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# Sensitivity of the Sand Crab *Emerita analoga* to a Weathered Oil

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Sand crabs or mole crabs (Crustacea:Decapoda:Hippidae) are small suspension feeding crabs that live in the swash zone of high energy beaches throughout the world, including the Pacific, Atlantic, and Gulf coasts of North America (Efford 1976). Sand crabs go through a planktonic zoea larval stage that lasts weeks to months, then metamorphose into a transitional megalopae stage that lasts several weeks, and then molt and develop into juvenile crabs (Efford 1970). Sand crabs are potentially very important ecological receptors for contaminant exposure in many coastal areas. Sand crabs may constitute the largest fraction of the intertidal biomass at high energy sandy beaches, and may serve as important forage for shore birds and near-shore fish species (Dugan et al. 1994; Dugan et al. 1995). In California, the sand crab *Emerita analoga* occurs intertidally on almost every type and length of ocean beach and is often the dominant species of macrofauna (Dugan et al. 1995). Sand crabs occur at densities of up 52,000 crabs per meter$^2$ and are easily collected in large numbers (Dugan et al. 1995). Populations of *E. analoga* have been used as bioindicators (Wenner 1988) and have been reported to accumulate metals and hydrocarbons (Burnett 1971; Rossi et al. 1978; Wenner 1988).

Numerous studies have identified crustaceans as particularly sensitive to a wide range of contaminants, including petroleum (e.g., Barron et al. 1999). Despite the large number of toxicity studies performed with crustaceans, sand crabs have received relatively limited toxicological investigation. Siegel and Wenner (1984) reported abnormal reproduction of sand crabs in the vicinity of a nuclear power facility, and Boese et al. (1997) reported that sand crabs were of intermediate sensitivity to the acute toxicity of fluoranthene and cadmium relative to six other species of marine crustaceans. The objective of this research was to determine the toxicity of water accommodated fractions (WAF; water soluble components of oil) prepared from a field-collected weathered middle distillate oil to the sand crab (*E. analoga*), and compare the relative sensitivity to a standardized test species, the mysid shrimp (*Mysidopsis bahia;* Barron et al. 1999). The megalopae life stage of the sand crab, an early life stage, was tested because it is the first stage to settle on beaches, and is easily field collected in high numbers during the spring and fall.

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## MATERIALS AND METHODS

The dilution water was natural seawater collected from the University of California Bodega Marine Laboratory system near Bodega Bay, California. The seawater was passed through a 0.45 µm filter before use. Sand crabs were acclimated and tested at their preferred temperature (15°C) and salinity (full-strength seawater; 34‰). Megalopae (post-larval, first settlement stage) and early crab (first or second molt) life stages of sand crabs were field collected from the swash zone of Refugio State Beach in California by sifting sand. Sand crabs were acclimated and tested in 1 to 2 cm depth of prewashed commercial sandblasting sand (Silver Sand #30) because of their requirement for a substrate. Sand crabs were acclimated for nine days under 48-hour static renewal conditions and fed *Artemia* nauplii *ad libitum* each day. Immediately before use in the toxicity tests, the sand crabs were sorted to avoid using organisms that exhibited abnormal behavior (e.g., loss of equilibrium), were injured, or did not appear to be at the megalopae life stage (e.g., long protruding eye stalks indicative of juvenile sand crabs molted from megalopae).

Toxicity tests were adapted from EPA guidance on short-term static renewal testing with mysids (described in Ban-on et al. 1999). Nominal test concentrations were control (0% WAF), 2.5, 5, 10, 20, and 40% WAF. The test vessels for sand crabs were 1 L glass beakers filled with 1 to 2 cm depth of prewashed sand and 500 mL of test solution. Test solutions were not aerated. The initial dry weight of test organisms crabs was determined using three pooled samples of 10 sand crabs (30 total). Samples were dried at 100°C overnight then weighed to the nearest 0.01 mg. Ten sand crabs were distributed into each of the three replicates for each of the test concentrations. All test vessels were loosely covered with a clear polyethylene film. Test vessels were then randomly placed within a 15 °C waterbath under standard laboratory fluorescent lighting (minimal ultraviolet light) on a 16 h light: 8 h dark photoperiod. Sand crabs were fed newly hatched *Artemia* nauplii once per day. Test vessels were monitored daily for temperature, pH, dissolved oxygen, and salinity.

Test solutions were prepared with an environmentally weathered oil in full strength filtered seawater (described in Barron et al. 1999). Measurement endpoints were daily mortality, survival to six days, growth (as the change in dry weight over the test period), number of emergent sand crabs (crabs not buried in the sand substrate), and the number of shed carapaces (indicative of the number of molts). Samples were collected for quantification of TPH (all treatment levels) as described in Barron et al. (1999). Initial (newly prepared WAF) and final (pooled test solution from replicate vessels sampled 12 h after renewal) test solutions were sampled at both test initiation and at test end.

Statistical analyses were performed using ToxCalc (Tidepool Scientific Software, McKinleyville, California) and S-Plus (Mathsoft, Seattle, Washington) using the mean TPH concentrations. No observed (NOEC) and lowest observed (LOEC) effect concentrations for survival to day 6, incremental growth (change in mean dry weight per individual between test initiation and test end), emergence (number of sand crabs not buried in the sand substrate), and molting (indexed as the number of observed

carapaces) were determined using analysis of variance (ANOVA) and Dunnett's multiple comparison tests (alpha = 0.05). LC50s and LC20s (concentrations causing 50% and 20% mortality) were estimated using Maximum Likelihood Probit method, and EC50s and EC20s (concentrations causing 50% and 20% growth reduction) were estimated as described in Barron et al. (1999).

## RESULTS AND DISCUSSION

Petroleum exposure concentrations are presented as mean measured concentrations of TPH, and ranged from nondetectable (< 0.05 mg/L) in the control to 6 mg/L in the highest treatment level (40% WAF) (n = 3 or 4; Figure 1). In general, measured exposure concentrations corresponded to the nominal WAF dilution, and were similar at test initiation and termination. Temperature and salinity were within the desired ranges (15°C; 33 to 34‰). pH declined from approximately 8.1 to 7.3 with increasing test concentration of TPH, possibly due to the weak acid properties of alkyl phenols in the test oil. Dissolved oxygen concentrations were above 5 mg/L.

Survival of control sand crabs was 97%, mortality increased with increasing test concentration, and occurred sooner at higher TPH concentrations (Figure 2). The test concentrations estimated to cause 20% and 50% mortality (LC20, LC50) in the test population of sand crabs was 3.5 and 7.1 mg/L TPH, respectively (Table 1). The 96-hour LC50 for sand crabs was estimated to be 7.7 mg/L TPH. Sand crabs in the control treatment weighed 3.19 ± 0.14 mg (mean dry weight per individual ± SD) at test initiation and 3.69 ± 0.36 mg at test end. Growth was significantly reduced ($p \leq 0.05$) at 3.4 mg/L TPH (Table 1; Figure 1). Growth was less affected at the highest test concentration, a typical observation in laboratory bioassays at lethal test concentrations. The test concentrations estimated to cause 20% and 50% reductions in growth (EC20, EC50) of the test population of sand crabs were 0.65 and 1.2 mg/L TPH, respectively (Table 1). Other endpoints assessed in the sand crab test were molting (indicated by the number of observed shed of the carapaces) and impaired behavior [indicated by the number of emerged (unburied) sand crabs]; these endpoints did not show statistically significant ($p \leq 0.05$) effects during the test period (Figure 2).

The relative species sensitivity of sand crabs and mysids (Barron et al. 1999) to the test oil WAF was estimated using a sensitivity ratio: effect or no effect concentration for sand crabs divided by the effect or no effect concentration for mysids (e.g., $NOEC_{sand\ crab}$/ $NOEC_{mysid}$) (Table 1). Sand crabs and mysids exhibited similar sensitivity to the WAF of the test oil. Sensitivity ratios ranged from 0.6 to 5, depending on the test endpoint (Table 1). Sand crabs exhibited a more limited scope for growth than mysids. For example, sand crab mass increased a maximum of 20% during the six-day test compared to a maximum increase in mysid mass of greater than 200% (Barron et al. 1999). Sand crab tests incorporated two additional endpoints: molting and emergence. Sand crab molting was limited during the 6 day test, consistent with previous studies of the molt frequency of megalopae (Dugan 1990). Molting was indexed by the number of carapaces that were easily distinguished from dead organisms and confirmed by the mass balance of organisms

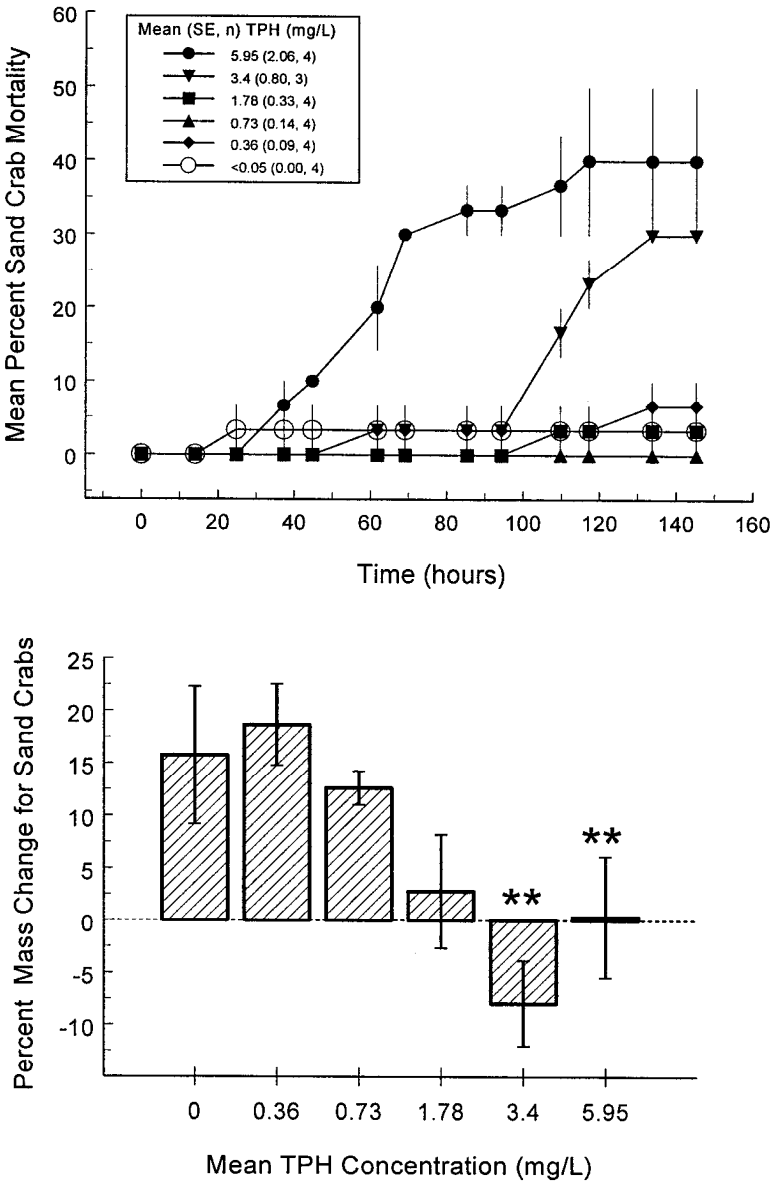


**Figure 1.** Mean cumulative percent sand crab mortality (top panel), and mean percent change in sand crab mass (bottom panel). Symbols are the mean cumulative percent mortality (±SE) at each test concentration. Bars are the mean difference (±SE) from the initial and final weights of organisms at each test concentration. Asterisks (**) indicate a significant reduction in growth and survival.

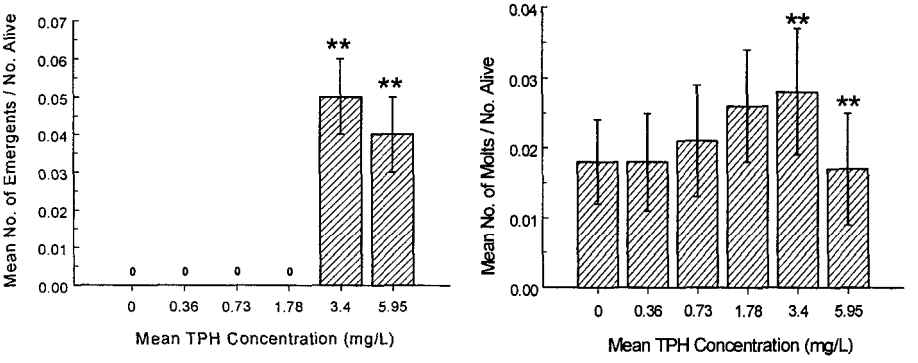


**Figure 2.** Mean number (±SE) of emergent (non-buried) sand crabs per number alive (left panel) and molts per number live sand crabs (right panel). Asterisks (**) indicate a significant reduction in growth and survival.

**Table 1.** Effect and No Effect Concentrations for Short-Term Survival and Growth Endpoints

| | Survival Reduction (mg/L TPH) | | | | Growth Impairment (mg/L TPH) | | | |
|---|---|---|---|---|---|---|---|---|
| Test Species | LC20 | LC50 | NOEC | LOEC | EC20 | EC50 | NOEC | LOEC |
| Sand Crab | 3.5 | 7.1 | 1.8 | 3.4 | 0.65 | 1.2 | 1.8 | 3.4 |
| Mysid | 1.1 | 1.5 | 0.91 | 1.8 | 1.1 | 2.1 | 0.91 | 1.8 |
| Sensitivity Ratio[b] | 2.9 | 4.7 | 2.0 | 1.9 | 0.6 | 0.6 | 2.0 | 1.9 |

a. Mysid data from Barron et al. (1999).
a. Ratio of sand crab:mysid effect or no effect concentration.

in the test vessels. However, this endpoint may be more sensitive during longer-term petroleum exposures because of the importance of endocrine regulation in crustacean molting and the potential for mixed function oxidase induction by PAHs in oil (Singer and Lee 1977). Sand crab test vessels contained a 1 to 2 cm depth of sand to accommodate the organism's normal behavior of partial burial; exposure to interstitial water may be minimal for sand crabs because of their 'head-up' orientation. The coarse grain sand (Silver Sand #30) quickly settled in the test vessels and allowed observation of the number of live organisms and the swimming, feeding, and burial behavior of the sand crabs. Sand crab emergence, indexed by the number of unburied organisms, preceded mortality but was not significantly affected during the six-day test. Impaired burial by sand crabs would likely cause adverse effects such as stranding on outgoing waves and possible increased predation. The burial speed of sand crabs, not assessed but ecologically important, may be a more sensitive endpoint than emergence.

Sand crabs should be considered as a monitoring or test species in toxicity assessments of spilled oil in the proximity of sandy beaches. They may be important ecological receptors for spilled oil because of their high density (>52,000 $m^2$), predominant intertidal biomass at high energy beaches, and importance as a forage species for near-shore fish and shore birds (Dugan et al. 1995). Also, sand crabs have the capacity to accumulate petroleum hydrocarbons (Rossi et al. 1978). *Mysidopsis bahia* and *Holmesimysis costata* are among the most widely used marine crustacean test species and are representative of estuary and near-shore sub-tidal waters. Sandcrabs have greater ecological relevance for evaluating contaminant effects on high energy beaches, although mysids may serve as reasonable surrogates based on similar sensitivity to the test oil.

Several aspects of the behavioral ecology of sand crabs make them suitable as a monitoring species. Post-larval stages of sand crabs have a limited home range and aggregate in the mid-intertidal area (Cubit 1969). Sand crabs are at risk from oil washing ashore because they move vertically with the tides through a continuous process of emergence (incoming wave) and reburial (outgoing wave) (Cubit 1969). Sand crabs are suspension feeders (Cubit 1969; Efford 1976), which provides an exposure route to dispersed or particle-bound petroleum hydrocarbons in addition to dissolved phase oil. Additionally, sand crabs may be exposed to contaminated groundwater discharges below the beach effluent line from up gradient sources. In the laboratory, sand crab megalopae exhibited high survival and substantial growth, and additional toxicity endpoints may be assessed. Establishing bioindicator species or sentinel organisms to monitor the health of coastal environments is highly desirable. Developing a bioassay that utilizes early life history stages of sand crabs, a known bioaccumulator (Burnett 1971; Rossi et al. 1978; Wenner 1988) and one of the most common intertidal species of sandy beaches will provide a needed tool for monitoring and assessing the effects of anthropogenic impacts in the coastal environment.

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