Environmental Science & Technology

Article

pubs.acs.org/est

Effects of *Deepwater Horizon* Oil on the Movement and Survival of Marsh Periwinkle Snails (*Littoraria irrorata*)

T. Ross Garner,[†] Michael A. Hart,[‡] Lauren E. Sweet,[†] Hanna T. J. Bagheri,[†] Jeff Morris,[§] James A. Stoeckel,[‡] and Aaron P. Roberts*[,†]

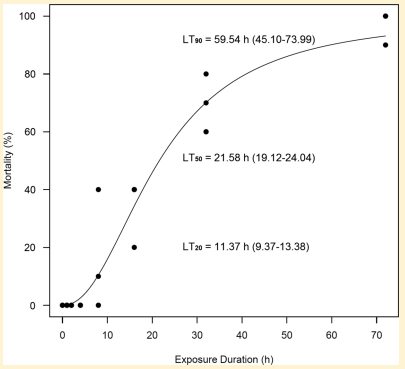
[†]University of North Texas, Department of Biological Sciences & Advanced Environmental Research Institute, Denton, Texas 76203, United States

[‡]Auburn University, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn, Alabama 36849, United States

[§]Abt Associates, Boulder, Colorado 80302, United States

Supporting Information

ABSTRACT: The *Deepwater Horizon* (DWH) oil spill resulted in the release of millions of barrels of oil into the Gulf of Mexico, and some marsh shorelines experienced heavy oiling including vegetation laid over under the weight of oil. Periwinkle snails (*Littoraria irrorata*) are a critical component of these impacted habitats, and population declines following oil spills, including DWH, have been documented. This study determined the effects of oil on marsh periwinkle movement and survivorship following exposure to oil. Snails were placed in chambers containing either unoiled or oiled laid over vegetation to represent a heavily impacted marsh habitat, with unoiled vertical structure at one end. In the first movement assay, snail movement to standing unoiled vegetation was significantly lower in oiled chambers (oil thickness ≈ 1 cm) compared to unoiled chambers, as the majority (~75%) of snails in oiled habitats never reached standing unoiled vegetation after 72 h. In a second movement assay, there was no snail movement standing unoiled structure in chambers with oil thicknesses of 0.1 and 0.5 cm, while 73% of snails moved in unoiled chambers after 4h. A toxicity assay was then conducted by exposing snails to oil coated *Spartina* stems in chambers for periods up to 72 h, and mortality was monitored for 7 days post exposure. Snail survival decreased with increasing exposure time, and significant mortality (~35%) was observed following an oil exposure of less than 24 h. Here, we have shown that oil impeded snail movement to clean habitat over a short distance and resulted in oil-exposure times that decreased survival. Taken together, along with declines documented by others in field surveys, these results suggest that marsh periwinkle snails may have been adversely affected following exposure to DWH oil.



INTRODUCTION

Marsh periwinkle snails (*Littoraria irrorata*) are intertidal gastropod mollusks that are abundant in salt marshes along the Gulf and Atlantic coasts of North America.[1] They are important to coastal environments, serving as a link between primary and secondary production through their fungiculture activities, and are also critical for ecosystem function as significant components of saltmarsh food webs.[2−4] Periwinkles reside on the marsh floor and move up *Spartina alterniflora* stalks during high tide to avoid predation and obtain oxygen.[5,6] Lateral movement, while localized, can extend up to 2 m over a 4 month period.[7] Studies suggest that directional movement of periwinkle snails occurs in response to phototactic, geotactic, and visual cues, as well as to numerous chemical stimuli, including food sources, predators, and conspecifics.[8,9]

Due to direct contact and interactions with the benthic community of salt marshes, a related species, the common periwinkle snail (*Littorina littorea*), has been termed a sentinel species for evaluating the sublethal effects of persistent contaminants, including polycyclic aromatic hydrocarbons (PAHs).[10] PAHs are common toxic components of petroleum products, including crude oil, as well as products of incomplete combustion of organic material and fossil fuels.[11−13] Potential sources of PAH contamination in marsh habitats include runoff of vehicle exhaust from highways and parking lots, recreational boating, atmospheric deposition, creosote oil leachates from historical industrial sites, and fuel/oil spills.[14−18] A decline in snail populations, due to mortality, has been documented in marsh periwinkles following a simulated fuel oil spill, with subsequent recolonization by juveniles.[19]

On the morning of 20 April 2010, BP's semisubmersible Mobile Offshore Drilling Unit (MODU) *Deepwater Horizon* (DWH) exploded and sank, resulting in the release of millions of barrels of oil into the Gulf of Mexico.[20] Current estimates suggest that as much as 1100 km of wetland/marsh shoreline

Received: April 11, 2017
Revised: June 28, 2017
Accepted: June 29, 2017
Published: June 29, 2017

DOI: 10.1021/acs.est.7b01565
Environ. Sci. Technol. 2017, 51, 8757−8762

was oiled, and as much as 32% of these habitats were impacted with "heavier" oiling.[21] Marshes represent one of the most productive ecosystems and serve as primary nursery habitats and feeding grounds for many species of fish, shellfish, and birds.[2,12,22]

Heavy oiling of salt-marshes following DWH resulted in near complete mortality of *S. alterniflora* and *Juncus roemerianus*, the two dominant flora utilized by marsh periwinkle snails.[23−26] Oiling also increased erosion rates and further increased the loss of some of these habitats.[25,27,28] Of impacted coastal marsh shoreline, 351 km was designated as heavily oiled, and oil thicknesses in these areas was greater than 0.1 cm.[21,29] These critical ecosystems were simply described as "oiled vegetation mats", with normally tall standing grasses laid over dead, horizontal, and covered by oil.[30,31] For months following the spill, oil remained in these habitats at thicknesses of 2−3 cm and showed no significant signs of weathering or degradation.[30,31]

Surveys of impacted salt marshes following the DWH oil spill also observed an absence of marsh periwinkle snails; a cause of concern as populations of this species are normally in extremely high abundance in these ecosystems.[27,31,32] In an effort to determine snail fate during the aftermath of the spill, this study examined the effects of oil on periwinkle behavior, specifically movement to nearby unoiled standing grasses and structures, and survivorship following exposure to oiled marsh grasses. Two movement assays were utilized to observe and quantify snail movement in oil. The first contained laid over natural vegetative substrate with varying oil thickness, while the second contained laid over vegetative substrate to more accurately achieve two different oil thicknesses. A toxicity assay was utilized to determine snail survivorship in oil following increasing exposure durations. Here, we show that movement of snails to standing structure was impeded by oil, and snail survival in oil was dependent upon length of exposure.

EXPERIMENTAL METHODS

Collection and Holding. Marsh periwinkles were collected from the coastline on Dauphin Island, AL near Mobile Bay (~30°25′16.91″N 88°08′27.46″W). Stalks of *S. alterniflora* were collected from the coastline southwest of Bayou La Batre, AL near Sandy Bay (~30°22′48.93″N 88°18′45.65″W). Snails and *S. alterniflora* were transported in containers with a small amount of water (for humidity) from the collection site and sent to the Auburn University Shellfish Laboratory (AUSL) (Dauphin Island, AL) for Movement Assay I, the University of North Texas (Denton, TX) for Movement Assay II, and to Auburn University (Auburn, AL) for the Toxicity Assay.

Movement Assay I. Mesocosms (1 oil treatment and 1 control treatment × 5 replicates each = 10 total chambers) were fabricated as experimental chambers using 24 L plastic storage containers (Sterilite, 15.5 cm × 38.4 cm × 61.9 cm) (Figure 1a). Two open-air recreational tents were erected outdoors to shield chambers from direct sunlight, and experimental chambers were placed in an alternating pattern (oiled vs control) atop a large tarp. Containers were filled with roughly 5 gal of sand so that a metal tray (2.5 cm × 33.3 cm × 45.4 cm) would lay flush with the edges of the test chambers. Each tray was filled with trimmed *S. alterniflora* shoots and leaves (~200 g), and left in filtered seawater overnight and until the start of the test. Prior to exposure, the metal trays were drained of water and placed inside experimental chambers to mimic "laid over" *S. alterniflora* beds. Standing *S. alterniflora*

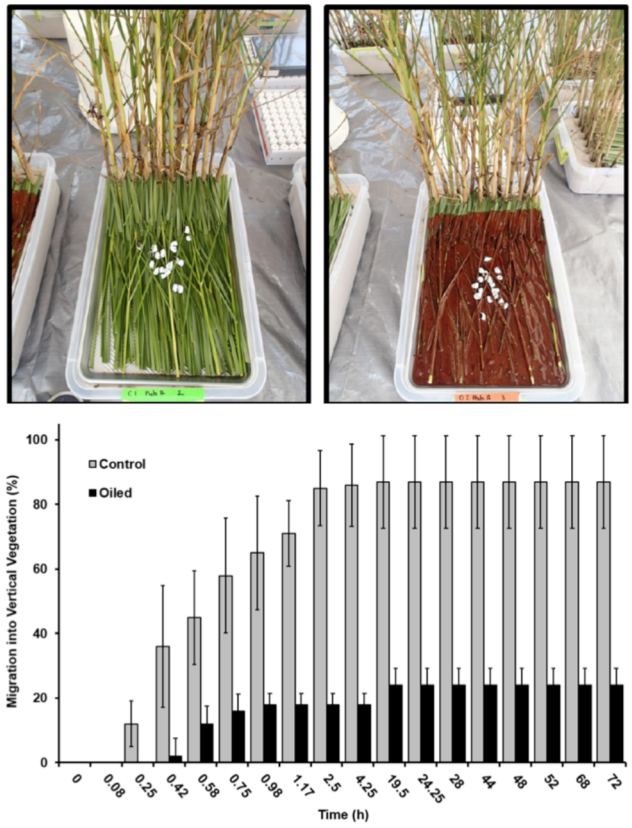
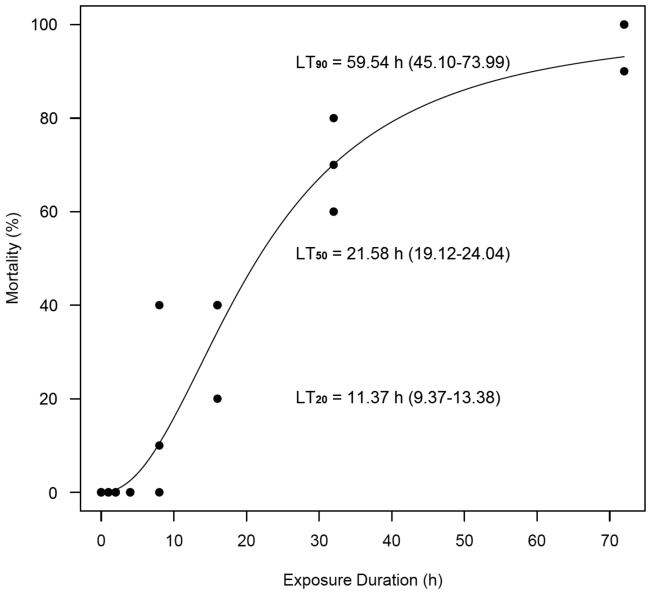


Figure 1. Dimensions of experimental chambers for Movement Assay I (a) and Movement Assay II (b). Black dot indicates location where snails were placed at the beginning of each assay.

shoots were inserted into the sand at the rear of the exposure chamber to mimic unaffected habitats. Trimmed blades of *S. alterniflora* were inserted between the standing and laid over shoots to tie the habitats together and facilitate movement into vertical vegetation. Oil was added to each of the 5 replicate oiled treatment chambers to a thickness of approximately 1 cm (~900 g of oil) to represent recent heavy oiling of the vegetation and substrate. The oil was a weathered surface slick oil, referred to as Slick B, collected close to shore on July 19, 2010 by the skimmer vessel *USCGC Juniper* and is routinely used in testing as part of the DWH Natural Resource Damage Assessment.[33−35]

Snails (20 per chamber) were marked with Bic White-Out and added to the center of the horizontal ("laid over") vegetation, roughly 23 cm from the vertical ("standing") vegetation, for each of the 10 exposure chambers (5 oiled; 5 control). Snail movement was photographed and documented at time-points of 0, 0.08, 0.25, 0.42, 0.58, 0.75, 0.98, 1.17, 2.5, 4.25, 19.5, 24.25, 28, 44, 48, 52, 68, and 72 h. Snails that successfully traversed horizontal vegetation into vertical vegetation, as well as those that exited the chamber from one of three directions other than that of the vertical vegetation, were removed from test and placed indoors in individual scintillation vials for mortality monitoring. At the end of the movement assay (72 h), snails that remained in the horizontal vegetation were transferred into individual vials indoors. Vials were transferred by vehicle to Auburn University (~4 h transit time) at ambient temperature for the 9-d total postexposure mortality monitoring period.

DOI: 10.1021/acs.est.7b01565
Environ. Sci. Technol. 2017, 51, 8757−8762



Snails were individually placed into labeled (according to order of removal from experimental chamber) 20 mL glass scintillation vials with a small amount of artificial salt water added to provide moisture. A small stalk of *Spartina* (approximately 2″) was placed inside of each vial to allow the snail to crawl up and out of the water. Snails in vials were monitored for mortality on a daily basis. Observations began 2 days post removal from exposure chambers and continued for an additional 7 days (9-d total postexposure observation period). Live snails were noted by observing whether or not they were attached to the glass or *S. alterniflora* by their foot. If they were attached to the glass or *S. alterniflora* by mucous (instead of their foot), or not attached and at the bottom of the container, then the snails were removed and assessed for mortality by gently prodding their operculum with a bamboo skewer to see if they responded to physical stimuli. Snails that did not respond to prodding were placed in 15 ppt salt water for 5−10 min. If they did not emerge from their shells within this time period, then they were considered dead.

Movement Assay II. Following Movement Assay I, the exposure system was modified to allow thinner, more consistent oil exposure thicknesses and standardize the oil exposure and standing vegetation portions of the experimental chamber. As such, mesocosms (2 oil treatments and 1 control treatment × 3 replicates each = 9 total chambers) were fabricated as experimental chambers and placed inside a portable greenhouse dome (FlowerHouse) (Figure 1b). Metal trays (2.5 cm × 33.0 cm × 45.7 cm) were placed atop fiberboard, and each tray bottom was lined with a banana leaf to serve as laid over vegetative substrate to allow for testing at two relatively constant oil thicknesses. Wooden dowel rods (1.27 cm diameter) were inserted into fiberboard at the rear of the exposure chamber to mimic unaffected, standing *S. alterniflora*. A fiberboard ramp was fabricated and placed under the banana leaf at the rear of the exposure tray to facilitate movement into vertical structure. Slick B oil was added to each of the 2 oiled treatments to thicknesses of approximately 0.1 cm (∼110g of oil) and 0.5 cm (∼560g) to represent two different thicknesses of oiled habitat.

Snails (20 per chamber) were marked with Bic White-Out and added to the center of each banana leaf, roughly 23 cm from the vertical structure, for each of the 9 exposure chambers. Snail movement was documented at time-points of 0, 0.25, 0.5, 0.75, 0.75, 1, 1.25, 1.5, 1.75, 2, 3, and 4 h. Snails that successfully traversed horizontal vegetation into vertical vegetation were removed from test and not used further.

Toxicity Assay. Microcosms (7 oil treatments and 7 control treatments × 4 replicates each = 56 total chambers) were fabricated as experimental chambers using 0.9L plastic storage containers (0.15 m × 0.15 m × 0.045 m). Each chamber contained a single layer of trimmed *S. alterniflora* (∼30g) and was fitted with a stainless steel wire bristle brush (OD = 2 cm; bristle diameter = 0.15 mm) situated around the perimeter to prevent experimental animals from exiting the chamber. Slick B oil (∼200g) was added to respective chambers resulting in a thickness of approximately 1 cm.

Experimental chambers were placed in an indoor water bath inside a room with temperature and photoperiod control. The water bath consisted of two shallow fiberglass trays (2.4m x 0.91m x 0.15m) situated on top of a metal stand. Both trays were fitted with standpipes that maintained a constant water depth of 9.5 cm and drained into a common sump (1.2 m × 0.4 m × 0.5 m). Total volume was ∼654 L. Two heaters (combined wattage = 1800 W) placed in the sump maintained water temperature between 31−32 °C. Water was pumped from the sump back into the two trays via a submerged pump and manifold.

Foam board insulation (14 mm thick) was floated on the top of the water in each tray to reduce heat loss. Fifty-six, evenly spaced slots were cut into the foam board to accommodate experimental chambers. Each slot was randomly assigned to one of seven exposure time treatments (1, 2, 4, 8, 16, 32, and 72 h). One chamber was placed in each slot for a total of 4 replicate oil and 4 replicate control chambers per time treatment. Each chamber side extended above the water surface by 21 mm, with the remaining 24 mm submersed.

Water was constantly circulated below and between chambers to ensure an even temperature throughout the water bath. A fluorescent light was suspended above the water bath and equipped with a timer to maintain a constant light:dark photoperiod of 14:10. Exposure chambers also received indirect filtered sunlight through a north facing window at an ambient light:dark cycle of ∼14:10.

Once all of the exposure chambers were situated in the water bath, snails were added, alternating between treatments and ascending with treatment exposure duration, until all exposure chambers contained 10 snails. Daily min/max temperatures were recorded for (a) the water in each water bath tray, (b) air of the room, (c) oil within one of the experimental chambers from 0 to 72 h, and (d) air within one of the subsequent holding chambers from 72 to 240 h. Following exposure, snails were held in the same water bath in respective clean glass jars for 7 d to monitor mortality post exposure.

Snails (10 per chamber) were randomly assigned to test chambers and were exposed to oil for periods of 0, 1, 2, 4, 8, 16, 32, and 72 h (n = 4 chambers per exposure period). At the end of each time period, all snails were removed from the appropriate chambers and checked for initial mortality by methods previously described (Experimental Methods: Movement Assay I).

Surviving snails were transferred into clean glass jars by replicate (one jar per replicate), and held in the same water bath as the oil exposure chambers. Each jar had a small amount of artificial salt water added to provide moisture for the animals throughout the monitoring portion of the experiment. A small

DOI: 10.1021/acs.est.7b01565
Environ. Sci. Technol. 2017, 51, 8757−8762

stalk of *S. alterniflora* (approximately 5″) was placed inside of each jar to allow movement up and out of the water, and a small square of window screening was screwed onto the top of each jar to let air in while preventing snails from escaping. Snail mortality was monitored daily for 7d following removal from exposure chambers by methods previously described (Experimental Methods: Movement Assay I).

Statistical Analysis. Median effect times (ET_{50}) in the movement assay and lethal times of exposure (LT_{50} and LT_{90}) in the Toxicity Assay were calculated with the *drc* package in R (version 3.1.2).[36] Effect time and lethal time models were generated using regression analysis with a logistic fit and are reported as the predicted value with their associated 95% confidence intervals. Results from Movement Assay I are reported as mean ±95% confidence intervals, and comparisons between confidence intervals were used to determine differences among treatments. All other values are reported as mean ± standard deviation, unless otherwise noted. Statistically significant differences in mortality in the Toxicity Assay were determined by a one-way analysis of variance (ANOVA) in conjunction with Tukey-Kramer's test for post hoc analysis using $\alpha = 0.05$ (JMP Student Edition 10, SAS Institute, Cary, NC).

RESULTS

Movement Assay I. Snail movement into vertical vegetation from horizontal vegetation occurred quickly and was observed in control treatments before oiled treatments (Figure 2). There was a significant difference between control

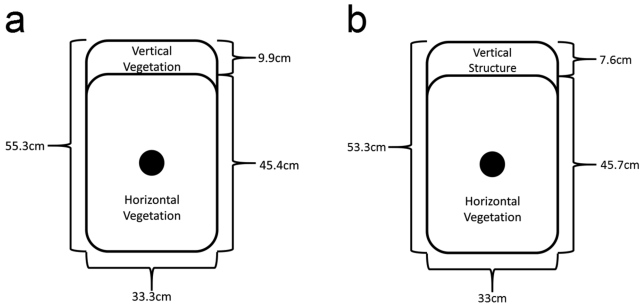


Figure 2. Movement Assay I: Graph of snail movement out of oiled (1 cm thickness) horizontal vegetation into vertical vegetation. Values are progressive over time and represent the mean ±95% confidence intervals ($n = 5$).

snail movement into vertical vegetation, after less than 1 h, when compared to all measured time-points for oiled treatments over the course of 72 h. More than 80% of the snails reached vertical vegetation in 2.5 h in control treatments, while only 24% of oiled snails reached the vertical vegetation during the entire experiment (72 h). Approximately 9% of control snails and 1% of oiled snails exited the exposure chambers in a direction other than toward the vertical vegetation. These snails were removed from the experiment but included in calculations. The calculated median effect time (ET_{50}) for movement of control organisms into the vertical vegetation was 0.67 h (0.57−0.78), and the ET_{20} was 0.24h (0.14−0.33). The ET_{50} for movement out of oil into nonoiled vertical vegetation was not reached, however the ET_{20} was 12.62 h (8.84−16.39).

Snails from control exposure chambers experienced no mortality throughout the test. All of the oiled snails that successfully moved into the clean vertical vegetation also survived for the remainder of the experiment. However, mortality was observed in 77 ± 4% of the snails that remained on oiled vegetation when examined 2d post exposure. No additional mortality was observed during 3−9 d post exposure.

Movement Assay II. As in the Movement Assay I, snail movement into the vertical structure occurred quickly in control treatments (between 0 and 0.25 h) (Table 1). After

Table 1. Movement Assay II: Snail Movement from Laid over Vegetation into Vertical Structure[a]

	snails in vertical structure (%)		
time (h)	control	0.1 cm oil	0.5 cm oil
0.00	0 ± 0	0 ± 0	0 ± 0
0.25	67 ± 20	0 ± 0	0 ± 0
0.50	73 ± 16	0 ± 0	0 ± 0
0.75	73 ± 16	0 ± 0	0 ± 0
1.00	73 ± 16	0 ± 0	0 ± 0
1.25	73 ± 16	0 ± 0	0 ± 0
1.50	73 ± 16	0 ± 0	0 ± 0
1.75	73 ± 16	0 ± 0	0 ± 0
2.00	73 ± 16	0 ± 0	0 ± 0
3.00	73 ± 16	0 ± 0	0 ± 0
4.00	73 ± 16	0 ± 0	0 ± 0

[a]Values represent the average ± one standard deviation ($n = 3$).

0.25 h, 67% of control snails had moved into vertical structure, and movement increased to 73% after 0.5 h. No control snails exited the chamber in any direction other than the vertical structure, and no increase in movement was witnessed in control treatments after 0.5 h. Over the course of the experiment, no snails moved out of oil and into vertical structure in either of the two oil treatments (0.1 cm oil thickness and 0.5 cm oil thickness). Mortality was not assessed on snails from this assay.

Toxicity Assay. All snails from control chambers that contained no oil survived throughout the test. Snails exposed to oil for 1, 2, and 4 h, also experienced no mortality during the post exposure monitoring period. Mortality was first observed in snails exposed to oil for at least 8 h (13 ± 19%), and mortality increased significantly following oil exposures of 16 h (35 ± 10%; $p \leq 0.0289$), 32 h (68 ± 10%; $p \leq 0.0021$), and 72 h (98 ± 5%; $p \leq 0.0021$) (Figure 3).

These data were then analyzed as a function of oil exposure duration, and lethal time of exposure estimates were calculated (Figure 3). The calculated median lethal time of exposure (LT_{50}) was 21.58 h (19.12−24.04), while 20% and 90% time to death estimates (LT_{20} and LT_{90}) were predicted after exposures of 11.37 h (9.37−13.38) and 59.54 h (45.10−73.99), respectively.

DISCUSSION

Periwinkle snails are critical components of coastal ecosystem habitats, and while much work has been conducted on their ecological roles, little to no work has examined movement following an oil spill. Periwinkles are a highly tolerant family, existing worldwide, and are able to withstand conditions of low oxygen and temperature, as well as tissue concentrations of PAHs into the ppm range.[13] This work tested the hypothesis that oil impedes the movement of snails from disturbed horizontal vegetation into standing unoiled vertical vegetation.

DOI: 10.1021/acs.est.7b01565
Environ. Sci. Technol. 2017, 51, 8757−8762

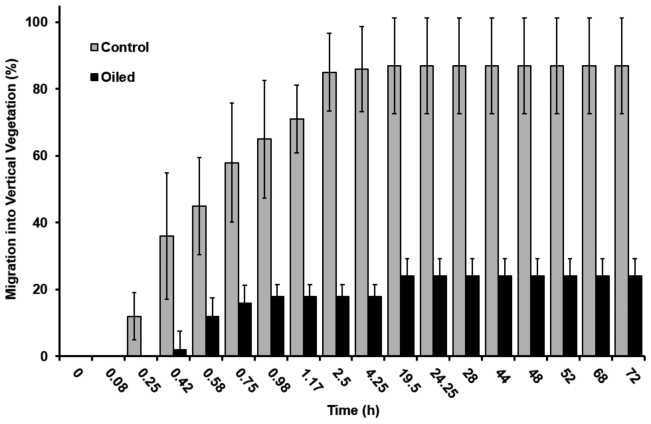


Figure 3. Toxicity Assay: Logistic regression of snail mortality following oil exposures of 0,1, 2, 4, 8, 16, 32, and 72 h, when examined 7 d post exposure (n = 4).

Results from this study support this hypothesis, as only 24% of oiled snails reached vertical vegetation in Movement Assay I (87% in control treatments), and no snails reached vertical structure in Movement Assay II (73% in control treatments) . Furthermore, snails that successfully moved to standing vegetation survived, while the majority of those that did not move from the oiled vegetation perished (Movement Assay I: 0% mortality for snails that reached standing vegetation and 77% mortality for snails in oiled treatments that did not).

Results from both movement assays revealed that all snail movement, if any, occurred within 19.5 h. While all snails that successfully left oiled habitat in Movement Assay I survived (oil exposure <19.5 h), subsequent toxicity testing observed significant mortality following a shorter oil exposure (Figure 3: 35% mortality following a 16h exposure). Additionally, the distance that snails had to traverse in order to reach standing vegetation in these movement assays was very short (~23 cm) in comparison to distances of oiled shoreline they may have encountered in the field (up to 10−15 m inshore).[29] These results, coupled with those from the Toxicity Assay, suggest that snails in impacted areas in the field likely died rather than moved out of oiled habitats. As mortality was a function of exposure duration in the Toxicity Assay (Figure 3: LT_{90} = 59.54 h), it is likely that periwinkles were not, and are not, able to survive in systems with high percent cover of persistent oil.

Although marsh periwinkles are believed to be a tolerant species, they were nearly absent from impacted marshes following the DWH oil coming ashore (~4 months) when compared to normal conditions in which they are generally abundant.[27] Surveys of periwinkle densities observed declines ranging from 50 to 90% 16 months post oiling and reported "little recovery more than three years following oiling".[31,32] Despite the fact that periwinkle snail recolonization has been documented less than a year following a simulated fuel oil spill on the Atlantic Coast,[19] the reestablishment of populations in Gulf of Mexico salt marshes following DWH will likely take much longer.

While *S. alterniflora* populations may potentially benefit from a period of reduced grazing stress, the prolonged absence of periwinkles could have a severe impacts on marsh food web dynamics and ecosystem function.[24,37] Many biota rely on marsh periwinkles as an important food source, including numerous species of birds, crabs, and the diamondback terrapin.[32] The downstream effects of periwinkle loss could include, but are not limited to, alterations in niche partitioning, increases in competition for resources, and subsequent indirect mortality in these systems; however, further research examining potential detriment to ecological fitness is warranted.

Overall, this study suggests that a critical window of opportunity existed for snails following the release of oil from DWH, and their survival was dependent on two factors: movement out of oiled vegetation and the rate of that movement. In one assay, only 24% of oiled snails traversed the 23 cm necessary to reach the clean vertical vegetation, coupled with high rates of mortality for those that failed to move out of the impacted area. In a second assay, no snails moved the necessary 23 cm when exposed to oil thickness of 0.1 and 0.5 cm. These data suggest that oil had a negative impact on snail populations in heavily oiled marshes, which would have been further exacerbated by an increase in distance necessary to reach clean habits. Information obtained from this study elucidate that massive population declines in marsh periwinkle snails following the DWH oil spill may have been due to mortality as opposed to movement out of affected ecosystems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b01565.

Additional information on snail movement and schematics/images of the microcosms are included (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 940-891-6957; fax: 940-565-4297; e-mail: aproberts@unt.edu (A.P.R.).

ORCID

Aaron P. Roberts: 0000-0003-4530-465X

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported as part of the *Deepwater Horizon* Natural Resource Damage Assessment (NRDA). The findings and conclusions in this publication are those of the authors and do not necessarily represent the view of NOAA or of any other natural resource Trustee for the BP/*Deepwater Horizon* NRDA. Data presented here are a subset of a larger toxicological database that was generated as part of the *Deepwater Horizon* NRDA, and these data may be included in additional analysis and interpretation which may include interpretation in the context of additional data not presented in this publication. The authors thank the Auburn University Shellfish Laboratory, specifically Dr. Bill Walton, Scott Rikard, and Glen Chaplain for their patience and use of facilities. Additionally the authors thank the following people for assistance in planning and the completion of this project: Steve Crockett (Bayou La Batre, AL); Dr. Matthew Alloy (University of North Texas); Rebekah Borgert (Auburn University); Dr. Michel Gielazyn and Marla Steinhoff (NOAA); Dr. Steven Pennings (University of Houston); Dr. Scott Zengel (Research Planning, Inc.); Dr. Sean Powers (University of South Alabama & Dauphin Island Sea Lab); Dr. Claire Lay, Dr. Michelle Krasnec, and Andrew McFadden (Abt Associates).

DOI: 10.1021/acs.est.7b01565
Environ. Sci. Technol. 2017, 51, 8757−8762

REFERENCES

(1) Silliman, B. R.; Zieman, J. C. Top-down control of Spartina alterniflora production by periwinkle grazing in a Virginia salt marsh. *Ecology* **2001**, *82* (10), 2830−2845.

(2) Gustafson, D. J.; Kilheffer, J.; Silliman, B. R. Relative effects of Littoraria irrorata and Prokelisia marginata on Spartina alterniflora. *Estuaries Coasts* **2006**, *29* (4), 639−644.

(3) Silliman, B. R.; Newell, S. Y. Fungal farming in a snail. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100* (26), 15643−15648.

(4) Stagg, C. L.; Mendelssohn, I. A. Littoraria irrorata Growth and Survival in a Sediment-Restored Salt Marsh. *Wetlands* **2012**, *32* (4), 643−652.

(5) Hovel, K. A.; Bartholomew, A.; Lipcius, R. N. Rapidly entrainable tidal vertical migrations in the salt marsh snail Littoraria irrorata. *Estuaries* **2001**, *24* (6a), 808−816.

(6) Henry, R. P.; McBride, C. J.; Williams, A. H. Responses of the marsh periwinkle, Littoraria (Littorina) irrorata to temperature, salinity and desiccation, and the potential physiological relationship to climbing behavior. *Mar. Behav. Physiol.* **1993**, *24* (1), 45−54.

(7) Vaughn, C. C.; Fisher, F. M. Dispersion of the salt-marsh periwinkle Littoraria irrorata: Effects of water level, size, and season. *Estuaries* **1992**, *15* (2), 246.

(8) Wollerman, L.; Duva, M.; Ferrier, M. D. Responses of Littoraria irrorata say (Mollusca: Astropoda) to water-borne chemicals: A comparison of chemical sources and orientation mechanisms. *Mar. Freshwater Behav. Physiol.* **2003**, *36* (3), 129−142.

(9) Petraitis, P. S. Occurrence of random and directional movements in the periwinkle, Littorina littorea. *J. Exp. Mar. Biol. Ecol.* **1982**, *59*, 207−217.

(10) Noventa, S.; Pavoni, B.; Galloway, T. S. Periwinkle (Littorina littorea) as a Sentinel Species: A Field Study Integrating Chemical and Biological Analyses. *Environ. Sci. Technol.* **2011**, *45* (7), 2634−2640.

(11) Cram, S.; Siebe, C.; Ortiz-Salinas, R.; Herre, A. Mobility and persistence of petroleum hydrocarbons in peat soils of southeastern Mexico. *Soil Sediment Contam* **2004**, *13* (5), 439−458.

(12) Garner, T. R.; Weinstein, J. E.; Sanger, D. M. Polycyclic Aromatic Hydrocarbon Contamination in South Carolina Salt Marsh-Tidal Creek Systems: Relationships Among Sediments, Biota, and Watershed Land Use. *Arch. Environ. Contam. Toxicol.* **2009**, *57* (1), 103−115.

(13) Ololade, I. A.; Lajide, L.; Oladoja, N. A.; Olumekun, V. O.; Adeyemid, O. O. Occurrence and Dynamics of Hydrocarbon in Periwinkles Littorina littorea. *Turk J. Fish Aquat Sc* **2011**, *11* (3), 451−461.

(14) Sanger, D. M.; Holland, A. F.; Scott, G. I. Tidal creek and salt marsh sediments in South Carolina coastal estuaries: II. Distribution of organic contaminants. *Arch. Environ. Contam. Toxicol.* **1999**, *37* (4), 458−471.

(15) Menzie, C. A.; Hoeppner, S. S.; Cura, J. J.; Freshman, J. S.; LaFrey, E. N. Urban and suburban storm water runoff as a source of polycyclic aromatic hydrocarbons (PAHs) to Massachusetts estuarine and coastal environments. *Estuaries* **2002**, *25* (2), 165−176.

(16) Walker, S. E.; Dickhut, R. M.; Chisholm-Brause, C.; Sylva, S.; Reddy, C. M. Molecular and isotopic identification of PAH sources in a highly industrialized urban estuary. *Org. Geochem.* **2005**, *36* (4), 619−632.

(17) Weinstein, J. E.; Garner, T. R. Piperonyl butoxide enhances the bioconcentration and photoinduced toxicity of fluoranthene and benzo[a]pyrene to larvae of the grass shrimp (Palaemonetes pugio). *Aquat. Toxicol.* **2008**, *87* (1), 28−36.

(18) Neff, J. M. *Polycyclic Aromatic Hydrocarbons in the Aquatic Environment*; Applied Science: London, 1979; p 255.

(19) Lee, R. F.; Dornseif, B.; Gonsoulin, F.; Tenore, K.; Hanson, R. Fate and effects of a heavy fuel oil spill on a Georgia salt marsh. *Mar. Environ. Res.* **1981**, *5*, 125−143.

(20) McNutt, M. K.; Camilli, R.; Crone, T. J.; Guthrie, G. D.; Hsieh, P. A.; Ryerson, T. B.; Savas, O.; Shaffer, F. Review of flow rate estimates of the Deepwater Horizon oil spill. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (50), 20260−20267.

(21) Nixon, Z.; Zengel, S.; Baker, M.; Steinhoff, M.; Fricano, G.; Rouhani, S.; Michel, J. Shoreline oiling from the Deepwater Horizon oil spill. *Mar. Pollut. Bull.* **2016**, *107* (1), 170−178.

(22) Ross, S. W. The relative value of different estuarine nursery areas in North Carolina for transient juvenile marine fishes. *Fish B-NOAA* **2003**, *101* (2), 384−404.

(23) Lin, Q. X.; Mendelssohn, I. A. Impacts and Recovery of the Deepwater Horizon Oil Spill on Vegetation Structure and Function of Coastal Salt Marshes in the Northern Gulf of Mexico. *Environ. Sci. Technol.* **2012**, *46* (7), 3737−3743.

(24) Mendelssohn, I. A.; Andersen, G. L.; Baltz, D. M.; Caffey, R. H.; Carman, K. R.; Fleeger, J. W.; Joye, S. B.; Lin, Q. X.; Maltby, E.; Overton, E. B.; Rozas, L. P. Oil Impacts on Coastal Wetlands: Implications for the Mississippi River Delta Ecosystem after the Deepwater Horizon Oil Spill. *BioScience* **2012**, *62* (6), 562−574.

(25) Lin, Q.; Mendelssohn, I. A.; Graham, S. A.; Hou, A.; Fleeger, J. W.; Deis, D. R. Response of salt marshes to oiling from the Deepwater Horizon spill: Implications for plant growth, soil surface-erosion, and shoreline stability. *Sci. Total Environ.* **2016**, *557*, 369−377.

(26) Hester, M. W.; Willis, J. M.; Rouhani, S.; Steinhoff, M. A.; Baker, M. C. Impacts of the Deepwater Horizon oil spill on the salt marsh vegetation of Louisiana. *Environ. Pollut.* **2016**, *216*, 361−370.

(27) Silliman, B. R.; van de Koppel, J.; McCoy, M. W.; Diller, J.; Kasozi, G. N.; Earl, K.; Adams, P. N.; Zimmerman, A. R. Degradation and resilience in Louisiana salt marshes after the BP-Deepwater Horizon oil spill. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (28), 11234−11239.

(28) McClenachan, G.; Turner, E. R.; Tweel, A. W. Effects of oil on the rate and trajectory of Louisiana marsh shoreline erosion. *Environ. Res. Lett.* **2013**, *8* (4), 044030.

(29) Michel, J.; Owens, E. H.; Zengel, S.; Graham, A.; Nixon, Z.; Allard, T.; Holton, W.; Reimer, P. D.; Lamarche, A.; White, M.; Rutherford, N.; Childs, C.; Mauseth, G.; Challenger, G.; Taylor, E. Extent and Degree of Shoreline Oiling: Deepwater Horizon Oil Spill, Gulf of Mexico, USA. *PLoS One* **2013**, *8*, 6.

(30) Zengel, S.; Bernik, B. M.; Rutherford, N.; Nixon, Z.; Michel, J. Heavily Oiled Salt Marsh following the Deepwater Horizon Oil Spill, Ecological Comparisons of Shoreline Cleanup Treatments and Recovery. *PLoS One* **2015**, *10* (7), e013232410.1371/journal.-pone.0132324

(31) Zengel, S.; Rutherford, N.; Bernik, B.; Nixon, Z.; Michel, J. Salt Marsh Remediation and the Deepwater Horizon Oil Spill, the Role of Planting in Vegetation and Macroinvertebrate Recovery. *International Oil Spill Conference Proceedings* **2014**, *2014* (1), 1985−1999.

(32) Zengel, S.; Montague, C. L.; Pennings, S. C.; Powers, S. P.; Steinhoff, M.; Fricano, G.; Schlemme, C.; Zhang, M. N.; Oehrig, J.; Nixon, Z.; Rouhani, S.; Michel, J. Impacts of the Deepwater Horizon Oil Spill on Salt Marsh Periwinkles (Littoraria irrorata). *Environ. Sci. Technol.* **2016**, *50* (2), 643−652.

(33) Forth, H. P.; Mitchelmore, C. L.; Morris, J. M.; Lay, C. R.; Lipton, J. Characterization of dissolved and particulate phases of water accommodated fractions used to conduct aquatic toxicity testing in support of the Deepwater Horizon natural resource damage assessment. *Environ. Toxicol. Chem.* **2017**, *36* (6), 1460−1472.

(34) Forth, H. P.; Mitchelmore, C. L.; Morris, J. M.; Lipton, J. Characterization of oil and water accommodated fractions used to conduct aquatic toxicity testing in support of the Deepwater Horizon oil spill natural resource damage assessment. *Environ. Toxicol. Chem.* **2017**, *36* (6), 1450−1459.

(35) Alloy, M. M.; Boube, I.; Griffitt, R. J.; Oris, J. T.; Roberts, A. P. Photo-induced toxicity of Deepwater Horizon slick oil to blue crab (Callinectes sapidus) larvae. *Environ. Toxicol. Chem.* **2015**, *34* (9), 2061−2066.

(36) Ritz, C.; Streibig, J. C. Bioassay analysis using R. *J. Stat Softw* **2005**, *12* (5), 1−22.

(37) Silliman, B. R.; van de Koppel, J.; Bertness, M. D.; Stanton, L. E.; Mendelssohn, I. A. Drought, snails, and large-scale die-off of southern US salt marshes. *Science* **2005**, *310* (5755), 1803−1806.

DOI: 10.1021/acs.est.7b01565
Environ. Sci. Technol. 2017, 51, 8757−8762