Bull. Environ. Contam. Toxicol. (1999)62:266-271


Bulletin of Environmental Contamination and Toxicology

# Sensitivity of the Mysid *Mysitlopsis bahia* to a Weathered Oil

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Received: 12 October 1998/Accepted: 25 January 1999

Numerous studies have identified crustaceans as particularly sensitive to a wide range of contaminants, including petroleum (e.g., Markarian et al. 1995). The objective of this research was to determine the sensitivity of the mysid shrimp (*Mysidopsis bahia*) to an environmentally weathered oil as a component of a study evaluating the comparative sensitivity of crustaceans to petroleum. Mysids were selected for evaluation because they are a standardized test species and have been extensively used in determining the toxicity of a variety of oils (e.g., Anderson et al. 1974; Rice et al. 1976). In the current study, juvenile mysids were evaluated in short-term bioassays using water accommodated fractions (WAF; water soluble components of oil) prepared from a field-collected weathered middle distillate oil. The toxicity of environmentally weathered oils has received only limited investigation (e.g., Anderson et al. 1987). The analytical chemistry of the WAF (designated 5x for the field collection location) used in this study is reported in Barron et al. (1999a) and the toxicity to sand crabs (*Emerita analoga*) is reported in Barron et al. (1999b).

## 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. Mysids were acclimated and tested at their preferred temperature and salinity: 25°C in 20‰ seawater prepared by diluting filtered seawater with deionized water. Six-day-old mysids were obtained from a commercial supplier (Aquatic Indicators, St. Augustine, Florida); the source culture of mysids was maintained at 20‰ natural seawater at 26°C. Mysids were fed newly hatched *Artemia* nauplii *ad libitum* during a one day acclimation period under static conditions and were seven days old at test initiation.

Oil samples (designated 5x for the sampling location) were collected using a hand bailer from an underground plume at a coastal California oil field. Residual water in the oil sample was removed by allowing the oil and water to separate during refrigeration in the dark. The dewatered oil samples were then composited and stored in the dark at 4°C until used. Test solutions were prepared daily from volumetric dilutions of newly prepared WAF of the test oil (Figure 1); WAF was prepared in full

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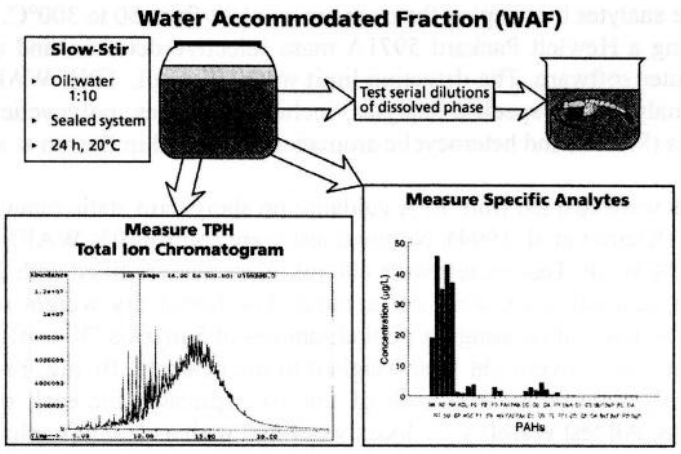


**Figure 1.** Schematic representation of the preparation, sampling, and testing of a water accommodated fraction. Example total ion chromatogram in 20% WAF and PAH concentrations in 100% WAF are shown.

strength seawater, then diluted to the test salinity. The WAF preparation procedures produced a solution of dissolved hydrocarbons similar in composition and concentration to groundwater collected in the vicinity of the oil plume. Each day, 100% WAF (no dilution) was prepared in a 3.8 L glass jar containing a Teflon-coated stir bar and a < 2 cm diameter glass tube for WAF sampling. Test solutions were prepared as follows: 330 mL (~280 g) of oil were gently poured onto 3.3 L of seawater to achieve a surface layer of oil (oil was excluded from the inside of the glass sampling tube). The glass jar was sealed and mixed at approximately 200 rpm at 19 to 20°C for 24 hours. The mixing regime produced a minimal vortex, with no obvious introduction of particulate oil into the WAF. WAF was removed within several minutes of cessation of mixing by inserting a Teflon siphon tube through the glass sampling tube to collect WAF from below the oil layer. The test solutions were prepared once per day by diluting 100% WAF to 20‰ filtered seawater. Test solutions were gently mixed and loosely covered before use in renewals or by gently pouring from the test vessel. New test solution at test temperature was then added to the test vessel by gently pouring to the original test solution volume. Test solutions were renewed every 12 h by first removing approximately 75% of the initial test solution.

Total petroleum hydrocarbons (TPH) samples were analyzed by neutral extraction and a GC/MS combination method. One L of sample was removed from the sample bottle and transferred to a separatory funnel. The sample was spiked with surrogate (o-terphenyl) and extracted two times with 90 mL of methylene chloride. The methylene chloride extract was dried with sodium sulfate, transferred to a Zymark Turbovap 500 Concentrator, and concentrated to 1 mL. The concentrated extract was then spiked with internal standard (p-terphenyl) and placed in the Hewlett Packard 5890 Series II GC autosampler. One µL of extract was injected into the GC that

separated the analytes by ramping the oven temperature from 50 to 300°C. TPH was detected using a Hewlett Packard 5971A mass selective detector, and quantified using computer software. The detection limit was 0.05 mg/L TPH. WAF samples were also analyzed for specific analytes, including alkanes, polycyclic aromatic hydrocarbons (PAHs), and heterocyclic aromatics (described in Barron et al. 1999a).

Toxicity tests were adapted from EPA guidance on short-term static renewal testing with mysids (Klemm et al. 1994). Nominal test were control (0% WAF), 1.25, 2.5, 5, 10, and 20% WAF. Test vessels were 400 mL glass beakers filled with 200 mL of test solution; test solutions were not aerated. The initial dry weight of the test organisms was determined using six pooled samples of 5 mysids (30 total). Samples were dried at 100°C overnight then weighed to the nearest 0.01 mg. Five mysids were randomly distributed into each of the six 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 26°C waterbath under standard laboratory fluorescent lighting (minimal ultraviolet light) on a 16 h light: 8 h dark photoperiod. Mysids were fed three times per day to minimize cannibalism. Test vessels were monitored daily for temperature, pH, dissolved oxygen, and salinity. Measurement endpoints were daily mortality, survival to six days, and growth (as the change in dry weight over the test period). Samples were collected for quantification of TPH (all treatment levels). 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 mean TPH concentrations. No observed (NOEC) and lowest observed (LOEC) effect concentrations for survival to day 6 and incremental growth (change in mean dry weight per individual between test initiation and test end) were determined using analysis of variance (ANOVA) and Steel's Many-One Rank Test (survival data) and Bonferroni t-test (growth data) (alpha = 0.05). LC50s and LC20s (concentrations causing 50% and 20% mortality) were estimated using the Trimmed Spearman-Karber method. EC50s and EC20s (concentrations causing 50% and 20% growth reduction) were estimated using ordinary least squares regression where growth increment was modeled as a function of the log TPH concentration. Growth measurements of organisms exposed to less than 0.25 mg/L TPH were not used in regression modeling because preliminary inspection of the results suggested that the threshold concentration for growth inhibition was greater than that value.

## RESULTS AND DISCUSSION

The test oil was collected from an underground oil plume at an abandoned oil field and had the characteristics of a weathered middle distillate oil. WAF prepared from the test oil exhibited a large unresolved complex mixture (chromatogram hump) with few resolved alkane peaks, a hydrocarbon range of 10 to 30 carbons, BTEX

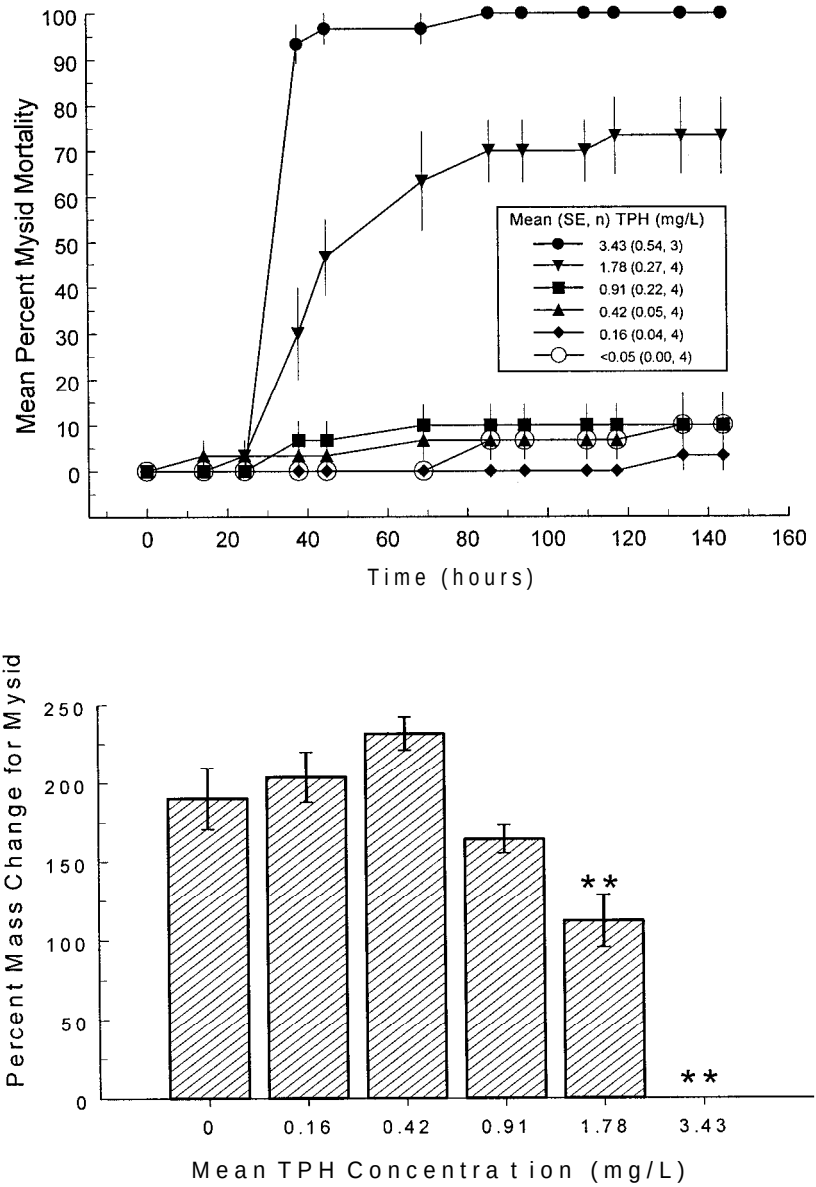


**Figure 2.** Mean cumulative percent mysid mortality (top panel), and mean percent change in mass (bottom panel). Symbols are the mean cumulative percent mortality (±SE) at each test concentration. Bars are the mean difference (±SE) from the initial and final weights of organisms at each test concentration. Asterisks (**) indicate a significant reduction in growth and survival.

concentrations less than 1% of TPH, alkyl napthalenes as the predominant PAHs, and an absence of four- and five-ring PAHs (Figure 1 and Barron et al. 1999a).

Petroleum exposure concentrations are presented as mean measured concentrations of TPH (mg/L, n = 3 or 4; Figure 2). TPH concentrations ranged from nondetectable (< 0.05 mg/L) in the control to 3.4 mg/L in the highest treatment level (20% WAF). 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 (25 to 26°C; 19.6 to 21.3‰). pH declined from approximately 8.2 to 7.3 with increasing test concentration of TPH, possibly due to the weak acid properties of alkyl phenols in the oil. Dissolved oxygen concentrations were above 5 mg/L.

Survival of control mysids was 90%, mortality increased with increasing test concentration, and mortality occurred sooner at higher TPH concentrations (Figure 2). Survival was significantly ($p \leq 0.05$) reduced at 1.8 mg/L TPH. The LC20 and LC50 at six days were 1.1 and 1.5 mg/L TPH, respectively. The 96-hour LC50 for mysids was estimated as 1.6 mg/L TPH. Mysids weighed 0.06 ± 0.01 mg at test initiation and 0.18 ± 0.03 mg at test end. Growth was significantly ($p \leq 0.05$) reduced at 1.8 mg/L TPH (Figure 2). The EC20 and EC50 for mysid growth were 1.1 and 2.1 mg/L TPH, respectively.

LC50 values for mysids were consistent with other short-term studies on the toxicity of similar petroleum products (middle distillate oils) to crustaceans (Anderson 1977). For example, Anderson et al. (1974) reported that 48- to 96-hour LC50s of WAF prepared from Fuel Oil #2 ranged from 0.9 to 5 mg/L for three species of crustaceans. Markarian et al. (1995) summarized the results of 129 short-term toxicity tests of middle distillate petroleum products to various invertebrates; the median LC50 was 3.36 mg/L TPH (range of 0.21 to 71.9 mg/L TPH). Eggs were the most sensitive invertebrate life stage (median LC50: 0.43 mg/L TPH), followed by larvae (1.3 mg/L), adults (3.4 mg/L), and juveniles (6.6 mg/L).

*Acknowledgments*. We thank John MacMurphy and Guy Denoux for petroleum analyses, Melissa Boggs for providing the oil, Jeff Cotsifas for laboratory and data analyses, and David Hubbard for manuscript review. We also thank Mr. Dave Cacela for providing additional statistical analyses and advice, and Josh Lipton for review and recommendations. This article is based on studies undertaken through contract with the California Department of Fish and Game Office of Oil Spill Prevention and Response. Contract. No. FG 4427 OS.

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