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SHORT-TERM TOXICITY OF 1-METHYLNAPHTHALENE TO *AMERICAMYSIS BAHIA* AND 5 DEEP-SEA CRUSTACEANS

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Abstract: There are few studies that have evaluated hydrocarbon toxicity to vertically migrating deep-sea micronekton. Crustaceans were collected alive using a 9-m^2 Tucker trawl with a thermally insulated cod end and returned to the laboratory in 10 °C seawater. Toxicity of the polycyclic aromatic hydrocarbon 1-methylnaphthalene to *Americamysis bahia*, *Janicella spinacauda*, *Systellaspis debilis*, *Sergestes* sp., *Sergia* sp., and a euphausiid species was assessed in a constant exposure toxicity test utilizing a novel passive dosing toxicity testing protocol. The endpoint of the median lethal concentration tests was mortality, and the results revealed high sensitivity of the deep-sea micronekton compared with other species for which these data are available. Threshold concentrations were also used to calculate critical target lipid body burdens using the target lipid model. *Environ Toxicol Chem* 2017;36:3415–3423. © 2017 The Authors. Environmental Toxicology and Chemistry published by Wiley Periodicals, Inc. on behalf of SETAC

Keywords: Deep-sea crustacean Marine toxicity test Polycyclic aromatic hydrocarbon 1-Methylnaphthalene Passive dosing

INTRODUCTION

Despite intense research effort into the ecological consequences of the Deepwater Horizon spill, questions remain regarding the current health of the ecosystem and what can be learned to respond to subsequent spills. One important component of the Gulf ecosystem that has received relatively little attention is the deep-water column (pelagic) micronekton (e.g., shrimp) and plankton (amphipods, copepods) inhabiting depths from 200 to 1000 m [1]. These organisms are key trophic intermediates in deep-sea food webs and represent a major trophic link between deep-water and shallow-water ecosystems [2]. These animals are involved in one of the largest animal migrations on earth, during which huge populations of animals migrate from the mesopelagic zone into the epipelagic zone on a nightly basis, forming massive sonic scattering layers that can be picked up on shipboard sonars [3]. These migrators serve as major sources of nutrition for cephalopods, commercially important epipelagic fishes, and cetaceans [4–7]. Although some commercially important fish, such as the bigeye tuna, follow their prey up and down in the water column, adults and juveniles of other species, such as yellowfin tuna and blue marlin, spend up to 90% of their time in the epipelagic zone [8]. Because micronektonic crustaceans, such as euphausiids, appear to be an important component of the diet of juvenile bluefin tuna [9], any disruption in the population density or migratory behavior of this community could have profound impacts on commercially important fisheries. In addition, because of their migratory behavior, they could serve as vectors of deep-sea contaminants into surface waters and the surface food web.

Previous studies on the micronekton and zooplankton response to the Deepwater Horizon spill have focused on process-level phenomena in surface-dwelling species, even though this massive spill occurred at 1500 m depth. Field studies have not documented large-scale changes in micronekton and zooplankton diversity and abundance in surface dwellers, although there is evidence of variable, yet distinct, petroleum/dispersant incorporation into zooplankton through polycyclic aromatic hydrocarbon (PAH) accumulation and isotopic carbon depletion [10–12].

Although studies have investigated the lethal effects of oil spills in terms of zooplankton decline in the euphotic zone [1], lethal effects on deep-sea species are less well known. To fully understand the magnitude and variability of these latter processes, a much clearer mechanistic understanding of the lethal effects of these compounds is needed at the level of the organism, particularly those inhabiting deeper water. Given their vertical range overlap with the subsurface hydrocarbon plumes resulting from the depth of the Deepwater Horizon spill and the deep-water application of dispersants [13–15] and their importance as a source of nutrition for shallow-water species, the deep-sea micronekton and their zooplankton prey need to be included in any studies on the ecosystem effects of deep-water oil spills.

Oil is a complex mixture of several thousand compounds, with significant variation in chemical composition between different oils and between different refined products. The relative solubility and persistence of constituent aromatic hydrocarbons result in crude oils with different impacts on organisms [16,17]. A major issue in toxicity studies with oil and oil dispersant is often the lack of quantitative chemical analysis, with results frequently based on nominal concentrations of hydrocarbons [18,19]. There are many physical and chemical processes that play an important part in bioavailability and thus toxicity [20].

An alternative to whole oils in toxicity studies is the use of individual hydrocarbons [21,22]. Generally, the toxicity of

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specific oils results from the additive toxicity of constituent hydrocarbons, especially aromatics [16,21,23]. The target lipid model (TLM) provides a quantitative framework for describing the toxicity of dissolved hydrocarbons based on the hypothesis that toxicity results when organismal tissue lipid concentrations of a specific hydrocarbon exceed the critical threshold for the organism in question, leading to mortality [24]. The hydrophobicity of PAHs promotes partitioning across permeable membranes into organismal tissue lipids until equilibrium is reached. Uptake and persistence of hydrocarbons during and after exposure may depend significantly on type and duration of exposure, as well as specific characteristics of the exposed species.

Modeling the toxicity of individual hydrocarbons based on lethality and sublethal effects permits prediction of the toxicity of any complex hydrocarbon mixture [16] while limiting experimental and analytical challenges by having to measure and interpret mixtures. Single hydrocarbons, such as 1-methylnaphthalene (1-MN) and phenanthrene, are often substantial contributors to the PAH content of water-accommodated fractions of petroleum substances and are therefore commonly used in toxicological studies [25,26]. Furthermore, alkylated PAHs are usually more abundant than parent PAHs [25,27] and demonstrate increased toxicity as a result of increased lipophilicity [28]. Consequently, alkylated derivatives may be more useful than the parent PAH compound in toxicity studies. There are no such studies reporting concentrations that elicit mortality in 50% of the test animals (LC50) for deep-sea organisms. The present laboratory experiments utilized a single compound, 1-MN, and a passive dosing protocol [22] to establish toxic threshold concentrations for use in the TLM.

METHODS

Deep-sea crustacean collection

Mechanistic studies on the effects of petroleum and dispersants have not been conducted on deep-water organisms, because of the difficulties in collecting live deep-sea animals that will survive in the laboratory for any length of time. We describe the use of unique equipment and expertise for collecting live mesopelagic species in excellent condition to conduct physiological experiments [29,30]. The mysid shrimp *Americamysis bahia* was obtained commercially (MBL Aquaculture) and used to set up the experimental systems and laboratory regime prior to collecting offshore organisms.

Deep-sea crustaceans were collected in the Straits of Florida, from depths ranging from 600 m (during the day) to 150 to 200 m (at night). A 9-m^2 Tucker Trawl with a thermally insulated, light tight cod end was used for animal collections. The primary net is 45 feet long and composed of one-fourth–inch knotless nylon. The secondary net is 12 feet long and composed of 303-μm Nitex. The net is designed to gently funnel the animals from a 9-m^2 mouth opening to the 10-cm opening at the end of the secondary net, which is attached to the cod end. An opening/closing timer, coupled with real-time depth data from a conductivity, temperature, and depth recorder mounted on the net frame, ensures that collections occur at optimal depths. With this proven system, the cod end can be closed at depth, ensuring that the organisms are brought to the surface in cold water and never exposed to damaging surface temperatures. For animals collected above 1000 m, pressure changes are not an issue for those without swim bladders (crustaceans do not have a swim bladder), but temperature changes can be lethal [31]. On net recovery, the closed cod end was detached from the net, and animals were sorted into maintenance chambers containing chilled seawater at their normal day depth ambient temperature (10 °C). Once sorted, organisms were returned to the laboratory in light, tight containers in Koolatrons to maintain temperature at 10 °C. In the laboratory, organisms were stored in subdivided acrylic containers (to prevent cannibalism) within temperature-controlled refrigerators inside of the dark room.

Experimental conditions

Determining threshold concentrations (i.e., LC50) for use in the TLM must be completed using a constant concentration of toxicant throughout the exposure to provide reliable data to generate dose–response curves [16,17,32]. Most petroleum PAHs are sparingly soluble, and obtaining constant exposure concentrations can be challenging as a result of loss mechanisms (sorption, volatilization, and degradation) [16,33]. As an example, exposure vessels with 10% headspace resulted in a 35 to 55% evaporation of total PAH over 24 h [26], whereas others saw a 64% decline in total PAHs over 84 h [34]. The passive dosing technique was developed to combat the issue of degradation whereby the chemical is partitioned from a solvent solution into a biocompatible polymer, such as polydimethylsiloxane (PDMS) [16]. A key criterion for successful use of the passive dosing system is to ensure excess mass of hydrocarbon in both the loading solution and PDMS O-ring reservoirs to prevent small amounts of depletion from affecting the target concentrations [35]. The excessive amount of hydrocarbon loaded into the PDMS O-rings has been proven to produce an accurate and precise constant aqueous concentration for the exposure duration, despite potential losses that occur in the test system [16,22,33].

O-ring loading

Polydimethylsiloxane O-rings (O-Rings West) were loaded with 1-MN (Acros Organics; 97%) in a methanol solvent (Fisher Scientific; HPLC grade), using partition coefficients, determined for the environmental conditions used in each experiment (see *Partition coefficients*). The 1-MN concentrations utilized for the mysid experiment were based on LC50 estimates made using the TLM because data for mysid shrimp were available from previous experiments. Results of the mysid shrimp assay and critical target lipid body burdens (CBBs) available for the most similar species were used to establish a range of concentrations to use in the deep-sea crustacean assays.

Partition coefficients ($K_{PDMS\text{-}Water}$)

The partition coefficient of 1-MN at ambient temperature for each of the exposure conditions (mysids = 23.3 °C and deep-sea crustaceans = 10 °C) was determined in the laboratory for use in the described assays. Precise $K_{PDMS\text{-}Water}$ values were essential to reach target dosing concentrations in dosing vessels. A predetermined amount of 1-MN was dissolved in 500 mL of methanol (MeOH) and mixed for 24 h. A total of 80 precleaned O-rings were then added to the 1-MN/MeOH solution in a 2-L glass bottle. A magnetic stir bar was added, and the solution was stirred for 72 h to reach equilibrium. Thirty loaded O-rings were added to each 2-L aspirator bottle, which was filled with seawater at the appropriate salinity (mysid = 27 practical salinity units [PSU] and deep-sea organisms = 35 PSU). O-rings were stirred by a stir bar for another 72 h. A time-series of water samples was then taken for measurement of 1-MN in seawater. Water samples were extracted with dichloromethane

(DCM; Sigma-Aldrich) before measurement with a Horiba Aqualog Scanning Spectrofluorometer (excitation = 260 nm and emission = 372 nm; model Aqualog-UV-800). For 1-MN concentrations in loaded O-rings, one O-ring was extracted with 20 mL DCM, and measurements were completed on the Aqualog fluorometer with an appropriate dilution to keep the measurement within the calibration curve. The partition coefficient between PDMS and seawater (SW) was calculated by Equation 1

$$K_{PDMS:SW} = \frac{C_{PDMS}}{C_{SW}} \quad (1)$$

where $K_{PDMS:SW}$ is the partition coefficient between PDMS and seawater, C_{PDMS} is the concentration of 1-MN in the loaded O-ring, and C_{SW} is the concentration of 1-MN in the equilibrated seawater.

Aqualog Horiba measurements

Concentrations of 1-MN were calculated from total scanning fluorescence, which was measured with an Aqualog Horiba Fluorometer. First, the total scanning fluorescence was measured for standard 1-MN, and the optimal wavelengths for maximum intensity were established (in this case, excitation = 260 nm and emission = 372 nm). A 5- to 7-point calibration curve was made using diluted DCM to provide a 1-MN standard with concentration ranges from 1 to 0.05 mg/L. Then, a 10-mL sample was extracted with DCM and measured for total scanning fluorescence. Maximum intensity at the established optimal wavelengths was used to calculate the concentration using the equation of best fit from the calibration curve.

Gas chromatography-mass spectrometry

The concentrations of 1-MN measured with the fluorometer were verified by gas chromatography-mass spectrometry (GC-MS; Agilent 6890N GC/5975C inert MSD). At the end of dosing experiments, 1-L water samples from each concentration were extracted 3 times with 100, 50, and 50 mL DCM. In the laboratory, surrogate standards (e.g., d8-naphthalene, d10-acenaphthene, d10-phenanthrene, d12-chrysene, and d12-perylene) were added to the sample, and the final extract volume was reduced to 1 mL. Extracts were then spiked with appropriate amounts of deuterated compounds as internal standards (e.g., d10-fluorene and d12-benzo[*a*]pyrene). Samples were measured in selected ion monitoring mode using a 30 m × 0.25 mm i.d. (0.25 μm film thickness) DB-5 fused silica capillary column (J&W Scientific) fitted in an Agilent model GC-MS. The oven temperature was programmed to an initial temperature of 60 to 300 °C at 12 °C/min and held at this temperature for 6 min. Selected samples were measured in a full scan to investigate any metabolized product from 1-MN by these deep-sea organisms.

Experimental design

To determine lethal exposure thresholds in organisms exposed to individual high purity aromatic hydrocarbons, in this case 1-MN, 48-h constant exposure assays were carried out using a continuous-flow exposure system as recommended by the Chemical Response to Oil Spills Ecological Effects Research Forum [20] working group. This fits studies where organisms, such as corals, have been exposed to single hydrocarbons [22]. The *A. bahia* test utilized 5 concentrations (nominally, 200, 400, 800, 1600, 3200 μg/L 1-MN) and a seawater control, tested in replicates of 4, with 10 mysids, 4 to 5 d old, in each exposure chamber. This test was completed at ambient laboratory temperature (25 °C) and 27 PSU to test the functionality of the dosing system and verify the measured partition coefficients. Deep-sea crustacean assays utilized 4 concentrations (nominally, 300, 600, 1200, 2400 μg/L 1-MN) and a seawater control, tested with as many replicates as possible based on number of organisms obtained (Table 1). For these experiments, the exposure system was constructed in temperature-controlled refrigerators and maintained at 11.3 ± 0.18 °C for experiment 1 (*Janicella* and euphausiids) and 12.0 ± 0.18 °C for experiment 2 (*Systellaspis*, *Sergestes*, and *Sergia*); salinity-matched ambient seawater (35 PSU) for both experiments. The entire system was built in a dark room, lit by only red lights to avoid any additional stress on the organisms.

Exposure system

A continuous-flow, recirculating exposure system (Figure 1) was used for the constant exposure experiments. This system is based on the novel passive dosing system designed to determine toxicity of the individual components of oil on test species [16]. Individual exposure chambers (750-mL Pyrex bottles) were connected to corresponding 2-L dosing vessels by Viton tubing and a Cole-Parmer multihead peristaltic pump, with a flow rate of 7.5 mL/min. Each exposure chamber was supplied by an individual dosing vessel, whereas the peristaltic pump intake line for each chamber was placed in the dosing vessel corresponding to the concentration being tested. Effluent lines from each chamber were placed in the same vessel as the intake line. All chambers and vessels were sealed by a cap with Teflon-lined septa with connectors to attach Viton tubing. The entire exposure system was built within temperature-controlled refrigerators to maintain temperature within the test organism's range. An image of the exposure system is available in the Supplemental Data (Figure S1).

The exposure system was filled with natural seawater sourced from Nova Southeastern University's coral nursery, aerated, ultraviolet (UV)-sterilized, and filtered to 1 μm (Polymicro). Dosing vessels were aerated prior to addition of O-rings to ensure that the seawater was 100% saturated with oxygen; no aeration took place during the exposure, to prevent volatile loss of 1-MN. Following loading, O-rings were rinsed (3 times in seawater), added to the dosing vessel of the exposure system, and equilibrated for 20 h. Water chemistry samples were collected, organisms were added to exposure chambers, and the test was initiated. Concentrations of 1-MN were monitored by fluorescence at the initiation and completion of the 48-h exposure. Samples for water chemistry (20 mL) were collected from the exposure chamber effluent line in certified volatile organic analyte vials (Thermo Scientific; with no headspace). Fluorescence was measured on the Horiba Aqualog scanning spectrophotometer calibrated with known compounds. Mortality was recorded every hour for the first 8 h and every 12 h thereafter to facilitate the calculation of 24- and 48-h LC50.

LC50 and CBB calculation

To determine the LC50, the geometric means of the 0- and 48-h concentrations of each chamber were utilized. Mortality in each chamber at 24 h was used to calculate a 24-h LC50 for each deep-water species, and 24- and 48-h mortality were used to calculate LC50s at both times for mysid shrimp. In cases with no partial mortality, the standard graphical method for

Table 1. Number of organisms in each replicate and treatment

Organism	Organisms per replicate	Total number per treatment[a]
Americamysis bahia	10	40
Janicella spinacauda	8	24
Euphausiidae	1 (controls = 2)	3 (controls = 6)
Systellaspis debilis	1	3 (2400 μg/L = 2)
Sergestes sp.	1	1 (no 2400 μg/L)
Sergia sp.	1	1

[a]"Treatment" refers to nominal target concentration groups.

determination of LC50 was used [36]. For organisms which exhibited partial mortality in some chambers, logistic regression was used. To calculate the LC50s for all organisms, individual chamber concentrations and mortality were utilized. This establishes a relationship between percentage of mortality and chamber concentration, which can be used to solve for the concentration causing 50% mortality in the test organism.

Following calculation of an LC50 for each species, CBBs were calculated using the TLM. Equation 2 gives the TLM

$$\log \mathrm{LC50} = \log CBB - 0.936 \times \log K_{\mathrm{ow}} + \Delta c \qquad (2)$$

where the LC50 (millimoles per liter) can be used to calculate a CBB (micromoles of chemical per gram of lipid) using the universal narcosis slope (–0.936), the chemical's octanol–water partition coefficient ($\log K_{\mathrm{OW}}$, 3.781 for 1-MN), and a chemical class correction factor (Δc, 0.352 for polyaromatics) [32].

Quality assurance and quality control

All data were tested for normality (Shapiro-Wilk) and homoscedasticity (Bartlett/Levene) and transformed to meet these assumptions where applicable, or nonparametric methods were used. One-way analysis of variance (ANOVA, $\alpha = 0.05$) or a Kruskal-Wallis ANOVA ($\alpha = 0.05$) on ranks of untransformed data was used to compare water quality data between treatments. All statistical analyses were completed using R (Ver 3.3.1).

The *A. bahia* assay only showed a significant difference in dissolved oxygen between treatments ($p = 0.031$) because of elevated levels in the 200 μg/L treatment compared to the 3200 μg/L treatment ($p = 0.030$). However, dissolved oxygen in either of these treatments was not significantly different from control levels. Ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), and phosphate (PO_4) concentrations were not statistically different between treatments ($p > 0.5$).

No significant differences ($p > 0.05$) in dissolved oxygen, NH_3, NO_2, NO_3, PO_4, or temperature were found between treatments for any of the deep-sea crustacean assays. *Sergestes* sp. and *Sergia* sp. assays did not have multiple replicates per treatment, preventing statistical comparisons. However, the water quality parameters did not show a pattern with 1-MN concentration, and the lack of significant differences in any of the species tested suggested that these water quality parameters were not different between treatments.

All experiments conducted in the present study included negative controls, where O-rings containing no 1-MN were added to the dosing vessel. These replicates provide a baseline level of mortality in the absence of a contaminant. However, controls of the mysid shrimp test resulted in measurable amounts of 1-MN, although O-rings were never loaded. This is assumed to be the result of contamination during sampling or analysis.

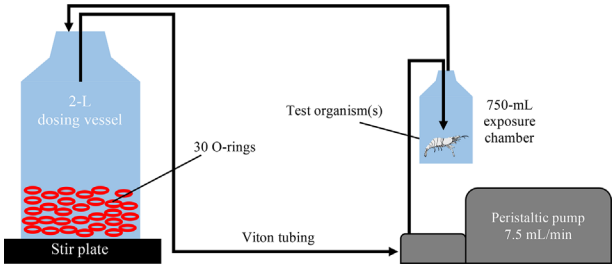

Figure 1. Schematic of the recirculating-flow exposure system. The 750-mL glass chamber is connected to a multichannel peristaltic pump with Viton tubing at a flow rate of 7.5 mL/min. Each chamber is supplied by a separate 2-L dosing vessel containing 30 O-rings.

RESULTS AND DISCUSSION

Organism collection

The mesopelagic taxa collected make up a critical component of the Gulf ecosystem, most notably in their role of connecting biological processes occurring at depth with those in the surface waters because of their diel vertical migrations. The oplophorid *Janicella spinacauda* dominated the trawl collections and is one of the most abundant caridean species in the vicinity of the Deepwater Horizon oil spill [37]. *Janicella spinacauda* is the smallest species tested that fit into the laboratory dosing chambers and is a robust species that can survive for up to 3 wk under laboratory conditions (T. Frank, Nova Southeastern University, Dania, FL, USA, personal observation). It is also a strong vertical migrator, with daytime depths of 400 to 600 m and nighttime depths of 0 to 200 m [14,37]. The original target was 25 organisms, but this was exceeded with 160—most of these were collected in the night trawls. *Acanthephyra purpurea* and *Systellaspis debilis*, also oplophorids, are the most abundant and third most abundant oplophorid near the Deepwater Horizon oil spill site [37] and are also strong vertical migrators, with daytime depths between 600 and 1000 m and nighttime depths between 0 and 200 m [14,37]. *Acanthephyra* were not collected, but 24 *Systellaspis* were returned to the laboratory alive.

Euphausiids are part of the midwater ecosystem in virtually all oceanic ecosystems and are often major components of the zooplankton biomass in the pelagic zone [38,39]. The euphausiids collected in the present study were all vertical migrators, with daytime depths between 300 and 500 m and nighttime depths between 0 and 150 m [40]. Thirty euphausiids were collected and returned to the laboratory alive. *Sergestes* sp. are relatively fragile species, and very few survived long enough to test after experiments with *J. spinacauda* and euphausiids were completed. These were caught on the first day of the cruise, with no further opportunity for capture.

Partition coefficient of 1-MN

Successful assessment of specific chemicals through passive dosing mainly depends on the precise measurement of the chemical's partition coefficient between the polymer and water ($K_{\mathrm{Polymer\text{-}water}}$; in this case $K_{\mathrm{PDMS\text{-}water}}$). In general, researchers use $K_{\mathrm{Polymer\text{-}water}}$ measured at room temperature and for fresh/distilled water for toxicity testing [41–44]. The aqueous solubility of hydrophobic organic compounds (HOCs) decreases with a decrease in temperature and an increase in salinity [45]. Thus, the partitioning of HOCs between the polymer (e.g., PDMS) and water ($K_{\mathrm{Polymer\text{-}water}}$) will vary with

temperature and salinity; specifically, $K_{Polymer\text{-}water}$ increases with a decrease in temperature and an increase in salinity [46,47]. Muijs and Jonker [47] determined $K_{Polymer\text{-}water}$ values for 9 PAHs (phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*e*]pyrene, benzo[*b*]fluoroanthene, and benzo[*k*]-fluoranthene) at the 5 to 30 °C temperature range and found that they vary widely (42–65%) for these HOCs. Thus, it was crucial for the present study to determine the $K_{PDMS\text{-}water}$ values at ambient conditions used in each toxicity test. For preliminary work with mysids, $K_{PDMS\text{-}water}$ values at room temperature (23.3 °C) and 26.8 PSU salinity were measured; and for deep-sea crustaceans, they were measured at 7 °C and 35 PSU (Supplemental Data, Table S1). These data can be found in the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) data repository (DOI: 10.7266/N7PR7TB8).

The partitioning experiments were completed in duplicate (sets A and B), and the log *K* values agree within 5% of each other. As expected, log $K_{PDMS\text{-}water}$ changes 0.11 units (from 3.09 to 3.20) when salinity changes from 0 to 26.8 PSU (both at 23.3 °C). However, it changed 0.30 units (from 3.09 to 3.39) when temperature changed from 23.3 to 7 °C and salinity from 0 to 35 PSU. From these data, it is apparent that both temperature and salinity affect partitioning of HOCs between the polymer and water phases. Jonker et al. [46] also determined that there were significant effects of both temperature and salinity in the partitioning of HOCs, and these effects were determined to be independent of each other.

Dosing concentrations

The target dosing concentrations were calculated based on the measured partition coefficients. To verify whether dosing chambers had reached target concentrations, aqueous concentrations were measured at 0 and 48 h. At the end of the experiments, water samples were also taken for GC-MS analysis to verify the fluorometric data from the Aqualog Horiba. Figure 2 shows a satisfactory agreement ($R^2 = 0.93$) between the data from Aqualog Horiba and the GC-MS.

Mean measured concentrations for each treatment during the *A. bahia* assay can be found in Table 2. Concentrations agreed well with predicted values and were on average 14.1% lower than target concentrations. The difference between the temperature during partition coefficient determination and the temperature during the exposure could be the reason for the difference in concentrations between target and measured values. Throughout the 48-h exposure, there was an average fluctuation of 9.7% for all chambers, with most of the variation attributable to fluctuations in the low concentration and control. The 1-MN concentration in half of the chambers decreased over 48 h, whereas the other half increased. The controls averaged 99.7 ± 9.2 μg/L 1-MN in all replicates. This could be attributable to contamination of samples during collection; therefore, the measured concentrations of 1-MN in controls were included in calculation of threshold concentrations. Individual chamber concentrations can be found in the GRIIDC data repository (DOI: 10.7266/N7WS8RN2).

The mean measured concentration for the deep-sea organism assays can be found in Table 3. Overall, 1-MN concentrations deviated from target concentrations by an average of 13.2%. The percentage of change over time for these assays was high (mean = 46.9%), as a result of increases in concentration of 1-MN in all chambers except 3, which showed slight decreases over 48 h. The passive dosing system relies on kinetics associated with the partitioning of hydrocarbon from the polymer to the aqueous phase until equilibrium is reached, and it is possible that the equilibration time in these assays was inadequate at the low temperature and that more time was required to reach target levels. The fluctuation over time was not attributable to volatile loss or degradation of the toxicant as observed in previous studies using petroleum hydrocarbons. The standard deviations for each treatment were also high; therefore, the individual chambers were treated as separate treatment concentrations and plotted against percentage of mortality. Individual chamber concentrations at each time point are available in the GRIIDC data repository (DOI: 10.7266/N7S46QBC).

LC50 and CBB

For each assay, the proportion of dead organisms was plotted against the concentration of 1-MN for each chamber individually to determine the LC50 for that organism. *Americamysis bahia* exhibited partial mortality at both 24 and 48 h of exposure, promoting determination of LC50 using a 3-parameter logistic regression. Figure 3 shows a satisfactory fit between the logistic models and observed mortality, with R^2 values of 0.947 and 0.930 at 24 and 48 h, respectively. In addition, *p* values of the 3 predicted model parameters were significant ($p < 0.001$) for both 24- and 48-h mortality data. Therefore, the logistic models from each time point were used to predict a 24-h LC50 of 441.02 μg L^{-1} (95% CI 384.90–474.77 μg L^{-1}) and a 48-h LC50 of 355.19 μg L^{-1} (95% CI 287.51–379.69 μg L^{-1}) following exposure to 1-MN. Type 1 narcotic compounds, such as 1-MN, exhibit nonpolar narcosis

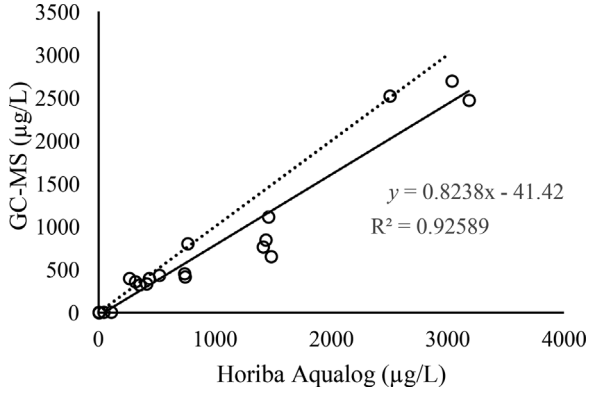

Figure 2. 1-Methylnaphthalene concentration using Horiba Aqualog and gas chromatography-mass spectrometry. Dotted line indicates a 1:1 relationship; solid line indicates the data trendline. GC-MS = gas chromatography-mass spectrometry.

Table 2. Mean measured concentrations of 1-methylnaphthalene in each nominal target concentration group of the *Americamysis bahia* exposure

Target (μg/L)	Mean concentration ± SD (μg/L)	48-h change (% of initial)[a]
Control (0)	99.7 ± 9.2	16.9
200	287.6 ± 4.2	14.8
400	440.8 ± 23.9	6.6
800	811.1 ± 28.7	6.2
1600	1551.5 ± 109.8	6.4
3200	3448.3 ± 312.6	7.2

[a]Absolute value of the percentage change.
SD = standard deviation.

Table 3. Measured mean concentration of 1-methylnaphthalene for each nominal target treatment group for the deep-sea crustacean exposures

Species	Treatment (*n*)	Mean concentration ± SD (μg/L)	48-h change (% initial)[a]
Janicella spinacauda	Control (3)	0[b]	NA
J. spinacauda	300 (3)	305.2 ± 149.6	33.2
J. spinacauda	600 (3)	564.1 ± 65.0	74.0
J. spinacauda	1200 (3)	1295.6 ± 7.1	19.3
J. spinacauda	2400 (3)	2802.2 ± 96.8	17.5
Euphausiidae	Control (3)	0	NA
Euphausiidae	300 (2)	325.4 ± 9.4	15.1
Euphausiidae	600 (3)	497.1 ± 105.7	27.8
Euphausiidae	1200 (2)	1274.4 ± 23.8	32.3
Euphausiidae	2400 (3)	2680.9 ± 185.4	41.7
Systellaspis debilis	Control (3)	0	NA
S. debilis	300 (3)	341.5 ± 91.3	88.3
S. debilis	600 (3)	549.0 ± 23.7	79.7
S. debilis	1200 (3)	1144.4 ± 138.5	58.2
S. debilis	2400 (2)	2297.4 ± 390.1	36.3
Sergestes sp.	Control (1)	0	NA
Sergestes sp.	300 (1)	343.0	60.8
Sergestes sp.	600 (1)	519.5	56.2
Sergestes sp.	1200 (1)	1064.6	92.9
Sergia sp.	Control (1)	0	NA
Sergia sp.	300 (1)	247.8	11.4
Sergia sp.	600 (1)	598.8	63.2
Sergia sp.	1200 (1)	1122.3	54.9
Sergia sp.	2400 (1)	2364.3	27.7

[a]Absolute value of the percent change.
[b]No standard deviation calculated for controls.
n = number of replicates for that group; NA = not applicable; SD = standard deviation.

as their toxic mode of action. These compounds partition into tissue lipids, specifically cell membrane lipids, and disrupt normal function [48]. For smaller organisms, equilibrium should be reached in the tissue lipid after roughly 24 h [24], which is why the 24- and 48-h LC50s for 1-MN are similar. The chemical had reached the organism's toxic threshold after 24 h, and mortality increased in only a few of the lower concentrations after an additional 24 h, causing a slight decrease in LC50.

Because of the elevated mortality in most chambers, including controls, of the deep-sea organism assays, only 24-h LC50 calculations were reliable for all organisms. The deep-sea organism exposures did not result in partial mortality in any species except for *J. spinacauda*. Therefore, mortality for *J. spinacauda* was plotted against the concentration of 1-MN, and the 3-parameter logistic model was used (Figure 4). The R^2 for this relationship was 0.908, and all 3 parameters of the logistic model were significant ($p < 0.001$). The 24-h LC50 for *J. spinacauda* following exposure to 1-MN was calculated at 889.75 μg/L^{-1} (95% CI 694.02–979.36 μg L^{-1}).

The 24-h LC50s for the remaining 4 species were estimated using the graphical method, which is the standard method for LC50 determination when mortality is either 0 or 100%. This process uses the highest concentration eliciting no mortality and the lowest concentration causing 100% mortality and prevents any calculation of confidence intervals because only those 2 points are used to create a line. The 24-h LC50s for all remaining organisms can be found in Table 4, along with those already discussed. The 48-h LC50 of the copepod *Oithona davisae* when

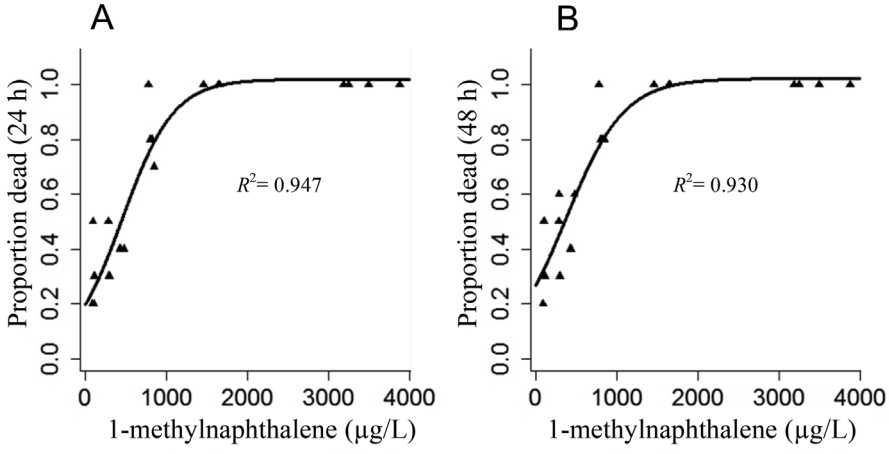

Figure 3. Proportion of *Americamysis bahia* dead at 24 h (**A**) and 48 h (**B**) following exposure to 1-methylnaphthalene. Fitted line represents the 3-parameter logistic regression based on the data. $n = 10$ for each point.

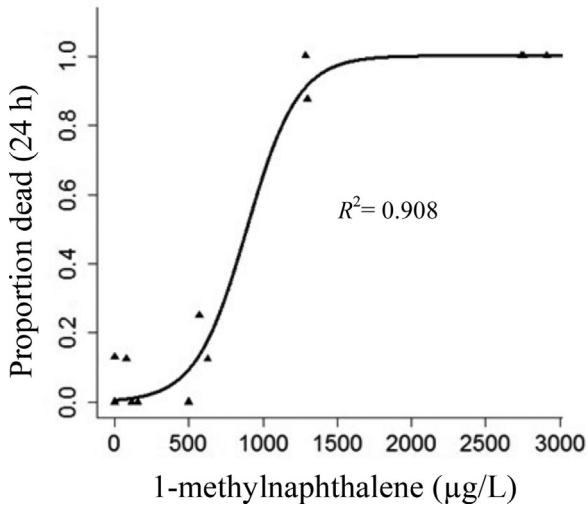

Figure 4. Proportion of *Janicella spinacauda* dead at 24 h following exposure to 1-methylnaphthalene. Fitted line represents the 3-parameter logistic regression based on the data. $n = 10$ for each point.

exposed to 1-MN was calculated at 2652 μg/L [49]. This is over 2-fold higher than the highest value calculated in the present study, indicating increased sensitivity for the deep-sea crustaceans tested. Another study on the effects of a very similar compound, 2-methylnaphthalene, on arctic species resulted in a range of 350 and 5420 μg/L for 96-h LC50s [50]. The range of LC50s calculated in the present study is 171.53 to 889.75 μg/L. The deep-sea crustaceans were slightly more sensitive compared with the arctic species tested, even at the shorter duration used in the present study. A possible source of this difference is the use of nominal concentrations by Olsen et al. [50], which would have resulted in an overestimation of LC50 because of a lack of measured concentrations for this volatile compound.

The 24-h LC50s for each organism tested in the present study were utilized to calculate a CBB using the TLM. The CBB is a comparable, interspecific measure of the toxic threshold for organisms exposed to narcotic chemicals that act via nonpolar narcosis. The range of CBBs previously calculated [32] is from 24.1 to 500 μmol/g lipid and represents a variety of species, including both freshwater and saltwater species that inhabit the water column and benthic zones. These values are also representative of a variety of monocyclic, polycyclic, and chlorinated aromatic hydrocarbons. The CBBs calculated in the present study were compared with those compiled previously (Figure 5). Although CBBs for most crustaceans are lower than those for other organisms, measured CBBs of all species tested

Table 4. Measured 24-h median lethal concentrations and associated critical target lipid body burdens for all organisms following exposure to 1-methylnaphthalene

Organism	24-h LC50 (μg/L; 95% CI)[a]	CBB (μmol/g lipid)
Sergestes sp.	171.53	9.39
Euphausiidae	325.38	17.80
Americamysis bahia	441.02 (384.90–474.77)	24.13
Systellaspis debilis	560.83	30.69
Sergia sp.	860.53	47.09
Janicella spinacauda	889.75 (694.02–979.36)	48.68

[a]95% CI included when applicable.
CBB = critical target lipid body burden; CI = confidence interval; LC50 = median lethal concentration.

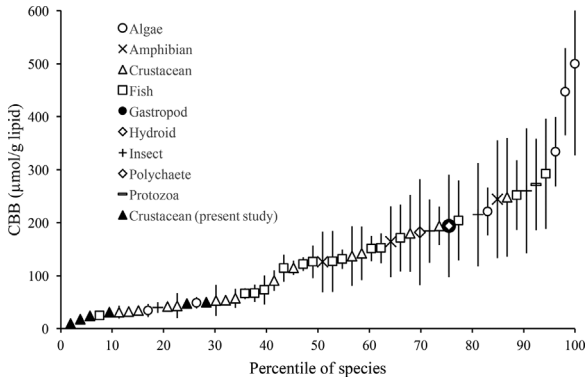

Figure 5. Percentile of previous and measured critical target lipid body burdens. Error bars indicate ± standard error. CBB = critical target lipid body burden.

in the present study were <70% (4 of the 6 species <90%) of the species for which these data are available, indicating higher sensitivity. Furthermore, the CBBs calculated for the deep-sea crustaceans tested also indicate higher sensitivity to narcotic chemical exposure when compared with other crustaceans with previously calculated CBBs.

In contrast to larger animals, micronekton and macrozooplankton present physiological and behavioral vulnerabilities that will influence the magnitude and duration of their exposure to petroleum/dispersant-derived substances localized in deep-water plumes. Most notably, their small size confers a large surface area relative to their volume, which, coupled with high permeability of the body surface and at the gills, results in increased exchange between the environment and the animal's extracellular fluid [51]. For some species, the size of prey will also overlap with oil droplets, which may be consumed [52]. For many species of micronekton and zooplankton in a spill zone, their vertical migration behavior would result in their residing in depths of relatively high hydrocarbon and dispersant concentration (e.g., deep-water plumes [15,53]) during the day and migrating into shallower waters at night. If petroleum/dispersant exposure inhibits neural function and/or motor activity in these organisms to such an extent that their migratory abilities are impaired, this would have a substantial impact on higher–trophic level predators in shallower waters, such as larval and juvenile bluefin tuna, which rely on these vertical migrators for a significant portion of their diets [9].

CONCLUSIONS

The purpose of the present experiment was to determine the partition coefficients of 1-MN at various environmental conditions and to utilize those values to reach target concentrations during passively dosed toxicity tests with a common laboratory organism and 5 deep-sea micronekton organisms. The partition coefficient work showed effects from both temperature and salinity, which aided in achieving only a 13 to 14% deviation from target concentrations during all assays. The concentrations obtained during the *A. bahia* exposures resulted in an average change of <10% over 48 h. Deep-sea organism exposure concentrations varied much more over the 48-h exposure, likely attributable to inadequate equilibration time at the lower temperatures utilized for these species, as concentrations in each chamber increased over time.

Mortality following a 24-h exposure to 1-MN was used to measure LC50s as inputs to the TLM to calculate CBBs for each species. The CBBs measured for *A. bahia* and each deep-sea

crustacean indicated high sensitivity to the narcotic chemical, 1-MN. The deep-sea micronekton utilized in this exposure are vertical migrators and a key trophic linkage between the mesopelagic and surface waters. If plumes of oil or dispersed oil remain within the boundaries of these vertical migrations, they will be traversed multiple times a day and toxicity at low levels may be the result. The measured sensitivity of these organisms could lead to disruption of this connectivity following exposure to petroleum hydrocarbons and eventually to effects on commercially important fish which rely on these animals for food.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3926.

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Data availability—Data are publicly available through the GRIIDC at https://data.gulfresearchinitiative.org (DOI: 10.7266/N7WS8RN2, 10.7266/N7S46QBC, and 10.7266/N7PR7TB8).

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