



Spills of fuel oil #6 and Orimulsion can have indistinguishable effects on the benthic meiofauna

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Abstract

Fuel oil #6 is used for the production of electrical power in the United States. Orimulsion® is being considered as an alternative fuel, but its value and risk compared to fuel oil #6 need to be assessed. Our study examined the relative impact of accidental spills of the two hydrocarbons on the meiofaunal community. To do so, we maintained microcosms of the shallow, sandy, subtidal environment for three months. Treatment microcosms received a single application of hydrocarbon-coated sand. As indicators of effect, we used copepod and nematode abundance and copepod species diversity, sex ratio, fecundity, age structure, and neutral-lipid content. A comparison of the hydrocarbon treatments showed no significant differences. The tests had adequate power to detect ecologically significant changes. Our results indicate that a spill of Orimulsion would have approximately the same impact as a spill of fuel oil #6 on the meiofauna.

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1. Introduction

The decision to use a new or an alternative fuel and the creation of regulations for its use depend on an accurate estimate of its costs and benefits relative to the fuel it is to replace. If the new/alternative fuel is found to be a greater environmental risk than the current fuel, that fact must be considered in the decision.

Fuel oil #6 is used for the production of electrical power in the United States (EIA, 1999). It consists of the heavy residue remaining after crude oil is fractionally distilled plus low-viscosity blending stocks added to adjust its viscosity (Neff et al., 1994). It is produced primarily in the Middle East and transported to the United States by ship.

Orimulsion®, an alternative fuel available since 1994, is currently used in Canada, Denmark, Italy, and Japan and is being considered for use in the United States. It consists of 70% bitumen (a viscous, highly degraded hydrocarbon, Tissot and Welte, 1978; Ostazeski et al., 1997), 30% water, and 0.17% surfactant. It is produced

in the Orinoco Basin of Venezuela and can be transported by ship and burned in plants that use fuel oil #6 (after minor refitting). It has the potential to reduce fuel, operating, and maintenance costs by over 40% (www.orimulsionfuel.com). Although Orimulsion is readily available and less costly than fuel oil #6, the environmental risks associated with its use are not known. In particular, the consequences of a spill during marine transport have not been studied.

The acute lethality of a petroleum hydrocarbon is determined primarily by its concentration of low-molecular-weight components. Fuel oil #6 contains 1.9% benzenes and 5.6% naphthalenes, compared to ~0% and 0.11%, respectively, in bitumen (Potter, 1995). Another indicator of toxicity is the concentration of polycyclic aromatic hydrocarbons (= PAH), some of the most toxic, carcinogenic, mutagenic compounds in the marine environment (Kennish, 1992). Fuel oil #6 contains ~23% PAH (Potter, 1995); Orimulsion contains only ~6% (Jokuty et al., 1995). On this basis, one would predict that a spill of Orimulsion would have less environmental impact than a spill of fuel oil #6. Here, we report experiments designed to compare the environmental damage caused by equivalent spills of Orimulsion and fuel oil #6.

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Our research was part of a larger project, which included bacteria (Leitman and Proctor, personal communication), zooplankton (Suderman and Marcus, in press), meiofauna, and microalgae. Our focus was on benthic copepods and nematodes.

These two taxa comprise ~60–90% and ~10–40%, respectively, of the total preservable estuarine meiofauna (Coull, 1999). They are ubiquitous in the marine environment and comprise an important link in the food chain, feeding on microalgae and bacteria and in turn being preyed upon by larger predators such as fish (Motta et al., 1995). They would be expected to be especially susceptible to sediment-associated contaminants because they live and feed in the sediments. Any effects of contaminants on them are likely to be passed through the system in that any reduction in their abundance will reduce the food available to other levels of the food chain, and any bioaccumulation in them could be passed to higher trophic levels (Street et al., 1998).

Benthic copepods and nematodes are also well suited to a microcosm experiment because they are small, abundant, easily maintained, sediment-bound throughout their life history, quick to reproduce (on the order of weeks, Itô, 1970), and sensitive to many toxicants (Coull and Chandler, 1992; Long, 1992). Using such small organisms allowed us to reduce the size of the microcosm containers without sacrificing their fidelity to the environment. Because smaller microcosms are less resource intensive to use than larger ones, we were able to increase replicate numbers and thereby increase statistical sensitivity.

We sought to answer three questions: (1) Do the effects of the two hydrocarbons differ? (2) If so, which has the greater impact? (3) Is a spill of either fuel environmentally important?

2. Methods

2.1. Locality

The study site was in a shallow inlet, adjacent to North Beach in Fort DeSoto Park, Tampa Bay, Florida, USA (27°39'N, 82°44'W). It was selected to represent the unvegetated, shallow, sandy, subtidal environment that makes up ~35% of Tampa Bay. The substrate was well-sorted, fine sand with <1% silt; the arithmetic mean grain size (Folk, 1980) was 176 μm . On sampling days, average salinity was 27 g l^{-1} , average water temperature was 29 °C, average wave height was <20 cm, estimated current speeds were <20 cm s^{-1} , and underwater visibility was 2–3 m. Sharp-edged sediment ripples indicated recent reworking of the sediment by waves. If an accidental oil spill occurred in Tampa Bay, modeling studies

predict that this site would experience heavy contamination (Harwell et al., 1995).

2.2. Sediment collection

The study area was marked with two parallel, 140-m transect lines, laid 3 m apart, along the 1-m isobath. The transect lines defined a corridor for transporting equipment. The sediment for the microcosms was collected outside this corridor at 1-m depth.

The microcosm containers were acrylic boxes 29 cm \times 19 cm \times 17 cm (1 \times w \times h, inside dimensions) filled with ~5 cm of sediment and ~10 cm of seawater (Fig. 1A). To preserve physical and chemical gradients, SCUBA divers collected the sediment with as little disturbance as possible by inserting a thin, metal sleeve 5 cm into the sediment, sliding an acrylic plate across its lower opening, wrapping a string under the bottom, and lifting the entire assembly into a microcosm container (Fig. 1B, see also Chandler, 1990; Carman et al., 1994). The string and the sleeve were removed. Some slumping occurred around the outer 1–2 cm of the sediment block, but the center remained undisturbed. The water overlying the sediment was sealed into the container with a 1.4-cm-thick, Styrofoam™ cover before the microcosm was removed from the water. Each unit (microcosm, lid,

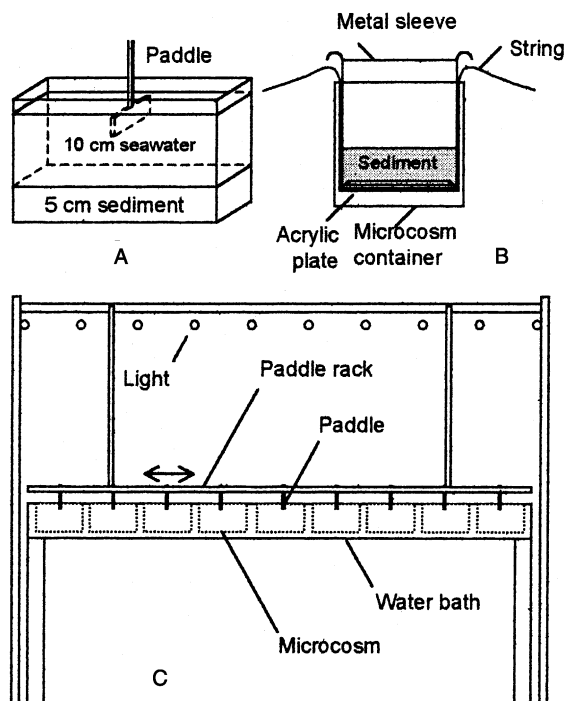


Fig. 1. (A) A microcosm, showing sediment and water height and location of paddle. (B) Side view of the metal sleeve containing the sediment sample being lowered into the microcosm container. (C) Side view of a single water bath showing the lights and the microcosms. The paddle rack holds the individual paddles. It oscillates in the direction of the two-headed arrow.

sediment, and its associated water) was transported by climate-controlled van to the Florida State University Marine Laboratory and placed in the environmental-control facility (described below). For logistical reasons, we conducted the experiment in four parallel runs (= replicates) at 48-h intervals.

2.3. Environmental-control facility

The environmental-control facility consisted of three water baths, each 3.1 m × 1.4 m × 0.2 m (Fig. 1C). The temperature within the water baths was maintained at 29 °C. Seawater in the microcosms was not exchanged; distilled water was added at least every 2 d to compensate for evaporation.

Each water bath was equipped with ten 110-W, high-output, cool-white, fluorescent lamps that were suspended 130 cm above the water surface in the microcosms, which provided 32.0 (±4.7) μmol photons m⁻² s⁻¹ (measured at the water surface, *n* = 30) for 6 h d⁻¹. This light regime permitted healthy growth of microalgae but minimized algal overgrowth on the sediment surface and microcosm walls (Perez et al., 1977).

To prevent anoxia in the sediments, we simulated natural pore-water circulation. Pore-water circulation is driven by inequalities in pressure on the sediment as waves traverse the air–water interface (Riedl et al., 1972). We installed paddles (18.0 cm × 2.5 cm × 0.6 cm) that oscillated horizontally through 8 cm at 35 strokes min⁻¹ creating small (<0.5 cm high) waves on the surface of the water that drove pore-water circulation. The water motion was not sufficient to change the sediment topography or redistribute the surface sediment.

2.4. Treatments

When a hydrocarbon is spilled into the marine environment, hours to days of weathering alter the composition of the hydrocarbon by breaking down labile molecules and removing volatile and soluble molecules, generally rendering it less toxic than the parent material (Jordan and Payne, 1980).

To simulate the weathering a spilled hydrocarbon would experience before contacting the sediment (Jordan and Payne, 1980; Chin et al., 1995), we placed 100 g of each hydrocarbon in its own 20-l, glass carboy with 10 l of autoclaved, 0.2-μm-filtered seawater and 1.5 kg of sand collected from the study site. Two additional carboys each received 10 l of filtered seawater and 8 kg of sand and were processed in parallel with the hydrocarbon-sand carboys. We had combusted the sand at 500 °C for 24 h to remove organic carbon and sieved it to remove silt (<62 μm) and very coarse material (>1 mm). The carboys were placed outdoors on an oscillating shaker table (~60 rpm) under natural light for 48 h (for photodegradation and evaporation of the hydrocar-

bons). The water was then siphoned off (taking with it the water-soluble fraction of the hydrocarbons). To ensure even distribution of the hydrocarbons, a hexane:acetone:cyclohexane mixture (4:4:1 by volume, Potter, pers. comm.) was added to each of the four carboys, and the contents were stirred until the weathered hydrocarbon was evenly mixed through the sand (or for an equivalent time for the hydrocarbon-free sand). The organic solvents were evaporated off by gentle heating in a 100 °C water bath, during which we took care to keep the sand saturated with water. (Drying the hydrocarbon-sand mixture may cause the hydrocarbon to “glaze,” potentially reducing bioavailability, Johnson, personal communication) The hydrocarbon content of the sand was determined gravimetrically as total hexane-extractable material and adjusted to 7000 mg kg⁻¹ by addition of hydrocarbon-free sand to the hydrocarbon-coated sand. This hydrocarbon concentration was selected after preliminary microcosm experiments in which a 1-mm-thick layer of 7000 mg kg⁻¹, Orimulsion-sand mixture caused >50% mortality of benthic copepods over the first 4 d of exposure (unpublished data). In the main experiment, an equal concentration of fuel oil #6 was used for comparison. The mixture was homogenized, and the organic-solvent mixture was removed as before. The Orimulsion-coated sand, the fuel oil #6-coated sand, and the hydrocarbon-free sand were divided into 88-g aliquots and stored at 5 °C until added to the microcosms. Each aliquot was sufficient to cover the sediment surface of one microcosm to a depth of 1 mm.

Microcosms treated with the hydrocarbon-free sand (= carrier sand) served as a control for the effects of the carrier sand in the Orimulsion and fuel oil #6 treatments. A second control, consisting of unmanipulated microcosms (= control), was also used to monitor the effects of containment in the microcosm system.

2.5. Sampling

Sediment samples were collected from the microcosms 0, 8, 16, 41, 60, 74, and 88 d after the treatments were administered. Before sediment sampling began, a 50-ml water sample was taken for determination of dissolved inorganic carbon concentration (= [DIC]) on a Shimadzu TOC-5050A TOC analyzer. As a result of dye-tracing studies assessing pore-water circulation, sampling was confined to the center of the microcosm, where the pore-water circulation was primarily oscillatory, similar to natural circulation (Riedl et al., 1972). To avoid any possible edge effects, we confined the working section to an area 15 cm × 15 cm, centered in the microcosm, which allowed for nine potential 5-cm-diameter coring sites. Three 19-cm² cores were taken from randomly selected coring sites. One core was analyzed for neutral-lipid content, abundance, species

diversity, sex ratio, fecundity, and age structure of copepods. The remaining two cores were analyzed only for abundance of copepods and nematodes.

2.6. Neutral-lipid analysis

During sample collection, one core per microcosm was sliced at a depth of 1 cm, and meiofauna were extracted by decantation and frozen at -80°C . For analysis, the sample was thawed, and benthic copepods were removed from the sediment, placed in groups of 20 individuals, and stained with Nile Red for 30 min (Carman et al., 1991; Thistle et al., 1995). Adults were placed with their right sides uppermost under a Nikon Optiphot compound microscope equipped with an acridine orange filter set (450–490-nm excitation filter, 510-nm dichroic mirror, 520-nm barrier filter). Images were captured with a COHU 4915-2010 video camera, Scion LG-3 frame-grabber card, and NIH Image software on an Apple Macintosh 7200/120 computer. For each animal, a bright-field image of the urosome was captured, and its area was determined. The copepod was then illuminated with epifluorescent light (100-W mercury lamp), and an image of the brightly glowing neutral-lipid droplets was captured. We applied the binarization function of the software, which converts all values above a threshold to white and all values below the threshold to black, and adjusted the threshold to match the area of the neutral lipids in the image to those visible under the microscope. The copepod was repositioned, and a similar pair of images was captured for the prosome. The neutral-lipid score was calculated as neutral-lipid area divided by total area. For samples with >20 adult copepods, a subsample of 20 randomly selected adults was processed. For each microcosm, the neutral-lipid-content metric was the mean of the neutral-lipid contents of all nongravid females in a single core.

2.7. Nematode and copepod abundance

The abundance of nematodes in a microcosm was estimated from two cores and that for copepods from three cores.

2.8. Other metrics

Benthic copepods from the core used for neutral-lipid analysis were identified to sex, stage, and species (24 species of harpacticoids and one cyclopoid). From these data, we calculated the sex ratio (the proportion of the adult copepod population that was female), fecundity (the proportion of the adult females that was gravid), age structure (the proportion of the copepod population that was juvenile), and species diversity (H' , Shannon and Weaver, 1949).

2.9. Statistical analysis

A treatment-by-block ANOVA was performed on each metric. The factor “Treatment” had levels of Control, Carrier, Fuel oil #6, and Orimulsion. The blocking factor “Time” had seven levels corresponding to the seven time points. Data were transformed with $\log_{10}(n + 1)$ transformations to meet the assumptions of ANOVA.

To be certain that our tests had sufficient statistical power to detect ecologically meaningful differences between the treatments if they occurred, we carried out statistical power analyses (Cohen, 1988). To determine the appropriate alternative hypothesis for the power analyses (in our case, the largest difference between the treatment levels that we did not consider ecologically important), we searched the literature. We found that changes of $<50\%$ of the control mean are not considered ecologically meaningful in a dynamic and variable environment like the shallow subtidal (Southwood, 1978; Shaw et al., 1994), so we adopted that standard. Because a power analysis cannot be readily interpreted when a factor has more than two levels, as did our overall ANOVAs, we performed power analyses on three individual, two-level, treatment-by-block ANOVAs that were of interest: One comparing the fuel oil #6 treatment to the Orimulsion treatment, one comparing the fuel oil #6 treatment with the carrier treatment, one comparing the Orimulsion treatment with the carrier treatment.

3. Results

3.1. Treatment main effects

The ANOVAs with treatment levels of Control, Carrier, Fuel oil #6, and Orimulsion did not detect a significant treatment effect for any metric (i.e., nematode and copepod abundance, copepod species diversity, fecundity, sex ratio, age structure, and neutral-lipid content). The means at each time point are plotted in Fig. 2.

3.2. Comparison of the fuel oil #6 and Orimulsion treatments

The treatment-by-block ANOVAs comparing the fuel oil #6 treatment with the Orimulsion treatment detected no significant differences between the two hydrocarbon treatments ($p > 0.05$). The power of each test (i.e., the probability that the test would detect a difference at least as great as half the mean of the carrier treatment) was >0.995 .

3.3. Effect of the hydrocarbon treatments

The treatment-by-block ANOVAs comparing the fuel oil #6 to the carrier treatment and the Orimulsion

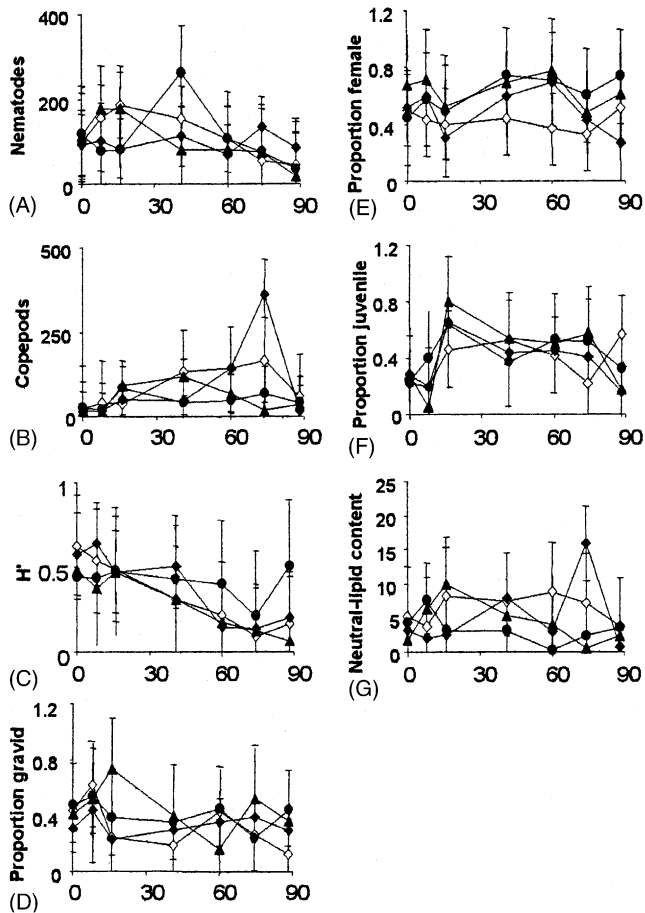


Fig. 2. Meiofaunal metrics at seven times during the experiment. Open diamonds = Control. Filled diamonds = Carrier control. Triangles = Fuel oil #6. Circles = Orimulsion. In all panels, the abscissa is time in days and the error bars are pooled 95% confidence intervals. (A) Abundance of nematodes, (B) abundance of copepods, (C) copepod species diversity, (D) copepod sex ratio, (E) copepod fecundity, (F) copepod age structure and (G) copepod neutral-lipid content.

treatment to the carrier treatment detected a significant difference only in the sex ratio of copepods subjected to the fuel oil #6 treatment ($F = 5.544$, $p = 0.024$, Fig. 2D). None of the other tests detected a significant effect of either hydrocarbon treatment ($p > 0.05$). The power of each test was >0.995 .

4. Discussion

Our study was conducted to determine the damage to the shallow, sandy, subtidal environment that a spill of Orimulsion would cause relative to that caused by an equivalent spill of fuel oil #6.

Because of its greater concentration of PAH and low-molecular-weight components, we anticipated that fuel oil #6 would have a greater impact than Orimulsion on the nematodes and copepods, but our results revealed no differences large enough to be likely to be of eco-

logical importance (i.e., larger than 50% of the control mean). At the beginning of the experiment, fuel oil #6 contained four times the PAH and many times the benzenes and naphthalenes of Orimulsion. We suspect that the 48-h weathering process removed the lighter, more bioavailable materials in the fuel oil #6, leaving components more similar to the highly degraded hydrocarbons in the Orimulsion (Jordan and Payne, 1980).

The effects of both hydrocarbon treatments on meiofauna were less intense than anticipated. We determined that any changes in nematode abundance or copepod abundance, species diversity, fecundity, age structure, or neutral-lipid content were smaller than differences regarded as ecologically important (Southwood, 1978; Shaw et al., 1994).

The only effect that might have been ecologically important was that of fuel oil #6 on copepod sex ratio. Given that we performed 28 significance tests at the $\alpha = 0.05$ level, the probability that one or more tests would be significant by chance alone is 0.76, so the significant change in sex ratio may well have been due to chance. Even if the difference is real, it appears to be unimportant. By manipulating the number of males in copepod populations, Woods and Coull (1992) found that populations with equal numbers of females grew at the same rate whether they had a 0.50 or a 0.83 sex ratio. The smaller difference we detected (sex ratio of 0.49 in the carrier treatment and 0.64 in the fuel oil #6 treatment) seems unlikely to cause lasting effects in the field, a prediction supported by the lack of significant effect on copepod abundance.

The levels of contamination we used were expected to affect the meiofauna significantly. Contamination with as little as 55 mg kg^{-1} PAH (from diesel fuel) has significantly affected benthic-copepod abundance (Carman et al., 1997). The concentration of PAH in the microcosm sediment was not measured. To estimate the amount of PAH in our treatments, we assumed that the proportion of PAH in the hydrocarbons did not change during weathering and that the added hydrocarbon-coated sand was mixed to a depth of 1 cm by bioturbation, which yields a concentration of 161 mg kg^{-1} PAH for fuel oil #6 and 42 mg kg^{-1} PAH for Orimulsion. These levels are comparable to those of Carman et al. (1997).

One possible explanation for the difference between our results and Carman et al.'s (1997) is that the PAH in our experiment were less bioavailable and toxic. Those in Carman et al.'s experiment were derived from diesel fuel and therefore likely to be of lower molecular weight (Jordan and Payne, 1980) than those in fuel oil #6 and bitumen-derived PAH. The PAH concentrations used in our experiment may therefore have been below the threshold for ecological effects. Alternatively, during weathering, the lighter and potentially more toxic PAH are most likely to be removed from the hydrocarbon

(Jordan and Payne, 1980). It seems likely that the weathered hydrocarbons we used were less toxic than the calculated PAH concentration would indicate.

In sum, our results suggest that a spill of either fuel oil #6 or Orimulsion that takes at least two days to reach the sediment and does not exceed a concentration of 7000 mg kg⁻¹ will have minimal effect on the meiofaunal community of the shallow, sandy, subtidal environment.

Ideally, we would like to compare our treatments (7000 mg kg⁻¹ of 48-h-weathered hydrocarbons) with typical oil-spill conditions. Hydrocarbon concentrations in subtidal regions after major oil spills have been shown to range from below detection (i.e., background levels) to 28,000 mg kg⁻¹, but most sites contained <500 mg kg⁻¹ hydrocarbons (Lee and Page, 1997). At 7000 mg kg⁻¹, our treatments were within this broad range but were more than an order of magnitude higher than the typical concentrations. Sediment hydrocarbon concentrations, however, are generally derived from the top 1–2 cm of sediment, representing 15–20 years of deposition in many environments. Sediment traps (which collect only the most recently deposited sediment) deployed after the *Tsesis* spill detected concentrations of 7300 mg kg⁻¹ (Boehm et al., 1982), very similar to those in our treatments.

Determining the degree of weathering sustained in a typical spill is difficult because the time between release into the environment and contact with the shore (i.e., weathering sensu our experiment) is not routinely reported. Generalizations can be made. Many spill models (e.g., Anderson et al., 1997) attempt to define the spill's trajectory at 3, 10, and 30 days, suggesting that a considerable portion of the spill remains in the water for at least 3 days, i.e., takes more than 48 h to make landfall. In many of the spills for which detailed reports are available (e.g., *Amoco Cadiz*, *Torrey Canyon*, *Exxon Valdez*, *Metula*, *IXTOC*), oil continued to make landfall for several days after the release of oil ceased, again suggesting that some portion of the oil experienced at least 48 h of weathering.

Our treatments, therefore, appear somewhat more severe than might typically be expected. The concentration of hydrocarbons exceeds that in a typical spill, and the duration of weathering seems typical or shorter than average. A site experiencing less contamination or contamination with hydrocarbons that are more weathered should experience similar or less-severe impact. We conclude that our findings are representative of the effects expected in the subtidal regions of the majority of oil-spill sites.

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