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Elizabeth M. Robinson

Louisiana State Univ, Dept Oceanog & Coastal Sci, elizrobin6@gmail.com

Nancy N. Rabalais

Louisiana State Univ, Dept Oceanog & Coastal Sci, nrabalais@lumcon.edu

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The effects of oil on blue crab and periwinkle snail interactions: A mesocosm study

Elizabeth M. Robinson^{a,*}, Nancy N. Rabalais^{a,b}

^a Department of Oceanography and Coastal Science, Louisiana State University, Baton Rouge, Louisiana 70803, USA

^b Louisiana Universities Marine Consortium, Chauvin, Louisiana 70344, USA

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ABSTRACT

We examined the sub-lethal effect of Macondo oil from the Deepwater Horizon oil spill on predator-prey interactions using blue crabs (*Callinectes sapidus*) and periwinkle snails (*Littoraria irrorata*). A 2 × 2 factorial mesocosm design determined the effect of oil (no oil vs. oil) and blue crabs (no blue crab predator vs. one blue crab predator) on periwinkle snail climbing and survival. Sixteen mesocosm tanks were used in the experiment, which were replicated three times. Each tank contained water, sand, and *Spartina* marsh stems. The sixteen tanks were divided between two, temperature-controlled chambers to separate oil treatments (no oil vs. oil). Oil was buried in the sand to prevent direct coating of mesocosm organisms. Half of the tanks contained only snails, while the other half contained snails and (one) blue crab in each chamber. Snail climbing behavior and survival were documented every 12 h over 96 h. Snails exposed to oil without a blue crab predator survived as well as snails not exposed to oil and no blue crab predator. Oil reduced snail survival in the presence of a blue crab predator. The increase in snail mortality can be attributed to changes in snail climbing behavior. Oil significantly reduced snail climbing height in the presence and absence of a blue crab predator. This change in behavior and subsequent decrease in snail survival could be beneficial for *Spartina* during recovery after an oil spill. A decrease in snail populations would reduce grazing stress on *Spartina*. However, field research immediately after an oil spill would be more useful in determining predator-prey interactions and further food web effects.

1. Introduction

Salt marshes are productive coastal ecosystems and generate multiple ecosystem services. They provide important habitat for numerous aquatic and terrestrial species, trap sediments, mitigate shoreline erosion, and attenuate flood effects. These services, which humans have become reliant upon, are impacted by multiple stressors on marsh vegetation such as eutrophication (Deegan et al., 2012), food web alteration (Altieri et al., 2012; Bertness et al., 2014; Carlsson et al., 2004), climate change (Gedan et al., 2009), and habitat destruction (Silliman and Bertness, 2004).

Food web alteration includes the removal of a foundational species, i.e., keystone predator or structural species (Ellison et al., 2005), and can lead to changes in ecosystem services and habitat type. Smooth cordgrass (*Spartina alterniflora*), the dominant foundation species of a northern Gulf of Mexico salt marsh, provides sheltering habitat for periwinkle snails (*Littoraria irrorata*, an intermediate consumer), blue crabs (*Callinectes sapidus*, a top predator), and numerous other organisms. Periwinkle snails and marsh crabs (*Sesarma reticulatum*) may

overgraze *Spartina*, when blue crabs are absent, changing the ecosystem from marsh to mudflat (Gittman and Keller, 2013; Holdredge et al., 2009; Silliman and Bertness, 2002; Silliman and Ziemann, 2001). Predators, like blue crabs, may be absent in an environment due to mortality associated with disease (Shields and Squyers, 2000), lethal concentrations of pollutants (Wendel and Smee, 2009), over-fishing (Guillory et al., 1998; Sharov et al., 2003), and changes in abiotic conditions (Rome et al., 2005).

Research, subsequent to the 2010 Deepwater Horizon (DWH) oil spill, indicates juvenile blue crabs are fairly resilient to oil (Anderson Lively and McKenzie, 2014; Pie et al., 2015; Robinson, unpublished data). Snails are more sensitive to direct oil exposure with high mortality two days after direct exposure to unweathered oil (Garner et al., 2017). The sub-lethal effects of oil on crabs and snails are less well known. Periwinkle snails climb on marsh grass stems to avoid predation and adverse abiotic conditions (Raymond et al., 1993; Warren, 1985), and direct oil exposure makes them less likely to move into vertical vegetation and migrate up stems (Garner et al., 2017). Juvenile blue crabs have restricted movements when exposed to high concentrations

* Corresponding author.

E-mail address: elizrobin6@gmail.com (E.M. Robinson).

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of water-accommodated fractions (WAFs) of oil. The ability of juvenile crabs to successfully detect and forage for food was hindered when exposed to undiluted WAFs. The dilution of WAFs did not impact the foraging success of crabs, but increased the foraging time of juvenile crabs in high WAF concentrations (< 50%; Robinson, unpublished data). The effects of oil on the interactions between the two organisms is unknown, however, and could affect marsh vegetation, food webs, and ecosystem health.

A mesocosm experiment was conducted for two common marsh organisms, blue crabs and periwinkle snails, to examine the effect of oil on marsh predator-prey interactions. Snail climbing height and snail survival were measured in response to the presence of buried oil and blue crabs, or control conditions. Snails and crabs were not coated with oil, but were exposed to WAFs and volatiles derived from oil buried within the mesocosm substrate. Here, we show that snails climbed to a lower elevation on *Spartina* stems when exposed to oil and succumbed to increased predation.

2. Material and methods

2.1. Mesocosm experiment

A mesocosm experiment was conducted at the Louisiana Universities Marine Consortium Marine Center in Cocodrie, Louisiana. The experiment had a 2 × 2 factorial design, with two oiling treatments (unoiled vs. oiled) and two predator conditions (no blue crab vs. one blue crab). The treatments were: unoiled with snails; unoiled with snails and one blue crab; oiled with snails; oiled with snails and one blue crab. Oiling treatments were separated between two environmental chambers (12-h light:dark, 28 °C). Four replicates of each treatment were randomly placed inside the chambers and maintained for 96 h. The experiment was replicated three times, once in 2015 and twice in 2016.

Mesocosm tanks in each chamber were constructed of round, plastic tubs (volume = 64 l; diameter = 55 cm, height = 41 cm). Purchased sand was cleaned and sieved (0.5 mm) prior to experiment construction. A layer of sand was distributed at the bottom of all tanks to a thickness of 8 cm. Oiled treatments contained a sand layer comprised of a mixture of 1 l of sand and 3 ml of DWH surrogate crude oil below the 8 cm layer of sand. The addition of the oiled layer did not add a measurable difference in sediment height. BP Exploration and Production Inc. provided the surrogate oil to the Gulf Coast Restoration Organization for distribution. Surrogate oil was collected from the GOM Dorado Field, Marlin Platform. The surrogate oil was similar to the Mississippi Canyon block 252 (MC-252) oil released during the DWH spill. Data for the chemical analysis of the surrogate oil is available at the Gulf of Mexico Research Initiative Information Data Cooperative (GRIIDC; BP Gulf Science Data (2014), doi: <https://doi.org/10.7266/N7R78CM9>). Oil was not weathered prior to the experiment. Four stalks of *Spartina alterniflora* (average height = 90 cm) and a measuring stick were planted in the center of the tank while sediments were being added to the tank. *Spartina* was not in direct contact with oiled sediments during planting. Sea water (unfiltered, salinity 15) was added to a depth of 10 cm above the substrate and was not changed to simulate low tide or high tide conditions during the experiment. An aerator was placed in each tank to prevent stress from oxygen depletion (Fig. 1).

Crabs and snails were collected from marshes around LUMCON using baited traps and hand collection, respectively, 48 h prior to the start of the experiment. Crabs were kept in glass aquaria with sea water (unfiltered, salinity 15), and were food deprived until the start of the experiment. Snails were kept in a mesh-covered, plastic bucket containing sea water (salinity 15, depth = 2.5 cm). Organisms in the lab were kept on a 12-h light:dark schedule resembling the natural light-dark cycle. These light conditions were also used in the experimental chambers. Snails were numbered prior to the start of the experiment.

Five snails (average length = 21.5 ± 1.5 mm) were added to each

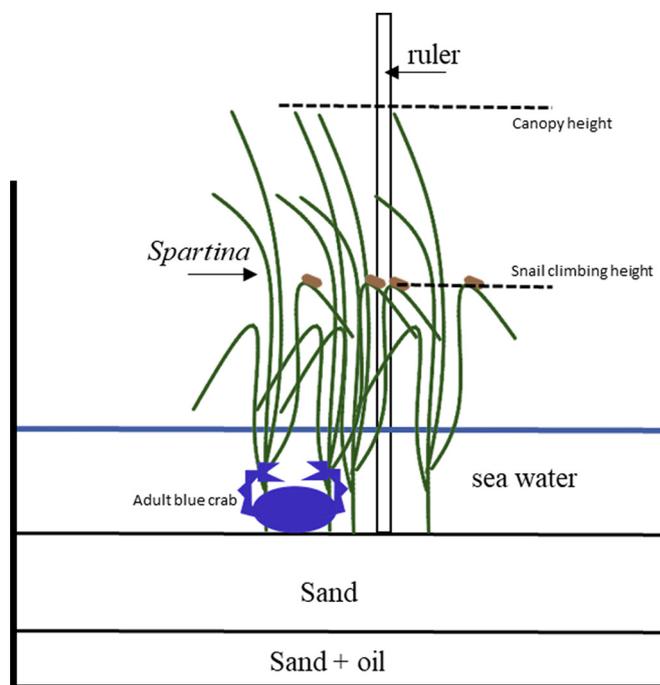


Fig. 1. Mesocosm tank design with potential location of snails. Figure is not drawn to scale.

tank near the *Spartina* stems to encourage climbing on the grasses. The density of snails used in this experiment (5 snails per 4 stalks of *Spartina*) represents observed densities of snails around LUMCON. One adult blue crab was added to predator treatments 60 min after the initial snail introduction. Snail climbing height was recorded one hour after crab introduction and every twelve hours (08:00 and 20:00) afterwards over 96 h for a total of nine data points. The experiment ended at 96 h due to noticeable changes in *Spartina* health. Sampling after 96 h could have introduced a plant health effect not intended for in this study. Snail climbing height was defined as the height on the main stem of a *Spartina* blade above the sediment surface, or the height of the snail above the sediment surface on a bent *Spartina* blade, i.e., how much more elevated than a potential blue crab predator (Fig. 1). Snails that were preyed upon were not replaced. Snail survival (%) was recorded at the end of the four-day period.

Predicted natural tidal height for Cocodrie, Louisiana was documented during the experiment using tidal data provided by the National Oceanic and Atmospheric Administration (Center for Operational Oceanographic Products and Services, 2016). Predicted natural tidal height was used to determine if snails maintained tidal climbing rhythms even though low and high tides were not simulated in experimental tanks. Predicted natural tidal height was averaged among the three, experimental replicates.

2.2. Polycyclic aromatic hydrocarbons (PAHs)

Water samples were collected from the oil contaminated tanks at the end of the experiment. One liter of water was sampled from four randomly chosen tanks using a submerged amber, glass bottle. Samples were collected from two of the three experiment replicates ($n = 8$). Water samples were frozen (−20 °C) until analysis. Samples were analyzed using Gas Chromatography/Mass Spectrometer – Selective Ion Monitoring (GC/MS-SIM). Forty-two polycyclic aromatic hydrocarbons (PAHs, Table 1) were detected by GC/MS-SIM. Total PAH concentrations in the mesocosm tanks ranged between 0.1013 μg L^{−1} to 2.554 μg L^{−1}. Data for concentrations of individual PAHs can be found at GRIIDC (doi: <https://doi.org/10.7266/n7-2hgq-jh13>).

Table 1
Polycyclic aromatic hydrocarbons analyzed in WAF samples.

List of analyzed polycyclic aromatic hydrocarbons		
Anthracene	C2- Chrysenes	C4- Chrysenes
Benzo (a) Anthracene	C-2 Naphthobenzothiophenes	C4- Pyrenes
Benzo (a) Pyrene	C2-Pyrenes	C4-Naphthalenes
Benzo (b) Fluoranthene	C2-Dibenzothiophenes	C4-Phenanthrenes
Benzo (e) Pyrene	C2-Fluorenes	Chrysene
Benzo (g,h,i) perylene	C2-Naphthalenes	Dibenzothiophene
Benzo (k) Fluoranthene	C2-Phenanthrenes	Fluoranthene
C1- Chrysenes	C3-Chrysenes	Fluorene
C-1 Naphthobenzothiophenes	C3-Dibenzothiophenes	Indeno (1,2,3 - cd) Pyrene
C1- Pyrenes	C3-Fluorenes	Naphthalene
C1-Dibenzothiophenes	C-3 Naphthobenzothiophenes	Naphthobenzothiophene
C1-Fluorenes	C3-Pyrenes	Perylene
C1-Naphthalenes	C3-Naphthalenes	Phenanthrene
C1-Phenanthrenes	C3-Phenanthrenes	Pyrene

2.3. Statistics

Differences in snail climbing height among treatments were assessed using a least squares linear model in the JMP® statistical package by SAS®. Oil treatment, predator treatment, and sampling time were fixed factors. All two-way and three-way interactions were included in the analysis. Random factors, sampling year (2015 and 2016) and tank nested in oil treatment, were not included in the statistical analysis. Preliminary analysis revealed the variance between years and among tanks was not significant (year, Wald p-value = 0.48; tank (oil treatment), Wald p-value = 0.07). A Tukey's post-hoc test was used to compare the significant effects among treatments. Snail survival was assessed using a Kruskal-Wallis ANOVA for nonparametric data. Oil and predator treatments were combined into one factor, and Wilcoxon comparison tests were used identify significant comparisons among the treatments. The concentrations of PAHs were not statistically analyzed due to the low sample size and statistical power (n = 8, power = 0.065).

3. Results

3.1. Snail climbing height

The results from a least squares linear model analysis indicated that oil treatment, predator treatment, sampling time, and interactions among factors explained 20.7% of the variation in snail climbing behavior (R² = 0.207; F_(35,1533) = 11.46, p < 0.0001). Snails in unoiled treatments (n = 809, height mean (± SE) = 44.5 (0.52)) climbed higher than snails in oiled treatments (n = 760, height mean (± SE) = 34.7 (0.54); F_{1, 1533} = 171.1, p < 0.0001). Snails exposed to blue crabs (n = 839, height mean (± SE) = 43.2 (0.55)) climbed higher than snails not exposed to blue crabs (n = 730, height mean (± SE) = 35.9 (0.51); F_{1, 1532} = 97.0, p < 0.0001).

There was a significant interaction between blue crab predator and oil treatments (F_{1, 1532} = 43.6, p < 0.0001, Fig. 2). Snails climbed highest in the unoiled, blue crab predator treatment (n = 388, height mean (± SE) = 45.7 (0.75)). This treatment was not significantly different from the unoiled, no blue crab treatment (n = 421, height mean (± SE) = 43.3 (0.71); p = 0.08), but was significantly higher than the oiled, no blue crab treatment (n = 421, height mean (± SE) = 28.6 (0.72); p < 0.0001) and oiled, blue crab treatment (n = 342, height mean (± SE) = 40.9 (0.80); p < 0.0001). Snails climbed the lowest in the oiled, no blue crab treatment. This treatment was significantly lower than the other three treatments (Fig. 2).

Sampling time (every 12h) was significant as a factor (F_{8, 1532} = 6.0, p < 0.0001) and significantly interacted with oil treatments (F_{8, 1532} = 1.9, p < 0.04, Fig. 3). Snail climbing height did not change significantly over time within unoiled treatments after 12 h. Climbing

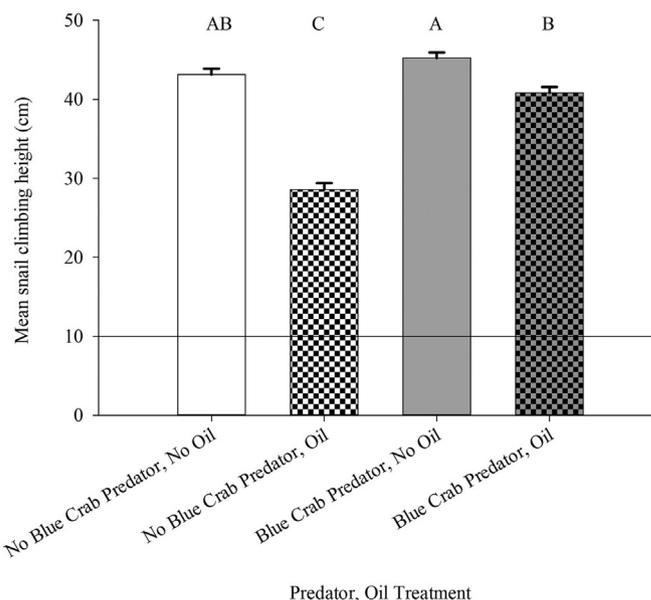


Fig. 2. Mean snail climbing height (± SE) with interaction between oil and predator treatments (p < 0.0001). Horizontal line at 10 cm represents water level inside the mesocosm tanks.

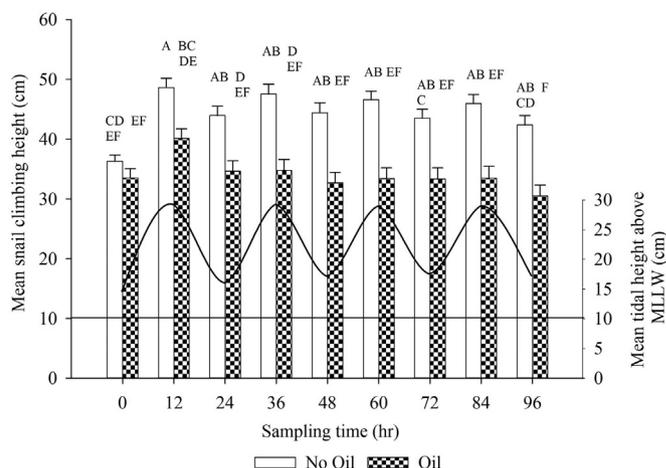


Fig. 3. Mean snail climbing height (± SE) with interaction between sampling time and oil treatment (p < 0.04). Horizontal line at 10 cm represents water level inside the mesocosm tanks. Predicted, natural mean tidal height above mean low low water (MLLW, cm) is represented by the sinusoidal, solid line. Tidal height overlaps snail climbing height and coincides with sampling time.

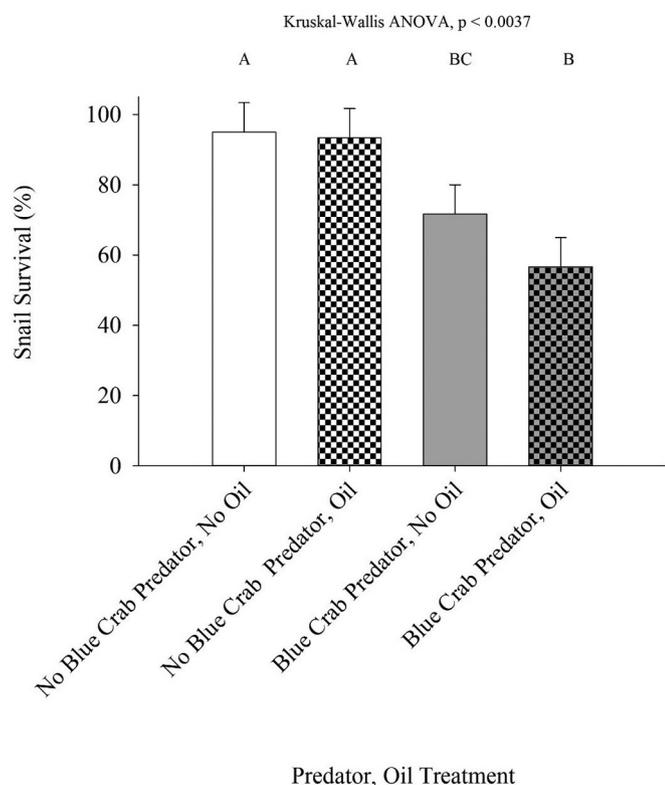


Fig. 4. Snail survival by predator, oil treatment. Data were analyzed using a Kruskal-Wallis rank sums test for nonparametric data. Different letters designate the pairs of treatments that are ranked as statistically significant using Wilcoxon comparison tests.

height was not significantly different over time within oiled treatments throughout the 96-h experiment. The interaction appears to be influenced by the oil treatment. Snail climbing height between unoiled and oiled treatments became significantly different from each other at 12 h and remained significantly different until the end of the 96-h experiment (Fig. 3). The two-way interaction between sampling time and predator treatment ($p = 0.0919$) and the three-way interaction between oil treatment, predator treatment, and sampling time ($p = 0.9092$) were not significant.

3.2. Snail survival

Snail survival was significantly different among treatments (Chi-Square = 13.50, $p < 0.004$, Fig. 4). Snail survival was $> 90\%$ in the no blue crab predator treatments. There was no significant difference between the unoiled, no blue crab treatment and the oiled, no blue crab treatment (93 to 95%; $p = 0.84$). Snail survival was significantly reduced with the addition of a blue crab predator in unoiled (72%, $p < 0.05$) and oiled treatments (57%; $p < 0.005$). There was no significant difference between unoiled and oiled, blue crab predator treatments ($p = 0.29$).

3.3. Polycyclic aromatic hydrocarbons

Total PAHs in oiled treatments ranged between $0.1013 \mu\text{g L}^{-1}$ to $2.554 \mu\text{g L}^{-1}$. Snails climbed to lower heights when no predator was present and total PAHs increased. Snails exposed to blue crabs climbed higher at low PAH concentrations than those exposed to high concentrations (Fig. 5).

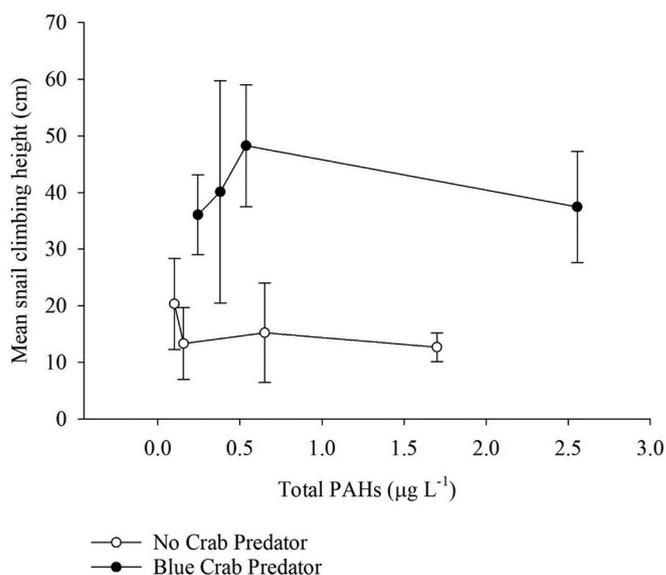


Fig. 5. Snail climbing height by total PAH concentration and predator treatment. Water samples were collected from the oil contaminated tanks at the end of the experiment. Values are mean snail climbing height (\pm SE). Statistics were not performed on the values due to the low sample size of PAH concentrations.

4. Discussion

Snails were able to tolerate PAH exposure throughout the 96-h experiment. Snails encountered PAHs through direct contact with WAFs and volatile PAHs. There is little evidence based on literature that snails encountered significant concentrations of PAHs through contact with *Spartina* leaves. Watts et al. (2006) showed that *Spartina* leaves from plants grown in contaminated soils have very low concentrations of PAHs, and there is no direct linear relationship to soil PAH concentrations. There was also no direct evidence of correlation between PAH concentrations in leaves and exposure through WAFs. Kassenga (2017) has shown that *Spartina* can uptake PAHs through stomatal gas exchange. Plants can accumulate volatile PAHs into their leaf cuticles due to the similarity of lipophilic properties of PAHs and the waxes and cutin in plant cuticles (Desalme et al., 2013; Kassenga, 2017). PAH accumulation could have occurred in this experiment due to the noticeable presence of volatile PAHs in this experiment, but the concentrations of PAHs in these leaves is unknown. It was not possible to determine which pathway impacted snail survival and climbing behavior due to the interconnectedness of WAFs, volatiles, and plant interactions. It is unlikely that any of these pathways would significantly impact snail mortality since the combined pathways of exposure did not have a significant lethal impact on the snails in this study. Snail survival in oiled treatments without the presence of a blue crab predator averaged 93% suggesting WAFs, volatiles, and plant interactions had no effect on snail mortality in a relatively short time period (96 h).

Snails in this experiment did not come into direct contact with buried oiled. There was no direct oil coating observed on the sand surface, snails, crabs, or *Spartina* leaves. Contact with the buried oil would have caused a significant decrease in snail survival. Field studies after the DWH oil spill showed snail mortality up to 50–90% along highly oiled marsh edges (Zengel et al., 2014, 2016). Lab experimentation demonstrated that snail survival was 23% after a 48-h exposure to *Spartina* coated with weathered oil at a thickness of 1 cm (Garner et al. (2017)). The application of oil in this mesocosm experiment did not mirror heavily-weathered oiled conditions, thus a high mortality rate did not occur in the oiled, no predator treatments. Snail survival was 93% in oiled, no blue crab treatments.

Oil exposure in this experiment may be more revealing of oil

impacts occurring throughout marsh recovery. PAHs did mix into the ambient water and concentrations ranged from $0.1013 \mu\text{g L}^{-1}$ to $2.554 \mu\text{g L}^{-1}$. These concentrations are lower than the PAH concentrations found in oil slick samples during the oil spill in 2010 (doi:<https://doi.org/10.7266/N71C1TV1>; Aeppli, 2014) and in the marsh sediments following initial exposure to oil residue (Turner et al., 2014). Concentrations of PAHs are variable in the field, and this experiment may represent PAH concentrations occurring throughout marsh recovery, particularly after the redistribution of buried oil after a strong weather event. The variability among PAH concentrations in this experiment could have been due to a number of factors such as volatility, solubility, oxygen availability, and temperature (Ghosal et al., 2016). Crabs did burrow into the sand in the mesocosms possibly impacting the solubility and volatility of the PAHs. However, it is unknown how this behavior affected the range of PAH concentrations due to the small sample size of WAFs collected. PAHs analyzed in the water were composed mainly of naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes, and anthracenes. These PAHs have smaller molecular weights, which results in greater solubility and volatility.

Crabs did not show any lethal or sub-lethal signs of toxicity (i.e., inability to right themselves, paralysis) in response to the PAHs. The crabs were aggressive and detected movement extremely well in oiled treatments (personal observation). The aggressive behavior shown by the blue crabs is not an uncommon response to pollutants (Reichmuth et al., 2009, 2011).

This is the first study to demonstrate that snail climbing behavior is altered by the presence of WAFs and volatile PAHs. Snails exposed to oil climbed significantly lower on *Spartina* grasses. Unoiled snails exposed to blue crabs climbed the highest on *Spartina*. Blue crab predators were able to consume 28% of snails in the absence of oil. Oil significantly decreased climbing height in the presence of a blue crab; the average climbing height was reduced to 40.9 cm. The 4-cm reduction in snail climbing height and the increased aggressiveness of blue crabs in oiled treatments increased snail mortality although not significantly. Further replication of this experiment may make snail survival in this treatment significant, but overall, blue crabs were able to consume 43% of the snails in oiled conditions compared to 28% in unoiled conditions. Snails did not exhibit climbing behaviors mirroring low and high tides occurring simultaneously in their natural environment past 12 h into the experiment. This suggests that snails adjusted to the controlled mesocosm conditions and reacted to the presence or absence of oil and a blue crab predator. This trainable behavior in regards to snail vertical migrations has also been documented by Hovel et al. (2001).

The initial absence of snails in oil-impacted areas is beneficial for *Spartina* recovery due to the release of grazing pressure. As oil breaks down through weathering or microbial degradation, snails from unaffected areas are able to recolonize impacted areas. Lingering PAHs in these areas may alter snail behavior, as shown in this experiment, making snails exposed to the PAHs susceptible to crab predators. Snail populations recovered after a fuel oil spill in Georgia salt marshes one year after exposure (Lee et al., 1981). However, snail populations along the Louisiana coast have not recolonized as quickly and showed little recovery three years after the spill (Zengel et al., 2014, 2016). The lag in snail recovery may be influenced by a persistent population of blue crabs, which can move in and out of marsh habitats. The reproductive biology of the blue crab allows the species to experience population reduction and recover rapidly (Guillory et al., 2001). If crabs were not avoiding impacted marshes, then recovering snail populations may encounter heightened predation thus allowing *Spartina* the opportunity to recover and grow. However, the absence of periwinkles could have negative impacts on further biodiversity, food web dynamics, and ecosystem function (Mendelssohn et al., 2012). Many secondary consumers, such as fish, birds, terrapins, and mammals, prey upon snails and may experience population reductions due to a slow recovery in snail abundances (McCann et al., 2017; Silliman and Bertness, 2002). Mesocosm studies, such as ours, are useful in predicting the impact of

stressors on food webs and can validate effects seen in the field. Immediate field research after an oil spill would help verify the results of this study and provide more insight into the effects of oil on predator-prey interactions and possible food web alterations.

Declarations of interest

None.

Acknowledgements

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