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Toxicity of Refugio Beach Oil to Sand Crabs (*Emerita analoga*), Blue Mussels (*Mytilus* sp.), and Inland Silversides (*Menidia beryllina*)

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Abstract: Monterey formation crude oil spilled from an onshore pipeline and entered the surf zone near Refugio State Beach, Santa Barbara County, California (USA) on 19 May 2015. During this season, early life stages of many marine fish and invertebrates were present. Surf zone water and beach porewater samples were collected during the 4 mo after the spill and 2 yr later for chemical analyses. Elevated polycyclic aromatic hydrocarbon (PAH) and total petroleum hydrocarbon concentrations were observed in surf zone water and porewater near the release point, declining with distance and time. Early life stage toxicity was investigated by conducting 6- and 7-d static renewal bioassays with sand crab (*Emerita analoga*) post larvae (megalopae) and inland silverside larvae (*Menidia beryllina*), respectively, and a 48-h blue mussel (*Mytilus* sp.) embryo development bioassay. Dilutions of a high-energy water accommodated fraction of the Refugio Beach oil and a seawater control were prepared to simulate surf zone PAH concentrations (nominal PAH_{45}; 0, 0.5, 1, 5, 10, 50, 100, and 500 µg/L). The PAH_{45} median lethal concentrations (LC50s), based on measured concentrations, were 381 µg/L for *Mytilus* sp., 75.6 µg/L for *Menidia*, and 40.9 µg/L for *Emerita*. Our results suggest that PAH concentrations in coastal waters of the spill-affected area were potentially lethal to early life stages of fish and invertebrates. *Environ Toxicol Chem* 2021;40:2578–2586. © 2021 SETAC

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INTRODUCTION

A pipeline carrying heated Monterey formation crude oil ruptured on 19 May 2015 in an upland habitat near Refugio State Beach, Santa Barbara County, California (USA). An estimated 2934 barrels (466 469 L) of oil, from several offshore platforms, were released before the pipeline was shut down (Pipeline and Hazardous Materials Safety Administration 2016). The fresh oil flowed a short distance, approximately 0.2 km, and then poured off a cliff face directly into the rocky and sandy intertidal zone, where the oil was mixed and dispersed into the water column by wave action. The oil was then transported by currents offshore into the Santa Barbara Channel and along the Gaviota coastline (Figure 1), becoming known as the Refugio Beach Oil Spill. Numerous dead aquatic organisms were found on the spill-affected shorelines in the days following the release. Due to the direct release to the shoreline, the spill caused significant impacts even though the spill volume was much smaller than the 1969 Santa Barbara Channel offshore platform oil spill (Foster et al. 1971).

Following the spill, a response Unified Command was established to direct the cleanup of oil on the ocean surface and stranded along the shoreline. State and federal trustee agencies also pursued a cooperative natural resource damage assessment (NRDA) with the responsible party, Plains All American Pipeline (Refugio Beach Oil Spill Trustees, 2021). The goal of the assessment was to quantify injuries to wildlife, habitat, and lost uses of those resources, and then to determine how to best restore the resources and compensate for the losses. The present study was initiated by the Trustees to

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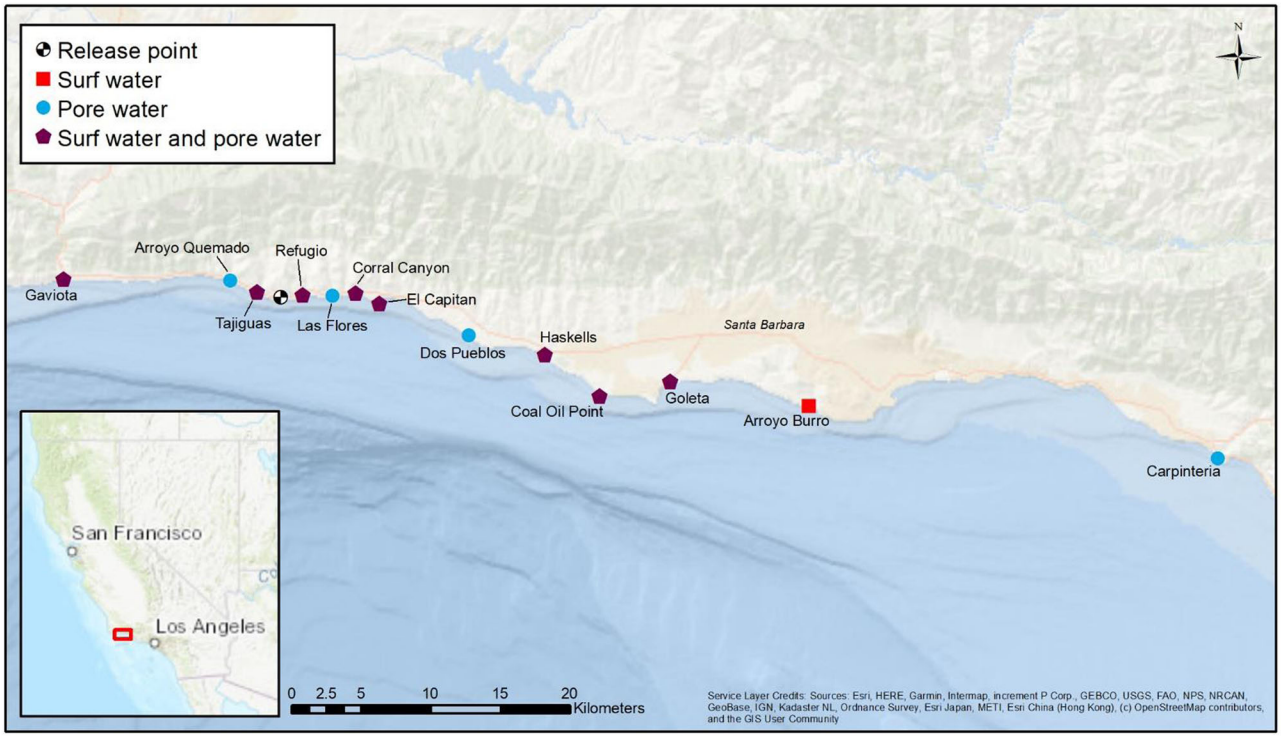

FIGURE 1: Refugio Beach Oil Spill release point and sampling locations for surf water and porewater.

help assess and quantify shoreline biological injuries caused by the spill. Several natural oil seeps are known to occur in the Santa Barbara Channel, especially near Coal Oil Point (Lorenson et al. 2009). To distinguish between Refugio Beach oil and seep oil, spatial and temporal water monitoring, accompanied by forensic chemistry analysis and oil transport modeling (MacFadyen 2017) were required.

The Santa Barbara Channel is known for high biodiversity and productivity due to its location in the transition zone between marine biogeographic provinces where the cool south-flowing California Current intersects the warm Southern California Countercurrent and Eddy. Sandy beaches dominate the shoreline habitat in this area, but intertidal boulder cobble fields and rock bench platforms are also present. The sandy beaches support highly diverse invertebrate communities, including bivalve mollusks, polychaete worms, beach endemic insects, and crustaceans, such as sand crabs (*Emerita analoga*), and talitrid amphipods (Dugan et al. 2003; Dugan and Hubbard 2016). On rocky intertidal substrates, mussel (*Mytilus* sp.) beds are limited to larger rock outcrops above sand burial depths. A variety of fish species inhabit the surf zone in this area, including silversides (Atherinopsidae), such as grunion (*Leuresthes tenuis*) and topsmelt (*Atherinops affinis*), and surfperches (Embiotocidae) that forage on the burrowing intertidal invertebrates (Allen and Pondella 2006).

Oil spills have been shown to reduce the abundance and diversity of sandy beach macrofaunal invertebrates (Bejarano and Michel 2016). Following the spill, field studies were conducted to evaluate responses of sandy beach and rocky intertidal invertebrates to the spill (Refugio Beach Oil Spill Trustees 2021). Mortality of early life stages from oil spills can be challenging to quantify in field studies due to the small size of these organisms and the settlement dynamics. Results of laboratory bioassay studies on early life stages can be used to assess thresholds of injury for different levels of oil exposure for these taxa. In the present study, the potential effects of Refugio Beach oil on early life stages, known to be sensitive to oil exposure (Barron et al. 1999; Incardona et al. 2015; Lee et al. 2015; Hodson 2017), were evaluated using toxicity tests on one fish (*Menidia beryllina*) and 2 invertebrate (*E. analoga* and *Mytilus* sp.) species.

Sand crabs, *E. analoga*, were identified as an indicator species because they are abundant in the swash zone along the Gaviota Coast and are important prey for shorebirds and surf zone fish. Sand crabs inhabit the swash zone and would be exposed to oil in surf and swash zone water and intertidal porewater because they burrow into sediment and use plumose antennae to filter feed in the wave wash zone (Efford 1966; Dugan and Hubbard 2016). They have been used for monitoring bioaccumulation of contaminants, such as petroleum and polycyclic aromatic hydrocarbons (PAHs; Rossi et al. 1978; Dugan et al. 2005) and have similar sensitivity to petroleum as mysids (Barron et al. 1999, 2013). In addition, *E. analoga* were monitored for polycyclic aromatic hydrocarbon (PAH) bioaccumulation during the spill. The megalopa postlarval life stage was chosen for a 6-d survival, growth, and emergence bioassay because it is the first stage to settle on sandy beaches and can be collected from beaches in high numbers during the spring when the spill occurred (Efford 1965; Barron et al. 1999).

Blue mussels, *Mytilus* sp., were selected as representative bivalve filter feeders of the rocky intertidal habitat. Mussels have frequently been used to evaluate bioaccumulation of contaminants in California marine waters (Kimbrough et al. 2008) and were monitored for PAHs during the spill. Embryo and larval stages are more sensitive to pollutants than later life stages and have been recommended for use in standard toxicity tests (US Environmental Protection Agency 1995). Bivalve embryo-larvae toxicity tests have been commonly used to evaluate impacts from other oil spills (Geffard et al. 2004; Saco-Alvarez et al. 2008; Stefansson et al. 2016) and PAH exposures (Pelletier et al. 1997; Bellas et al. 2008). Hence, the standardized *Mytilus* sp. 48-h embryo development bioassay was conducted with Refugio Beach oil.

The inland silverside (*Menidia beryllina*) was utilized in the present study because of the taxonomic similarity to other surf zone fish along the Gaviota Coast and the availability of the larvae for the standardized 7-d survival and growth bioassay (US Environmental Protection Agency 2002). This bioassay has been frequently used to evaluate petroleum toxicity to fish early life stages (Barron et al. 2013), allowing for an evaluation of the relative toxicity of Refugio Beach oil compared with other crude oils. Because invertebrates and fish were exposed to oil in the surf zone during the spill, a high-energy water accommodated fraction (HEWAF) of the oil was used in the bioassays to mimic these conditions. An additional objective of our study was to compare PAH and total petroleum hydrocarbon (TPH) concentrations measured in the surf water and sandy beach porewater after the spill to bioassay effect concentrations.

MATERIALS AND METHODS

Bioassay HEWAF preparation

A sample of Refugio Beach oil was collected from the pipeline on 21 May 2015. The HEWAFs from this oil sample were made daily during the experiment with 0.45-μm filtered seawater (collected from the University of California Granite Canyon Marine Laboratory, Carmel, CA, USA), following the protocol developed for the *Deepwater Horizon* Oil Spill (Carney et al. 2016). Precleaned commercial stainless-steel blenders were filled with 3.75 L of seawater and 1.0 g/L of Refugio Beach oil. The solution was blended on low for 30 s, and the contents were transferred to a 4-L separatory funnel, and were allowed to separate for 1 h. The resulting HEWAF was decanted and composited into a 10-L glass container. This HEWAF stock solution (nominal PAH_{45} concentration of 500 μg/L; 100% HEWAF) was combined with seawater to make a PAH dilution series of 100 (20% HEWAF), 50 (10% HEWAF), and 10 μg/L (2% HEWAF). The 10-μg/L solution was diluted to prepare the 5 (1% HEWAF), 1 (0.2% HEWAF), and 0.5 μg/L (0.1% HEWAF) test solutions. A blended control was made by blending 3.75 L of seawater on low for 30 s. A seawater control consisted of the seawater used to make the dilutions. A sample of each dilution was collected in a 1-L amber bottle after daily solution preparation for PAH analysis.

Bioassay conditions and endpoints

All bioassays were conducted with a 16:8-h light: dark photoperiod under ambient laboratory illumination (50–100-ft candles). Salinity, dissolved oxygen, and pH were monitored daily. The *E. analoga* megalopae post larval stage 6-d survival, growth, and emergence test followed Barron et al. (1999). Megalopae were collected from Salmon Creek Beach, Sonoma, California (USA) and were maintained in seawater at 15 °C for 3 d. At test initiation, 10 megalopa were randomly selected and placed in a 1-L glass beaker containing 1 to 2 cm of 212- to 300-μm grain size white quartz sand (Sigma-Aldrich) and 500 mL of a HEWAF dilution as the treatment. There were 4 replicates/HEWAF dilution. These beakers were randomly positioned in a temperature-controlled room. Approximately 80% of the test solutions were renewed daily, and the survival of megalopae and the number of shed exuvia were recorded daily. Replicate treatments and controls were fed freshly hatched brine shrimp ad libitum twice daily. Potassium chloride dilutions (0.125, 0.25, 0.5, 0.75, 1, and 2 g/L) were used as a reference toxicant.

Emerita analoga mortality, biomass, dry weight, length, incremental growth, and mean emergence were recorded at test termination. Replicate biomass was calculated as the dry weight of surviving organisms/replicate divided by the initial number of organisms ($n = 10$). Replicate dry weight was calculated by dividing the dry weight of the surviving organisms by the number of surviving organisms. Replicate percentage incremental growth was determined by subtracting the initial dry weight from the final dry weight, and then dividing by the initial dry weight and multiplying the quotient by 100. Mean emergence for each replicate was calculated as the sum of the number of sand crabs emerging from the substrate daily in each replicate divided by the number of testing days ($n = 6$).

The mussel, *Mytilus* sp., 48-h embryo development bioassay followed the US Environmental Protection Agency (1995) protocol. Gravid mussels were obtained from Taylor Shellfish Farms (Seattle, WA, USA) and were induced to spawn by placing them in seawater at 20 °C. Gametes were collected and examined microscopically to evaluate viability and quality and then used to prepare freshly fertilized embryos. The test was initiated with the random inoculation of approximately 150 to 300 embryos into each 30 mL vial containing 10 mL of HEWAF dilution, with 4 replicates/dilution. Potassium chloride dilutions (0.5, 1, 2, 3, and 4 g/L) were used as a reference toxicant. The vials were loosely covered with a plastic lens cover and placed in an incubator to maintain a water temperature of 15 °C. After 48 h, embryos were fixed by the addition of 5% glutaraldehyde and then examined microscopically to determine the percentage of embryos exhibiting normal development. Normal development was considered when mussels reached the D-hinge stage. Those that had misshapen or malformed shells were also considered normal if they had completed development.

The fish, *M. beryllina*, larval 7-d survival and growth bioassay followed the US Environmental Protection Agency (2002) protocol. Organisms were obtained from a commercial supplier (Aquatic Indicators, St. Augustine, FL, USA) and were

maintained at 25 °C in seawater. There were 4 replicates for each treatment, consisting of 400 mL of HEWAF dilution in a 600-mL glass beaker. Ten-10-d old larval fish were randomly allocated to each replicate. Potassium chloride dilutions (0.5, 1, 1.25, 1.5, and 2 g/L) were used as a reference toxicant. Each replicate was fed freshly hatched brine shrimp nauplii ad libitum twice daily. Approximately 80% of the test solution was replaced daily with freshly prepared HEWAF after mortality was noted and dead fish were removed. The number of live fish was recorded at test termination, and the dry weight was determined. The total dry weight was divided by the initial number of fish/replicate ($n = 10$) to determine biomass and by the surviving number of fish/replicate to determine the dry weight value.

Field water sampling

Unified Command response staff collected surf zone water samples from 9 locations from 20 May to 20 July 2015 (-Figure 1) by wading into the surf zone and filling a 1-L amber glass bottle for PAH analysis and a 1-L amber glass bottle for TPH analysis. The NRDA staff collected triplicate surf water samples on 27 May 2015 at Gaviota State Beach, and Refugio State Beach (Figure 1). On 17 June 2017, single surf zone water samples were collected at these locations. Samples were collected in 1-L amber glass bottles for PAH analysis, immediately placed on ice, and then transported to the laboratory for analysis.

The NRDA staff also collected intertidal beach porewater samples at up to 12 locations on 29 May, 10 June, 23 August, 8 September 2015 and 27 to 28 June 2017 (Figure 1). At each location, 3 sampling pits spaced 10 m apart were dug in the intertidal zone of the beach. Tidal elevation for these pits was selected to be landward of the saturated zone or effluent line but above the swash zone. Porewater was allowed to seep into the newly dug sampling pit for several minutes before collecting it in a precleaned 250-mL glass jar. Porewater from each of the 3 sampling pits was composited into a 1-L amber glass bottle. Oil sheen in the sampling pits was noted, if present, but was not specifically avoided when sampling.

Chemical analyses

Unified Command response surf zone water samples were analyzed by Pace Analytical® (Los Angeles, CA, USA) or GCAL Analytical Laboratories (Baton Rouge, LA, USA). By using US Environmental Protection Agency (2021) method 8272SIM, extracts were analyzed for 37 PAHs: naphthalene (N0), 1-methylnaphthalene, 2-methylnapthalene (summed as N1), C2-naphthalenes (N2), C3-naphthalenes (N3), C4-naphthalenes (N4), acenaphthylene (AY), acenaphthene (AE), fluorene (F0), C1-fluorenes (F1), C2-fluorenes (F2), C3-fluorenes (F3), anthracene (A0), phenanthrene (P0), C1-phenanthrene/anthracenes (PA1), C2-phenanthrene/anthracenes (PA2), C3-phenanthrene/anthracenes (PA3), C4 phenanthrene/anthracenes (PA4), fluoranthene (FL0), pyrene (PY0), C1-fluoranthene/pyrenes (FP1), C2-fluoranthene/pyrenes (FP2), C3-fluoranthene/pyrenes (FP3), benz[*a*]anthracene (BA0), chrysene (BC0), C1-chrysenes (BC1), C2-chrysenes (BC2), C3-chrysenes (BC3), C4-chrysenes (BC4), benzo[*b*]fluoranthene (BBF), benzo[*k*]fluoranthene (BJKF), benzo[*e*]pyrene (BEP), benzo[*a*]pyrene (BAP), perylene (PER), indeno(1,2,3-c,d)pyrene (IND), dibenz[*a,h*]anthracene (DA), and benzo[*ghi*]perylene (GHI), When we calculated the total PAH concentration (PAH_{37}), nondetects were assumed to be zero. Diesel (C_{10}–C_{28}) and motor oil (C_{24}–C_{36}) range TPH was analyzed by US Environmental Protection Agency (2019) method 8015. These ranges were summed to estimate TPH, assuming nondetects were equal to the detection limit (0.02–0.025 mg/L).

The California Department of Fish and Wildlife, Water Pollution Control Laboratory (Rancho Cordova, CA, USA) analyzed NRDA surf zone water, intertidal beach porewater and HEWAF samples for 45 PAHs by US Environmental Protection Agency (2021) method 8272SIM. In addition to the 37 PAHs just listed, biphenyl (B), dibenzothiophene (DBT0), C1-dibenzothiophenes (DBT1), C2-dibenzothiophenes (DBT2), C3-dibenzothiophenes (DBT3), C4-fluoranthene/pyrenes (FP4), C1-dibenz[*a,h*]anthracenes (DA1), C2-dibenz[*a,h*]anthracenes (DA2), and C1-dibenz[*a,h*]anthracenes (DA3) were measured, and C1-napthalenes (N1) were reported as one value. When we calculated the total PAH concentration (PAH_{45}), nondetects were assumed to be zero.

A prebioassay experiment was conducted to evaluate the relationship between PAH_{45} and TPH in the HEWAF, and to evaluate changes in HEWAF PAH concentrations between the daily renewals. Two HEWAFs were prepared, diluted to 5 nominal PAH_{45} concentrations (500, 100, 10, 1, and 0.5 μg/L), with a seawater control, and analyzed for TPH (C_{20}–C_{36} in mg/L) and PAHs (PAH_{45} in μg/L). One set of dilutions was placed in the *E. analoga* experimental chambers and incubated at 15 °C for 24 h. The second set of dilutions was placed in *M. beryllina* experimental chambers and incubated at 25 °C for 24 h. After 24 h, the HEWAF dilutions were analyzed for PAHs. Regression equations, using measured PAH_{45} and TPH concentrations, were used to estimate bioassay endpoints based on TPH ($TPH = 0.0176 \times PAH_{45} + 0.5019$ for 15 °C and $TPH = 0.087 \times PAH_{45} + 0.1522$ for 25 °C). For the bioassay HEWAF chemistry data, the grand mean of the ratio of PAH_{37} to PAH_{45} was 0.84. This ratio was applied to adjust PAH_{45} bioassay endpoints to PAH_{37} equivalents.

Statistical analyses

Statistical analyses of bioassay data were performed using CETIS® statistical software (TidePool Scientific). Treatments that exhibited a significant reduction in survival were excluded from hypothesis testing for sublethal endpoints. Daily PAH_{45} concentrations were averaged over the bioassay duration for dose–response modeling.

RESULTS

Effects of Refugio Beach oil HEWAF on survival and growth

Early life history stages of the 3 species were sensitive to the Refugio Beach oil, but results varied among taxa. Percentages

TABLE 1: *Mytilus* sp. percentage normal development and *Emerita analoga* and *Menidi beryllina* mortality results for high-energy water accommodated fraction (HEWAF) dilutions[a]

	Mytilus sp.		*E. analoga*		*M. beryllina*	
Nominal PAH_{45} (μg/L)	HEWAF PAH_{45} (μg/L)[b]	% Normal embryos	HEWAF PAH_{45} (μg/L)[b]	Mean % mortality	HEWAF PAH_{45} (μg/L)[b]	Mean % mortality
Control[c]	0.01	90.2	0.01	0.0	0.01	12.5
0.5	0.61 ± n.c.	89.7	0.59 ± 0.05	0.0	0.59 ± 0.04	15.0
1	1.08 ± n.c.	89.3	1.12 ± 0.07	2.5	1.12 ± 0.06	7.5
5	6.03 ± n.c.	88.9	5.86 ± 0.32	2.5	5.87 ± 0.29	7.5
10	11.24 ± n.c.	87.5	11.87 ± 0.92	2.5	11.85 ± 0.84	7.5
50	61.66 ± n.c.	86.3	61.69 ± 2.99	57.5*	61.76 ± 2.73	40.0*
100	123.47 ± n.c.	85.5*	125.71 ± 4.83	100.0*	125.96 ± 4.46	87.5*
500	668.65 ± n.c.	0.0*	676.45 ± 25.56	100.0*	677.06 ± 23.39	100.0*
EC/LC20(CL)	208 (183–223)		NA		50.9 (31–63)	
EC/LC50 (CL)	381 (365–390)		40.9 (32–55)		75.6 (60–89)	

[a]Polycyclic aromatic hydrocarbon (PAH) concentrations are the sum of 45 analytes (PAH_{45}). Results are presented as the mean ± standard deviation (μg/L).
[b]First-day concentration for *Mytilus*, 6-d average for *E. analoga*, and 7-d average for *M. beryllina* of daily high-energy water accommodated (HEWAF) PAH_{45} concentrations.
[c]The LC20 and LC50 values based on the blended control were within the confidence limits of those calculated using the seawater control and were not included.
*Significantly different from seawater control at $p < 0.05$.
EC/LC20 = 20% effect/lethal concentration; EC/LC50 = median effect/lethal concentration; CL = 95% lower and upper confidence limits; NA = not applicable, unable to calculate; n.c. = not calculated.

of normal development (*Mytilus* sp.) and survival (*E. analoga* and *M. beryllina*) are presented in Table 1. At the highest HEWAF concentration, 100% mortality occurred within 48 h for inland silverside (Supplemental Data, Figure S1) and within 24 h for the sand crab (Supplemental Data, Figure S2). Reference toxicant (KCl) median lethal concentration (LC50) values for *Mytilus* sp. (2.1 g/L) and *M. beryllina* (1.4 g/L) were within the established response range for these species, indicating species were responding to toxic stress in a typical manner. For *E. analoga*, the KCl LC50 was 0.8 g/L, but an established response range was not available for this species. Water quality parameters were within acceptable limits (Supplemental Data, Tables S1–S3).

For sand crabs, in those treatments that did not have a significant reduction in survival (0.59–11.87 μg/L PAH_{45}), mean dry weight, mean dry biomass, and incremental growth did not differ from the seawater control (Supplemental Data, Figures S3–S5). In the treatment with partial mortality (61.69 μg/L PAH_{45}), there was no significant difference in mean dry weight but mean individual length (Supplemental Data, Figure S6), biomass, and incremental growth were significantly reduced, compared with the control. Mean emergence (i.e., sand crabs not burrowed in sand substrate), was also significantly higher at this concentration level (Supplemental Data, Figure S7). For *M. beryllina*, decreases in mean dry weight (median effect concentration [EC50] = 57.0 μg/L PAH_{45}) and mean dry biomass were observed. However, there was an interrupted concentration response between the 1.12- and 11.85-μg/L PAH_{45} treatments (Supplemental Data, Figures S8 and S9). All bioassay results are provided in a report in the Refugio Beach Oil Spill Administrative Record (Refugio Beach Oil Spill Trustees, 2021).

Daily HEWAF PAH_{45} concentrations were consistent within each dilution, varying between 3 and 8% over the duration of the bioassays (Supplemental Data, Table S4). In the prebioassay experiment conducted, PAH_{45} concentrations in freshly made HEWAF compared with the HEWAF after 24 h showed a 21 to 38% loss at 15 °C and a 32 to 64% loss at 25 °C between the 5 dose levels (Supplemental Data, Table S5). The PAH composition of freshly made HEWAF (Figure 2 and Supplemental Data, Table S6) was predominated by naphthalenes, and to a lesser extent dibenzothiophene and phenanthrenes. Observed losses within 24 h in the prebioassay experiment were primarily due to volatilization of naphthalenes (Supplemental Data, Table S7). Porewater and surf water collected 9 to 10 d after the spill showed lower percentage composition in naphthalenes, compared with the HEWAF, but similar dominance of the lower molecular weight phenanthrenes and dibenzothiophenes (Figure 2), consistent with weathering. Polycyclic aromatic hydrocarbon composition in porewater and surf water samples, collected 2 yr after the spill, showed a lower percentage of PAHs compared with the initial water samples. Higher molecular weight PAH composition was more dominant in samples up to 2 yr after the spill, indicating oil weathering (Supplemental Data, Figure S10).

Comparison of bioassay results with surf zone water and beach porewater chemistry

Surf zone water was not sampled consistently until 5 d after the spill. Weekly ranges of surf zone water PAH_{37} and TPH concentrations from the western (Gaviota) to the eastern (Arroyo Burro) extent of the sampled area during the first 4 wk after the spill were calculated (Table 2). All chemistry data are available on the National Oceanic and Atmospheric Administration Data Integration Visualization Exploration and Reporting (DIVER) website, as described in the Refugio Beach Oil Spill Damage Assessment and Restoration Plan (Refugio Beach Oil Spill Trustees, 2021). At each sampling location, the maximum PAH_{37} concentration observed occurred during the first 2 wk after the spill, with the highest concentration measured at Corral Canyon (73 μg/L), an observed oil collection point approximately 4.4 km east of the release point. This maximum

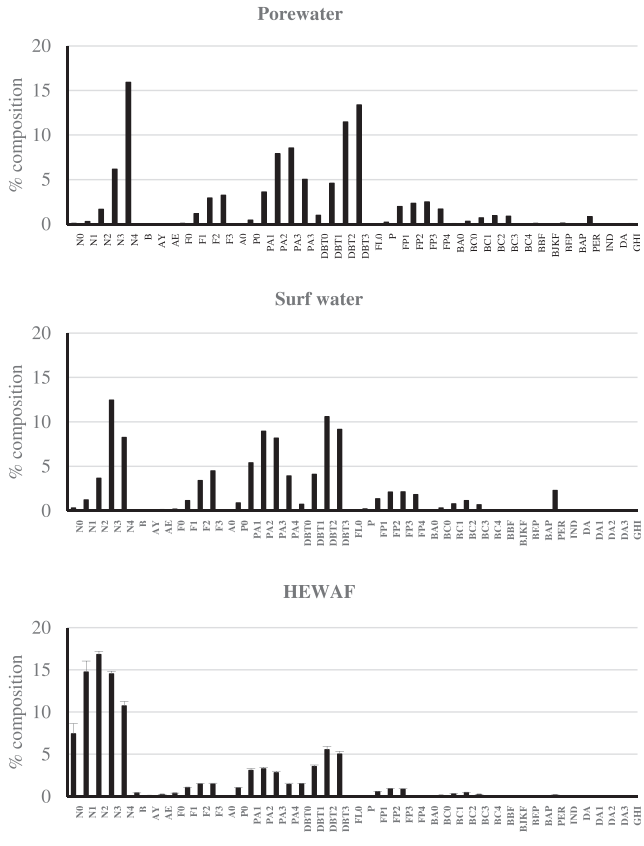

FIGURE 2: Percentage of polycyclic aromatic hydrocarbon (PAH) composition in beach porewater (top) and surf zone water (middle) collected from Refugio State Beach on 29 and 27 May 2015 (PAH_{45} = 10–13 μg/L), respectively, and compared with mean percentage composition (± standard deviation) of high-energy water accommodated fractions (HEWAFs) of Refugio Beach oil (PAH_{45} = 50 μg/L; n = 7). N0 = naphthalene; N1 = summed 1-methylnaphthalene and 2-methylnapthalene; N2 = C2-naphthalenes; N3 = C3-naphthalenes; N4 = C4-naphthalenes; B = biphenyl; AY = acenaphthylene; AE = acenaphthene; F0 = fluorine; F1 = C1-fluorenes; F2 = C2-fluorenes; F3 = C3-fluorenes; A0 = anthracene; P0 = phenanthrene; PA1 = C1-phenanthrene/anthracenes; PA2 = C2-phenanthrene/anthracenes; PA3 = C3-phenanthrene/anthracenes; PA4 = C4 phenanthrene/anthracenes; DBT0 = dibenzothiophene; DBT1 = C1-dibenzothiophenes; DBT2 = C2-dibenzothiophenes; DBT3 = C3-dibenzothiophenes; FL0 = fluoranthene; P = pyrene; FP1 = C1-fluoranthene/pyrenes; FP2 = C2-fluoranthene/pyrenes; FP3 = C3-fluoranthene/pyrenes; FP4 = C4-fluoranthene/pyrenes; BA0 = benz[*a*]anthracene; BC0 = chrysene; BC1 = C1-chrysenes; Bc2 = C2-chrysenes; BC3 = C3-chrysenes; BC4 = C4-chrysenes; BBF = benzo[*b*]fluoranthene; BJKF = benzo[*k*]fluoranthene; BEP = benzo[*e*]pyrene; BAP = benzo[*a*]pyrene; PER = perylene; IND = indeno(1,2,3-c,d)pyrene; DA = dibenz[*a,h*]anthracene; DA1 = C1-dibenz[*a,h*]anthracenes; DA2 = C2-dibenz[*a,h*]anthracenes; DA3 = C1-dibenz[*a,h*]anthracenes; GHI = benzo[*ghi*]perylene.

concentration exceeded the PAH_{37} adjusted Refugio Beach oil LC50 for *E. analoga* (34 μg/L) and *M. beryllina* (64 μg/L; Figure 3). Surf zone water samples collected in the first 2 wk after the spill exceeded these levels more frequently closest to the release point, declining with distance and time (Figure 3 and Table 2). Sampling occurred much less frequently 5 to 9 wk after the spill at the 9 locations. Surf zone water PAH_{37} concentrations at all the sampled locations ranged from not detected to 6.4 μg/L at Arroyo Burro during the 5- to 9-wk time period. Approximately 2 yr after the spill, surf water PAH_{45} concentrations ranged from 0.67 μg/L at Gaviota State Beach to 0.07 μg/L at Refugio State Beach.

Concentrations of TPH in surf zone water were not directly correlated to PAH_{37} concentrations because they were collected as separate samples and surf water was likely heterogeneous. The highest TPH concentration was measured at El Capitan (697 mg/L) 3 d after the spill (Table 2). The Refugio Beach oil LC50s, based on TPH (C_{20}–C_{36}), were 3.4 mg/L for *E. analoga* and 6.7 mg/L for *M. beryllina*. Table 2 presents TPH as the sum of diesel (C_{10}–C_{28}) and motor oil (C_{24}–C_{36}) ranges. When just the motor oil range surf water TPH concentrations were compared with the Refugio Beach oil LC50 values, exceedances for *M. beryllina* occurred at Refugio State Beach, Corral Canyon, and El Capitan during the first 2 wk after the spill, extending into the third week at Corral Canyon. Exceedances of the Refugio Beach oil LC50 for *E. analoga* occurred during the first 2 wk after the spill at Gaviota, Tajiguas, and Haskells, but not at points with greater distance from the release point. Motor oil range TPH averaged 64% of the total TPH in the surf water samples that exceeded LC50 benchmarks.

Concentrations of intertidal beach porewater PAH_{45} were within the range of surf water samples and showed a similar spatial and temporal pattern (Figure 4). Highest concentrations were measured during the first 2 sampling periods (11 and 23 d post spill) at beaches closer to the spill release point. The maximum porewater concentration was measured at Corral Canyon (41 μg/L), a site that was not sampled until 77 d after the spill. This concentration exceeded the *E. analoga* LC50 from the Refugio Beach oil bioassay (Figure 3). Two yr after the spill, porewater PAH concentrations were lower and comparable across sites, ranging from 0.05 to 0.3 μg/L.

DISCUSSION

Very few published studies have evaluated the toxicity of Monterey Formation crude oil to fish and invertebrates (see Straughan and Hadley 1978; Spies and Davis 1982). Although it is difficult to compare the present results with those of these older studies due to differences in analytical methods and exposure conditions, comparisons may be made with recent studies with other crude oils. The *M. beryllina* PAH_{45} LC50 (75.6 μg/L) in the present study was within the range of the PAH_{41} inland silverside and topsmelt larvae 96-h LC50s (30.3–128.2 μg/L) for 5 crude oils (Gala et al. 2001). The PAH_{45} LC50 (40.9 μg/L) for *E. analoga* from the present study was slightly higher than the range of *Americamysis bahia* 96-h PAH_{41} LC50s (19–36.2 μg/L) for 3 crude oils (Gala et al. 2001). Vignier et al. (2015) reported that the PAH_{50} EC50 for oyster (*Crassostrea virginica*) embryo abnormality was 342 μg/L for *Deepwater Horizon* crude oil, similar to the *M. edulis* PAH_{45} EC50 (381 μg/L) in the present study. On a TPH basis, the *M. beryllina* LC50 (6.7 mg/L) was within the range of 96-h inland silverside LC50s for a variety of crude oils (0.8–15.6 mg/L; Clark et al. 2001; Rhoton et al. 2001; Fuller et al. 2004; Hemmer et al. 2011). The *E. analoga* TPH LC50 (3.4 mg/L) was within the same order of magnitude as that reported by Barron et al. (1999) for a middle distillate petroleum mixture (7.1 mg/L) and was

TABLE 2: Ranges of surf zone water polycyclic aromatic hydrocarbon (PAH_{37}) and total petroleum hydrocarbon (TPH) concentrations 1–4 wk after the Refugio Beach Oil Spill

	PAH_{37} (μg/L)[a] Max Min *No.*				TPH (mg/L)[b] Max Min *No.*			
Station (distance from release, in km)	Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	Week 3	Week4
Gaviota[c] (12.9)	11.91	0.31	0.48	0.10	0.34	4.90	0.09	0.05
	ND	ND	ND	ND	0.05	0.05	0.05	0.04
	4	*7*	*7*	*7*	*4*	*7*	*7*	*7*
Tajiguas[c] (1.5)	13.27	14.87	1.86	2.31	5.50	4.60	0.10	16.60
	ND	ND	ND	ND	0.05	0.05	0.05	0.0
	4	*7*	*5*	*7*	*4*	*7*	*5*	*7*
Refugio[d] (1.2)	1.49	2.74	1.23	0.32	3.70	11.90	0.38	1.58
	0.45	ND	ND	0.02	0.26	0.05	0.09	0.05
	4	*7*	*5*	*6*	*4*	*7*	*5*	*6*
Corral Canyon[d] (4.4)	7.10	73.21	5.94	1.78	21.90	19.41	63.10	3.32
	1.50	1.02	ND	0.14	0.62	3.30	0.27	0.20
	4	*5*	*5*	*7*	*4*	*5*	*6*	*7*
El Capitan[d] (5.8)	21.11	5.96	1.50	0.98	697.00	1.16	2.07	2.30
	ND	ND	ND	ND	0.42	0.08	0.09	0.08
	6	*7*	*7*	*7*	*6*	*7*	*7*	*7*
Haskells[d] (15.8)	0.60	9.13	1.93	0.69	1.02	4.80	6.90	2.42
	ND	ND	ND	ND	0.05	0.05	0.09	0.05
	4	*7*	*7*	*7*	*5*	*7*	*7*	*7*
Coal Oil Point[d] (19.8)	5.60	0.28	1.37	0.41	1.96	0.38	2.28	0.20
	0.04	ND	ND	ND	0.05	0.05	0.05	0.05
	5	*7*	*7*	*7*	*5*	*7*	*7*	*7*
Goleta[d] (23.6)		5.27	1.41	0.28		0.57	1.90	0.43
		ND	ND	ND		0.07	0.08	0.04
	0	*6*	*7*	*7*	*0*	*6*	*7*	*7*
Arroyo Burro[d] (32.0)	1.65	0.91	0.58	0.30	0.05	0.12	0.83	5.10
	0.14	ND	ND	ND	0.05	0.05	0.05	0.05
	3	*7*	*7*	*7*	*3*	*7*	*7*	*7*

[a]PAH_{37} assumed ND = 0.
[b]Sum of diesel and motor oil ranges, detection limit used for nondetect values.
[c]West of release point.
[d]East of release point.
ND = not detected; *No.* = sample size.

comparable to mysid LC50s for a variety of crude oils (Barron et al. 2013). Our results contribute to the evaluation of Monterey Formation crude oil toxicity to early life stages of nearshore fish and invertebrates, indicating it ranks within the range reported for other crude oils.

The relative sensitivity of the species tested was consistent with previous reports that crustaceans, such as *E. analoga*, are more sensitive to the effects of crude oil, compared with fish (Barron et al. 2013) and mollusks (Stefansson et al. 2016), in these early life stage bioassays. Mysids (*A. bahia*) are a

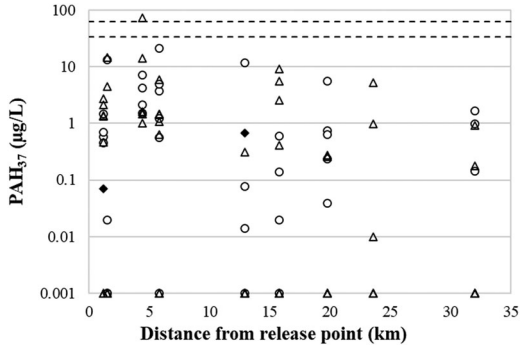

FIGURE 3: Surf zone water polycyclic aromatic hydrocarbon $(PAH)_{37}$ (μg/L) concentrations measured 1 wk (open circle), 2 wk (open triangle), and 2 yr (solid diamond) after the spill at varying distance from the spill release point (km). Data reported as PAH_{37} of 0.001 μg/L are considered nondetect because the detection limit was set to zero. *Emerita analoga* PAH_{37} median lethal concentration (LC50; 34 μg/L) and *Menidia beryllina* LC50 (64 μg/L) are shown as dashed lines; the *Mytilus* sp. LC50 (381 μg/L) is not shown on the figure.

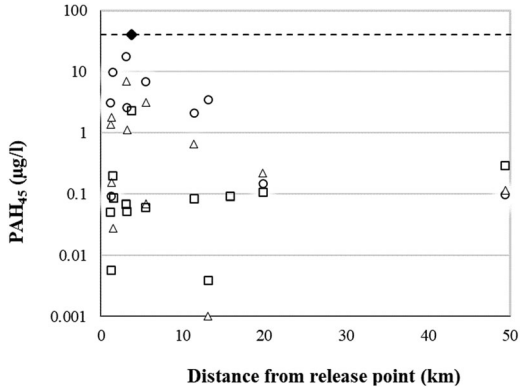

FIGURE 4: Beach porewater polycyclic aromatic hydrocarbon $(PAH)_{45}$ (μg/L) concentrations measured 11 (open circle), 23 (open triangle), 77 (solid diamond), and 113 (open square) d after the spill from sandy beaches with varying distance from the spill release point (km). *Emerita analoga* PAH_{45} median effect concentration (EC50; 41 μg/L) is shown as a dashed line.

standard bioassay species native to coastal estuaries and embayments of the eastern United States and have been widely used to evaluate petroleum toxicity (US Environmental Protection Agency 2002; Hemmer 2011). However, in the present study, *E. analoga* proved to be sufficiently sensitive to evaluate the effects of crude oil on sandy beach invertebrates. *Emerita analoga* and potentially other Hippidae crabs should be considered a suitable monitoring or bioassay species for the evaluation of the effects of oil spills that impact sandy beaches. Similarly, HEWAF exposures were representative of surf zone conditions that sand crabs would experience during an oil spill.

Trends in surf zone water PAH concentrations were consistent with shoreline oiling observations and oil trajectory modeling for the spill. Oiled shoreline was initially observed between Tajiguas and El Capitan State Beach, but within the first week it extended several miles eastward and then southward (MacFadyen 2017). Forensic analysis of NRDA surf zone water samples from May 2015 at Refugio State Beach indicated the chemical composition was consistent with Refugio Beach oil and not natural seep oil (Stout 2016). Declines in PAH water concentrations with distance from the release point and over time have been noted following other marine oil spills (Boehm et al. 2007, 2011). In the *Deepwater Horizon* oil spill, the maximum PAH_{50} concentration measured was 146 000 μg/L, and concentrations were <1.0 μg/L 15 to 20 miles away (Boehm et al. 2011). Although the maximum measured PAH_{45} concentration in surf zone water was lower (73 μg/L) during the spill, it is likely that peak oil concentrations were not measured because samples were not taken during the period of time in which thick oil was observed in the surf zone immediately after the spill. As has been noted for other oil spills, it can be challenging to obtain an adequate set of data to evaluate spatial and temporal trends (Boehm et al. 2011), especially considering the dynamic nature of the surf zone. Concentrations of PAHs in intertidal porewater, although lower than surf zone water peak concentrations, showed similar spatial and temporal trends. Intertidal beach porewater concentrations were generally less variable than those in surf zone water and persisted at detectable quantities for a long period of time after the oil spill, indicating its utility as an environmental monitoring media for sandy beach ecosystems (Refugio Beach Oil Spill Trustees 2021).

Following the Refugio oil release, hundreds of adult invertebrates, including sand crabs, were found coated with oil or dead on the beaches. At least 15 genera of adult fish washed up dead onto the shoreline. Because the spill occurred in the spring, early life stages of many fish and invertebrates were present in the surf zone and intertidal zones of beaches and rocky shores. The bioassays selected in the present study provided a means to assess impacts to these sensitive life stages by comparing surf zone water and beach porewater PAH concentrations with Refugio Beach oil LC50s. Exceedances of *E. analoga* megalopae and *M. beryllina* larval LC50s indicated that surf zone and beach porewater PAH concentrations during the first 2 wk after the spill were potentially lethal to fish and invertebrate early life stages.

In a recent literature review, Lee et al. (2015) reported that the PAH EC50 and LC50s ranged from 0.3 to 60 μg/L and from 0.03 to 11 mg/L on a TPH basis. The PAH-induced fish embryotoxicity such as pericardial and yolk sac edemas, and craniofacial, spinal, and cardiac deformities, have been reported to occur at the lower end of that range (0.3 μg/L; Incardona et al. 2015). Hodson (2017) concluded that PAH concentrations >0.1 μg/L following oil spills should be considered hazardous. Although the species and life stages tested in the present study provided valuable insight into the effects of the spill, other sensitive species, life stages, and ultraviolet light exposure conditions in this unique ecosystem may not have been adequately represented.

The impacts of oil spills on sandy beaches have not been widely evaluated (Bejarano and Michel 2016), creating gaps in our understanding of injury to these ecosystems. During the spill, field assessments of impacts to populations of selected sandy beach invertebrates and nearshore fish were conducted (Refugio Beach Oil Spill Trustees 2019). Inclusion of laboratory bioassays with a variety of species and surf zone water and intertidal beach porewater chemistry provided a valuable bridge to results of these field studies. Our toxicity test results have improved understanding of the acute effects of Monterey Formation crude oil and will inform future natural resource damage assessments.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.5148.

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Data Availability Statement—Data pertaining to this manuscript are deposited at: https://www.diver.orr.noaa.gov/web/guest/refugio-beach. Data, associated metadata, and calculation tools are also available from the corresponding author (Michael.Anderson@wildlife.ca.gov).

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