Effects of salinity on oil dispersant toxicity in the eastern mud snail, *Ilyanassa obsoleta*

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Abstract

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Chemical dispersants can be a beneficial method for breaking up oil slicks; however, their use in mitigation could pose potential toxic effects on the marine ecosystem. Dispersants may be transported to lower salinity habitats, where toxicity data for aquatic species have not been established. This study examined the effect of salinity on oil dispersant toxicity in the eastern mud snail, *Ilyanassa obsoleta*, using two dispersants authorized for oil spill response, Corexit® 9500A and Finasol® OSR 52. Median lethal toxicity values (LC50) and sublethal effects were examined at 10, 20, and 30 ppt salinity in adult and larval mud snails. Two biomarkers (lipid peroxidation and acetylcholinesterase) were used to measure sublethal effects. The 96-h static renewal LC50 values indicated significant differences in toxicity between dispersants and salinities. Larval snails were significantly more sensitive than adult snails to both dispersants, and both life

stages were significantly more sensitive to Finasol than to Corexit. Larval snails were more sensitive to dispersants at lower salinity, but adult snails were more sensitive at higher salinities. Dispersants increased lipid peroxidation and decreased acetylcholinesterase activity. These results demonstrate that dispersant toxicity varies among compounds and organism life stages, and that physicochemical properties of the environment, such as salinity, can affect the potential toxicity to estuarine species.

Keywords

Oil
Dispersants
Estuarine
Mud snail
Toxicity
Salinity

Responsible editor: Cinta Porte

Introduction

In response to open ocean oil spills, chemical dispersants are commonly applied during mitigation efforts to enhance oil dispersion (Coolbaugh and McElroy 2011). These chemicals reduce surface tension between oil and water, thus allowing for smaller oil droplets to form. By increasing the oil surface area, dispersants accelerate oil biodegradation by hydrocarbon-degrading bacteria (Lee 2012). Dispersants may lower the overall impact of an oil spill (Lessard and DeMarco 2000) but can also enhance the bioavailability of petroleum hydrocarbons (Ramachandran et al. 2006), increasing the risk to benthic and coastal habitats (Anderson Lively and McKenzie 2014). Current and tidal movement may transport dispersants into sensitive coastal habitats such as mangroves and salt marshes where dispersed oil molecules may become trapped and concentrate within semi-enclosed coastal areas.

Over 1.7 million gallons of chemical dispersants were applied during the 2010 Deepwater Horizon oil spill (Lee 2012), including Corexit® 9500A (Nalco Energy Services, Sugar Land, TX, USA). Finasol® OSR 52 (Total Fluides, Paris-La Defense, France) is another chemical approved by the EPA for oil spill response; however, there are few studies regarding its toxicity to estuarine species. Both

dispersant compounds consist of proprietary mixtures of petroleum distillates and surfactants. While components of Corexit are publicly available (OSAT 2010), the constituents of Finasol remain undisclosed.

The eastern mud snail, *Ilyanassa obsoleta*, is a common estuarine species found along marsh flats on the East Coast of the USA (Kelaher et al. 2003). Both the larval and adult life stages have served as a model gastropod species (Downs et al. 2001) for a variety of studies (Collier 2002) including fuel oil toxicology (Miller and Pechenik 1983). Eastern mud snails have non-selective feeding habits, depending more on opportunity than nutritional need (Curtis and Hurd 1981). The eastern mud snail plays an important ecological role in regulating estuarine intertidal soft-sediment community structure (Kelaher et al. 2003) as their foraging habits accelerate nutrient cycling (Connor et al. 1982) and modulate annelid densities (Kelaher et al. 2003). Female snails readily lay eggs under laboratory conditions in addition to egg deposition on natural substrate (Scheltema 1962). These eggs develop into veliger larvae that metamorphose into snails between 2 and 2.5 weeks after hatching (Dickinson and Croll 2003).

Estuaries can experience rapid and dynamic changes in their water quality conditions, causing physiological stress for estuarine organisms. Adaptation for organisms living in such a wide range of environmental factors may come at an energetic cost such as reduced growth (da Silva Rocha et al. 2005), metabolism (Lannig et al. 2010), and respiration (Fernandes and Rantin 1994). Adult and larval eastern mud snails tolerate salinities ranging from approximately 10 ppt to full strength (approximately 35 ppt) seawater (Scheltema 1965). Lower salinity levels have been shown to inhibit shell growth rate (Scheltema 1965), prevent metamorphosis (Scheltema 1965), and reduce velar ciliary beat frequency (Richmond and Woodin 1996).

Chemical toxicity might exacerbate salinity stress and cause an increase in mortality for estuarine organisms. Alternatively, salinity itself might not affect survival, but rather modify chemical toxicity to negatively impact the organism. Salinity has been shown to affect biotransformation rates and toxicity for several classes of chemicals (DeLorenzo 2015). An increase in chemical toxicity when combined with salinity stress has been attributed to decreased physiological functions, such as contaminant metabolism and detoxification processes (DeLorenzo 2015). Salinity has already been shown to influence the toxicity of chemical dispersants in grass shrimp, with increased toxicity at lower salinity (DeLorenzo et al. 2016), and several studies have examined the effect of salinity on

oil toxicity in species such as oysters (Zanette et al. 2011), amphipods (Tedengren et al. 1988), and mussels (Tedengren and Kautsky 1987). Salinity effects on oil toxicity varied with species, but toxicity generally increased at higher salinities. Dispersant effectiveness (Chandrasekar et al. 2006) and oil degradation rates also increased with higher salinities (Kuhl et al. 2013).

Several factors must be considered to evaluate mitigation techniques in the event of an oil spill, including the type of oil spilled (Michel and Rutherford 2014), presence of sensitive or protected species and habitats, spill distance from shore, weather, and wave action (Michel and Rutherford 2013). Establishing toxicity thresholds for oil dispersants in coastal species under various environmental conditions will allow more informed spill response decisions. The purpose of this study was to examine the effects of salinity on the toxicity of two oil dispersants, Corexit 9500A and Finasol OSR52, on both adult and larval life stages of the eastern mud snail, *I. obsoleta*. In addition to comparing mortality thresholds between chemicals, salinities, and life stages, this study also examined sublethal effects in adult mud snails. Two sublethal biomarkers were selected for study: lipid peroxidation and acetylcholinesterase activity. Both of these biomarkers have been shown to be altered in invertebrates after exposure to oil spill dispersants (DeLorenzo et al. 2017). These cellular biomarkers indicate impairment of important physiological functions, with lipid peroxidation indicating cellular membrane damage resulting from oxidative stress and acetylcholinesterase inhibition indicating chemical disruption of neurotransmission.

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Materials and methods

Toxicity testing

Adult *I. obsoleta* were collected from Leadenwah Creek (N 32° 38′ 50.89″; W 080° 13′ 18.05″), a tidal tributary of the North Edisto River, SC, USA, during low tide. This area has a tidal range of approximately 1.8 m and a salinity range of 0–36 ppt (DeLorenzo et al. 2009). Mud snails (15–18 mm in length) were acclimated for 3 days in the laboratory in 76-L aquariums, at a density of approximately 250 snails per aquarium, with holding conditions of 25 °C, 20 ppt salinity, and a 16-h light:8-h dark photoperiod. Adult mud snails were fed Tetramin® fish flakes daily ad libitum.

Three salinities were selected for testing: 10, 20, and 30 ppt. These salinities correspond to the standard estuarine toxicity test condition of 20 ppt, and

approximately the lower tolerance level for *I. obsoleta* (Scheltema 1965) and the upper bound of typical southeastern estuarine salt marsh habitat (DeLorenzo et al. 2009). Preliminary testing with both adult and larval snails at the three salinities selected was performed to assure adequate control survival (≥80%) before conducting the acute toxicity testing with the dispersants. Adult snails were acclimated to the exposure salinities for 7 days in 76-L tanks and were fed Tetramin® fish flakes ad libitum daily.

Adult snails were tested in 600-mL glass beakers with 400 mL of test solution, with ten snails per beaker and three replicate beakers per dispersant concentration. Beakers were covered with aluminum foil, aerated, and placed in an environmental chamber at the above light and temperature conditions. Corexit and Finasol were each tested using a control and four nominal concentrations (37, 111, 333, and 1000 mg/L), tested concurrently for each of the three salinities (10, 20, and 30 ppt), for a total of 45 beakers for each dispersant. The test concentrations were selected based on preliminary LC50 testing. Test solutions were prepared by adding the neat dispersant to seawater in a graduated cylinder, bringing the solutions to volume, and stirring vigorously before pouring into the test chambers. Every 24 h, water quality (temperature, dissolved oxygen, salinity, and pH) was measured and the test solutions were renewed. Adult snails were not fed during the exposure. At the end of the 96-h exposure, mortality was determined (no response to gentle prodding of soft tissue with a dissecting probe). Surviving snails were dissected and the tissues were stored frozen (−80 °C) for the lipid peroxidation and acetylcholinesterase assays. A 96-h median lethal concentration was determined for Corexit and Finasol at each of the three salinities tested.

Adult snails deposited egg capsules on the glass sides of the aquarium. Egg capsules were scraped from the side of the tank using a razor blade and transferred to a glass finger bowl containing filtered (0.22 μm) 20 ppt seawater, covered with aluminum foil and kept aerated in an environmental chamber, set at 25 °C and on a 16-h light:8-h dark photoperiod until larvae hatched. Mud snail larvae (≤24-h-old swimming veligers) were tested in 24-well polystyrene plates coated with hydrogel (Corning™) to reduce chemical adherence (Chandler et al. 2004), with 2 mL of test solution and one larva per well. Each dispersant was tested using a control and four nominal concentrations (4.1, 12.3, 37, and 111 mg/L), at each of the three salinities (10, 20, and 30 ppt), with three replicate 24-well plates per concentration (n = 72 individuals per treatment). The plates were placed on an orbital shaker (80 rpm) in a Percival environmental chamber set to the above laboratory conditions. Larval mortality was determined daily by visual inspection with a dissecting microscope.

At each daily treatment renewal, surviving larvae were transferred to new 24-well plates with new treatment solution and fed 12,000 cells/mL of the alga *Isochrysis galbana*. Water quality parameters (salinity, temperature, oxygen, and pH) were measured daily from the 24-h-old test solutions. A 96-h median lethal concentration was determined for Corexit and Finasol at each of the three salinities tested.

Lipid peroxidation assay

Assessment of lipid peroxidation for adult mud snails followed the malondialdehyde method of Ringwood et al. (2003), adapted to microplate format. Individual, whole snails (tissue removed from shell) were homogenized on ice in 50 mM K_2PO_4 buffer (4:1 volume: sample weight). Homogenates were centrifuged at 13,000 *g* for 10 min at 4 °C, and 100 µL of each supernatant was transferred to a new microcentrifuge tube. Lipid peroxidation standards consisted of malondialdehyde (MDA) (3200 mM in K_2PO_4 buffer, final concentration of 12.5–1600 mM), and a blank of 100 µL K_2PO_4. One thousand four hundred microliters of 0.375% thiobarbituric acid (TBA) and 14 µL of 2% butylated hydroxytoluene (BHT) were added to 100 µL of each sample, standard, and blank. Samples and standards were then vortexed and heated at 93 °C for 15 min. Samples and standards were centrifuged at 13,000 *g* for 5 min at room temperature. Supernatant was transferred to a 96-well plate, and absorbance was measured using a spectrophotometer at a wavelength of 532 nm. Results were reported as nanomoles MDA formed/grams wet tissue weight.

Acetylcholinesterase assay

Acetylcholinesterase (AChE) enzyme activity was assessed using methods of Key et al. (1998) in adult snails (tissue removed from shell). Tissues were individually homogenized on ice in Tris-HCl buffer (20:1 *w*/*v*). A 75-µL aliquot of homogenate was added to 1.425 mL Tris-HCl buffer in triplicate for each sample. A 15-µL aliquot of ethanol was added to the three replicate test tubes. Each tube was sealed with parafilm, then vortexed at 2-min intervals, and held in a 30 °C shaking water bath for 15 min while 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and acetylthiocholine iodide (ACTH) vials were removed from the freezer and allowed to thaw. After the first tube had incubated in the water bath for 15 min, 33 µL DTNB, 967 µL incubated homogenate, and 10 µL ACTH were combined in a cuvette, covered with parafilm, inverted to mix, wiped, and placed in the Ultrospec 5300 pro spectrophotometer. Absorbance was read on the spectrophotometer at a wavelength of 412 nm every 10 s for a total of 70 s. A sample incubated with 10 µM eserine sulfate was used to account for non-enzymatic, non-AChE

hydrolysis of the substrate. Protein content was determined using a modified Lowry protein assay (Lowry et al. 1951). Data were expressed as AChE nanomoles/milligrams protein/minute.

Statistical analysis

Median lethal concentrations (96-h LC50 values) with 95% confidence intervals (CIs) were determined based on nominal chemistry values using SAS Probit Analysis (PROC PROBIT, SAS V.9.4, Cary, NC, USA). Significant differences ($p < 0.05$) between LC50s of the different chemicals, life stages, and salinities were determined using the LC50 ratio test (Wheeler et al. 2006). William's Test for Monotonic Trend and Minimum Effective Dose was used to determine significant trends in the lipid peroxidation and acetylcholinesterase data.

Results

I. obsoleta larval toxicity

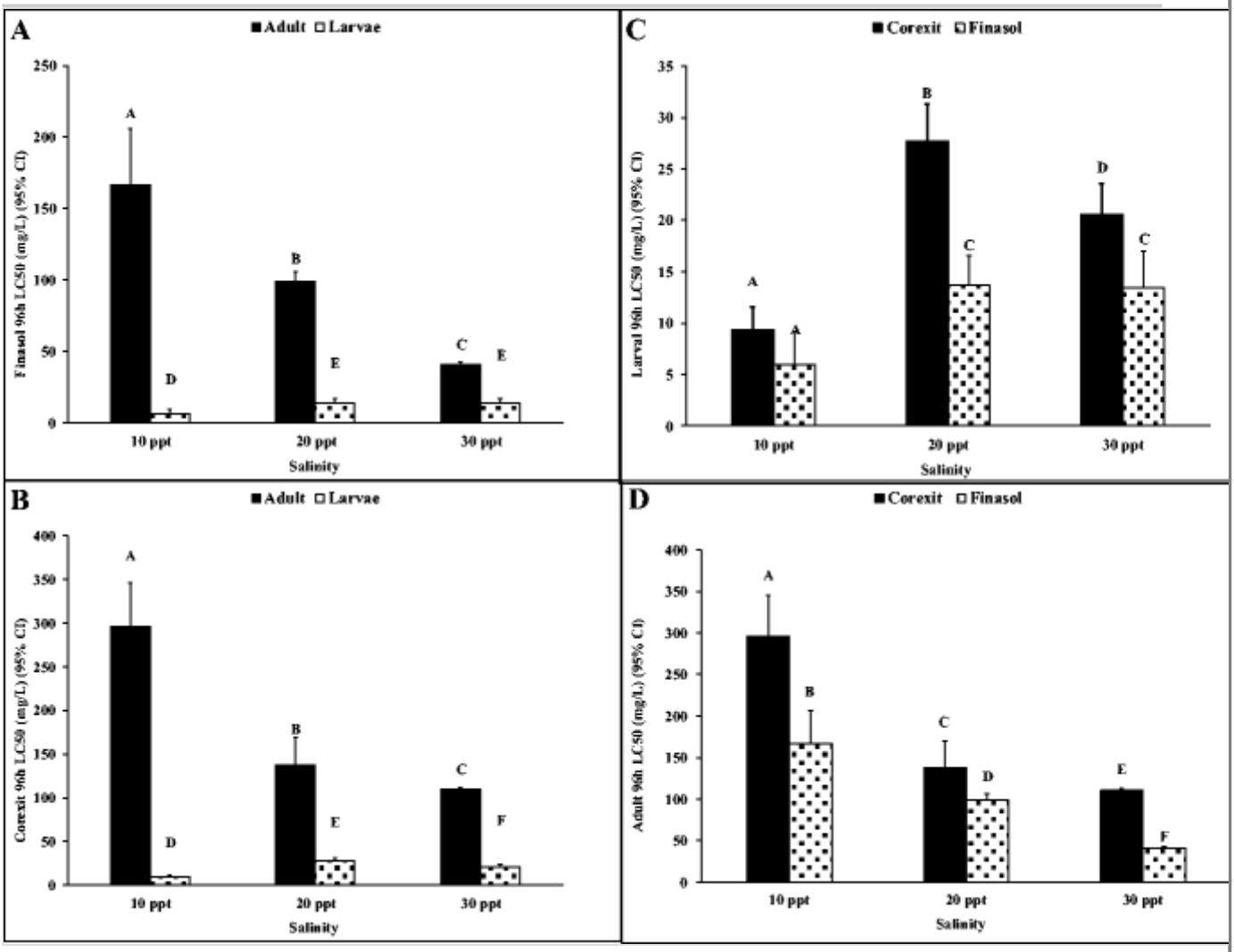
Larval mud snail mortality at 96 h for each of the three salinities tested averaged 9.7% at 10 ppt, 1.39% at 20 ppt, and 0.69% at 30 ppt in the control exposures. While mortality was higher in the 10 ppt, it did not exceed the test criteria of <20% control mortality.

There was a significant effect of salinity on Finasol toxicity, with greater toxicity at the lower salinity (Fig. 1a). There was a significant difference between the LC50 value at 10 ppt of 5.98 mg/L (95% CI: 2.95–9.19) and the LC50 value at 20 ppt of 13.65 mg/L ($p = 0.0036$). The LC50 value at 30 ppt (13.46 mg/L; 95% CI: 11.48–16.98) was also significantly different from the LC50 value at 10 ppt ($p = 0.0096$). There was not a significant difference between the LC50 values determined at 20 vs. 30 ppt ($p = 0.915$).

Fig. 1

Median lethal toxicity values (*bars* represent 96-h LC50 values and *error bars* represent 95% confidence interval) for adult and larval mud snails exposed to Finasol (*A*) and Corexit (*B*) for each salinity condition. Different letters indicate significant differences between salinities based on LC50 ratio test p value <0.05. These data are also shown as Corexit vs. Finasol LC50 values for larval snails (*C*) and adult snails (*D*). Different letters indicate significant differences between dispersants based on LC50 ratio test p value <0.05. There were also significant differences between adult and larval life stages (letters not shown), with larval snails significantly more

sensitive than adults for both Corexit and Finasol at all salinities tested (all p values <0.0001)



A similar trend in toxicity was observed with Corexit and larval snails, with the greatest toxicity found at the lowest salinity tested (Fig. 1b). The LC50 value at 10 ppt of 9.37 mg/L (95% CI: 7.76–11.55) was significantly lower than the LC50 value at 20 ppt of 27.69 mg/L ($p < 0.0001$). The LC50 values between 10 and 30 ppt (20.55 mg/L; 95% CI: 11.48–16.98) were also significantly different ($p < 0.0001$). There was a significant difference between the LC50 values determined at 20 vs. 30 ppt ($p = 0.0006$).

Finasol was significantly more toxic than Corexit to larval mud snails at the standard test salinity of 20 ppt (Fig. 1c), with 96-h LC50 values of 13.65 mg/L (95% confidence interval (CI): 11.71–16.58) for Finasol and 27.69 mg/L (95% CI: 24.47–31.33) for Corexit (LC50 ratio test $p < 0.0001$). Finasol concentrations of 4.1 and 12.3 mg/L yielded 18 and 40% mortality, respectively, whereas Corexit at the

same concentrations yielded 4 and 15% mortality, respectively. Finasol was also significantly more toxic than Corexit to larval mud snails when tested at 30 ppt (LC50 ratio test $p = 0.0003$), whereas at 10 ppt, there was no significant difference between the Corexit and Finasol LC50 values (LC50 ratio test $p = 0.4234$; Fig. 1c).

I. obsoleta adult toxicity

Adult mud snails tolerated all salinities tested, with <10% control mortality at 10, 20, and 30 ppt during both dispersant tests. Adult snails were significantly less sensitive than larval snails to both Corexit and Finasol at all salinities tested (all LC50 ratio test p values comparing adults vs. larvae <0.0001).

For adult snails, the trend in dispersant toxicity with salinity was the reverse of that observed for larval snails. Finasol was most toxic to adult snails at the highest salinity tested (Fig. 1a). The 96-h LC50 values determined were 166.04 mg/L (95% CI: 133.79–205.99) at 10 ppt, 99.09 mg/L (95% CI: 92.65–105.98) at 20 ppt, and 40.58 mg/L (95% CI: 38.59–42.66) at 30 ppt. Each LC50 value was significantly different from the others, with all LC50 ratio tests having p values <0.0001.

Corexit was also most toxic to adult snails at the highest salinity tested (Fig. 1b). The 96-h LC50 values determined were 295.86 mg/L (95% CI: 239.72–345.46) at 10 ppt, 137.61 mg/L (95% CI: 112.26–169.70) at 20 ppt, and 110.16 mg/L (95% CI: 108.00–112.36) at 30 ppt. All toxicity values were significantly different when compared among salinities tested (p values <0.0001).

Finasol was significantly more toxic than Corexit to adult mud snails at the standard test salinity of 20 ppt (Fig. 1d), with 96-h LC50 values of 99.09 mg/L (95% confidence interval (CI): 92.65–105.98) for Finasol and 137.61 mg/L (95% CI: 112.26–169.70) for Corexit (LC50 ratio test $p < 0.0001$). Finasol was also significantly more toxic than Corexit to adult mud snails when tested at 10 and 30 ppt (Fig. 1d; LC50 ratio test p values <0.0001).

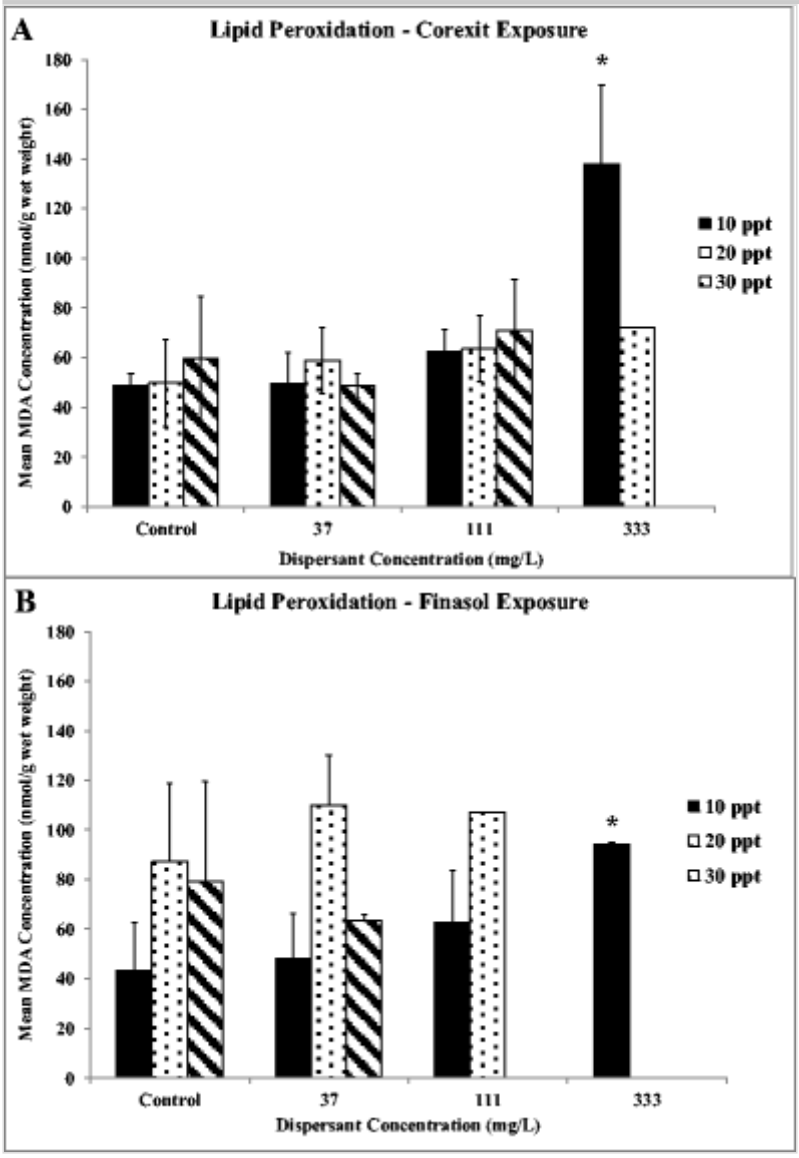
Lipid peroxidation

There was a significant trend of increasing lipid peroxidation (MDA concentration) with increasing dispersant concentration at the lowest salinity tested (William's Test for Monotonic Trend p values <0.0001). Adult mud snails in the 10-ppt salinity exposure had significantly higher lipid peroxidation at 333 mg/L Corexit (Fig. 2a) and 333 mg/L Finasol (Fig. 2b) compared to control ($p < 0.0001$). There were no significant differences among Corexit treatments at 20 ppt ($p = 0.9239$) or

30 ppt (p = 0.4046), or among Finasol treatments at 20 ppt (p = 0.5908) or 30 ppt (p = 0.4722).

Fig. 2

Lipid peroxidation in adult mud snails exposed to Corexit (**a**) and Finasol (**b**) at salinities of 10, 20, and 30 ppt. *Asterisks* indicate significant treatment differences from respective salinity control, Dunnett's test (n = 3 for all bars except 20 ppt, 333 mg/L Corexit and 20 ppt, 111 mg/L Finasol, where n = 1). *Missing bars* for a given salinity/concentration indicate there were not enough surviving snails to analyze



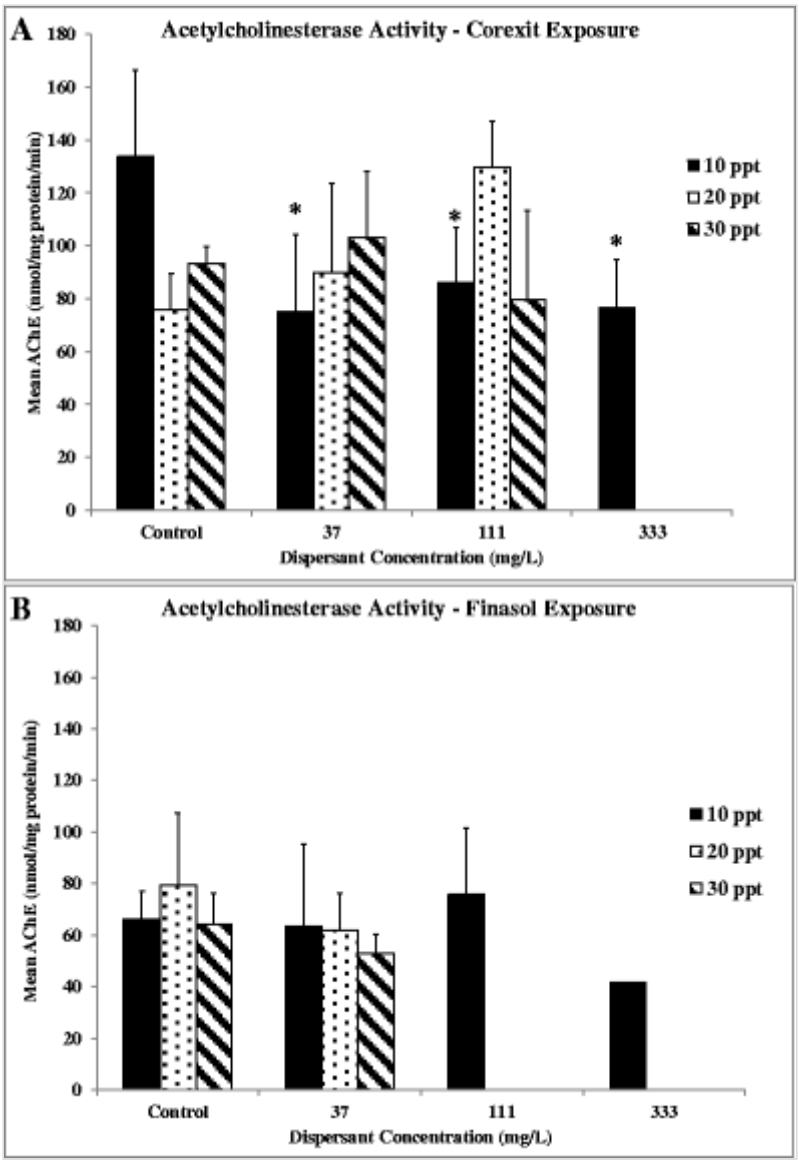
Acetylcholinesterase activity

Acetylcholinesterase activity decreased with Corexit exposure at the lowest salinity tested (William's Test for Monotonic Trend p = 0.0303). AChE activity was

significantly lower in the 10-ppt salinity exposure at Corexit concentrations ≥37 mg/L, relative to control ($p < 0.05$) (Fig. 3a). There was no significant difference in acetylcholinesterase levels among Corexit treatments at 20 ppt (William's Test for Monotonic Trend $p = 0.0992$) or 30 ppt (William's Test for Monotonic Trend $p = 0.4911$). In general, the snails in the Corexit exposures appeared to have slower foot muscle response to stimuli. There was no significant difference among Finasol concentrations at any salinity tested (William's Test for Monotonic Trend $p = 0.1039$ at 10 ppt, $p = 0.5127$ at 20 ppt, and $p = 0.2752$ at 30 ppt; Fig. 3b).

Fig. 3

Acetylcholinesterase activity in adult mud snails exposed to Corexit (**a**) and Finasol (**b**) at salinities of 10, 20, and 30 ppt. *Asterisks* indicate significant treatment differences from respective salinity control, Dunnett's test ($n = 3$ for all bars except 10 ppt, 333 mg/L Finasol, where $n = 1$). *Missing bars* for a given salinity/concentration indicate there were not enough surviving snails to analyze



Discussion

This study provides new toxicity threshold values for oil dispersants and a common estuarine gastropod species. The eastern mud snail larval life stage (96-h LC50 of 13.65 mg/L) was similar in sensitivity to Finasol compared with the mysid *Americamysis bahia* (48-h LC50 value of 9.37 mg/L; USEPA 2003), the silversides minnow *Menidia beryllina* (96-h LC50 of 11.7 mg/L; USEPA 2003), and larval grass shrimp *Palaemonetes pugio* (96-h LC50 of 16.8 mg/L; DeLorenzo et al. 2016) at their normal test salinity of 20 ppt. The eastern mud snail was more sensitive to Corexit (larval 96-h LC50 of 27.69 mg/L; adult LC50 of 137.61 mg/L) than grass shrimp (larval 96-h LC50 of 40.1 mg/L; adult LC50 of 419 mg/L; DeLorenzo et al. 2016) at 20 ppt. Both mud snail life stages were significantly more sensitive to Finasol than to Corexit, and both dispersants were more toxic to larval snails than to adults.

Consistent with the abiotic conditions of their tidal creek habitat, both adult and larval *I. obsoleta* were tolerant to the wide range of salinities tested. Adult snails were less sensitive to salinity stress, with no difference in survival at 10, 20, or 30 ppt, whereas larvae showed a slight increase in mortality at 10 ppt.

For larval mud snails, salinity combined with dispersants caused significantly greater toxicity than dispersants alone. The greatest interaction between salinity and dispersant was for Corexit, with larval snails exhibiting 4.6-fold greater toxicity at 10 ppt than at the standard test condition of 20 ppt. Lower salinity also influenced Finasol toxicity, but to a lesser degree: 1.4-fold greater toxicity at 10 ppt compared to 20 ppt. Increasing toxicity from 20 to 30 ppt had little effect on the toxicity of either dispersant to larval mud snails. Adult mud snail dispersant toxicity followed a different pattern with salinity. Toxicity of Finasol and Corexit was highest at the highest salinity tested.

I. obsoleta has very limited capacity for osmoregulation (Avens and Sleigh 1965; Prosser 1973). Adults retreat into the shell at low salinity and have been shown to reduce oxygen consumption to almost zero at 10 ppt (Avens and Sleigh 1965; Prosser 1973). This avoidance behavior may reduce water intake and therefore reduce chemical exposure, leading to lower toxicity at lower salinity for adult snails. The inconsistency in adult and larval responses could be due to the differences in physiology of the pelagic, swimming veliger larvae, and the benthic, shelled adult. A previous study also documented differences in the effects of low salinity on larval and adult life stages of the intertidal gastropod *Crepipatella peruviana* (Montory et al. 2014). In that study, the adult snails were able to withstand low salinity conditions by clamping their shells shut against substrate, whereas the veliger larvae experienced negative physiological effects (decreased swimming activity, microalgal clearance rates, and oxygen consumption) and increased mortality rates with low salinity stress.

Increased toxicity at lower salinities were also reported for the grass shrimp with Finasol and Corexit (DeLorenzo et al. 2016), and for the Gulf killifish (Kuhl et al. 2013) with Corexit. The interactive effect of salinity on dispersant toxicity may be related to reduced degradation of the dispersant, increased solubility and bioavailability, or increased sensitivity due to physiological stress at lower salinity. Other studies have shown chemical toxicity to increase with decreasing toxicity, for example in larval oysters with metal exposure (Calabrese et al. 1973), in grass shrimp with insecticide exposure (Breken-Folse et al. 1994), and in blue mussels with diesel oil exposure (Tedengren and Kautsky 1987). Additional research is

needed to identify the mechanism of how salinity affects dispersant toxicity in these life stages.

Sublethal effects of dispersants were also identified in this study, including increased lipid peroxidation and acetylcholinesterase activity inhibition. Lipid peroxidation is an indicator of damage to cellular membranes, occurring when free radicals react with lipids to produce cytotoxic products that may damage DNA and enzymes (Kehrer 1993). Corexit and Finasol increased lipid peroxidation in adult mud snails. This same effect on cellular membrane damage was seen with grass shrimp (DeLorenzo et al. 2016). Cellular membrane damage can lead to negative effects on respiration, osmoregulation, acid–base balance, and nitrogen excretion (Henry et al. 2012). Acetylcholinesterase inhibition was seen with other surfactants similar to the surfactant components of Corexit and Finasol. For example, sodium dodecyl sulfate inhibited AChE activity levels in daphnia (Guilhermino et al. 2000) and exposure to sodium dodecylbenzenesulfonate inhibited AChE in the freshwater cladocera *Moina macrocopa* (Martinez-Tabche et al. 1997). The snails in the Corexit exposure had noticeable decrease in foot muscle response to stimuli. This could indicate potential for chronic effects due to neurological impairment, such as inhibited predator avoidance ability. In addition, energy to counter cellular membrane damage may come at the cost of reduced growth or fecundity.

Measured levels of oil dispersants in offshore waters were reported by Kujawinski et al. (2011) as ranging between 0.4–12 μg/L dioctylsulfosuccinate (DOSS) (a marker for Corexit 9500) during and after the DWH event. Another marker for Corexit 9500 (dipropylene glycol n-butyl ether (DPnB)) was detected in both offshore and nearshore waters after the spill at concentrations ranging from 0.0170–114.4 μg/L (OSAT 2010). The nominal dispersant LC50 values reported here are much higher (5.98–295.86 mg/L); however, the lack of quantitative data for the dispersant itself, particularly in coastal areas, makes the comparison difficult. Moreover, in the environment, organisms will likely be exposed to dispersants in an even more complex chemical mixture of dispersed oil. Previous testing with Corexit and Finasol applied to Louisiana Sweet Crude oil demonstrated increased hydrocarbon exposure and subsequent toxicity in the dispersed oil preparations, with larval mud snail LC50 values well below measured total petroleum hydrocarbon surface water concentrations (DeLorenzo et al. 2017). Should dispersed oil be present in coastal areas, the enhanced toxicity of oil dispersants at lower salinities determined for sensitive larval life stages suggests an increased risk to estuarine biota. The results of this study provide response managers with toxicity data for two dispersants under a range of salinity conditions to guide oil spill

mitigation decisions specific to estuarine habitats. Further research is needed to elucidate mechanisms of chemical toxicity under different abiotic conditions and to incorporate those factors into toxicity testing for risk assessment.

Acknowledgements

We thank Blaine West and James Daugomah for the assistance with field collections. We appreciate helpful manuscript reviews by Len Balthis, Paul Pennington, and Natasha White. Funding for Brittany Evans' participation was provided in part by the NOAA Ernest F. Hollings Undergraduate Scholarship Program. The NOAA, National Ocean Service does not approve, recommend, or endorse any proprietary product or material mentioned in this publication.

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