Effect of Deepwater Horizon Oil on Growth Rates of Juvenile Penaeid Shrimps

Lawrence P. Rozas · Thomas J. Minello · M. Scott Miles

Abstract Marsh shoreline, an important habitat for juvenile penaeid shrimps, was extensively oiled in coastal Louisiana by the Deepwater Horizon oil spill of 2010. The effect of this spill on growth was examined for brown shrimp *Farfantepenaeus aztecus* and white shrimp *Litopenaeus setiferus* held for 7 days in field mesocosms in Barataria Bay during May and August 2011, respectively. The experiments each had 10 treatment combinations, five apparent oil levels, each one with and without added food. Mesocosms were placed in northern Barataria Bay along shorelines that varied in oiling (designated as heavy, moderate, light, very light, or none based on NOAA surveys), and shrimp in half the mesocosms received additional food. Polycyclic aromatic hydrocarbon (PAH) concentrations determined from sediment cores collected at each mesocosm were significantly higher at heavy and moderate than very light shorelines and also higher at moderate than light and none shorelines. Brown shrimp grew more slowly at heavy than very light or none shorelines, and a statistically significant negative relationship was detected between brown shrimp growth rates and sediment PAH concentrations. In August, PAH sediment concentrations had decreased significantly from the values measured in May, no significant difference in white shrimp growth rates was detected among oiling levels, and no relationship was detected between white shrimp growth and sediment PAH concentrations. Both brown shrimp and white shrimp grew more rapidly in mesocosms where food was added. Our study shows that exposure to nonlethal concentrations of petroleum hydrocarbons can reduce growth rates of juvenile penaeid shrimps.

Keywords Field experiment · Growth comparison · *Farfantepenaeus aztecus* · *Litopenaeus setiferus* · Food addition

Introduction

The Deepwater Horizon (DWH) oil spill that began on April 20, 2010 released an estimated 4.2 million barrels of oil over 87 days into the Gulf of Mexico (McNutt et al. 2011). Although the spill originated from a wellhead on the seafloor 80 km southeast of the Mississippi River delta, in the weeks and months that followed the well blowout, oil from the spill was eventually driven inshore by coastal winds into the delta wetlands of Louisiana (Walker et al. 2011). An estimated 728 km of marsh and mangrove shoreline in Louisiana were oiled by this spill, and the degree of oiling for 40 % of this shoreline was classified as heavy or moderate, the two highest categories (NOAA 2012). Some of the most severe oiling occurred in the Bay Jimmy area of northern Barataria Bay, where 7 months after the spill, Lin and Mendelssohn (2012) observed near complete mortality of the marsh vegetation along heavily oiled shorelines.

Estuarine wetlands in coastal Louisiana provide valuable nursery habitat for young shrimps, crabs, and fishes (Baltz et al. 1993; Minello 1999). The shallow wetland habitats of the Barataria Bay system, especially the marsh shoreline (edge),

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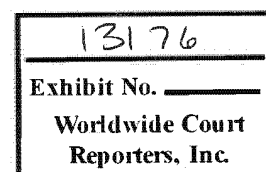
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support high densities of ecologically and economically important penaeid shrimps (Roth 2009; Rozas and Minello 2010). The marsh edge is also particularly vulnerable to oiling because the vegetation there is the first to come into contact with floating oil from adjacent waterways (Mendelssohn et al. 2012).

The oil that coated the shorelines of Louisiana estuaries had weathered in the Gulf of Mexico and lost most of its volatile constituents before making landfall (Mendelssohn et al. 2012). Even so, this oil had lethal and sublethal effects on some marsh animals. Fiddler crab and terrestrial arthropod (insects and spiders) populations were suppressed in oiled marshes, even though the plants in these marshes appeared unaffected by the oil (McCall and Pennings 2012). The weathered oil also caused sublethal damage to genes, enzymes, and the gills of gulf killifish *Fundulus grandis*, an abundant resident fish of coastal marshes (Whitehead et al. 2011).

Nekton and other mobile organisms may temporarily leave and avoid heavily oiled habitat (Roth and Baltz 2009), but many animals continue to use contaminated shoreline. Nekton densities on the marsh surface were not reduced by low levels of degraded petroleum hydrocarbons in marsh sediments following spills in Galveston Bay (Rozas et al. 2000). Those organisms that continue to use contaminated habitat could suffer sublethal, chronic health effects and reduced rates of growth and survival (Moles and Norcross 1998; Whitehead et al. 2011).

Our objective was to establish whether contamination by petroleum hydrocarbons affected the nursery function of estuarine habitats for shrimp by examining the relationship between the amount of petroleum hydrocarbons in shoreline sediments and the growth rates of juvenile brown shrimp *Farfantepenaeus aztecus* and white shrimp *Litopenaeus setiferus*. We measured growth of shrimps held in mesocosms placed in shallow water along marsh shorelines that were designated by National Oceanic and Atmospheric Administration's (NOAA) Shoreline Cleanup and Assessment Technique (SCAT) surveys as having different amounts of oiling. We tested the null hypotheses that the amount of oil in the sediments and the growth rates of shrimp were not significantly different among these oiling treatment levels. We also more directly examined the relationship between growth and the amount of oil in sediments using linear regression. Because the effect of oil may be through impacts on available food, we also measured the biomass of benthic infauna (used as food by shrimp) in mesocosms, and we tested for a food effect on growth by adding food to half of the mesocosms.

Methods

Field Experiments

Our study area was located in northern Barataria Bay and included marsh shorelines in and around the Bay Jimmy area

(Fig. 1). Tides in the study area are predominantly diurnal and have a mean daily range of 0.3 m (Orlando et al. 1993). We selected shoreline locations each of approximately 1 km in length within the study area that varied by oiling level (heavy, moderate, light, very light, none) based on compiled data from NOAA SCAT surveys of oiling intensity conducted before April 24, 2011. Our experiments were conducted in 2011 when each species was locally abundant in the study area: brown shrimp in May and white shrimp in August. Oil from the DWH spill first made landfall in the study area in June 2010 (Lin and Mendelssohn 2012). Therefore, our experiments were initiated about 11 (May) and 14 (August) months following this initial oiling, although oil continued arriving on these shorelines for months after June 2010. For the May experiment, five locations were randomly selected along each shoreline within each level of the oiling treatment, and a pair of mesocosms was placed at each location for a total of 50 mesocosms (Fig. 1). Mesocosms in August were located near the initial sites in undisturbed areas.

Mesocosms were deployed 1–2 days before initiating an experiment. Rozas and Minello (2011) provide a detailed description of these mesocosms, which were constructed of 3.2-mm mesh nylon netting and enclosed 0.89 m^2 of shallow nonvegetated bottom. Each enclosure was set in place by pushing it through the water to the bottom substrate, and no effort was made beforehand to remove potential competitors or predators from the mesocosm site. The bottom edge of the mesocosm was pushed 10–15 cm into the substrate to prevent escape by experimental shrimp or entry by organisms after the initiation of an experiment. We then collected five (2.5-cm deep × 5.0-cm diameter) benthic cores from undisturbed sediment around the outside perimeter of each mesocosm. Four of these core samples were pooled (combined sample area= 78.5 cm^2) and used to measure potential prey (benthic infauna) availability at each mesocosm site. These samples were washed through a 0.5-mm mesh sieve, and the material retained was preserved in formalin, labeled, and returned to the laboratory for processing. The fifth core was used to measure sediment grain size and organic content. We used the method of Folk (1980) to determine proportions of sand, silt, and clay in the sediment samples. The sand fraction that remained after sieving the sediment samples contained organic material that could be removed only by combustion. Therefore, this method was modified slightly for the sand fraction by first combusting this material as described below to remove any organic material. The combustion process left an oily film on the inside of the containers (glass beakers) of some samples. The weight of this oily residue (if present) and the organics was subtracted from the sample weight before computing the proportion of sand in these samples. Sediment organic content was determined by combusting a 2–10-g sediment subsample in a muffle furnace at 550 °C for 1 h (Dean 1974). Additional sediment cores (2.5-cm

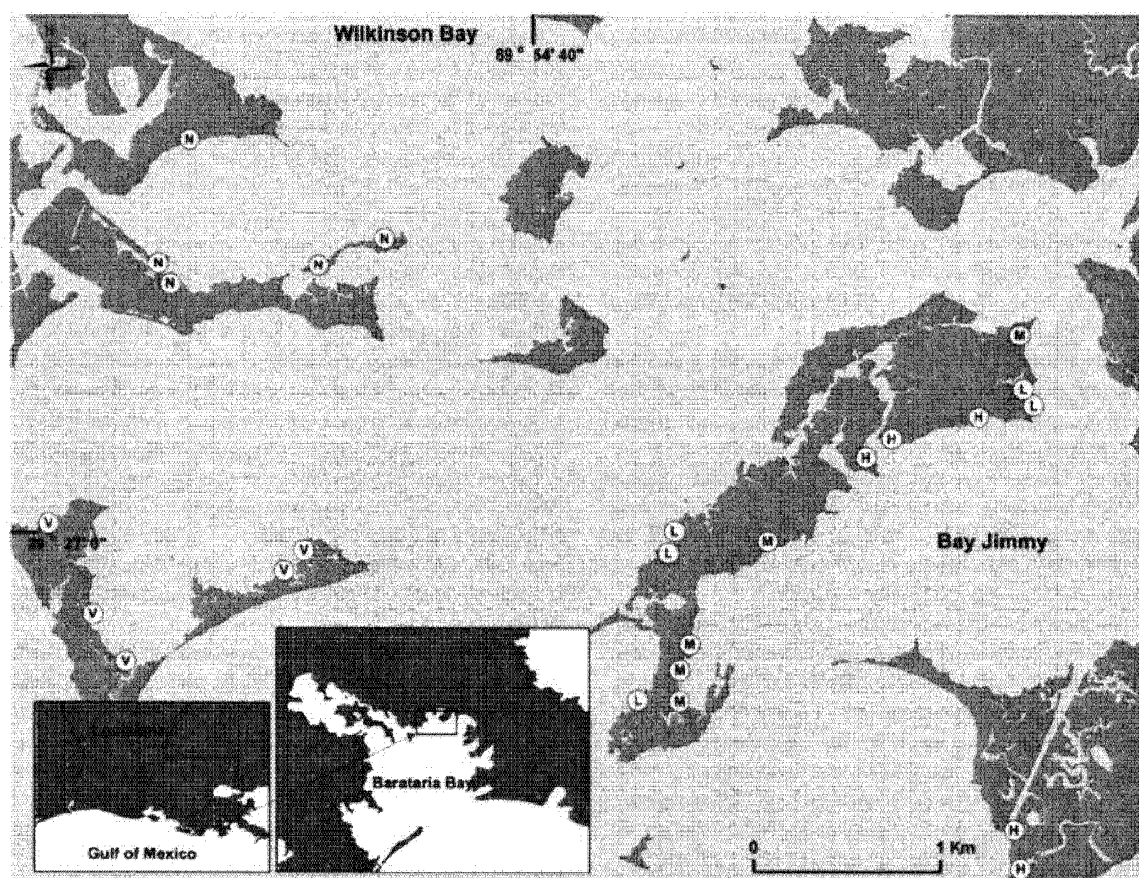BP-HZN-2179MDL09222130

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Fig. 1 Map of study area and experimental mesocosm locations within Barataria Bay estuary, southeastern Louisiana. Experimental mesocosms were located in pairs at 25 locations (*yellow circles*) in Bay Jimmy area. *Letters in circles* indicate oiling treatment levels (H = heavy, M = moderate, L = light, V = very light, and N = none)

deep×3.8-cm diameter) were taken adjacent to the four infaunal cores and were pooled, placed into pre-cleaned glass jars covered with Teflon-faced lids, transported to Louisiana State University (LSU) on ice, and used for hydrocarbon analysis.

The experimental design incorporated both oiling and food treatments with five replicate mesocosms per oiling–food treatment combination. At each of 25 locations, we placed a pair of mesocosms spaced approximately 10 m apart in shallow water along the shoreline (mean distance from marsh= 1.2 m). We included a food treatment to examine possible indirect effects (through food availability) of the oil on shrimp growth. One of these shallow water mesocosms was randomly assigned to receive additional food (0.78 g Rangen Shrimp Production Formula 35^{TM} enclosure^{-1} day^{-1}) during an experiment, whereas no food was added to the other mesocosm. Rangen 35 is commercially available and has been shown to sustain growth and survival of penaeid shrimps (Davis and Arnold 1994).

We collected shrimp for the experiments from two locations outside oiled areas of Barataria Bay (based on SCAT surveys) using small bag seines and immediately transferred them to aerated containers. Experimental shrimp for the May experiment were collected just west of Wilkinson Bay approximately 3 km northwest of our study area. In August, the white shrimp population at this location was too low to stock mesocosms within the time allotted for setting up this experiment. Therefore, we collected shrimp the day before initiating the August experiment near Caminada Bay approximately 36 km southwest of the study area. Experimental shrimp were tagged, measured to the nearest millimeter in total length (TL), and then assigned randomly to a mesocosm. We used five individuals per mesocosm in each experiment. This stocking density (5.6 shrimps m^{-2}) allows us to compare our results with previous work using a similar density (Rozas and Minello 2009, 2011; Baker and Minello 2010), and it is within the range of naturally occurring densities (individuals per square meter) measured at high tide for shrimp in salt marsh

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ponds of Barataria Bay (Rozas and Minello 2010). These experimental densities are likely lower than those expected at low tide when shrimp are concentrated within subtidal areas. We used Visible Implant Elastomer (VIE™) tags injected into the abdominal muscle tissue to individually mark the experimental shrimp within each mesocosm. Retention for VIE tags is high in shrimp (Godin et al. 1996), and our unpublished laboratory experiments indicate that these tags do not affect shrimp growth or survival. No effect on growth was observed using VIE tags on juvenile blue crabs (Davis et al. 2004).

We measured the TL of shrimp at the beginning and end of an experiment along with the final wet weight. We estimated initial weights of experimental shrimp using length–weight relationships derived from other shrimp collected at the beginning of each experiment; this approach was used to reduce handling effects on experimental animals. We derived these length–weight relationships (equations) after first log transforming the size and weight data to ensure a linear relationship and then regressing Log_{10} weight by Log_{10} TL.

We measured environmental variables that might affect growth both within and outside of mesocosms to determine whether experimental artifacts affected our results. Selected mesocosms were instrumented with 11 Onset™ recorders (for water temperature) and six Hydrolab™ Datasonde 3 multiparameter water quality loggers (for water temperature, salinity, DO = dissolved oxygen concentration) to continuously measure environmental conditions near the bottom during the experiments. Water depth, water temperature, salinity, and DO also were measured at each mesocosm during the day approximately daily ($n=6$) during each experiment. We measured water temperature, salinity, and DO along the outside edge of each mesocosm with a handheld meter, which was calibrated each day before use to ensure accuracy. These variables also were measured inside mesocosms on days 2 and 6 of each experiment. The data from this daily monitoring were used to assess the reliability of the instruments used to continuously monitor selected mesocosms. Water depth was measured with a meter stick just outside each mesocosm at the point farthest from the marsh. The water depth measured at each mesocosm during daily monitoring was used with continuously recorded water level data from a NOAA tide gauge and two temporary tide gauges to calculate flooding durations for mesocosms at each location.

Each growth experiment was run for 7 days. At the end of an experiment, we collected the shrimp by carefully lowering a drop sampler (2.6-m diameter fiberglass cylinder; Zimmerman et al. 1984) over the mesocosm and removing the water inside the sampler with a gasoline-powered centrifugal pump. Once the area within the drop sampler was drained, we carefully removed the mesocosm and collected by hand all nekton located within the mesocosm area, the area enclosed during the experiment. Marked shrimp were immediately placed on ice

within pre-cleaned 125-ml clear glass jars covered with Teflon-faced lids, as these organisms were later analyzed for hydrocarbon contamination. Any organisms within the drop sampler but not within the mesocosm area were released and not enumerated. The protocol we used to recover experimental shrimp allowed us to drain the area around each mesocosm before removing it to recover shrimp and other organisms. Therefore, we are confident that the organisms recovered with the experimental shrimp were present inside the mesocosms during the experiments. We weighed and measured each tagged shrimp within 12 h to determine their final size. Because TL could not be measured for shrimp with broken rostrums, we estimated the TL of these shrimp based on their final weight from length–weight equations derived as described above for initial lengths. We determined growth rates for each recovered experimental shrimp by subtracting the initial size measurement (TL or wet weight) from the final size measurement and dividing this difference by the duration (in days) of the experiment. After these data were collected, marked shrimp were returned to pre-cleaned sample jars, frozen, and transported whole to LSU for analysis.

Unmarked fishes and decapod crustaceans recovered when we removed the experimental shrimp from the mesocosms could have affected the growth or survival of experimental shrimp through competition or predation. Therefore, nekton recovered from each mesocosm was identified to the lowest feasible taxon. We measured the size of each unmarked organism and pooled individuals of each species in a sample to determine biomass (wet weight).

Analytical Methods for Hydrocarbon Analyses

Sediment and shrimp samples were analyzed for petroleum hydrocarbon contamination using gas chromatography/mass spectrometry (GC/MS) at the LSU Department of Environmental Sciences (DES). The GC/MS analysis, operated in selective ion monitoring (SIM) mode, has previously been used by the DES for both oil spill response activities and fate and effect studies throughout the USA (Henry and Overton 1993; Hoff et al. 1993; Sauer et al. 1993; Overton et al. 2008; Miles et al. 2011; Gao et al. 2012).

Sediment samples were extracted according to the SW-846 Method 3540C—Soxhlet extraction (U.S. Environmental Protection Agency 1996). The prepared sediment sample was spiked with a 1.0-ml aliquot of surrogate solution (20 µg ml^{-1}) and allowed to flux for approximately 12 h. The concentrated sediment extract was spiked with a 10-µl aliquot of internal standard (1,000 µg ml^{-1}) immediately prior to analysis.

Shrimp tissue samples were extracted using a matrix solid-phase dispersion method. Prior to extraction, whole shrimp samples were hand-washed with deionized water to remove debris or contamination from the shrimp exoskeleton. Shrimp

tissue and C18 media were homogenized, spiked with a 1.0-ml aliquot of surrogate (20 μg ml⁻¹), and allowed to set for approximately 10 min. The spiked mixture was then transferred into a pre-cleaned polypropylene 10-ml syringe barrel that contained a pre-cleaned filter disk and extracted as described in Crouch and Barker (1997). The resulting elution was collected in a 15-ml glass extraction thimble and concentrated to a final volume of 1.0 ml using a nitrogen concentration apparatus. The tissue extract was spiked with a 10-μl aliquot of internal standard (1,000 μg ml⁻¹) immediately prior to analysis.

An Agilent 7890A gas chromatograph fitted with a HP-5MS high-resolution capillary column (30-m long, 250-mm diameter, and 0.25-mm thick film) and equipped with an Agilent 5975C inert XL mass selective detector (MSD) was used to analyze sediment and tissue extracts. The carrier gas used was ultrahigh purity helium (Air Liquide, Houston, TX) operating at a constant flow rate of 1 ml min⁻¹. The injection port was set at 250 °C and operated in splitless mode. The oven temperature program was as follows: initial temperature was set to 60 °C and was held for 3 min, and temperature was then increased to 280 °C at a rate of 5 °C min⁻¹ and held for 3 min. The oven was then heated from 280 to 300 °C at a rate of 1.5 °C min⁻¹ and held at 300 °C for 2 min. The temperature of the MSD interface to MS was set at 280 °C. The MSD was operated in the SIM mode for quantifying specific alkanes and polycyclic aromatic hydrocarbons (PAHs). Employing the internal standard method, a five-point calibration curve containing saturated alkanes in the range of nC10 through nC35 and parent PAHs was used to calculate final alkane and PAH concentrations. Alkylated PAH homologues were quantified from response factors derived from the un-alkylated parent compounds, as many alkylated homologue standards were not commercially available.

Statistical Analyses

We considered the mean growth rate (millimeters per day or milligrams per day) from multiple individuals of shrimp recovered from each mesocosm as a single observation in our analyses. We used a two-way ANOVA to test the null hypothesis that growth rates of experimental shrimp were similar across oiling treatment levels (heavy, moderate, light, very light, none) and food treatment levels (food added, no food added). Sites were initially blocked by geographical location in the ANOVA to explore the possibility that the analytical results would be confounded by the spatial arrangement of mesocosms in the study area. Five geographical groups used in this analysis included Wilkinson Bay = N21–N25; St. Mary's Point = V16–V20; Wilkinson Bayou = L11, L12, L14, L15, M10; Northwest Bay Jimmy = M8, M9, H1, H2, H5; and South Bay Jimmy = L13, M6, M7, H3, H4. In this analysis, the blocking term was not significant (all

p values > 0.950). Therefore, this term was dropped from the final ANOVA model. Plots of residuals, boxplots of growth rates within each oiling level, and tests (Levene, Bartlett) for unequal variances did not indicate heterogeneity of variances in the data; therefore, growth rates were not transformed prior to analyses (Quinn and Keough 2002). We also used this ANOVA to test for a significant interaction between treatments. When the main effect of oiling was significant at the 0.05 level, we used Tukey's HSD post hoc tests to compare growth rates among the five levels of this treatment, which allowed us to compare growth of experimental shrimp between all possible pairs of oiling levels while controlling for family-wise type 1 error (Quinn and Keough 2002). We used this same analysis to test for treatment effects in the biomass of potential benthic prey, PAH and total alkane concentrations in shrimp tissue, and the percent of penaeid shrimp recovered (a measure of survival) from each mesocosm experiment. A two-way ANOVA also was used to test the null hypothesis that sediment PAH and total alkane concentrations were similar among oiling levels and between experimental months. In this analysis, we considered food and no food measurements at each location as replicates.

We examined scatter plots and used regression analysis to explore potential relationships between shrimp growth rates and the environmental and biological conditions in mesocosms. Potential relationships between shrimp growth and concentrations of PAH and total alkanes in sediments were examined. In addition, growth rates in biomass were compared with penaeid biomass, crustacean biomass, and total biomass measured from both marked and unmarked organisms recovered from the experimental mesocosms. We also compared the number of recovered marked shrimp (survivors) with predator biomass to test for a possible relationship between shrimp survival and predation risk. We used regression analysis to look for possible size-related differences in shrimp growth rates and to examine the potential relationship between shrimp growth rates and the biomass of potential benthic prey. Statistical analyses were conducted using JMP (version 10.0, Cary, NC, 2012), and we considered alpha levels ≤0.05 to be significant in all results.

Results

Aquatic Environment

Tides were relatively high during both experiments, and mesocosms remained constantly flooded. An analysis of tide gauge data showed that even in the shallowest mesocosm, the water depth never fell below 10 and 5 cm during the May and August experiments, respectively.

Water temperature was measured continuously during each experiment at three selected locations. Continuously recorded water temperature data from the Onset™ recorders were

considered reliable, as these data appeared to match the data we collected through daily monitoring. Based on these continuous data, the temperature range was 21.1–32.6 °C in May (mean=26.9±0.17 SE) and 27.5–35.8 °C in August (mean=31.3±0.10 SE). When we continuously monitored water temperatures inside and outside of the same mesocosm, the temperatures inside the mesocosm tracked the outside temperature.

Salinity and DO data were collected continuously during the experiments at four selected mesocosms. Based on reliable data from continuous measurements, mean salinities ± SE in May were 13.4±0.11 at N24 and 14.5±0.13 at H4 and in August 5.5+0.08 at N24 and 8.3+0.13 at H4. Salinity measured inside the experimental mesocosms tracked the salinity measured on the outside during these experiments.

The DO from continuous measurements was high relative to daily measurements used to check reliability. Therefore, we adjusted the continuous data using the mean difference between six daily values and measurements from the datasondes taken at the same time (May: H4–1.4, N24–2.3; August: H4–1.1, N24–1.2). Based on these adjusted values, mean DO ± SE was 3.1 ± 0.07 mg l^{-1} at H4 and 4.0 ± 0.07 mg l^{-1} at N24 in the May experiment and 4.4 ± 0.14 mg l^{-1} at H4 and 5.5 ± 0.08 mg l^{-1} at N24 in the August experiment. Diel fluctuations were large, and these daily swings in DO were greater at H4 than N24 (Fig. 2). At the same site, fluctuations in DO inside mesocosms generally tracked those outside.

Sediment Characteristics

The oil from our sediment samples was confirmed to be from the DWH spill based on terpane and sterane (biomarker) double ratio plots of samples from mesocosm sites (N24, V16, L14, M9, H5) and MC252 source oil (Wang et al. 2006). The amount of oil in the sediment varied among the shoreline areas used in our experiments (oiling levels) and between the May and August experiments (Table 1, Fig. 3, and Online Resource Tables 1 and 2). We did not expect hydrocarbon contamination to be different between the food and no food treatment mesocosms because hydrocarbons were measured at the initiation of the experiments before food was added, and the food treatment levels were randomly assigned to paired mesocosms. Initial statistical analyses confirmed this conclusion. For hydrocarbon analyses, therefore, we considered the food/no food measurements as site replicates when testing for seasonal and oiling level effects. The concentration of PAHs was significantly higher at heavy and moderate than very light shorelines and higher at moderate than light and none shorelines (Table 1). The concentration of total alkanes was higher at heavy and moderate sites than the other three oiling treatment levels (Table 1). Hydrocarbon concentrations at heavy and moderate sites were not statistically different. By August, concentrations of PAHs and total alkanes in the
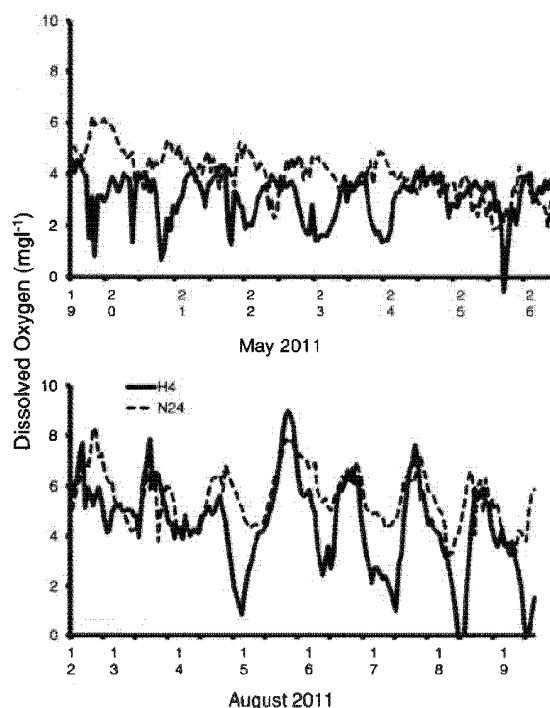
Fig. 2 Hourly dissolved oxygen concentration (in milligrams per liter) measured during May and August 2011 experiments at locations N24 and H4

sediment had decreased significantly from the values we measured in May. Differences in PAHs among oiling treatment levels were less apparent in August, as indicated by a significant interaction in the ANOVA (Table 1 and Fig. 3).

The concentration of oil in sediments did not appear to be related to either sediment grain size or organic content. Sediments in the study area consisted mostly of fine particles (silt and clay content >90 % in May and >80 % in August) with an organic content of ~20 % (mean, 19.4 % in May and 21.9 % in August). In linear regression analyses, no significant relationships were detected between PAH concentration and sediment grain size or organic content (all p values>0.164).

Experimental Shrimps

The brown shrimp and white shrimp used in the experiments ranged in size from 30 to 45 (mean=37.6±0.23 SE) and 28 to 59 (mean=41.2±0.41 SE)mm TL, respectively. These juvenile shrimp reflected the size of shrimp most abundant in the study area when the experiments were conducted. The mean initial size of shrimp within each experiment was not significantly different among the treatment combinations.

Mean recovery rates varied between 36 and 92 % among treatment combinations in the two experiments, and recovery rates were generally higher for brown shrimp than white

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Table 1 Comparison of mean (SE) petroleum aromatic hydrocarbons (PAH) as micrograms per kilogram and total alkanes as milligrams per kilogram from benthic cores taken at mesocosms located among five oiling levels (heavy, moderate, light, very light, none) and between two experimental months (May and August 2011). PAH and total alkanes were extracted from sediments collected in cores taken before initiating each experiment. Each mean was estimated from 20 and 50 samples for the oiling and month treatment levels, respectively. ANOVA results (*p* values) are given for main effects of oiling and month treatments and oiling × month interaction. Significant results of Tukey's HSD post hoc tests comparing growth rates among five oiling levels also are given

	Oiling treatment main effect						Tukey's HSD	Month treatment main effect			Interaction
	Heavy	Moderate	Light	Very light	None	ANOVA	Post hoc comparison	May	August	ANOVA	Oiling × month
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	*p* value	Results	Mean (SE)	Mean (SE)	*p* value	
PAH (µg kg^{-1})	403 (55.5)	468 (55.2)	294 (58.6)	210 (38.2)	277 (21.7)	0.0001	[H=M]>V, M>[L=V=N]	479 (32.2)	182 (13.7)	0.0001	0.0233
Total alkanes (mg kg^{-1})	5.6 (0.34)	5.9 (0.48)	3.4 (0.40)	2.7 (0.23)	3.5 (0.22)	0.0001	[H=M]> [L=V=N]	4.8 (0.29)	3.7 (0.25)	0.0002	0.1809

H heavy, *M* moderate, *L* light, *V* very light, *N* none

shrimp (Online Resource Table 3). For both experiments, however, there was no significant effect of oiling or food treatments on recovery rates of experimental shrimp (ANOVA: brown shrimp: MS=0.135, $F_{9, 40}$=1.497, p= 0.182; white shrimp: MS=0.070, $F_{9, 40}$=0.679, p=0.723). Potential predators recovered when the mesocosms were emptied included hardhead catfish *Ariopsis felis*, silver perch *Bairdiella chrysoura*, sand seatrout *Cynoscion arenarius*, spotted seatrout *Cynoscion nebulosus*, pinfish *Lagodon rhomboides*, speckled worm eel *Myrophis punctatus*, southern flounder *Paralichthys lethostigma*, and blue crab *Callinectes sapidus*. Regression analyses, however, indicated that the number of shrimp recovered was not related to the biomass of predators in the mesocosms at the end of the experiments (May: p=0.182; August: p=0.224).

Mean growth rates differed significantly among oiling treatment levels, but only for brown shrimp (Table 2, Fig. 4, and Online Resource Fig. 1). Mean daily growth of brown shrimp was reduced from 0.9 mm and 38.3 mg day^{-1} at the none sites to 0.4 mm and 15.3 mg day^{-1} at the heavy sites. This reduced growth was equivalent to a 60 % reduction in daily biomass increase, one measure of shrimp production. Brown shrimp growth in mean percent increase in body weight (in percent per day) for the food treatment ranged from 4.3 % (1.7) at the heavy sites to 13.4 % (2.0) at the none sites (Online Resource Table 4). For the no food treatment, these values ranged from 3.0 % (0.9) at the heavy sites to 8.5 % (1.9) at the very light sites.

Growth rates also differed significantly between food treatment levels for both brown shrimp and white shrimp (Table 2, Fig. 4, and Online Resource Fig. 1). Both species consistently grew more rapidly in mesocosms where food was added. The lack of a significant interaction in these analyses suggests that food was limiting in all of the mesocosms and that the oiling effect on growth was not strongly related to the availability of food. For white shrimp, however, there was a trend suggesting that oil negatively affected growth in mesocosms with no added food.

No effect of initial shrimp biomass on growth rates (in milligrams) was detected in these experiments for either brown shrimp (p=0.136) or white shrimp (p=0.216). Initial length, however, was significantly related to growth for both brown shrimp (p=0.002) and white shrimp (p=0.004), but these relationships were weak (R^2s<6 %). The white shrimp
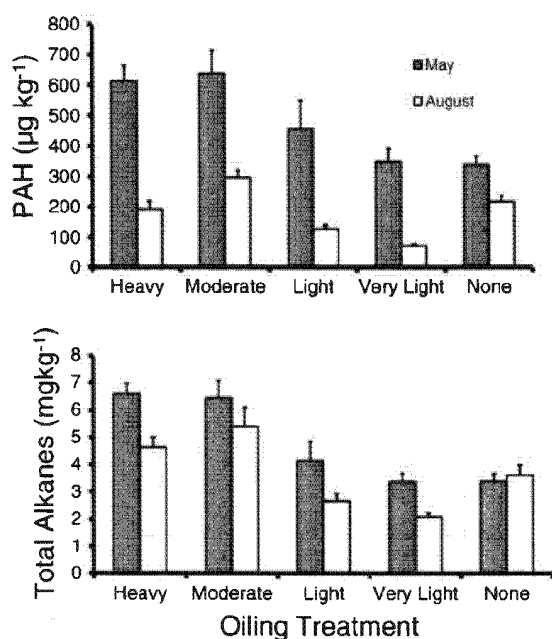

Fig. 3 Comparison of concentrations of PAH (*above*) and total alkanes (*below*) in sediments among oiling treatment levels (heavy, moderate, light, very light, and none) in May and August 2011 experiments

Table 2 Comparison of mean (SE) daily growth rates of shrimp as millimeters per day total length and milligrams per day wet weight biomass among five oiling (heavy, moderate, light, very light, none) and between two food (no food, food added) treatment levels. Data were derived from field experiments conducted in May and August 2011. Each mean was estimated from 10 samples among oiling (except for: brown shrimp: heavy = 9, light = 9, none = 9, light = 7, none = 8; white shrimp: light = 7, none = 9 and 24 samples between food (except for: brown shrimp: food=22; white shrimp: food=23; no food=23) treatment levels, and each sample was determined by calculating the mean growth rate of experimental shrimp recovered from a field enclosure. ANOVA results (p values) are given for main effects of oiling and food treatments and oiling × food interaction. Significant results of Tukey's HSD post hoc tests comparing growth rates among five oiling levels also are given

| | Oiling treatment main effect | | | | | | Tukey's HSD | Food treatment main effect | | | Interaction |
	Heavy Mean (SE)	Moderate Mean (SE)	Light Mean (SE)	Very light Mean (SE)	None Mean (SE)	ANOVA p value	Post hoc comparison Results	No food Mean (SE)	Food added Mean (SE)	ANOVA p value	Oiling × food
Growth (mm day⁻¹)											
May 2011											
Brown shrimp	0.4 (0.08)	0.6 (0.09)	0.7 (0.15)	0.8 (0.12)	0.9 (0.09)	0.0178	[N=V>H, [M=L=V=N], [H=M=L]	0.5 (0.06)	0.8 (0.09)	0.0161	0.9349
August 2011											
White shrimp	0.5 (0.17)	0.6 (0.09)	0.7 (0.13)	0.8 (0.10)	0.6 (0.08)	0.5517		0.5 (0.06)	0.8 (0.08)	0.0158	0.5061
Growth (mg day⁻¹)											
May 2011											
Brown shrimp	15.3 (3.72)	21.6 (4.05)	29.9 (7.21)	38.6 (5.37)	38.3 (5.01)	0.0065	[N=V]>H, [M=L=V=N], [H=M=L]	22.4 (2.66)	35.3 (4.23)	0.0075	0.8216
August 2011											
White shrimp	21.9 (7.65)	21.1 (4.59)	31.8 (6.34)	34.3 (6.79)	26.8 (4.16)	0.3443		17.4 (3.15)	36.4 (3.55)	0.0004	0.5432

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data met assumptions of ANCOVA, but removing the effect of initial size in this secondary analysis did not affect our results.

In a linear regression analysis, a statistically significant relationship was detected between mean brown shrimp growth rate in a mesocosm and the concentration of contaminants in sediments. This negative relationship between brown shrimp growth and sediment PAH explained approximately 22 % of the variability in the data (Fig. 5), and the relationship with total alkanes was similar ($R^2=23.4$ %). The range of PAH values in August was much lower (no values above 400 µg kg⁻¹, Online Resource Table 2), and there was no significant relationship between hydrocarbons and white shrimp growth (Fig. 6).

We measured hydrocarbon burdens in whole shrimp samples from experimental organisms removed from mesocosms, but there was no clear pattern apparent in these data. Tissue burdens of contaminants for brown shrimp differed among oiling treatment levels, with PAH concentrations higher at very light sites than other sites (ANOVA: MS=456, $F_{4,45}=19.837$, $p=0.000$) and total alkanes higher in shrimp caged at heavy sites than those at very light and light sites (ANOVA: MS=472, $F_{4,45}=4.535$, $p=0.005$). A significant food effect also was detected, and total alkanes were significantly lower for brown shrimp held in mesocosms where food was added (ANOVA: MS=2,282, $F_{1,45}=21.942$, $p=0.000$). Hydrocarbons in white shrimp were highest at very light sites (ANOVA: PAH: MS=2,105, $F_{4,45}=3.699$, $p=0.013$; alkanes: MS=249,581, $F_{4,45}=4.268$, $p=0.006$), and there was no apparent food effect (p values>0.250).
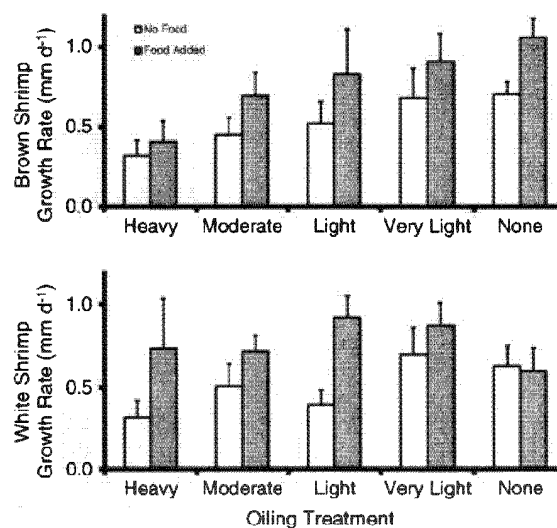

Fig. 4 Comparison of daily brown shrimp and white shrimp growth rates in length (in millimeters per day) from May and August 2011 experiments. Each mean and SE were calculated from five samples except in treatments where no experimental shrimp were recovered from some mesocosms (Online Resource Table 3)
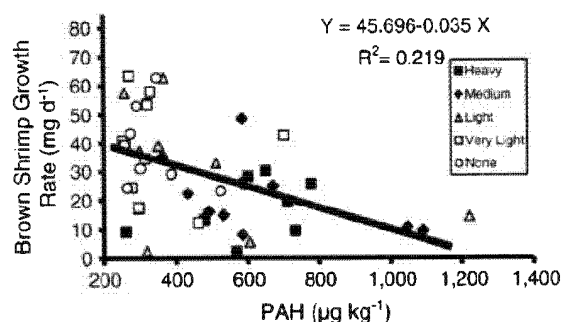
Fig. 5 Relationship between mean brown shrimp growth rate (biomass) in mesocosms and concentration of PAH in sediment collected from cores taken before initiating May 2011 experiment. Data from 46 mesocosms where experimental shrimp were recovered are included

Trophic Effect

We found little evidence for a trophic effect of oil on shrimp growth rates. Food apparently limited growth in all mesocosms as indicated by the significant food effect in our analysis, but there was no significant interaction between oiling and food. In addition, potential infaunal prey biomass (derived from benthic sediment cores) did not differ significantly among oiling treatment levels in either May or August (Online Resource Table 5). Regressions of benthic food biomass on individual shrimp growth in mesocosms also were not significant. Moreover, these differences in growth rates did not seem to be influenced by the biomass of other nekton competitors or predators (unmarked organisms) in mesocosms during the experiment. No significant relationships were detected in regression analyses between brown shrimp growth and total penaeid shrimp biomass ($p=0.205$), total crustacean biomass ($p=0.555$), or total biomass ($p=0.431$).

Discussion

Petroleum hydrocarbons, and PAHs in particular, are known to induce shrimp mortality in laboratory experiments (Tatem et al. 1978). We have shown that exposure to nonlethal concentrations of these hydrocarbons in the field can have sublethal physiological effects as well. In our study, growth rates were reduced for brown shrimp held for only 7 days at field sites oiled by the DWH spill. The greatest reduction in growth rates was observed at sites along the most severely oiled shorelines where brown shrimp grew at less than half the rate of shrimp at control (none) sites. Furthermore, brown shrimp growth was negatively related to concentrations of PAHs in sediments. Penaeid shrimps and other crustaceans can metabolize and excrete ingested oil when the level of contamination is not lethal (Lee et al. 1976; Neff et al. 1976), but depuration comes at a cost. These organisms must use metabolic energy

that otherwise would be used for growth to respond to these contaminants, and this reduces somatic growth rates (Edwards 1978). The hepatopancreas appears to be the primary organ in penaeid shrimps and other crustaceans used to detoxify contaminants (Lee et al. 1976; Neff et al. 1976). Exposure to petroleum hydrocarbons can cause cytological and histological damage to this organ (Sreeram and Menon 2005), and metabolic energy also would be required to repair this damage.

Oil spills also may impair the growth of shrimp and other estuarine organisms indirectly through effects on the food web (Suchanek 1993; Mendelssohn et al. 2012). Penaeid shrimps prey on annelids, small crustaceans, and other shallow-burrowing benthic infauna (McTigue and Zimmerman 1998; Fry et al. 2003; Beseres and Feller 2007), and petroleum hydrocarbons can reduce the abundance and biomass of these prey at severely oiled sites (Southward 1982; Fleeger and Chandler 1983; Decker and Fleeger 1984; Nance 1991; Teal et al. 1992). We measured initial infaunal prey biomass to examine this possibility, but prey biomass did not differ among the oiling treatment levels in our experiments; therefore, we cannot attribute the differences in brown shrimp growth to differences in prey biomass. However, the infaunal biomass available to brown shrimp in our experiments was <17 % of the biomass measured in spring 2007 under similar environmental conditions (water temperature, salinity, and DO) at a site (saline UE location) ~26 km southwest of our study area (Rozas and Minello 2011). The relatively low biomass of available infauna may have suppressed shrimp growth rates across all oiling treatment levels in our experiments. The significant effect of adding food to the mesocosms and the lack of an interaction with oiling indicates that food was limiting growth in all mesocosms. Although not significant, the data for white shrimp suggested that oil may have negatively affected growth in mesocosms where no food was added. Oil may also indirectly affect growth through changes in behavior. For example, the presence of oil has been shown
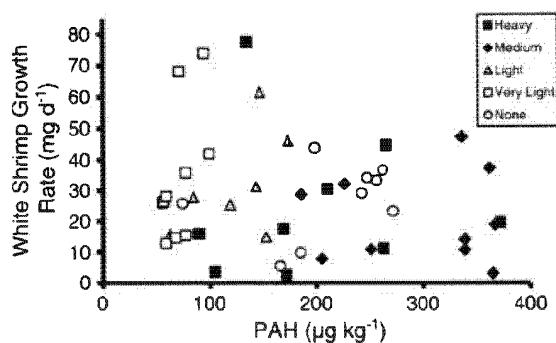

Fig. 6 Relationship between mean white shrimp growth rate in mesocosms and concentration of PAH in sediment collected from cores taken before initiating August 2011 experiment. Data from 46 mesocosms where experimental shrimp were recovered are included

to reduce foraging activity in marine organisms (Southward 1982; Suchanek 1993), and it is possible that concentrations at our heavily oiled sites reduced feeding by shrimp enough to affect their growth rates.

The route of contaminate intake for the experimental shrimp in our study is unknown, but shrimps and other crustaceans can take up oil from food, sediment, or water (Lee et al. 1976; Neff and Anderson 1981; Meador et al. 1995). Penaeid shrimps are omnivorous and feed by probing the bottom substrate for benthic infauna and other food with their pereiopods, which they use to select, pick up, and move food to their mouths (Dall et al. 1990; McTigue and Zimmerman 1998; Fry et al. 2003; Beseres and Feller 2007). The shrimps in our experiments may have ingested oil by consuming contaminated food, or they may have taken up oil adsorbed to sediment particles or detritus inadvertently eaten along with their prey. Feeding mode can clearly influence the uptake of contaminants (Meador et al. 1995). Brown shrimp may be particularly susceptible to oil-laden sediments because this species is a prodigious burrower and, while burrowed, would be in close contact with contaminated sediments. White shrimp burrow less than brown shrimp (Wickham and Minkler 1975). Fiddler crabs, which also burrow and forage on the sediment surface, were found to be contaminated 20 years after a spill severely oiled a New England salt marsh (Teal et al. 1992). Unlike many fishes, brown shrimp do not appear to avoid oiled habitat (Roth and Baltz 2009), which also increases their exposure to oil. Although we did not measure petroleum hydrocarbons in the water column, concentrations there were likely relatively low given the open nature of the shorelines in our study area. Waves and tidal flushing would likely dilute the concentration of water-soluble or suspended oil in the water column. Just several weeks after the DWH spill oiled a Grand Terre marsh in lower Barataria Bay, only trace amounts of hydrocarbons could be detected in the water column, even though the sediments there were highly contaminated (Whitehead et al. 2011). DWH oil did not appear to be taken up by filter feeders (barnacles, mussels) in Barataria Bay (Fry and Anderson 2014). Nonetheless, we occasionally observed small patches of rainbow sheens along the marsh shoreline indicating the presence of oil in the water at some of our experimental sites. Severely oiled sediments may continue to release oil and contaminate marsh fauna for years following a spill (Culbertson et al. 2007; Whitehead et al. 2011).

There are at least two possible explanations for the lack of a response we observed in our experiment with white shrimp. The different response by brown shrimp and white shrimp may indicate species-specific differences in sensitivity to oil. This is unlikely, however, because white shrimp are thought to be more sensitive to oil than brown shrimp (Neff and Anderson 1981). A more likely explanation is that the levels of exposure to PAHs were different for the two species. There

was a significant decrease in sediment contamination from May (brown shrimp experiment) to August (white shrimp experiment), and the PAH levels to which white shrimp were exposed may have been below the threshold required to elicit a detectable growth response. When we examined the relationship between brown shrimp growth and PAH concentrations below 400 μg kg^{-1}, there was no significant effect at these low levels ($p=0.537$).

We identified factors that may have influenced our results and that should be considered in planning future research. Although our field measurements of sediment contamination at the mesocosm sites were generally consistent with the SCAT classification we used to assign oiling treatment levels in our experimental design, contaminated sediments also were identified at our control (none) sites. These sites in Wilkinson Bay were labeled as not oiled in the SCAT classification, but airborne visible infrared spectrometer data later identified shoreline in Wilkinson Bay oiled by the DWH spill (Kokaly et al. 2013). The oil of Wilkinson Bay also may have reduced growth rates of experimental shrimp at none sites, and using these oiled sites as a control in our experiments likely made it more difficult to detect differences in growth rates with more contaminated sites. Mean growth rates measured at these sites were lower (brown shrimp, 0.9 vs. 1.2 mm day^{-1}, 38.3 vs. 101.9 mg day^{-1}; white shrimp, 0.6 vs. 0.8 mm day^{-1}, 26.8 vs. 60.9 mg day^{-1}) than those measured in 2007 under similar environmental conditions (water temperature, salinity, and DO) and ~26 km southwest (brown shrimp at saline UE location) and ~17 km northwest (white shrimp at brackish location) of Wilkinson Bay (Rozas and Minello 2011). Our experimental design also could have been improved by using fewer oiling treatment levels. We observed only small differences in contamination and shrimp growth responses between the light and very light oiling levels and our control (none). Therefore, including all of these levels may have reduced the efficiency of our experimental design (Peterson et al. 2001). Had we used fewer levels (e.g., heavy, moderate, none), more replication per level, and selected a control unaffected by the spill, the statistical power of our analyses and our resulting capability to discern differences among these levels likely would have been greater (Cox 1958; Peterson et al. 2001).

Oil spills can have either lethal or sublethal effects on penaeid shrimps, but questions remain about dose–response relationships. Contaminant concentrations within any spill area are highly variable both spatially and temporally. Therefore, levels of exposure also can be quite variable, making it difficult to compute the dose–response relationships using data from field experiments. Carefully controlled laboratory experiments offer a better approach to acquire data for revealing these relationships.

Field experiments such as ours, however, are essential to examine actual conditions following a spill. The measurement of abundance, growth, and mortality of experimental shrimp

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exposed to the contaminated environment provides a realistic assessment of impacts on shrimp production. Although we have no direct evidence of changes in shrimp abundance or mortality from these experiments, the daily reduction we measured in brown shrimp growth at the heavily oiled sites is equivalent to a 60 % decrease in shrimp production.

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