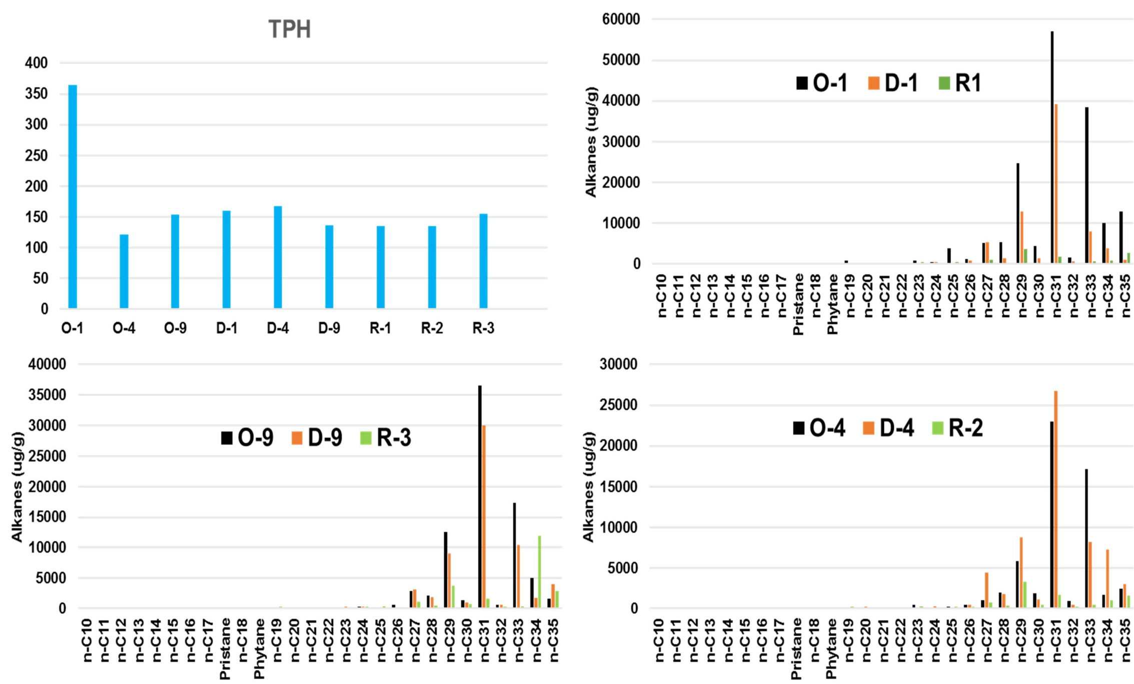

Figure 7. Total petroleum hydrocarbon (TPH) concentration (µg/g) in all the cores (left top corner) and comparison of alkanes concentration at Oil only (O), Dispersed oil (D), and Reference (R) sites.

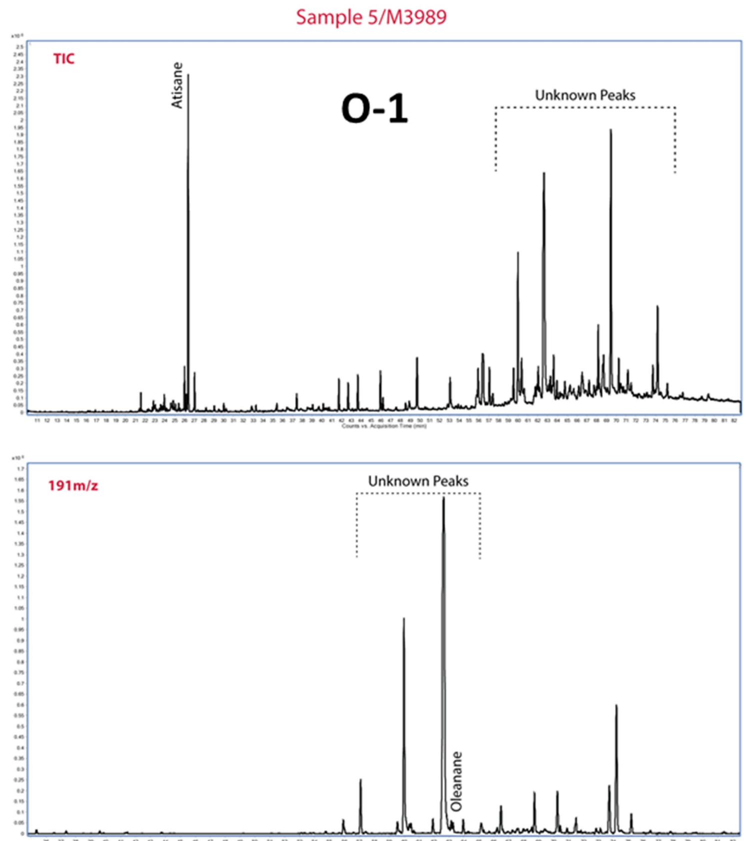

Figure 8. Biomarker chromatogram of sediment core O-1 where one of highest total PAHs concentration was found. Chromatogram indicates no presence of petrogenic biomarkers.

CONCLUSIONS

Thirty-two years after a significant oil release in a tropical marine near-shore ecosystem, seagrass density, mangrove canopy cover, and mangrove root density were found to be lower at the Oil only site compared to the Dispersed oil or Reference sites. No significant differences in occurrence of reef organisms between sites was found.

While there were some differences in methodology used in previous evaluations, qualitative comparison of the present results with those of the past can be made. Lower mangrove canopy cover and tree density was observed at the Oil only site as opposed to the Dispersed oil site, which is consistent with previous visits. Dodge et al. (1995) compared mean coverage of all corals, all animals, all plants, and all organisms, normalized by subtraction to

November 1984 pretreatment levels. The 32 year mean coverages were also normalized and the combined data are presented in Fig. 9.

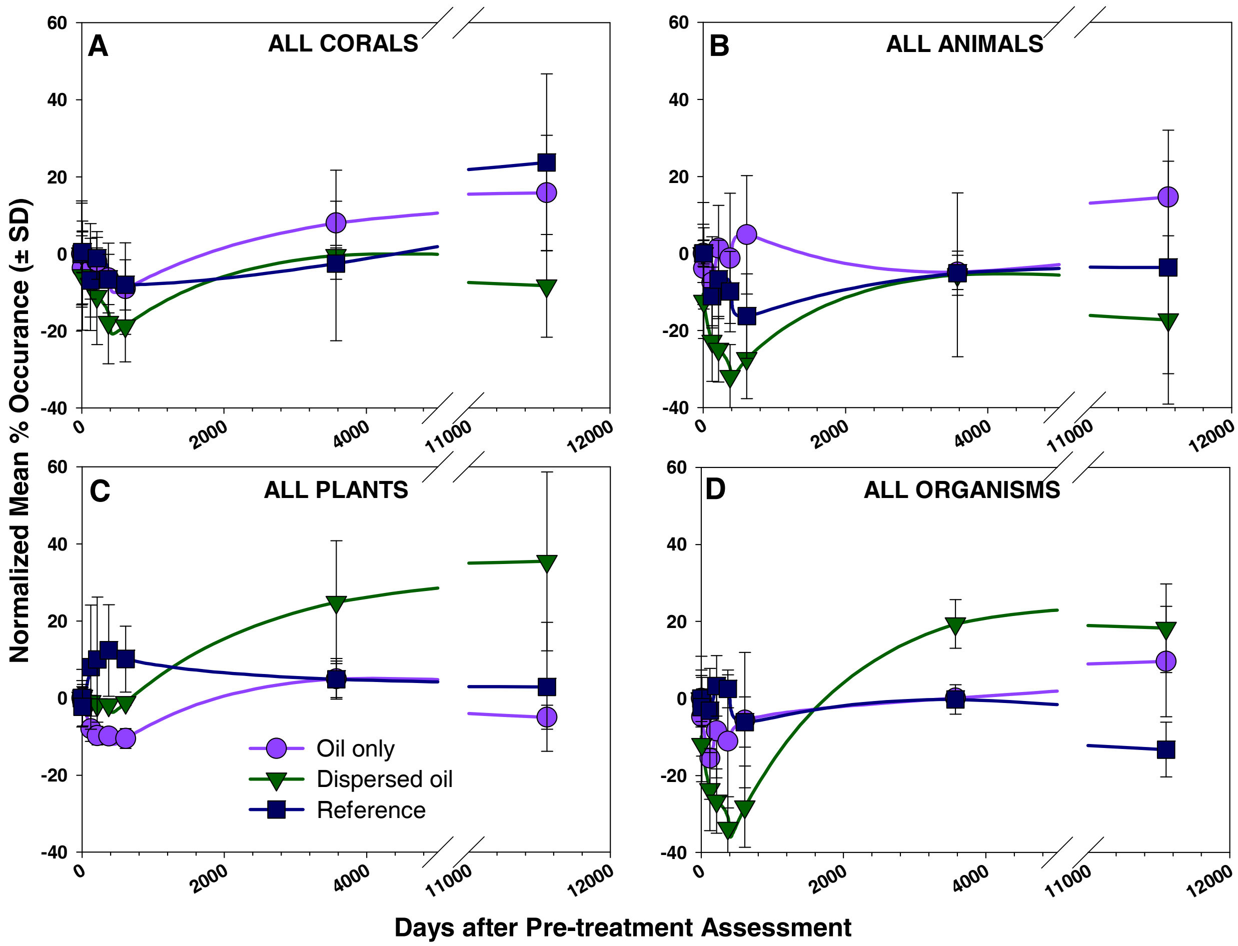

Figure 9. The normalized mean percent occurrence (±SD) of A) all corals, B) all animals, C) all plants) and D) all organisms on the coral reef at the Oil only, Dispersed oil and Reference sites. The percentages are normalized by subtraction to the Nov. 1984 pre-treatment mean for each site.

Qualitatively, the percentage of stony corals and all animals has increased or stayed the same at the Reference and Oil only sites, but has decreased at the Dispersed oil site since the 10 year measurements. The percentage of plants (seagrass/macroalgae) has increased at the Dispersed oil site, and decreased at the Reference and Oil only sites. Statistical comparisons are not possible due to the variability in number and placement of transects assessed (n=4 transects for the 32 year assessment, n=8 transects for previous assessments; original transect markers were not visible after 32 years). The large variation within sites suggests that site differences would not be significantly different from the 10 year observations. Similar sampling methods to past visits were used to evaluate oil persistence. Unlike other periods in the past evaluations, there was no indication of oil or biodegradation products in any of the sediment cores; however, the dense root composition in the substrate limited the depth of the cores, therefore petrogenic compounds may still be present in the deep sediments that could impact growth of seedlings into mature trees. Historically there had been oil and residue persisting at the Oil only site. The current absence of oil there is likely due to erosion of sediment from the lack of mangrove binding over three decades.

APPLICATION TO NEBA AND OIL SPILL PREPAREDNESS AND RESPONSE

In the late 1980s, TROPICS and follow up studies generated the Net Environmental Benefit Analysis (NEBA) concept and the graphics below (Fig. 10 and Fig. 11, courtesy of IPIECA 1992) (Knap, 1992). These depict the trade-offs of dispersant non-use versus use in a near shore tropical marine ecosystem. The 32 years of the TROPICS field study continues to serve as a valid and useful prototype for environmental and ecosystem trade-offs for the use or non-use of dispersants in near shore tropical marine ecosystems. From the initial dosing in 1984, through the 10 years of study by most of the original investigators, and further still through several other data collection visits, the experimental ecosystems have exhibited significantly different recovery regimes in mangroves, sea grass, and coral (and coral reef associated flora and fauna communities) depending on whether the sites were exposed to non-treated crude oil or dispersed crude oil. While this study does not reveal definitively whether to use or not use dispersants, the trade-offs from exposure to oil slicks on the surface versus water column exposure from chemically dispersed oil are clearly demonstrated.

Studies after the 10 year analysis (Dodge et al. 1995) continued to show long-term effects at the Oil only site, including overall disruption to the marine ecosystem and less recovery to the “pre-dosing” state. It was concluded that non-dispersed oil penetrated and remained at least partially trapped in the mangrove substrate, which presented a chronic leaching condition of low concentrations of oil over a number of years. This possible chronic leaching effected the mangroves’ ability to fully recover, as witnessed by a large amount of juvenile mangrove seedlings, and a significantly lower amount (compared to the Dispersed oil and Reference sites) of mature mangrove trees. While the current study did not confirm the persistence of oil in the sediments after 32 years, it is possible that non-treated crude oil remains trapped deeper and below the approximate 18 cm depth of the core samples collected and analyzed. If so, mangrove seedlings roots systems may reach this trapped oil, which could inhibit further growth and development.

Outside of the three study sites there are a number of observable depauparate areas (seen as blow-outs or holes) occurring in nearby mangrove forests visually similar to what is observed for the mangroves in the Oil only site. Both the Oil only site and the Dispersed oil site had similar mangrove densities and structures prior to dosing, yet only the mangroves in the Oil only site failed to recover. Mangroves are the land side component of the study ecosystem in which the exchange of sea water is much less frequent and efficient than the exchange and cleansing of the seaside environment of sea grass and corals. The corals at the Dispersed oil site were subject to a short (1-2 days) shock exposure, while corals at the Oil only site were subject to chronic oil exposure at low concentrations. While corals at the Dispersed oil site showed significantly greater decline compared to the other sites for more than 2 years following dosing, both coral sites generally appeared to have recovered to their pre-study state by year 10 (Dodge et al. 1995), although overall changes in the numbers and ratios of various corals were observed.

Previous hydrocarbon analysis indicated that aromatic hydrocarbons were detected for 25 years (but not after 29 years) at the Oil only site. Aromatic hydrocarbons were not detected after 3 years at the Dispersed oil or Reference sites. (Dodge et al 1995, Baca et al 2014). Mangrove substrate core samples taken after 32 years and analyzed using modern analytical methods indicated that there was no remaining oil or degradation products present to a depth of approximately 18 cm into the substrate. It is possible that either all oil was flushed from the mangroves at all sites, or that some oil remains below the sampling depth. In any case, the previously observed persistence of non-dispersed oil, some perhaps trapped in relatively deeper anaerobic mangrove substrate, appeared to be the principal cause of observed mangrove impacts at the Oil only site.

TROPICS has been the foundational and seminal field study, and historical antecedent for Net Environmental Benefit Analysis (NEBA). These studies have served as the basis for follow-on Spill Impact Mitigation Analysis (SIMA) and Comparative Risk Analysis (CRA) for oil spill planning, preparation, and response. The initial dosing at the Oil only and Dispersed oil sites represented relatively severe spill conditions. For this specific study [ie, the particular exposure scenario (type, amount, concentrations, length of exposure), the resources at risk, and other environmental factors] the observed results tended to favor the use of dispersants to preserve the mangrove habitat as the underpinning component for the health of the entire study ecosystem. This conclusion is based on neutral weighing of the ecosystem subcomponents (coral, sea grass, mangroves). Under different spill conditions and with weighing or prioritization of resources at risk under NEBA, SIMA, or CRA, the outcome as it informs response decisionmaking could be different. Based on the observations from TROPICS, mitigating impacts on a key habitat, such as the mangrove forest, allows a greater opportunity for repopulation and overall recovery of the ecosystem.

While government authorizations to spill oil in the environment and other practical realities limited the TROPICS comparative dosing experiment to one replicate of each treatment, the study is nonetheless valuable as a case study which demonstrates the trade-offs that must be made in responding to oil spills. Additional information that is currently being developed, such as toxicity thresholds for corals (Renegar et al. 2016) and a better understanding of the toxicity pathways and uptake for oil/dispersed oil in mangroves (Pardue et al. 2017) will enable higher fidelity NEBA, SIMA, and CRA analysis in marine tropical ecosystems in the future. Further investigations on mangrove repopulation and a comparison of the sea side components (sea grass, coral, and sea grass-coral interactions) of the three TROPICS sites to surrounding areas is warranted. TROPICS is one of the earliest and longest term field studies in the short history of oil spill science and should be part of any curricula on marine pollution and oil spill preparedness and response.

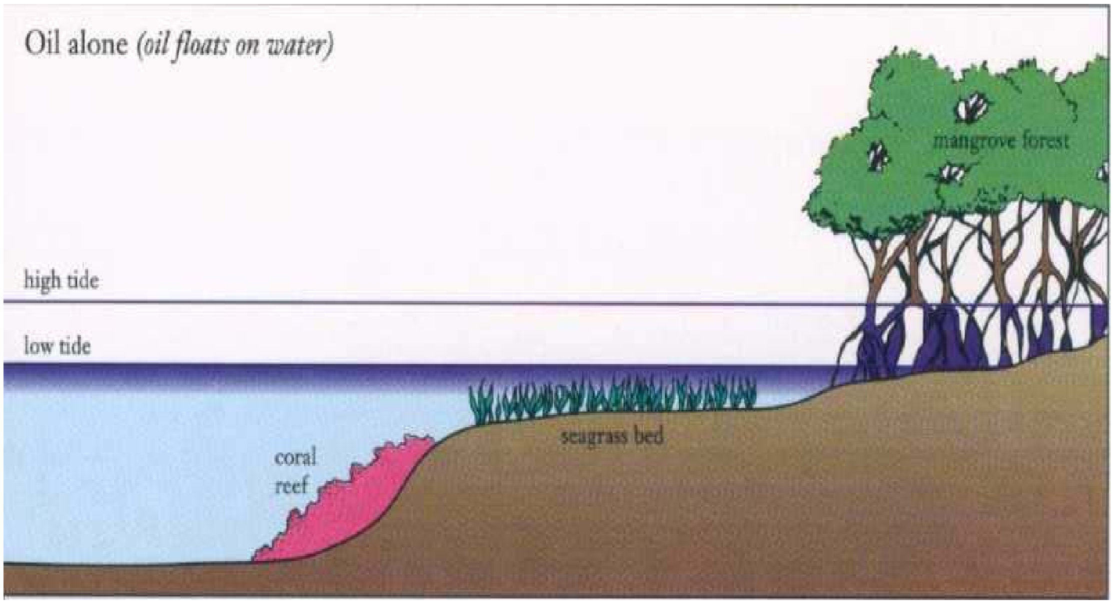

Figure 10. Exposure scenario created by floating oil in near shore tropical marine environment. IPIECA 1992

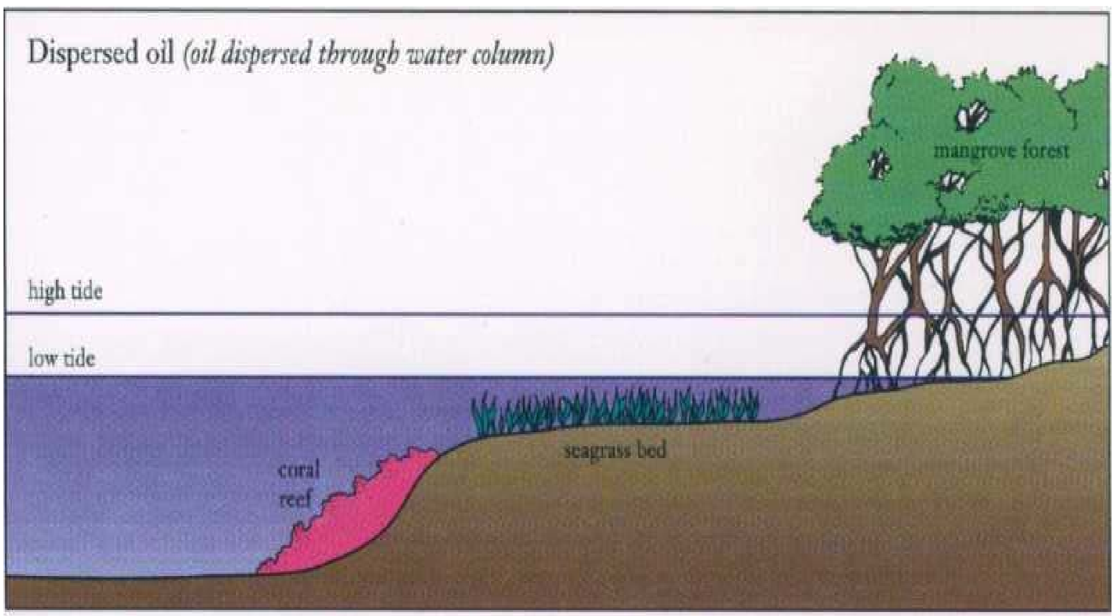

Figure 11. Exposure scenario created by dispersed oil in near shore tropical marine environment. IPIECA 1992

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