



Baseline

Uptake of polycyclic aromatic hydrocarbons via high-energy water accommodated fraction (HEWAF) by beach hoppers (Amphipoda, Talitridae) using different sandy beach exposure pathways

Bryand M. Duke^{a,*}, Kyle A. Emery^{b,c}, Jenifer E. Dugan^b, David M. Hubbard^b, Bruce M. Joab^d

^a National Oceanic and Atmospheric Administration, St. Petersburg, FL 33701, United States of America

^b Marine Science Institute, UC Santa Barbara, Santa Barbara, CA 93106, United States of America

^c Department of Geography, UC Los Angeles, Los Angeles, CA 90095, United States of America

^d Office of Spill Prevention and Response (OSPR), California Department of Fish and Wildlife, 95605, United States of America

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ABSTRACT

Sandy beach ecosystems are highly dynamic coastal environments subject to a variety of anthropogenic pressures and impacts. Pollution from oil spills can damage beach ecosystems through the toxic effects of hydrocarbons on organisms and the disruptive nature of large-scale clean-up practices. On temperate sandy beaches, intertidal talitrid amphipods are primary consumers of macrophyte wrack subsidies and serve as prey for higher trophic level consumers, such as birds and fish. These integral organisms of the beach food web can be exposed to hydrocarbons by direct contact with oiled sand through burrowing and by the consumption of oiled wrack. We experimentally evaluated the primary polycyclic aromatic hydrocarbon (PAH) exposure pathway via high-energy water accommodated fraction (HEWAF) for a species of talitrid amphipod (*Megalorchestia pugettensis*). Our results indicated that tissue PAH concentrations in talitrids were six-fold higher in treatments that included oiled sand compared to those with only oiled kelp and the controls.

Coastal ecosystems, at the interface between land and sea, are highly dynamic and vulnerable to the consequences of anthropogenic pressures on spatial scales ranging from global to local. Increasing coastal development and exploitation of natural resources puts these ecosystems at growing risk for detrimental impacts, such as pollution (Islam and Tanaka, 2004). For decades, the extraction of crude oil products has resulted in direct and indirect impacts to source and adjacent ecosystems, predominantly due to spills (Barron et al., 2020). Such spills may occur at drilling sites, on transport ships, or along pipelines (Peterson et al., 2003; Beyer et al., 2016; Donohoe et al., 2021). Oil spill effects may be vast and extend across multiple ecosystems, impacting biota from microbes to plants and animals and the ecosystem services and functions they provide (Teal and Howarth, 1984; Kennedy and Cheong, 2013; Brussaard et al., 2016). The effects of oil spills on ecosystems arise immediately and can persist for several years (Kingston, 2002). Due to the highly dynamic nature of coastal ecosystems, it is often difficult to gauge the impacts of spills via sampling after spills or through experimental efforts, especially for sandy beaches (Fegley and Michel, 2021).

Sandy beach ecosystems occupy a narrow intertidal zone between

terrestrial and marine environments and are the predominant coastline type globally (Luijendijk et al., 2018). Beaches are one of a handful of ecosystem types that are largely defined by their sediments. Sand is the principal substrate and habitat in this ecosystem, and it is constantly being transformed and transported by wind, water and organisms (Chapman, 1983; Quartel et al., 2008; Delgado-Fernandez and Davidson-Arnott, 2011). These dynamic ecosystems are impacted by a growing range of threats (Defeo et al., 2009). The natural supply of sand to beach ecosystems, largely through riverine inputs, has been threatened globally by construction of dams and sand mining (Defeo et al., 2009), and mitigation efforts which include beach filling are often detrimental to beach-dwelling organisms (Manning et al., 2014; Viola et al., 2014). Climate change, particularly sea level rise, and societal responses to erosion pose significant and growing threat to beach ecosystems (Dugan et al., 2008; Barnard et al., 2021). Similarly, the pollution of beach sand by toxic substances also has demonstrably negative effects on resident organisms (Defeo et al., 2009; León et al., 2019). For example, the exposure to and uptake of microplastics is strongly coupled to sediment grain size (Costa et al., 2019; Vermeiren et al., 2021) as is

* Corresponding author.

E-mail address: bryand.duke@noaa.gov (B.M. Duke).

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the prevalence of microbial pathogens (Yamahara et al., 2012; Abreu et al., 2016).

Ocean beaches have low levels of in situ primary production, but support complex, multi-trophic ecosystems. In the temperate zone, beach food webs are generally fueled by subsidies from adjacent ecosystems typically consisting of phytoplankton, carrion, and drift macrophytes that wash ashore as wrack (McLachlan and Brown, 2006; Colombini et al., 2003; Hyndes et al., 2022). Highly productive kelp forests are a major source of wrack to temperate beaches. The primary intertidal consumers of kelp wrack in many regions, talitrid amphipods, use freshly deposited wrack as food and habitat (Lastra et al., 2008; Michaud et al., 2019; Emery et al., 2021) and their abundance is highly correlated to the amount of wrack a beach receives (Dugan et al., 2003; Lowman et al., 2019; Page et al., 2021). The delivery of these mostly buoyant allochthonous drift kelp resources to beaches is largely driven by nearshore currents, tidal, and wave processes (Zobell, 1971; Orr et al., 2005; Liebowitz et al., 2016). Invertebrate wrack consumers typically burrow in the sand under and adjacent to wrack deposits along the 24-h high tide line (Dugan et al., 2013; Emery et al., 2022). Talitrid amphipods are adapted to life in a very dynamic environment; these organisms burrow rapidly along the ever-moving drift line (Dugan et al., 2013), are direct-developers that brood their young (Pavesi and De Matthaeis, 2009) and are generalist consumers (Bessa et al., 2014). Because of the zone these organisms inhabit and their dependence on wrack subsidies, their populations are highly vulnerable to development and urbanization (Dugan et al., 2003; Schooler et al., 2019; Jaramillo et al., 2020). Pollution can also have serious consequences for the beach food web. For example, an oil spill can contaminate beach sand as well as drifting kelp wrack, and both the oil and the oiled kelp wrack are likely to deposit along the high tide line of sandy beaches, where a major component of beach animals live (De la Huz et al., 2005; Bejarano and Michel, 2016). This type of disruption to habitat, basal resources and primary consumers is likely to have negative consequences for the beach food web (De la Huz et al., 2005; Michel et al., 2017).

Sandy beach macroinvertebrates can accumulate notable concentrations of polycyclic aromatic hydrocarbons (PAH). Post-settlement sand crabs (*emerita analoga*), a dominant macroinvertebrate species on California open coast beaches, are highly susceptible to PAH toxicity (Barron et al., 1999). This species likely experienced lethal PAH concentrations during the 2015 Refugio oil spill based on laboratory toxicity testing using observed concentrations from surf zone and porewater samples (Donohoe et al., 2021). Collectively, our study and prior research demonstrate PAH uptake by sandy beach macrofauna is mediated by seawater and sand, indicating that these organisms can be useful bio-monitors for oil spills that impact sandy beaches. To strengthen oil spill injury assessment in sandy beach ecosystems, improved knowledge of oil exposure routes and effects on sandy beach biota is needed.

To address the need to better understand exposure pathways, we conducted a laboratory experiment to evaluate the primary route of exposure of high-energy water accommodated fraction (HEWAF) derived PAHs to *Megalorchestia pugettensis*, an intertidal talitrid amphipod species endemic to open coast sandy beaches of the Eastern Pacific coast. Hydrocarbons may deposit on a beach after a spill in two forms, as floating oil or as dissolved oil that is well-mixed and suspended in seawater. Both forms can coat floating or stranded drift kelp and fill the interstitial spaces in the sediment with contaminants. We used a HEWAF because the mixture of oil and seawater is likely the predominant exposure mechanism over larger spatial areas when compared to pure crude oil because of oceanographic and surf-zone processes. Additionally, the HEWAF exposures may persist after the thicker deposits of oil are removed during cleanup operations. Although they inhabit the intertidal zone, beach hoppers generally avoid contact with seawater, undertaking daily migrations to follow the tides. For this reason, we hypothesized two possible primary routes of PAH exposure for talitrids on sandy beaches, 1) contact with oiled sand and 2) contact

with and/or consumption of oiled kelp wrack (food sources). We developed novel methods to expose talitrid amphipods to crude oil to evaluate the primary route of exposure to PAH for these important intertidal taxa.

We conducted two laboratory exposure experiments to assess the relative importance of animal contact with contaminated substrate (sand) and with a contaminated food source (kelp wrack) in the uptake of oil by talitrid amphipods. First, we conducted range finding trials to estimate sublethal hydrocarbon exposure levels. Using those values as a guide we then conducted the experiment to quantify exposure pathways. The exposure experiments were conducted at the University of California, Davis, Marine Pollution Studies Laboratory (MPSL) (Fig. 1).

- 1) Range finding trial: This trial was used to determine appropriate oil loading, specifically contamination concentrations that did not cause significant mortality within 96 h, for the subsequent experiment. Single replicates of each treatment (oiled sand, oiled kelp, oiled sand + oiled kelp) were tested at each of three oil loadings (0.25 g/L, 0.5 g/L and 1 g/L.) with an un-oiled control (10 total experimental chambers).
- 2) Experiment: The experiment used the maximum oil loading from the range finding trial (1 g/L) to evaluate the primary uptake pathway of PAHs for the talitrids. This 96-h experiment included three replicates for each treatment: oiled sand, oiled kelp, oiled sand + oiled kelp, un-oiled control (12 total experimental chambers).

The experimental chambers consisted of 16 L rectangular (0.51 m × 0.27 m) glass aquaria containing two liters of sand (median grain size of 390 μm) collected from the same zone of the beach as the talitrid amphipods. Three inverted glass crystalizing dishes (15 cm diameter) were placed in the sand to serve as pedestals for fresh kelp (*Macrocystis pyrifera* collected at MPSL, Fig. 1) which served as talitrid food during the experiment. Kelp blades were cut into 15 cm diameter circles that weighed approximately 45 g (Fig. 2). The experimental chambers were maintained at 20 °C (±3 °C) for 96-h with a 16:8 light/dark photoperiod.

Adult (20–25 mm) talitrid amphipods (*Megalorchestia pugettensis*; Fig. 2) were collected live at Asilomar State Beach (36°37'07.1"N, 121°56'29.2"W), Monterey County, California for use in the trial and experiment (Fig. 1). Asilomar State Beach is an intermediate morphotype open-coast beach which is part of the Asilomar State Marine Reserve, a marine protected area, and inshore of the Monterey Bay National Marine Sanctuary. The beach is unmanipulated, with no grooming activities or driving. The talitrids and intertidal sand were field collected and transported to the MPSL on August 31 and October 28, 2018, for the range finding trial and the experiment, respectively. For both the trial and the experiment, live organisms were added to the experimental chambers and observed to ensure no mortality and that all individuals burrowed into the sand. In the range finding trial approximately 100 organisms were added to each of the 10 experimental chambers. For the experiment, the number of talitrids was increased to 125 organisms/experimental chamber (12 chambers) to ensure that at least 10 g of live organism tissue (wet weight) would be available at the conclusion of the experiment for chemical analysis. The resulting experimental chamber density of 911 organisms m⁻² is within the range of observed field-measured densities in this region of 0 to 10,000 organisms m⁻².

Crude oil from the Santa Barbara Channel, obtained on July 18, 2018, was used in the trial and the experiment. The collected oil was free of produced water but was known to contain paraffins and hydrogen sulfide. Hydrocarbons were introduced to the experiment in the form of a HEWAF which was used to amend sand or kelp substrates in the treatments. The HEWAF was prepared using a standard methodology developed during research studies following the Deepwater Horizon spill (Carney et al., 2016). The HEWAF was prepared with seawater and crude oil in a Waring® CB15 commercial food blender (or equivalent).

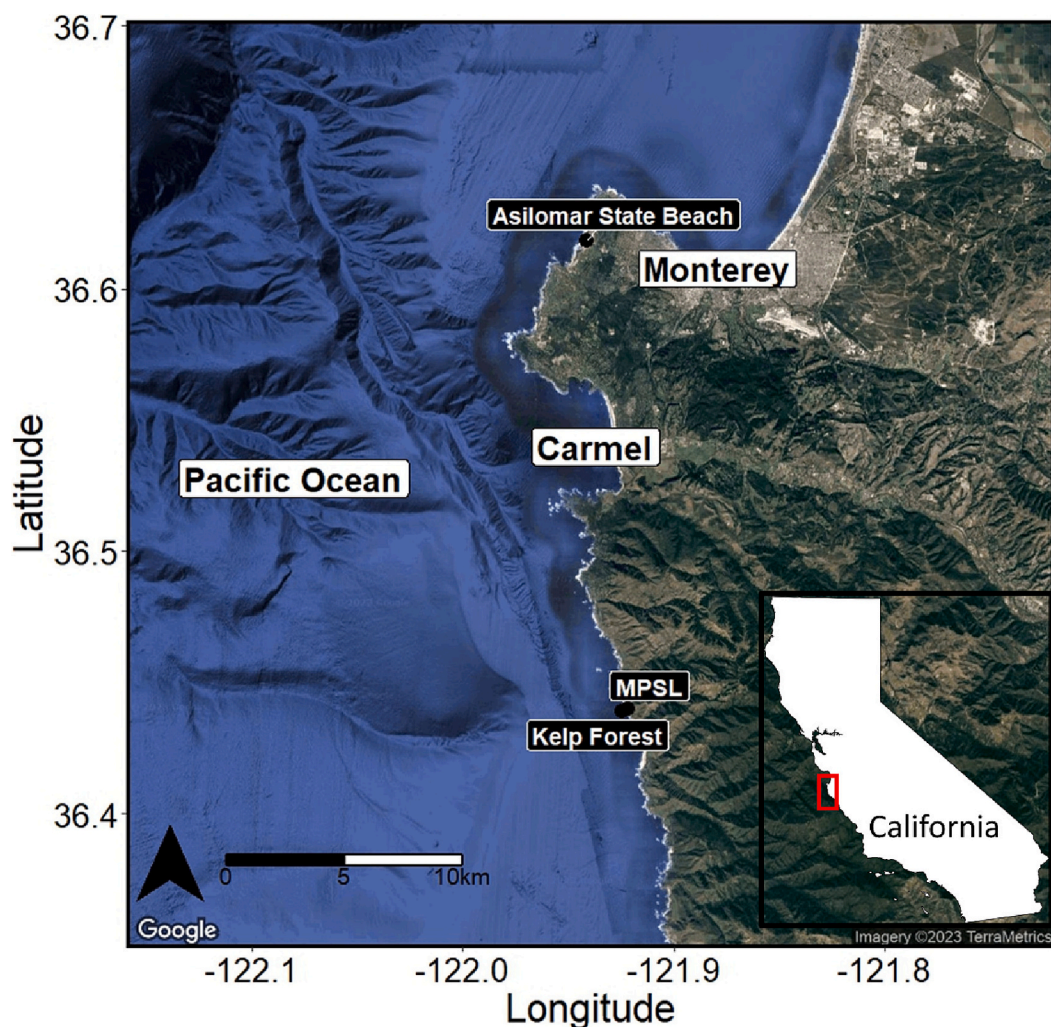


Fig. 1. A map indicating the collection location for talitrids and sand (Asilomar State Beach) and the kelp (Kelp Forest) used in the trial and experiment conducted at the Marine Pollution Studies Lab (MPSL). Inset shows the location of the expanded map on the California coast (red box). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The blender lid was lined with aluminum foil to prevent oil contact with anything other than the stainless-steel pitcher or the foil-lined lid. Crude 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. The oil-water mixture (1 g oil/L seawater) was blended on “low” for 30 s using 0.45 μm -filtered seawater from MPSL, and the contents were transferred to a 4 L separatory funnel. The HEWAF was allowed to settle and separate for 1 h. The bottom layer of the HEWAF was collected in a 4 L beaker, which was aliquoted into one-liter amber bottles for application to sand and kelp.

Sand in the oiled kelp treatments and the controls was pre-moistened by adding 300 mL of filtered seawater to 2 L of pre-sieved beach sand (1.5 mm mesh bag) collected from Asilomar State Beach (Fig. 1). The sand/water mixture was prepared in a 4 L glass jar and placed on a sediment rolling table. After 30 min, a polypropylene spoon was used to hand mix the sand and remove sand from the walls of the jar. The sand/water mixture was then placed on the rolling table for an additional 30 min. Oiled sand treatments were prepared in the same manner, but substituted MPSL seawater with 300 mL of HEWAF. Thoroughly mixed sand was then distributed to the appropriate experimental chambers for each treatment. Pre-weighed circles of kelp (15 cm in diameter) were placed on inverted crystalizing dishes in the oiled sand and control chambers. For oiled-kelp treatments, kelp circles were submerged in HEWAF for 60 s, then placed on crystalizing dishes (15 cm diameter).

Talitrids were added to each randomized treatment chamber by inverting a two-liter plastic collection jar containing organisms. The condition of the talitrids was observed and any dead talitrids were removed and recorded. Dead talitrids were not replaced as new organisms would have less time in a treatment and not accumulate the same amount of PAH in their tissues as the original talitrids. Replacement would lead to an artificial dilution of PAH concentrations in the pooled talitrid samples used for chemical analyses. Kelp and surface sand were re-hydrated daily using a spray bottle filled with fresh MPSL filtered seawater. At the termination of the 96-hour exposure, kelp was removed, and sand was sieved to capture surviving talitrids. Talitrids were enumerated, placed in foil pouches, and frozen for analysis of PAH in tissues.

Chemical analyses for 45 PAHs (PAH₄₅) were conducted using the US Environmental Protection Agency method 8272SIM at the CDFW-OSPR Water Pollution Control Laboratory in Rancho Cordova, CA. Composited talitrid experimental samples (~125 individuals) were rinsed with deionized water prior to homogenization to remove any matrix (e.g., sand) adhering to the organisms. A Brinkman Polytron homogenizer Model PT 10-35 was used for homogenization of the talitrid samples.

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 GCMS in selected ion monitoring mode (EPA 8270-SIM) for

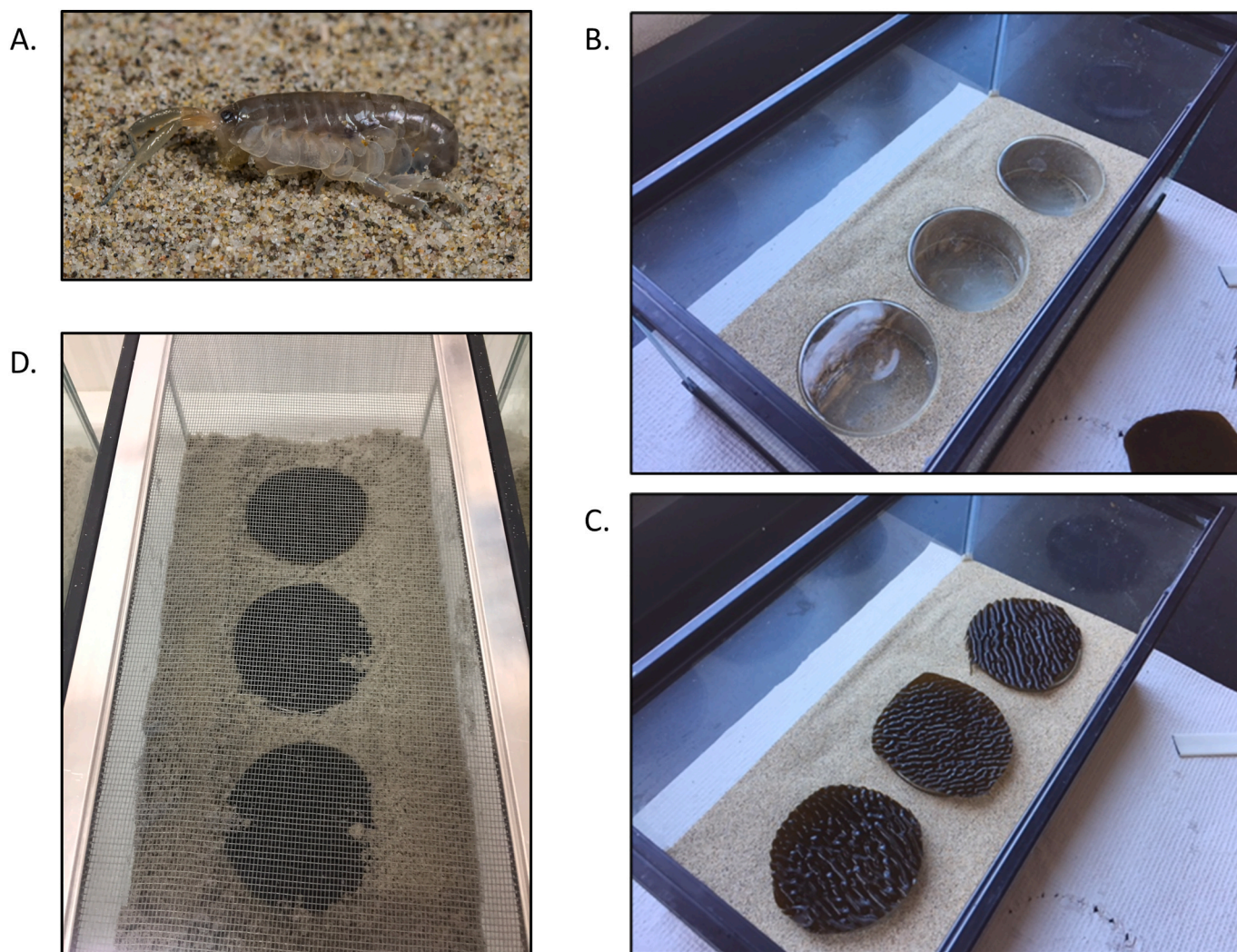


Fig. 2. Images (clockwise from top left) of a) Adult talitrid amphipod, *Megalorchestia pugettensis* (approximately 20–25 mm in length), used in uptake experiments (photo courtesy of Nicholas Schooler) and the experimental chambers with b) sand and glass crystallizing dishes, c) experimental chambers with kelp, *Macrocystis pyrifera*, and d) experimental chambers with screen cover.

quantification of individual parent and alkylated PAH₄₅ compounds. Alkylated PAH₄₅ were quantified using the response of the parent PAH₄₅. Results were reported as total PAH₄₅.

To determine whether treatment type had a significant effect on the number of dead organisms found as well as the amount of kelp consumed in each replicate of the experiment, a one-way analysis of variance (ANOVA) was performed. A one-way ANOVA was used to determine if untreated and treated kelp were significantly different and if untreated and treated sand were significantly different. One-way ANOVA was also used to determine the effect of treatment type on talitrid tissue PAH₄₅ concentrations followed by a post hoc Tukey HSD test. Analyses were conducted using R (v4.1.2; R Core Team, 2021) and the ‘tidyverse’ (Wickham et al., 2019) and ‘multcomp’ (Hothorn et al., 2008) packages.

No effects on survival of the talitrids were observed after exposure to three levels of oil loading (0.25 g/L, 0.5 g/L and 1 g/L) in the 96-hour range finding trial. No >6 dead individuals were found in any treatment chamber. Based on these results, the maximum oil loading (1 g/L) was used in the experiment.

In the 96-hour experiment, the mortality of talitrids did not differ significantly among treatments, including the un-oiled control (one-way ANOVA, $F = 1.0$, $p = 0.44$). The amount of kelp consumed by the talitrids was marginally nonsignificant among treatments (one-way

ANOVA, $F = 3.63$, $p = 0.06$) with the greatest amount of kelp consumed in the controls and the least in the oiled sand treatments.

The PAH₄₅ concentration of the crude oil used in the experiment was 9911.6 ppb and the HEWAF mixture was 194.3 ppb. The mean (\pm standard deviation) PAH₄₅ concentration was 1.6 ± 0.5 ppb for untreated sand and 9.6 ± 0.6 ppb for treated sand (Fig. 3). Concentrations of PAH₄₅ differed significantly between untreated and treated sand (one-way ANOVA, $F = 274.0$, $p < 0.0001$). The mean PAH₄₅ concentration was 0.7 ± 0.3 ppb for untreated kelp and 71.6 ± 8.0 ppb for treated kelp (Fig. 3). Untreated and treated kelp PAH₄₅ concentrations differed significantly (one-way ANOVA, $F = 238.2$, $p = 0.0001$). See Supplementary Table 1 for the PAH concentrations of these target analytes.

Mean values of PAH₄₅ concentrations, measured in talitrid tissues as total PAH₄₅, varied more than sixfold among treatments at the conclusion of the experiment. Mean values (\pm standard deviation) of PAH₄₅ in tissues were 203.8 ± 18.6 ppb for oiled sand, 33.7 ± 8.1 ppb for oiled kelp, 243.0 ± 29.4 ppb for sand and kelp and 35.0 ± 2.7 ppb for controls. See Supplementary Table 1 for the PAH concentrations of these treatments. The concentration of PAH₄₅ in talitrid tissues, differed significantly among treatments (one-way ANOVA, $F = 113.6$, $p < 0.00001$). Post hoc analysis revealed that these differences were driven by the treatments containing oiled sand, with or without oiled kelp (Fig. 4).

The concentrations of PAH₄₅ in talitrid tissues measured in our experiment suggest that oiled sand is the dominant route by which PAH₄₅ are taken up by these intertidal organisms. However, it is important to note that this was a controlled laboratory experiment, and exposure pathways in a natural setting may be more complex. On beaches, this process is likely driven by wave and tide action transporting hydrocarbons into the intertidal sand matrix that the talitrids inhabit (Huettel, 2022). The talitrid tissue PAH₄₅ concentration from the oiled kelp treatment alone did not differ from the concentrations in the control group. This indicates that, for this burrowing organism, exposure via contact with sand is the primary mechanism of oil contaminant uptake rather than consumption of oiled food resources.

Results show that PAH₄₅ concentrations were higher in the tissues of the talitrids in the oiled sand treatments compared to oiled kelp treatments. The behavior of talitrid amphipods who do not occupy permanent burrows and dig new burrows in intertidal sand every day (Dugan et al., 2013; Emery et al., 2022) may increase their exposure to hydrocarbons from oil spills. Aside from the organic materials present in phytoplankton and wrack washed ashore, the sandy substrate of open-coast beaches is relatively low in organic carbon content (Rodil et al., 2007). The PAHs that were measured in this research have reported

sediment organic carbon-water partitioning coefficients (K_{oc}) values that range from 2.45 to 8.91 for two to six ring molecules (Hawthorne et al., 2006), with mean Log K_{oc} values ranging from 4.40 to 7.19 for these compounds. These values demonstrate that PAHs will partition into the available organic carbon preferentially, including the talitrid amphipods burrowed into the sand on an oiled sandy beach. As oil ages, the composition of the PAHs changes, and one would expect that the proportions of the lighter end and heavier end PAHs would shift as well. Experimental evidence has shown that the decomposition of sediment-oil agglomerates in beach sand can take decades, whether on the beach surface or buried (Bociu et al., 2019). Whether such compounds accumulate in or on the carapace or into the tissues deeper within exposed organisms is a question that requires additional investigations to explore the mechanism of the cross-carapace transmission and the tissues that function as the primary reservoirs of these compounds. The results of this exposure experiment provide a needed foundation for further research designed to study the impacts of oil exposure for talitrid metabolism, depuration, toxicity and sublethal effects on growth and reproduction (Gesteira and Dauvin, 2000; Donohoe et al., 2021). Such sublethal bioassays are critical for identifying exposure impacts that are not as apparent as direct oiling or mortality (Emery et al., 1997; Gray

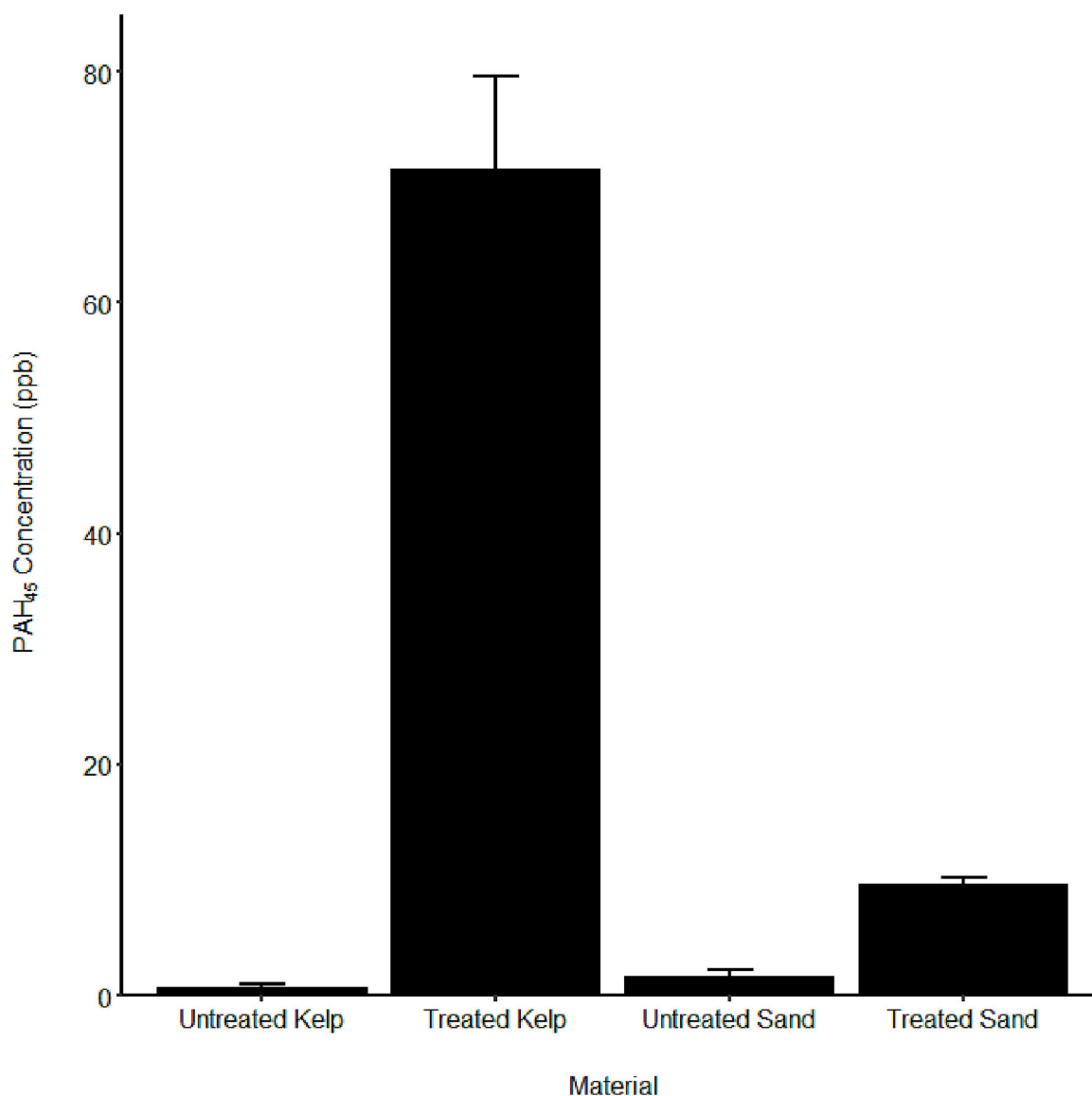


Fig. 3. Mean values ($n = 3$) \pm 1 standard deviation of PAH₄₅ concentrations for untreated and treated kelp and sand. Concentrations for treated sand and kelp were significantly higher than their respective untreated materials.

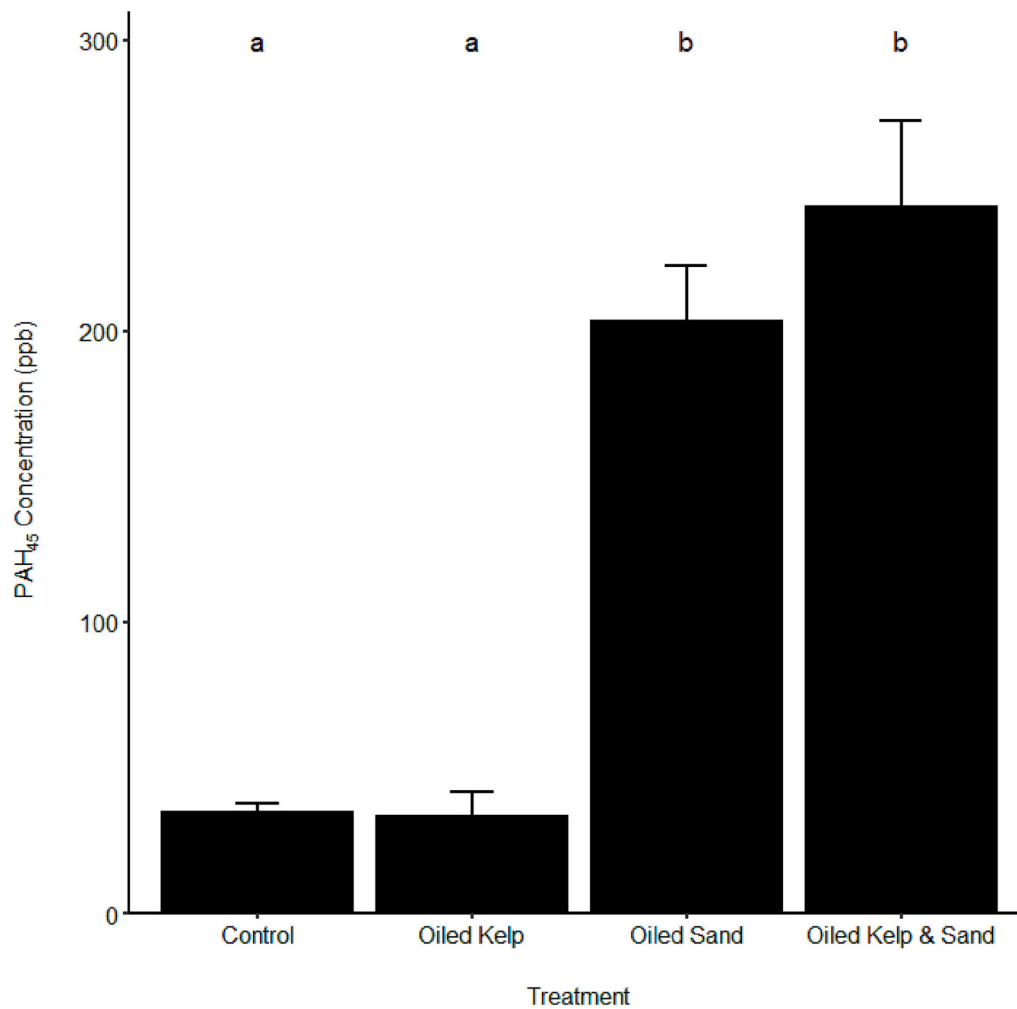


Fig. 4. Mean values ± 1 standard deviation of tissue PAH₄₅ concentrations (ppb) for talitrid amphipods in each experimental treatment (3 replicates; 100 organisms per replicate). Matching Letters displayed at the top of the bars indicate statistical differences as determined by results of Tukey HSD tests.

et al., 1998; Gilde and Pinckney, 2012; Powers et al., 2013). In addition, exploring the potential cascading effects of toxic substances, like oil, within the beach food web and across ecosystem boundaries would increase our understanding of how sediment contamination affects the beach ecosystem (Henkel et al., 2012; Bejarano and Michel, 2016).

Persistence of oil in beaches, if not manually removed, is highly dependent on beach type, oil type, tidal pumping, photooxidation, and biodegradation (Wang et al., 2021; Huettel, 2022). Oiled beach wrack is often the target of beach clean-up efforts after an oil spill, but removal can cause negative effects on wrack-associated organisms as loss of this basal resource directly impacts the beach food web (Dugan et al., 2003; De la Huz et al., 2005; Baker et al., 2017; Michel et al., 2017; Schooler et al., 2019). An example of this is the Prestige oil spill in Spain. During this spill, the upper beach zone was heavily impacted with oil deposition along the high tide strandline and further by efforts to remove oil and oiled wrack (De la Huz et al., 2005). Wrack is a key resource on sandy beaches and the propagation of this energy subsidy up the food web highlights the potential risk of oil exposure to the broader ecosystem (Dugan et al., 2003; Schlacher et al., 2017; Page et al., 2021). Talitrids, and the other wrack-associated invertebrates, play a vital role in sandy beach ecology (Lastra et al., 2008; Emery et al., 2021; Michaud et al., 2019; Lowman et al., 2019). If heavily oiled wrack remains on beaches, predators, including species listed as threatened (e.g., snowy plover, *Charadrius nivosus*), may also experience greater exposure to oil while searching for food (e.g., talitrids). These results demonstrate that while oiled wrack could lead to additional contamination over time, through

consumption or leaching into the sand, the potential additional contamination of wrack associated invertebrates from oiled wrack appears to be lower than that from oil contaminated sand. Cleanup of oiled wrack may be a comparable impact to the beach in terms of wrack removal as beach grooming (Dugan et al., 2003; Hubbard et al., 2014; Schlacher et al., 2016; Schooler et al., 2017, 2019). However, the impacts of this standard spill response activity are likely contingent on the amount of oil, namely thick layer deposits will have greater impacts than a coating of oil (Michel et al., 2013).

Determining the impacts of oil spills on natural coastal ecosystems is challenging because of the hazardous nature of the spill combined with their extent, access restrictions, and the limited ability of researchers to conduct surveys and experiments in such dynamic places in a very compressed time frame (Michel et al., 2013; Halanych and Westerholm, 2021). It is important to understand the impacts of oil spills to sandy beach ecosystems within the context of the ecological functions and ecosystem services that they provide, especially food and habitat provisioning and cultural elements (Fegley and Michel, 2021). The impacts may be extensive across space and time, but without strong baseline knowledge of beach ecosystems and an understanding of toxicity due to exposure, it will continue to be challenging to document the damage, injury, impacts, and recovery of these widespread and vulnerable open coast coastal systems from oil spills (Bejarano and Michel, 2016). Studies of oil spills have widely demonstrated that impacts to the beach ecosystem are rapid and recovery is prolonged (Schlacher et al., 2011; Huettel, 2022). Experiments, such as the one described in this study, are

important for identifying when and where oil spills may have their largest impacts on intertidal biota. Irrespective of the mechanism of uptake of PAHs, it is reasonable to infer from our results that oil spill cleanup techniques that target the removal of oiled wrack resources may not be effective at reducing exposure, especially relative to known negative impacts of wrack removal. These results also suggest that future toxicity testing of PAHs to talitrids may more effectively be performed by controlling the concentrations in the sand that they burrow into rather than by modifying the concentrations in their food source. Future studies that use the sand exposure techniques described here could contribute valuable information toward oil spill related injury determination during natural resource damage assessments. Future exposure experiments should explore the effects of direct oiling compared to HEWAF types of exposures for uptake of PAHs by organisms. Studies of the toxicity and sublethal effects of oil to biota could help improve quantification of the extent of impacts associated with oil spills in any ecosystem. Our results provide much needed new information on fundamental exposure and uptake pathways that can be used in determining appropriate responses to future oil spills that can reduce environmental degradation and shorten recovery times.

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CRediT authorship contribution statement

Bryand M. Duke: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Supervision, Project administration, Funding acquisition. **Kyle A. Emery:** Methodology, Investigation, Formal analysis, Writing – original draft, Visualization. **Jenifer E. Dugan:** Conceptualization, Methodology, Resources, Writing – original draft, Supervision, Project administration. **David M. Hubbard:** Conceptualization, Methodology, Writing – original draft. **Bruce M. Joab:** Conceptualization, Resources, Writing – original draft, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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