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Effects of sediment amended with Deepwater Horizon incident slick oil on the infaunal amphipod *Leptocheirus plumulosus*

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ABSTRACT

Crude oil released from the Deepwater Horizon disaster into the Gulf of Mexico posed potential impacts to infaunal invertebrates inhabiting near shore habitats. The effects of sediment-associated weathered slick oil on the amphipod *Leptocheirus plumulosus* was assessed using 28-d exposures to total PAH sediment concentrations ranging from 0.3 to 24 mg/kg (sum of 50 PAHs or tPAH50). Survival and growth rate were significantly decreased in the 2.6, 11.4 and 24.2 mg/kg treatments, but only growth in 5.5 mg/kg. Offspring production was dramatically decreased but was variable and significantly different only for 24.2 mg/kg. The concentrations associated with 20% decreases relative to reference were 1.05 (95% CI = 0–2.89) mg/kg tPAH50 for growth rate and 0.632 (95% CI = 0.11–2.15) mg/kg tPAH50 for offspring production. The concentrations of PAHs affecting amphipods are within the range of concentrations measured in marsh areas reportedly impacted by DWH oil after its release.
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1. Introduction

As a result of the Deepwater Horizon (DWH) disaster, crude oil was released into the Gulf of Mexico (GOM) from a depth of approximately 5000 ft in the Mississippi Canyon 252 (MC-252) lease block in 2010. The released oil reached beaches, wetlands, and barrier islands along the GOM coast, extending beyond shorelines and reportedly affected large portions of the southeastern Louisiana marshland (Wang and Roberts, 2013; Michel et al., 2013; Ramsey et al., 2014). Although concentrations of polycyclic aromatic hydrocarbons (PAHs) declined in the water column after the bulk of the release was halted, oiled sediment impacts in many marsh habitats may remain a concern for years or decades (Silliman et al., 2012; Liu et al., 2012; Turner et al., 2014). Oil washed into salt marshes or deposited in sediments may be subjected to variable weathering processes, which can significantly change the chemical composition and toxicity of the oil.

Exposure to oil or to PAHs has been reported to cause lethal and sublethal toxicity to a variety of marine and estuarine sediment-dwelling invertebrates (e.g., Engraff et al., 2011; Morales-Caselles et al., 2008; Kravitz et al., 1999; Wirth et al., 1998). In addition, oil trapped in sediment has been reported to have altered the macrofaunal community (Gomez Gesteira and Dauvin, 2000; Junoy et al., 2005; Hong et al., 2014). Potential impacts to fish and invertebrates exposed to water and sediment samples collected from the GOM and presumed impacted by the DWH crude oil release or amended with field-collected oil residues have been reported (e.g., Raimondo et al., 2014, in press; Dubansky et al., 2013; Brown-Peterson et al., 2015; Echols et al., 2015; Faksness et al., 2015).

Whole-sediment toxicity tests are commonly used in contaminated site assessments. Chronic whole-sediment toxicity tests represent a larger portion of the organism's life cycle and directly address sublethal endpoints (e.g., growth, reproduction) in addition to survival. The amphipod *Leptocheirus plumulosus* 28-d growth and reproduction test (U.S. EPA, 2001; ASTM, 2008) is one of the most frequently used sublethal test methods (Greenstein et al., 2008). Amphipods have high ecological importance, high abundance, and a widespread distribution as they are found in nearly all marine and freshwater sediments. *L. plumulosus* is a borrowing amphipod that lives in close physical contact with the sediment, builds semi-permanent tubes and is capable of surface deposit feeding. It has a wide distribution on the east coast of the United States, occurring from Cape Cod, Massachusetts, to northern Florida (ASTM, 2008). This infaunal amphipod is easily cultured in the laboratory and has been widely used in sediment toxicity assessments according to standard guidelines (U.S. EPA, 1994, 2001; ASTM, 2008). To assess the potential impact to infaunal invertebrates inhabiting near shore habitats affected by the Deepwater Horizon spill, we determined the effects of field-collected MC-252 oil on chronic survival,

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growth and reproduction of *L. plumulosus* using standard 28-d whole-sediment toxicity testing.

2. Material and methods

2.1. Sediment treatments preparation

Reference (non-oiled) sediment from the Gulf of Mexico (fine sand 1.13%, very fine sand 1.15%, silt 26.22%, clay 72.85%; total organic carbon [TOC] = 0.93%) was collected at Caminada Bay, LA (29.21070, 90.09827) on 3 December 2013. Negative control sediment (sand 18.9%, silt 66.6%, clay 14.2%; TOC = 1.7%) was collected from Sequim Bay, WA (Battelle, Sequim, WA, USA). This control sediment was used in laboratory cultures of *L. plumulosus* and provides a measure of test acceptability and evidence of test organism health (U.S. EPA, 2001; ASTM, 2008). To prepare the different oiled sediment treatments, DWH oil (weathered surface MC-252 slick oil "Slick B" collected under chain of custody on 19 July 2010 by the skimmer vessel *USCGC Juniper*) was amended to the reference sediment as described in Krasnec et al. (2015). Reference sediment was frozen for transport to the laboratory (Brown-Peterson et al., 2015) and was allowed to thaw for 5 days at room temperature before being homogenized by mixing for 2 min in a stainless steel mixer bowl (Cuisinart® SM-70 7-quart stand mixer) at low speed. Reference sediment (2.5 kg wet wt.) was mixed with the appropriate mass (0.74–23.79 g) of oil (Table S1) for 30 min using the mixer mentioned above to create seven oiled sediment treatments targeting increasing concentrations of sediment-associated total PAHs (Table S1). This preparation method creates a homogenous oil treatment and does not alter the composition of individual PAHs in the oil (Brown-Peterson et al., 2015; Krasnec et al., 2015). The reference and the negative control sediment treatments were mixed using the procedures described above, sans the addition of oil. Sediment treatments were added to exposure chambers following their preparation at which time a sample (~200 g) from each treatment was collected, kept at 4 °C and within 24 h was shipped overnight in ice for chemical analysis. The sediment was solvent extracted upon arrival.

2.2. Whole sediment toxicity test

Laboratory-cultured *L. plumulosus* were exposed to oil-dosed sediment according to the 28-d toxicity test protocol described in the U.S. EPA (2001) and in ASTM Method E1367-03 (Reapproved 2008). The 28-d protocol incorporates the assessment of two sublethal endpoints, growth and reproduction, in addition to lethality. For each treatment, each of seven 1-L beakers (five beakers for toxicity testing and two sham beakers for chemistry) received 200 mL of spiked sediment and 800 mL of 20‰ artificial seawater and was placed in a water bath maintained at a constant temperature of 25 °C. Seawater was reconstituted using dry sea salts (Crystal Sea® Marinemix, Marine Enterprise, International, Baltimore, MD, USA) and type I reverse osmosis water and was aged for at least 1 week prior to use. Each beaker received a plastic lid cover, trickle flow aeration and was left undisturbed for 24 h. After this period, twenty juvenile amphipods were added to each beaker (day 0). Juvenile amphipods were defined as those that passed through a 0.61-mm mesh and were retained on a 0.425-mm mesh (1.1–2 mm in length; average dry wt. = 30 μg). Beakers were maintained under fluorescent lights on a 16:8 light:dark regime to mimic a natural light cycle. Water quality parameters (temperature, pH, dissolved oxygen, salinity and total ammonia) were measured on days 0 and 28 for all replicates and prior to water exchange from one replicate per treatment during the test. Amphipods were fed ground Tetramin 3 times weekly (20 mg/beaker for the first two weeks, then 40 mg/beaker for the last two weeks). Porewater was obtained by centrifugation from one sham beaker per treatment on days 0 and 28 and analyzed for total ammonia, salinity, and pH. The overlying water of all beakers was renewed three times weekly (50% of volume). At the end of the 28-d exposure, sediment was sampled from one sham beaker, kept at 4 °C and within 24 h was shipped overnight in ice for chemical analysis. The contents of the five replicate beakers used for toxicity assessment were gently sieved (0.425- and 0.25-mm ASTM testing sieves, stacked) to collect individuals. Surviving adults retained on the 0.425 mm sieve were transferred to a glass bowl, enumerated, placed on a pre-dried and pre-weighed aluminum pan, dried in an oven at 60 °C for 24 h, and re-weighed for dry biomass determination. The offspring retained on the 0.25-mm sieve along with all material remaining after removal of surviving adult amphipods were transferred to 70% ethanol/Rose Bengal solution and later enumerated.

2.3. Chemical analysis of amended sediment

Sediments from each of the treatments were analyzed for polycyclic aromatic hydrocarbons (PAHs) including alkyl homologues at experiment initiation and termination by gas chromatography with low resolution mass spectrometry using selected ion monitoring (GC/MS-SIM). The analytical procedure is based on EPA Method 8270D. A sum of 50 different PAHs (Table S2) was quantified and expressed as total PAHs (tPAH50) on a dry-weight basis. All chemical analyses were performed by ALS Environmental (Kelso, WA, USA).

2.4. Data analysis

Mean amphipod replicate survival, growth (as the mean dry weight gain per day per adult amphipod surviving at test termination), and reproduction (as offspring per survivor) data determined at the end of the test were arc-sine transformed and then tested for homoscedasticity and normality using Levene's and Shapiro-Wilk's tests, respectively. Oil-spiked sediment treatments were compared with the reference treatment (non-spiked sediment) using one-way analysis of variance (ANOVA) using SigmaStat® statistical software (SPSS). For data that fulfilled assumptions of parametric statistics, significant differences in survival were evaluated via analysis of variance followed by pairwise contrasts between test sediments and the control using Fisher's least significant difference (1-tailed, $P = 0.05$). Data that failed to meet the normality assumption were evaluated using Steel's Many-One Rank Test, (1-tailed, $P = 0.05$).

For the reproduction and growth endpoints, effects concentrations that produced 10% and 20% reduction from controls (EC_{10} and EC_{20}) were derived by fitting log-logistic curves with the drc package (Ritz, 2010; Ritz and Streibig, 2005). Profile-likelihood based confidence intervals (Venzon and Moolgavkar, 1988; Faraggi et al., 2003) were estimated using the bbmle package (R development core team, 2013; Bolker and R Development Core Team 2013). This analysis was performed in R using version 3.1.1 (R Core Team, 2013). Point estimates (e.g., LC_{50}) were not calculated for amphipod survival because no method produced reliable point estimates for this endpoint.

3. Results and discussion

3.1. Sediment PAH characterizations and concentrations

The tPAH50 concentration for the control sediment was 0.4 mg/kg. Reference sediments (no oil added) had trace levels of tPAH50 (0.008 mg/kg). Proportionately increasing concentrations of tPAH50 in the treatments were successfully achieved using the mixing process. Measured concentrations of tPAH50 in spiked reference sediments at experiment termination were 0.3, 0.6, 1.0, 2.6, 5.5, 11.4, 24.2 mg/kg dry wt. The measured concentration of individual PAHs is presented in Table S3. Concentrations of tPAH50 measured at experiment initiation are not reported because of uncertainty resulting from an analytical sediment sample labeling error. However, we do not expect that the concentrations of tPAH50 decreased substantially, if at all, during this 28-d test based on the low level of tPAH50 decrease (from no decrease