Measuring Laboratory Exposure to Polycyclic Aromatic Hydrocarbons (PAH) from Oil Spills Using Talitrid Amphipods

Final Report
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Introduction

Following the Refugio Beach Oil Spill in 2015, some sandy beach invertebrates accumulated notable concentrations of polycyclic aromatic hydrocarbons (PAH). To strengthen oil spill injury assessment in sandy beach habitats, better knowledge of oil exposure routes and effects on sandy beach organisms is needed. Talitrid amphipods, also known as beach hoppers, include any member of the Talitridae family under the order Amphipoda. Although talitrids are crucial to beach ecology, very little is known about background PAH concentrations in talitrids, or the primary environmental exposure routes during spill events. Measurements of significant PAH uptake following the Refugio Beach Oil Spill event in southern California suggest that these invertebrates could be useful for monitoring oil spill impacts on sandy beaches (Refugio Beach Oil Spill Trustees, 2021).

Oil spill events that impact beach environments affect not only the sand, but stranded kelp and sea grass, or beach wrack. Oiled beach wrack is often the target of beach clean-up efforts, but removal can cause negative effects on wrack-associated biota, such as talitrids, which play a vital role in beach ecology. Talitrids are instrumental in the breakdown of wrack material and the nutrient cycling that occurs as a result (Dugan et. al, 2011). In addition, avian species (e.g., Sandpiper, *Calidris mauri*; Western Snowy Plover, *Charadrius nivosus*) often rely on talitrids as a food source.

Because talitrids have not previously been used in laboratory exposure studies, the route of exposure and the effect of oiled sand versus oiled wrack on PAH uptake by talitrids is unknown. The current study used novel methods to dose these organisms with crude oil and elucidate the primary route of exposure to PAHs. Determining the primary source of PAH exposure will inform researchers performing future experiments to evaluate other processes, such metabolism and depuration, and effects on survival, growth, reproduction, or other endpoints.

Methods

Test Organisms

Talitrids (*Megalorchestia pugettensis*) were collected at Asilomar State Beach, California on three occasions for experiments conducted at the University of California Davis Marine Pollution Studies Laboratory (MPSL). All collections were conducted by California Department of Fish and Wildlife personnel and collaborators from the University of California Santa Barbara. Talitrids were collected on August 31, October 4, and October 28, 2018, for a range-finding study and two definitive experiments, respectively. Exposure experiments were initiated on the day of collection. Talitrids were collected by excavating sand and sieving it through coarse

mesh. Talitrids were counted into 12-15 two-liter polyethylene jars containing approximately 100 milliliters (mL) of collection site sand and then immediately transported to MPSL.

Experimental Design

Experiments were designed to determine the primary route of exposure of talitrids to PAHs deposited on a beach during an oil spill. Hydrocarbons will be deposited on a beach after a spill in two forms - as oil, or oil dissolved in the surrounding water. Oiled sand and oiled kelp, the latter being their food source, are two possible routes of exposure for talitrids to PAHs. For these experiments, dissolved hydrocarbons were tested in the form of a high-energy water accommodated fraction (HEWAF), which was applied to sand or kelp (described below).

Exposure chambers were 16-liter (L) aquaria containing two liters of site collected sand that had been pre-moistened with 300 mL of MPSL seawater. Embedded in the sand were three inverted crystalizing dishes that served as pedestals for circles of fresh kelp (*Macrocystis pyrifera* collected at MPSL) weighing approximately 45 grams (Figure 1). Treatments consisted of three replicate aquaria of either oiled sand, oiled kelp, or both. Unoiled treatments served as controls. Test conditions are summarized in Table 1.

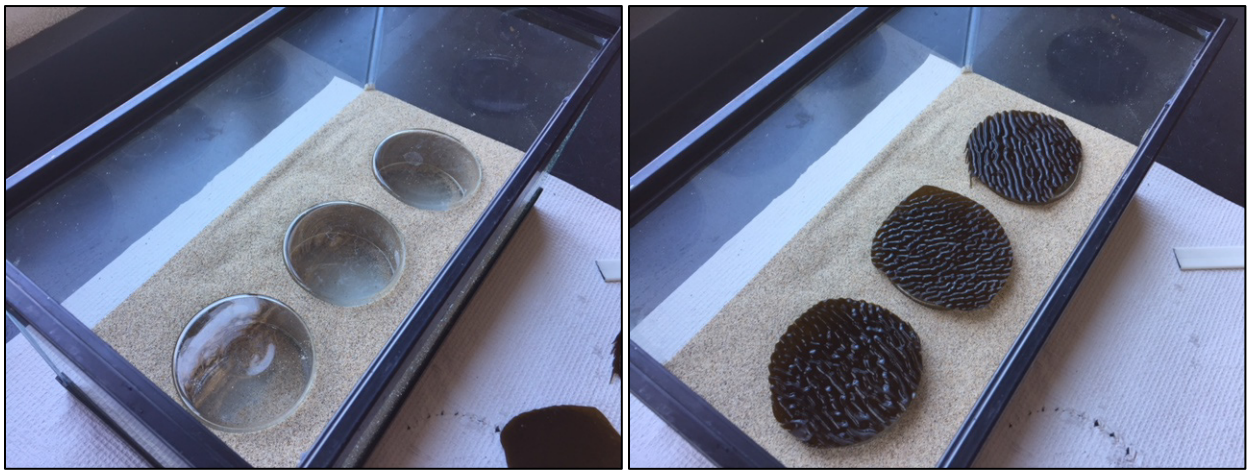


Figure 1. Exposure chamber with sand and inverted crystalizing dishes with and without kelp

High-Energy Water Accommodated Fraction (HEWAF) Preparation and Application

HEWAF was prepared using a standard method used in post Deepwater Horizon studies, described in Carney et al. 2016. The fraction was prepared in a Waring® CB15 commercial food blender (or equivalent). Blender lid was lined with aluminum foil to prevent oil contact with anything other than the stainless-steel pitcher, or the foil-lined lid. HEWAF was prepared by

adding 3.75L of 1 micron (µm) filtered MPSL seawater to the blender, followed by 3.75 grams of oil (1 gram per liter [g/L] loading).

Crude oil was sourced from Platform Irene (referred to as Monterey Formation Crude Oil), and was collected on July 18, 2018, from Freeport-McMoRan oil and gas field plant in Lompoc, California. The collected oil was free of produced water, but is known to contain paraffins and hydrogen sulfide, and was collected directly from a tank spigot.

Table 1. Summary of exposure conditions.

Parameter	**Conditions**
Test Organism	Talitrid amphipods (*Megalorchestia pugettensis*)
Source	Asilomar State Beach (Field collected by OSPR and UCSB)
Life Stage	Adult
Organisms per Replicate	100 (~30 grams) for the range-finding experiment and 125 (~38 grams) individuals for the definitive experiments.
Exposure Chambers	16 L Glass aquaria (20.3-centimeter (cm) (Width) x 40.6 cm (Length), 20.3 cm (Height)) – Three inverted 5 cm (Height) x 10 cm (Depth) crystallizing dishes to serve as platforms for kelp (*Macrocystis pyrifera*). Sand filled in to 5 cm.
Sand Source	Asilomar State Beach sand from talitrid collection site.
Kelp Source	Fresh collected from MPSL or as stranded beach wrack. Kelp was laid flat on three inverted crystalizing dishes in each aquarium (30 grams of kelp per aquarium). Food consumption studies estimate that 100 talitrids are expected to consume 1 grams of kelp in 96 hours.
Chamber Cover	Screen material (1-2 millimeter [mm]) that has been soaked in seawater for 1 hour
Treatments	1) Oiled Sand, 2) Oiled Kelp, 3) Oiled Sand and Oiled Kelp, 4) Control
Replicates per Treatment	1 (range-finding), 3 (definitive)
Oil Source	Platform Irene Oil (Santa Barbara, California)
Oil Preparation	High-Energy Water Accommodated Fraction (1 gram oil/1L seawater)
Temperature	20 ± 2° Centigrade (C)
Light Quality	Wide-spectrum fluorescent lights
Photoperiod	14 Hours Light: 10 Hours Dark
Test Duration	96 Hours
Daily Activities	Observe daylight feeding and burrowing. Record number of dead and remove. Re-hydrate sand with spray bottle of fresh seawater.

Source oil was added using a pre-cleaned and pre-oiled gastight glass syringe. The tare weights and final weights of the syringe were recorded to determine the exact oil loading rate. Oil-water mixture was blended on "low" for 30 seconds, and the contents were transferred to a 4L separatory funnel. HEWAF was allowed to settle and separate for one hour. The bottom layer of the unfiltered HEWAF was collected in a 4L beaker, which was aliquoted into one-liter amber bottles for chemical analysis for PAHs and application to sand and kelp.

Sand in oiled kelp treatments and controls was pre-moistened by adding 300 mL MPSL seawater to 2L of pre-sieved beach sand collected at Asilomar State Beach. The sand/water mixture was prepared in a 4L glass jar and placed on a sediment rolling apparatus. After 30 minutes, a polypropylene spoon was used to hand mix the sand and remove clotted sand from the walls of the jar. The sand/water mixture was then placed on the roller for an additional 30 minutes. Oiled sand treatments were prepared using the same procedure but substituted MPSL seawater with 300 mL of HEWAF. Thoroughly mixed sand was then distributed to the appropriate aquaria for testing. Pre-weighed circles of kelp (~20 cm in diameter) were placed on inverted crystalizing dishes in the oiled sand and control aquaria. For oiled-kelp treatments, kelp circles were submerged in HEWAF, allowed to drip dry for ten seconds, then placed on crystalizing dishes. Approximately 100 talitrids were added to each randomized treatment by inverting a two-liter plastic collection jar containing the test organisms to place them into the assigned aquaria.

Daily Observations and Exposure Termination

The condition of the talitrids was observed and a qualitative measurement of kelp consumption was recorded every day. Dead talitrids were removed and recorded. Kelp and surface sand was re-hydrated daily with fresh MPSL seawater using a spray bottle mister.

At the termination of the exposure, kelp was removed and weighed, and sand was sieved to capture surviving talitrids. Talitrids were placed in foil pouches and preserved for chemical analysis. Subsamples of oiled and unoiled sand and kelp were also saved for analysis of PAHs.

Chemical Analysis

All chemical analyses were conducted at the CDFW Water Pollution Control Laboratory in Rancho Cordova, California. Talitrid samples were rinsed with deionized water prior to homogenization to remove any matrix (i.e., sand) adhering to the test organisms. A Brinkman Polytron Model PT 10-35 was used to homogenize the talitrid samples.

A 10-gram aliquot of the tissue homogenate was extracted using pressurized fluid extraction (EPA 3545A mod), followed by gel permeation (EPA 3640A mod) and silica column (EPA Method 3630) cleanup. Final extracts were analyzed by gas chromatography – mass spectrometry (GCMS) in selected ion monitoring mode (EPA 8270-SIM) for quantitation of individual parent and alkylated PAH compounds. Alkylated PAHs were quantified using the response of the parent PAH. Results were reported as a list of individual compounds/homologue series and as Total PAH_{45}.

The results of the total PAH analysis of talitrid tissue from the three replicates for each treatment group were used to calculate a mean value, and a paired two sample for means t-test was used to compare each treatment with the control.

Exposure Results

Rangefinder Experiment

A range-finding experiment with three oil loadings was conducted to determine if oil exposure would cause significant mortality to talitrids. HEWAF was prepared as described, but at three different nominal loading rates: 0.25 g/L, 0.5 g/L and 1 g/L. Actual loading rates based on measured oil mass were 0.30 g/L, 0.45 g/L and 0.99 g/L.

This range-finding experiment was conducted with single replicates of each treatment concentration and control. No significant effects on survival were observed upon recovery of the talitrids after the 96-hour exposure (Table 2). The initial mass of kelp added to the exposures was not measured, but the final mass was weighed to the nearest tenth of a gram. Kelp appeared to be uniformly consumed among the test concentrations and treatments, with a slightly higher rate of consumption in the control. Except for the talitrids that died, all organisms remained buried during the observation periods.

Definitive Experiments

After the range-finding experiment, two definitive experiments were conducted with a HEWAF dose of 1 g/L. Actual loadings based on measured masses of oil were 1.03 g/L and 1.04 g/L for definitive tests one and two, respectively. No significant adverse effects on the talitrids were observed in the first definitive experiment (Table 3). As with the range-finding experiment, very few talitrids were observed on the surface of the sand during the daily observations. At termination, talitrids in this test batch were much less active during the sieving process than the range-finding test.

Table 2. Survival and kelp mass results from the rangefinder experiment.

Treatment	Final Kelp Mass (gram)	Number of Surviving Organisms	Number of Dead Organisms
Control	3.7	98	2
Kelp 0.25 g/L	6.3	97	1
Kelp 0.5 g/L	5.2	102	1
Kelp 1 g/L	7.3	103	1
Sand 0.25 g/L	7.8	91	6
Sand 0.5 g/L	6.3	95	1
Sand 1 g/L	7.1	92	2

Table 3. Survival and kelp mass results from the first definitive experiment.

Treatment	Net Kelp Consumption (gram)	Mean (SD) Kelp Consumption (gram)	Number of Surviving Organisms	Number of Dead Organisms
Control 1	8.2	8.5 (0.9)	121	1
Control 2	9.6		125	0
Control 3	7.8		119	0
Kelp 1	9.7	10.7 (1.4)	121	1
Kelp 2	12.3		109	3
Kelp 3	10.2		123	2
Sand 1	9.7	12.0 (2.2)	118	3
Sand 2	14.1		122	2
Sand 3	12.3		118	2
Kelp/Sand 1	13.3	10.4 (2.6)	121	0
Kelp/Sand 2	9.6		123	0
Kelp/Sand 3	8.4		133	3

The second definitive exposure included a treatment without talitrids to provide a baseline measurement for kelp weight to compare to kelp consumption in the control and oiled treatments. At the termination of the exposure, kelp was dried for 24 hours at 60 °C, cooled in a desiccator, and weighed to the nearest hundredth gram. Comparisons were made among replicate weights for all treatments with analysis of variance (ANOVA) and post-hoc Tukey-Kramer tests. The control and two of the oiled treatments were significantly different from the baseline, indicating a significant amount of kelp had been consumed during the exposure period (Table 4). Kelp was consumed in the sand treatment, but not a statistically significant amount. A greater amount of kelp was consumed in the control compared to the oiled treatments, but the only statistically significant difference was found with the comparison to

the oiled sand treatment. Differences between the mean kelp mass of the control and those of the oiled treatments indicate that the oil dose limited talitrid feeding behavior.

No significant mortality effects on the talitrids were observed in the oiled treatments in the second definitive experiment (Table 4). Talitrids in this experiment were active during the exposure, but their activity did not follow any pattern based on the treatments (Table 5). Activity increased significantly on Day 3 and further on Day 4. Talitrids were also very active during test termination. Percent moisture content of the sand was measured for each replicate and did not differ significantly among replicates or treatments. Average percent moisture was 8.21 ± 0.61 percent.

Table 4. Survival and kelp mass results from the second definitive experiment. [a] indicates significant difference from baseline treatment. [b] indicates significant difference from the control.

Treatment	Final Kelp Dry Mass (gram)	Mean (SD) Kelp Dry Mass (gram)	Number of Surviving Organisms	Number of Dead Organisms
Control 1	0.44	0.58 (0.30) [a]	111	0
Control 2	0.38		108	0
Control 3	0.93		119	0
Kelp 1	1.50	1.32 (0.16) [a]	111	0
Kelp 2	1.26		113	0
Kelp 3	1.20		119	0
Sand 1	1.17	1.62 (0.48) [b]	116	0
Sand 2	2.05		109	0
Sand 3	1.64		118	0
Kelp/Sand 1	1.82	1.19 (0.56) [a]	117	1
Kelp/Sand 2	1.06		112	0
Kelp/Sand 3	0.72		120	0
Baseline 1	2.49	2.38 (0.11)	N/A	N/A
Baseline 2	2.28		N/A	N/A
Baseline 3	2.38		N/A	N/A

Table 5. Approximate number of talitrids that were active outside their burrows during daily observations of the second definitive exposure.

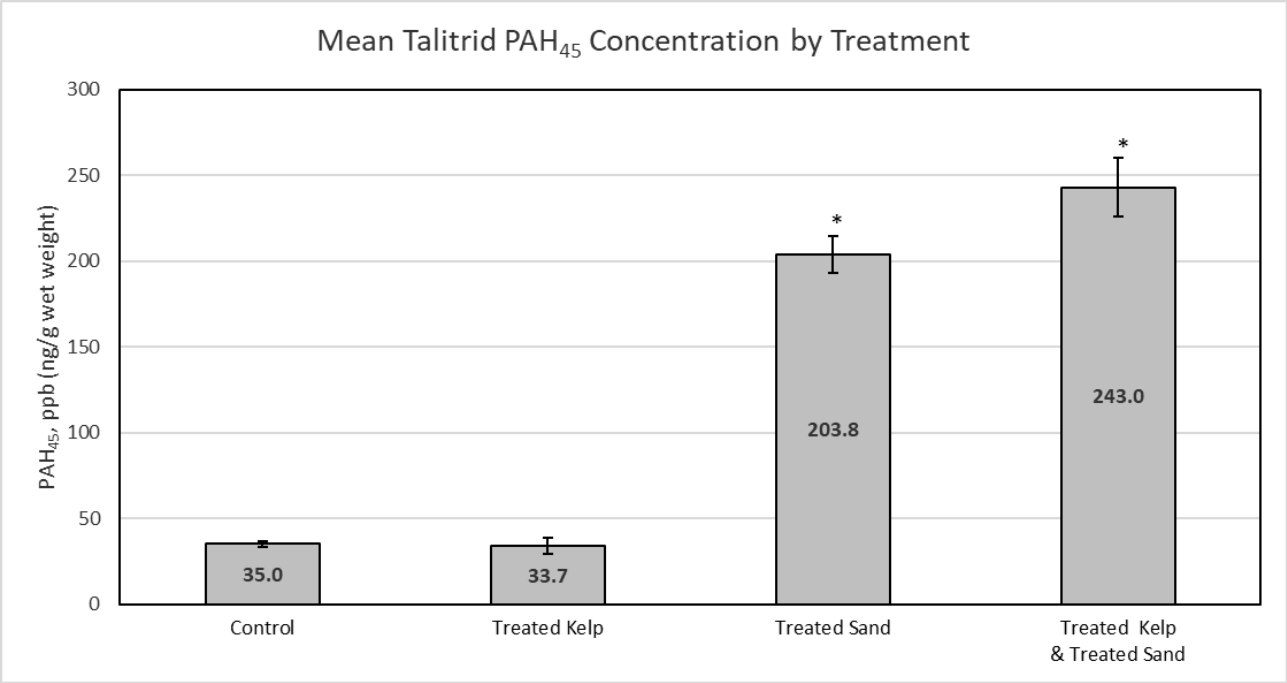
Treatment	Day 1	Day 2	Day 3	Day 4
Control 1	37	9	43	45
Control 2	0	0	2	14
Control 3	0	0	0	24
Kelp 1	0	0	41	38
Kelp 2	2	0	28	22
Kelp 3	0	0	27	55
Sand 1	1	0	27	25
Sand 2	0	0	55	45
Sand 3	0	8	55	33
Kelp/Sand 1	2	19	49	55
Kelp/Sand 2	0	0	9	10
Kelp/Sand 3	0	0	26	17

The concentration of PAHs by talitrids, measured as total PAHs, demonstrated that the oil-treated kelp treatments were not significantly different than the controls. The talitrid concentrations in the oil-treated sand group were significantly different (higher) than those of the control, as were the concentrations in the oil-treated kelp and sand group. Results are in Table 6 and Figure 1.

Table 6. Concentrations of total PAH for each treatment and replicate, in ppb (ng/g). Asterisks indicate significant difference from control. The paired two sample means t-Test result for P(T<=t) one-tail was 0.003 for the oil-treated sand, and 0.004 for the oil-treated kelp and sand.

Replicate	Control (Total PAH_{45} ppb)	Oil Treated Kelp (Total PAH_{45} ppb)	Oil Treated Sand (Total PAH_{45} ppb)	Oil Treated Kelp and Sand (Total PAH_{45} ppb)
1	37.59	26.45	187.0	216.7
2	35.18	42.48	200.6	274.8
3	32.24	32.15	223.7	237.4
Mean	35.00	33.69	203.8*	243.0*

Figure 1. Concentrations of total PAH for each treatment and replicate, in ppb (ng/g). Asterisks indicate significant difference from control.



Conclusions

The results demonstrate that talitrids were not acutely affected by the 1 g/L oil loading concentration. No significant mortality was observed in the oiled treatments. There were significant effects on feeding rate, but further testing is necessary to confirm these effects. Average feeding rates in all oil treatments were substantially lower than those in the control, but only the oiled sand treatment was statistically different (lower) compared to the control.

The PAH residues measured in these experiments suggest that treatment of the sand with oil leads to increased PAH concentrations in talitrid tissue. Oiled sand appears to be the dominant route by which PAHs were taken up by the organisms in these exposures, as the treatment of the kelp alone did not lead to a difference in talitrid PAH concentration, when compared to the control group.

The results of these exposures may allow for more informed answers to questions concerning talitrid exposures following oil spills, and potential impacts on beach ecology and ecosystems.

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