# Effects of shoreline oiling on salt marsh epifaunal macroinvertebrates

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The *Deepwater Horizon* oil spill resulted in varying degrees of oiling in the salt marshes of northern Barataria Bay, Louisiana, USA. This study examines the effects of oiling intensity and recovery on two conspicuous marsh-platform macroinvertebrates, *Uca* spp., fiddler crabs, and *Littoraria irrorata*, the salt marsh periwinkle, from 2.5 to 4.5 years after the spill. The dominant fiddler crab within these marshes, *Uca longisignalis*, was the only species observed in field collections, and no significant difference in burrow density or burrow size was found among oiling levels over the study period indicating recovery from any negative effects of oiling already occurred for this species. The highest density of *L. irrorata* was found at moderately oiled sites compared to both reference (without visible oiling) and heavily oiled stations. *Spartina alterniflora* density recovered within two years after the spill at the moderately oiled stations facilitating recovery of L*. irrorata* approximately one year later. *L. irrorata* average shell length and length-frequency distributions were equivalent at moderately oiled and reference stations but snails were shorter at heavily oiled stations because of a greater proportion of subadults.  Shell length data from the heavily oiled sites indicate that direct mortality due to oiling or oil-induced reductions in recruitment occurred in 2010 and that recovery was starting to occur at 48 months after the spill. The extent and duration of oil in the water during the spill and the biological responses we measured indicates that L. irrorata and *Uca* longisignalis were both affected in their ability to carry out their life cycle on the marsh and/or in the water column at all stations including the reference stations for some period following the entry of oil into the region.

1 **Title Page**

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14 Keywords: *Deepwater Horizon* oil spill; *Uca*, fiddler crab, *Littoraria irrorata*, marsh periwinkle

## Abstract

The *Deepwater Horizon* oil spill resulted in varying degrees of oiling in the salt marshes of

northern Barataria Bay, Louisiana, USA. This study examines the effects of oiling intensity and recovery

on two conspicuous marsh-platform macroinvertebrates, *Uca* spp., fiddler crabs, and *Littoraria irrorata*,

the salt marsh periwinkle, from 2.5 to 4.5 years after the spill. The dominant fiddler crab within these

marshes, *Uca longisignalis*, was the only species observed in field collections, and no significant

difference in burrow density or burrow size was found among oiling levels over the study period

indicating recovery from any negative effects of oiling already occurred for this species.  The highest

density of *L. irrorata* was found at moderately oiled sites compared to both reference (without visible

oiling) and heavily oiled stations. *Spartina alterniflora* density recovered within two years after the spill

at the moderately oiled stations facilitating recovery of L*. irrorata* approximately one year later. *L.

irrorata* average shell length and length-frequency distributions were equivalent at moderately oiled

and reference stations but snails were shorter at heavily oiled stations because of a greater proportion

of subadults.  Shell length data from the heavily oiled sites indicate that direct mortality due to oiling or

oil-induced reductions in recruitment occurred in 2010 and that recovery was starting to occur at 48

months after the spill.  The extent and duration of oil in the water during the spill and the biological

responses we measured indicates that *L. irrorata* and *Uca* spp. were both affected in their ability to

carry out their life cycle on the marsh and/or in the water column at all stations including the reference

stations for some period following the entry of oil into the region.

## Introduction

The release of a judge-ruled 3.19 million barrels of oil from the *Deepwater Horizon* (*DWH*) oil spill

(Malakoff, 2015) exposed the nation's largest and most productive wetland-estuarine environment to an

39    unprecedented potential for environmental damage. Oil spills can cause widespread impacts to the

40    structure, function, resilience, and sustainability of coastal wetlands depending upon the oil type, extent

41    of contamination of the vegetation and marsh soils, exposure to waves and currents, time of year of the

42    spill, and species sensitivity to oiling (Michel & Rutherford, 2014). Although the oil that made landfall in

43    the *DWH* oil spill was relatively "weathered" and consisted of emulsions of crude oil depleted of its more

44    volatile and toxic components, the spill resulted in the oiling of 796 km of coastal marsh shoreline, as

45    documented by Shoreline Cleanup Assessment Technique (SCAT) teams (Michel *et al*., 2013). Of that total,

46    approximately 135 km were described as heavy and 165 km as moderate marsh oiling (Michel *et al*., 2013).

47    Approximately 95% of the total marsh oiling occurred in coastal Louisiana, and the heaviest marsh oiling

48    was most widespread in northern Barataria Bay marshes dominated by *Spartina alterniflora* and to a lesser

49    extent *Juncus roemerianus* (Zengel *et al*. 2014, Michel *et al*. 2013). The plant communities in northern

50    Barataria Bay experienced strong responses to oiling, including heavy plant mortality that denuded

51    shorelines (Lin and Mendelssohn 2012; Silliman *et al*. 2012; Zengel and Michel 2013; Zengel *et al*. 2014;

52    Zengel *et al.*, 2015).

53         The marshes of the Mississippi River delta system provide a suite of environmentally and

54    economically important services including marsh platform elevation, the prevention of soil erosion, soil

55    nutrient cycling, food web support both grazing and detrital, and many others (McCall & Pennings, 2012;

56    Silliman *et al*., 2012). Most, if not all, of these ecosystem services depend upon a healthy, functioning

57    plant-microbial-benthic system. Oil spills can exacerbate wetland loss by destabilizing these interrelated

58    processes that control the capacity of coastal wetlands to function properly.  Initial impacts of the *DWH*

59    oil spill on coastal wetlands have been and are being reported (e.g., Whitehead, 2011, Lin and

60    Mendelssohn, 2012, McCall & Penning, 2012, Silliman *et al*., 2012). This study is a part of a program

61    documenting the longer term (currently 4 years) impacts and recovery of the oiled marsh systems and

62    their potential for sustainability.

63      Other members of the study team are analyzing and providing data on plant parameters, soil

64      parameters, soil bacteria, benthic microalgae, and benthic meiofauna (Lin *et al.*, unpublished data; Fleeger

65      *et al.*, 2015). This and other studies have shown that different marsh plant species respond differently to

66      oiling (Alexander & Webb, 1987; Baca *et al.*, 1987: Mendelssohn *et al.*, 1990, 2012; Hoff *et al.*, 1993; Lin

67      & Mendelssohn, 1996, 1998, 2008, 2009; Hester & Mendelssohn, 2000; Pezeshki *et al.*, 2000; Lin *et al.*,

68      2002; DeLaune *et al.*, 2003; Culbertson *et al.*, 2008; Michel & Rutherford, 2014), demonstrating impacts

69      to coastal wetland vegetation, e.g. reduced plant photosynthesis, transpiration, shoot height, stem

70      density, and above and below ground biomass. These impacts on vegetation and the marsh surface can

71      result in cascading effects on soil bacteria and macro-, meio-, and micro-faunal communities that inhabit

72      wetlands and depend on the plant organic matter as carbon sources or as foundation species. For

73      example, benthic microalgae and meiofauna are, both individually and when monitored together,

74      important indicators of ecosystem function regarding food-web support and a broad range of effects and

75      recovery from oil spills (Fleeger *et al.*, 2015).

76      Our sampling focused on two conspicuous and foundational marsh macroinvertebrates, *Uca* spp.,

77      marsh fiddler crabs (Montague, 1980), and *Littoraria irrorata*, the salt marsh periwinkle (Silliman &

78      Zieman, 2001).  Fiddler crabs are one of the most thoroughly-studied shore crab in North America, with

79      robust literature available that examines individual species population dynamics, life history and ecology

80      (Grimes *et al.*, 1989). Fiddler crabs greatly influence the marsh through burrowing and feeding activities,

81      in effect enhancing effects on vegetation productivity and biomass, altering sediment and nutrient

82      characteristics, altering biogeochemical cycles, altering microbial processes by aerating the marsh

83      sediment, increasing soil drainage, and facilitating nutrient transport.  Generally, the presence of fiddler

84      crabs has been noted as indicating greater diversity of other marsh organisms, and crab population

85      densities can reflect the productivity of a wetland (Montague, 1980; Mouton & Felder, 1996).  Fiddler

86   crabs have been shown to be sensitive to oil spills, making them a valuable environmental indicator

87   species (Burger *et al*. 1991, 1992; Burger & Gochfeld, 1992).

88        Various fiddler crab species inhabit the northern Gulf of Mexico coast. Two species in particular

89   are found in the Louisiana marshes, *Uca spinicarpa* and *U. longisignalis*.  *Uca spinicarpa* prefers clay-

90   dominated substrates in brackish marshes ranging from nearly fresh to hypersaline.  *Uca longisignalis* is

91   restricted to sediments of terrigeneous origin ((i.e. mucky soils) and found primarily in lower salinity

92   environments (upper estuaries).  The two species can be found in close proximity but have preferred

93   habitats based on elevation, vegetation and sediment character (Mouton & Felder, 1996; Zengel *et al*.,

94   2014). Although there may be more than one species of fiddler crab, we will refer to them collectively as

95   *Uca* except when we refer to a specific species.

96        *L. irrorata* also is an indicator species of the health of the salt marsh habitat (Silliman & Zieman,

97   2001).  In areas dominated by short-to-intermediate form *Spartina alterniflora*, the species has been

98   noted to occur in densities of at least 100 individuals/m$^2$ (Silliman & Zieman 2001; Stagg & Mendelssohn

99   2012).  *L. irrorata* is a rasping detritivore/herbivore specialist, feeding on organic matter on the marsh

100  surface during low tide and ascending the Spartina stems to feed upon standing-dead Spartina and its

101  associated microbial assemblages as the tide rises (Silliman & Zieman 2001). As a detritivore, *L. irrorata*

102  influences nutrient dynamics by expediting the decomposition of *Spartina alterniflora* and serves as an

103  important link between primary and secondary production (Stagg & Mendelssohn 2012). The presence of

104  *S. alterniflora* has been directly linked to increased abundance, growth and survival of *L. irrorata* in

105  marshes (Kiehn & Morris 2009; Stagg & Mendelssohn 2012).

106       Fiddler crabs (Krebs & Burns, 1977; Burns & Teal 1979; Burger *et al*., 1991; Teal *et al*., 1992;

107  Culbertson *et al*., 2007; Morris *et al*. 2015) and the marsh periwinkle (Hershner & Moore 1977; Hershner

108  & Lake, 1980; Lee *et al*., 1981; Bennett *et al*., 1999; Garner *et al*. 2015) both can be severely impacted by

109  oil spills, with direct effects including direct mortality, reduced population densities, and sublethal effects,

110 e.g. behavior alterations and reproduction effects. Pennings *et al.* (in review), using only sites without

111 substantial oiling from this and other studies, evaluated the potential impact of the *DWH* oil spill on

112 recruitment of *L. irrorata* in Louisiana comparing the proportion of subadults that would have recruited

113 before the oil spill (pre-2011) to the proportion in 2011 and the proportion occurring in subsequent years

114 to evaluate recovery.  They found that the proportion of subadults in *L. irrorata* populations in Louisiana

115 was reduced by 90% in 2011 suggesting recruitment failure in 2010. Effects of oiling can occur during the

116 portion of these species life spent on the marsh platform or, as indicated by the Pennings et al. (in review)

117 study, when they release fertilized eggs into the water and expect return of larvae to the marsh shoreline.

118 Recovery time following oil-induced impacts for these organisms can vary substantially based on a variety

119 of oiling and habitat conditions, from a year to several decades (Culbertson *et al.*, 2007).

120     In this study, we present the results of an investigation of the effects of the *DWH* oil spill on fiddler

121 crab and marsh periwinkle at different oiling levels between 30 and 54 months after the spill.  As has been

122 indicated, Lin et al. (unpublished data) studied the response of the plant community including the

123 dominant salt marsh plants *S. alterniflora* and *J. roemerianus* in the same sampling stations as used for

124 this investigation.

125

## Methods

127     The 21 shoreline sampling stations were established in 2011 within an approximate 8 km by 5 km

128 area in Wilkinson Bay and Bay Jimmy in northern Barataria Bay, Louisiana, USA (between coordinates N

129 29.44060° - 29.47459°, W 89.88492° - 89.94647°) that had been impacted to varying degrees by the *DWH*

130 oil spill (Fig. 1). Sampling stations were randomly selected using SCAT data and our own field observations,

131 and included seven locations each that received either no oiling (i.e., reference) (RF), moderate oiling

132 (MD), or heavy oiling (HV). Sampling occurred at various occasions (generally bi-annually) from 30 months

133 (November 2012) after the spill to 54 months (October 2014) after the spill. Sampling was generally in

134 the spring (April - June) and fall months (September – November). Total petroleum hydrocarbon and plant

135 community data were collected at the time of sampling (Lin *et al.*, unpublished data).

136

137 **Total Petroleum Hydrocarbon (TPH) analysis**

138

139      The 0–2 cm surface soils were collected from each station on each collection date, transported to

140 the Louisiana State University laboratory on ice, extracted with dichloromethane (DCM), and analyzed

141 gravimetrically (Lin & Mendelssohn, 2012). DCM extracts were transferred to pre-weighed dishes, where

142 the DCM was evaporated, and the unevaporated oil remaining in the dishes was weighed to the nearest

143 0.0001 g. TPH concentration was calculated and expressed as mg g$^{-1}$ dry soil. The results of the analysis

144 for 30 to 54 months after the spill are provided in Figure 8.

145

146 **Aboveground Biomass and Stem Density**

147

148      Plant aboveground biomass and stem density were taken at each station during each sampling

149 within a haphazardly located 0.25 m$^2$ quadrat. All plants rooted within the quadrat were then clipped to

150 the ground surface and separated into live and dead components by species. Stem density was

151 determined by counting the number of intact living stems for each dominant plant species. All

152 aboveground biomass was then dried to a constant mass at 60°C and weighed. Parameters reported

153 included total live and dead above ground biomass and above ground biomass and stem density for each

154 of the dominant species (*Spartina alterniflora* and *Juncus roemerianus*).

155

## Macroinvertebrate sampling

156

157

158    Sampling included fiddler crab (*Uca*) burrow density and size and marsh periwinkle (*Littoraria*

159    *irrorata*) density and size for both components of the study.  Sampling was conducted using three 0.25 m²

160    quadrats placed at random approximately 1 m inward from the marsh edge.    Data was converted to m⁻²

161    basis for analysis. Each quadrat was sampled for both *L. irrorata* abundance and *Uca* burrow abundance.

162    *L. irrorata* were found on the marsh surface or attached to the vegetation within the plot; juvenile *L.*

163    *irrorata* were often found hiding in the leaf bracts of *Spartina alterniflora*. *L. irrorata* shell length and *Uca*

164    burrow size (diameter) were measured to the nearest millimeter using calipers and a transparent ruler,

165    respectively. Shell lengths were taken as a total height, a measurement from the base of the aperture to

166    the top point of the shell. Once measured, *L. irrorata* were returned to the marsh within their specific

167    sampled quadrat. As fiddler crabs are mobile and difficult to quantify within a certain area, their burrows

168    were used as a proxy for their abundance on the marsh. We were not able to measure crab burrows at

169    two sampling occasions (36 and 42 months after the spill) because water was present on the marsh

170    platform, covering the marsh sediment in the sampling area. Photos were taken of the general site setting

171    and each quadrat, and general notes were made concerning site flora and fauna composition and

172    characteristics. *Uca* adults were identified to species level and released if found in the sampling site

173    vicinity.

174    Measured *L. irrorata* were grouped into putative age classes based on their size: 0-6 mm were

175    classified as juveniles, 7-13 mm were classified as sub-adults, and 14+ mm were classified as adults

176    (Hamilton, 1978; Stagg & Mendelssohn, 2012). Sizes were examined as a percentage of the total

177    abundance for each site type (RF, MD, and HV; Figs. 5-7). Size frequency distributions of *L. irrorata* are

178    often bi- or tri-modal with the modes representing age class (Hamilton, 1978; Zengel *et al*., 2014). The

179    largest snails in most populations range from 20 – 26 mm in shell length (Emerson and Jacobson, 1976;

180    Morris, 1975), though the largest reported size is 32 mm (Kaplan, 1988). Based on growth estimates

181    (Stiven and Hunter, 1976), juveniles would be <1 year of age, subadults would average about 1 year of

182    age, and adults would be 2 years and older in age. These designations were used in analyses to examine

183    life stage-specific parameters of *L. irrorata* abundance and distribution throughout the study.  Evaluating

184    size distribution by comparing size frequency histograms over time is helpful in determining how

185    populations were impacted and tracing recovery trajectories (Zengel *et al.*, 2014; Zengel *et al.*, 2015;

186    Pennings et al., in review).

187

188    **Statistical Analysis**

189

190         All statistical analyses were conducted using SAS (Statistical Analysis Systems, version 9.2, SAS

191    Institute, Cary, NC). *L. irrorata* abundance data were tested for normality and found to be skewed

192    [Shapiro-Wilk Test (alpha = 0.05)]. Several transformations were tested and the natural log transformation

193    was the best fit; therefore, all *L. irrorata* abundance data were transformed as the natural log prior to

194    analysis. We used repeated measures, two-way mixed-model Analysis of Variance (ANOVA) with oiling

195    level, sampling period, and their interactions to test for variation in *L. irrorata* abundance. We used

196    Mauchly's Test of Sphericity to examine the form of the common covariance on the assumptions of the

197    repeated measures ANOVA and found that the data do not meet sphericity assumptions (Pr>ChiSq =

198    0.0057). An adjusted Tukey's test was used to determine differences between *L. irrorata* abundances and

199    oiling level at each sampling period.

200         We tested the *L. irrorata* shell size data for normality and found these data to be skewed [Shapiro-

201    Wilk Test (alpha = 0.05)]. All attempted transformations (log, natural log, square root) were unsuccessful.

202   Kruskal-Wallis test was therefore used to examine shell size by oiling level and sampling period, e.g.

203   comparing shell size for the RF sites by sampling period.

204        The nonparametric Kolmogorov-Smirnov two-sample test was used to compare the total

205   abundance (total, adult, and subadult) and average shell length data between oiling levels across all

206   sampling periods with the null hypothesis that the two oiling levels have the same distribution.

207        *Uca* burrow abundance data were tested for normality and found to be skewed [Shapiro-Wilk Test

208   (alpha = 0.05)].  Transformations and removal of the 36 and 40 month data sets which were mainly zeros

209   (periods of high water) were unable to normalize data.  Oiling levels and abundance of burrows were

210   therefore tested using Kruskal-Wallis/Mann-Whitney (non-parametric) tests, both leaving in the 36 and

211   40 month datasets and removing those datasets from the analysis; however, no significant results were

212   found.

213        Correlations were performed, using Kendall's Tau non-parametric rank correlations with

214   dependence on variables, with oiling level and sampling period to 48 months after the spill because of the

215   lack of availability of plant and TPH data at the time of the analysis; *L. irrorata* abundance; *Uca* burrow

216   density; *S. alterniflora* stem density and biomass; *J. roemerianus* stem density and biomass; total live

217   biomass; total dead biomass; and TPH.

218        Significant difference was defined as p ≤ 0.05. P-values are reported to 2 decimal places and, in

219   cases where p = 0.00, the p-value is reported as p < 0.01. All error terms are expressed in standard error

220   (SE). Raw data can be found at the following link: doi:10.7266/N7FF3Q9S.

221

222   **Results**

223

224   *Littoraria irrorata* **abundance**

225      *L. irrorata* abundance varied among oiling levels when data were combined across all sampling

226    periods and size classes. *L. irrorata* were found to have the highest average density at MD (mean = 92.5

227    ind m$^{-2}$, n=3,888) compared to RF (mean = 38.1 ind m$^{-2}$, n =1604, Kolmogorov-Smirnov test, p < 0.01) and

228    HV sites (mean = 33.5 ind m$^{-2}$, n = 1404, Kolmogorov-Smirnov test, p < 0.01). RF sites were also found to

229    be statistically different than HV sites (Kolmogorov-Smirnov test, p = 0.02). This same trend was found

230    within adults as both RF and MD differed from HV sites (Kolmogorov-Smirnov test, p = 0.02 and <0.01,

231    respectively, Fig. 2). No difference was found among oiling levels for subadults. Juveniles were cryptic

232    and inconsistent in their distribution, and there was no significant difference between oiling levels and

233    abundance of juveniles. *L. irrorata* abundance also exhibited differences between size classes at all of the

234    oiling levels. Adults are significantly more abundant than both sub-adults (Kolmogorov-Smirnov test, p <

235    0.01) and juveniles (Kolmogorov-Smirnov test, p < 0.01), while sub-adults are significantly more abundant

236    than juveniles (Kolmogorov-Smirnov test, p < 0.01).

237        When sampling period and oiling level were analyzed together with ANOVA, oiling level had a

238    significant effect on *L. irrorata* total abundance (n = 7 and p < 0.01), but the sampling period and

239    interaction effect were not significant (Fig. 3). We examined within-subjects main effects tests to examine

240    changes in snail abundance over time with the null hypothesis that mean abundance did not change over

241    time. We rejected the null hypothesis using Wilk' test (Pr>F = 0.0139) and concluded that abundance

242    varied over time. We used the same method to test the interaction between time and oiling level. We

243    again rejected the null hypothesis and concluded that change in mean abundance across time depended

244    on oiling level (Pr>F = 0.0037). Significant differences in *L. irrorata* total density were noted between oiling

245    levels at different sampling periods. Significant differences existed between both MD (Tukey's test, p =

246    0.02) and RF (Tukey's test, p < 0.01) and HV sites at 36 months after the spill; between the MD and RF

247    sites (Tukey's test, p = 0.05) at 40 months after the spill; and MD and RF (Tukey's test, p = 0.05) and HV

248    sites at 36 months after the spill.

249

## *Littoraria irrorata shell size*

251      L. irrorata body size also varied among oiling levels when data were combined across all sampling

252    periods. *L. irrorata* average shell length was significantly less at HV sites (mean = 15.44 mm) when

253    compared to both MD sites (mean = 17.37, Kolmogorov-Smirnov test, p < 0.01; Fig. 4) and RF sites (mean

254    = 16.62 mm, Kolmogorov-Smirnov test, p < 0.01).  RF and MD sites showed no statistical difference in shell

255    length (Kolmogorov-Smirnov test, p = 0.43).

256      *L. irrorata* shell length was also used to examine size-frequency distributions at all oiling levels. RF

257    and MD sites exhibited similar proportions of juveniles, subadults, and adults, while the HV sites contained

258    a lower percentage of adults, in particular, large adults.  This low proportion of adults at the HV sites likely

259    corresponded to the direct mortality of all size categories of *L. irrorata* at the HV sites due to the oiling in

260    2010.  As indicated in methods, adults are two years or older; therefore, even at 30 months, smaller adults

261    would just be entering the populations and replacing these losses at the HV sites.

262      The null hypothesis of no difference in shell length across sampling periods was rejected (Kruskal-

263    Wallis test, p < 0.01) indicating that there was a difference in shell size among oiling levels (figure 8).

264    Periwinkles in all oiling categories averaged less than 15 mm in shell size 30 months after the spill.  The

265    HV sites continued to average less than 15 mm in shell size up to 48 months after the spill; whereas, the

266    RF and MD sites achieve an average shell size greater than 17 mm at 48 months after the spill.

267

## *Uca burrow density and size*

269      All of the fiddler crabs collected during all sampling periods were identified as *Uca longisignalis*.

270    No significant difference was found between oiling level and *Uca* burrow density. Because no significant

271    difference was found with density, burrow size was not tested.

272

## Correlations

274    With all oiling levels and all stations, *L. irrorata* abundance was positively correlated with most of

275    the plant parameters including *S. alterniflora* above ground biomass ($p = 0.05$); *J. roemerianus* above

276    ground biomass ($p < 0.01$) and stem density ($p < 0.01$); and total live ($p < 0.01$) and dead above ground

277    biomass ($p = 0.06$). *Uca* burrow density was negatively correlated with *S. alterniflora* stem density ($p = $

278    $0.03$).

279    For the RF sites, the only significant correlation occurred at 30 months after the spill between *L.*

280    *irrorata* abundance and *Uca* burrow density ($p = 0.04$). The MD sites showed a significant positive

281    correlation between *L. irrorata* abundance and *J. roemerianus* above ground biomass ($p = 0.02$) and stem

282    density ($p = 0.03$) at 40 months after the spill; a significant negative correlation between *L. irrorata* and

283    *Uca* burrow density ($p = 0.01$) 42 months after the spill; and a significant negative correlation with *S.*

284    *alterniflora* stem density ($p = 0.03$) and positive correlation with *J. roemerianus* stem density ($p = 0.02$) at

285    48 months after the spill.  The HV sites showed no significant correlations.

286    TPH did not correlate with either *L. irrorata* or *Uca* abundance; however, there was a positive

287    significant correlation between TPH and *S. alterniflora* stem density ($p = 0.04$, all data; $p < 0.01$, HV sites).

288    There were significant negative correlations between *J. roemerianus* above ground biomass ($p < 0.01$) and

289    stem density ($p < 0.01$) and total live and dead biomass (both, $p < 0.01$).

290

## Discussion

### *Littoraria irrorata*

293    As stated in the Results, *L. irrorata* were found to have the highest average density at MD

294    compared to RF and HV sites. RF sites were also found to be statistically different than HV sites. This same

295    trend was found within adults as both RF and MD differed from HV sites. The reduced abundance at HV

296    compared to MD was due to the oiling, either by direct mortality or effects on recruitment by the loss of

297    vegetation at the HV sites. Many studies (Kiehn & Morris, 2009; Silliman & Zieman 2001; Silliman &

298    Bertness 2002; Silliman & Newell, 2003; Silliman *et al.*, 2005. Kiehn & Morris (2009) have noted that marsh

299    periwinkle density is positively correlated with *S. alterniflora* stem density. Stagg & Mendelssohn (2012),

300    in a study of marshes restored using sediment from dredging operations, found that *L. irrorata* growth,

301    survival, and productivity were positively correlated to increasing *S. alterniflora* canopy cover.  High levels

302    of *J. roemerianus*, however, may be detrimental to *L. irrorata* density. Alber *et al*. (2008) noted that

303    densities of *L. irrorata* are generally low on *J. roemerianus* in their analysis of the possible reasons of

304    saltmarsh dieback. There may be a limit to the amount of *J. roemerianus* (biomass or stem density) within

305    the marsh that is beneficial compared to the amount that suppresses the *L. irrorata* population density.

306    Above ground biomass and stem density of *J. roemerianus* was equal to or greater at times than *S.*

307    *alterniflora* density at the RF stations (Lin *et al.*, unpublished data), potentially limiting the abundance of

308    *L. irrorata*.  *J. roemerianus* has been shown to be more sensitive to oiling than *S. alterniflora* (Lin *et al*.,

309    unpublished data, Lin & Mendelssohn, 2012), and its density and biomass was expectedly suppressed at

310    the moderately and HV stations (Lin *et al*., unpublished data).

311        Location may have also contributed to lower *L. irrorata* density at the RF sites.  All of the RF sites

312    are located northwest of the moderately and HV stations in a cove that is connected to larger marsh

313    islands. This location could have a larger predator population than the smaller marsh islands where the

314    other stations are located. The location is also further north into the estuary and may be influenced by

315    freshwater, potentially altering the recruitment of *L. irrorata* and/or the population dynamics of *J.*

316    *roemerianus* and *S. alterniflora* (Pennings *et al*., 2005). Alternatively, abundances in the MD sites may

317    have been higher than the RF sites because of an elevated stem density of *S. alterniflora* (Fleeger et al.,

318    2015).  Increased stem density at the MD sites may have been a response by *S. alterniflora* to reduced

319    competition from *J. roemerianus* or a compensatory response associated with oiling.

320         Although the density of L. irrorata was low at RF sites, length-frequency data are similar to those

321    found in other gulf coast areas not impacted by oiling (Pennings *et al.*, in review), suggesting that these

322    data adequately represent a true reference population for comparison with oiled sites.  Average *L. irrorata*

323    shell lengths (Fig. 4) of *L. irrorata* population at the MD and RF stations were equivalent indicating that

324    recovery at MD sites was achieved within the time period of this study.  As an example of recovery of

325    lightly oiled areas, McCall and Pennings (2012) estimated *L. irrorata* density, not shell length frequency,

326    at oiled and control sites in Louisiana and Mississippi four and 16 months (August 2010 and 2011) after

327    the *DWH* spill.  The sampling sites were located 1-2 m behind the HV zone in areas dominated by *Spartina*

328    *alterniflora*, and often partially coated with oil, with a very light (sheen) on the soil surface.  McCall and

329    Pennings (2012) reported no difference in the population density at 4 or 16 months after the spill.

330         A similar comparison of the average shell length (Fig. 4) between the RF and MD stations and the

331    HV stations indicates that body size was much lower at the HV stations and that they did not fully recover

332    within the time period of the study. Shell length also indicates that all of the oiling levels may have initially

333    been affected by oiling within Barataria Bay because the average shell length was initially smaller than

334    that which was eventually achieved at the RF sites (17.71 mm). These data indicate that recovery may

335    have been occurring at the RF and MD sites approximately 36 months after the spill and, at the HV sites,

336    48 months after the spill. Other studies have investigated more heavily-oiled sites associated with the

337    *DWH* spill using population density. Silliman et al. (2012) sampled *L. irrorata* density at three reference

338    and three impacted sites, approximately 3 m from the shoreline, in Barataria Bay approximately 6 months

339    (October 2012) after the *DWH* spill. Their group found no live individuals at impacted stations and

340    approximately 50 m$^{-2}$ at the reference stations.  Note that their reference stations had relatively low

341   densities (slightly greater than the density at our RF stations) and less than the average density of our MD

342   stations.

343       In a study of shoreline treatment options on a marsh island in northern Barataria Bay,

344   approximately 1 km south of our study sites, Zengel *et al*. (2015) monitored *L. irrorata* population densities

345   and shell size frequencies in relation to shoreline treatment options (manual, mechanical, and no

346   treatment; see Zengel and Michel (2013) 17 and 29 months (September 2011 and 2012) after the *DWH*

347   spill. This study provides data at reference and heavily-oiled stations between the time period of the

348   Silliman *et al.* (2012) and our study.  They found minimal recovery of *L. irrorata* at treatment sites. An

349   interesting result was that the reference station had less than 50 m$^{-2}$ *L. irrorata* in 2011 (17 months after

350   the spill) and greater than 150 m$^{-2}$ in 2012 (29 months after the spill). Zengel *et al*. (2015) mentions two

351   potential reasons for the differences in densities between the sampling periods; one, under-sampling the

352   juvenile *L. irrorata* in the first sampling period due to not searching leaf bracts for juveniles and, two, the

353   possible effect of widespread oiling in the water surface within Barataria Bay during the summer of 2010

354   and the effect that may have had on the larvae of *L. irrorata*.  We find the juvenile *L. irrorata* very patchy

355   in their distribution on the marsh platform because of the juveniles' preference, described in Zengel et al.

356   (2015) to aggregate and hide in areas on *S. alterniflora,* such as within leaf bracts.  We also find the

357   juveniles sporadic between years potentially because of their broadcast life cycle and we do mention

358   below that both *L. irrorata* and *Uca* were affected in their ability to carry out their broadcast life cycle in

359   2010 throughout northern Barataria Bay because of oil on the water.  This caused a potential recovery

360   effect at all of our stations including the RF stations.

361       The correlation of *L. irrorata* abundance with vegetation parameters is not unexpected. Kiehn &

362   Morris (2009) and Stagg & Mendelssohn (2012) found similar positive correlations between *S. alterniflora*

363   health (stem density, canopy cover) and *L. irrorata* health (density, growth, survival, productivity).

364    Additionally, Fleeger et al. (2015) found that the infauna at our study sites followed the recovery of *S.*

365    *alterniflora*. Of particular interest is the correlation of *L. irrorata* with *J. roemerianus* parameters (above

366    ground biomass, stem density, and total live and dead above ground biomass).  This may indicate the

367    importance of *J. roemerianus* to the recovery of *L. irrorata* within the marsh.  Hughes (2012) describes the

368    potential importance of *J. roemerianus* as alternative refuge for *L. irrorata*; as described above, however,

369    Alber *et al.* (2008) note that the densities of *L. irrorata* on *J. roemerianus* is generally low.  We, therefore,

370    may be underestimating the importance of *J. roemerianus* within a diverse marsh system.  The correlation

371    of *L. irrorata* and *J. roemerianus* at 40 months (above ground biomass and stem density) and 48 months

372    (stem density) at the MD stations, however, may indicate the coincidental recovery of the two species

373    with the reduction of oil at those stations (Figure 9).

374

## *Uca*

376        This study started 30 months after the *DWH* oil spill, and our data clearly show that that any effect

377    of the spill on fiddler crab, *Uca*, density passed prior to the beginning of this effort.  We found that average

378    density of crab burrows within the area of our sampling (approximately 1 m from the shoreline edge) was

379    low, between 5 and 10 burrows m$^{-2}$. This is not surprising, as Mouton & Felder (1996) noted the variation

380    in burrow density of *U. longisignalis* along a 15-m transect from the edge into the marsh.  The burrow

381    density was lowest near the marsh edge and highest in the middle and upper reaches of the transect with

382    no marked change in elevation noted beyond 3 m from the water's edge.  Several studies have found

383    similar results.  Silliman *et al*. (2012) sampled *Uca* sp. crab burrow density at the three reference and three

384    impacted sites described above and found no difference between the density of crab burrows

385    (approximately 10 m$^{-2}$) at the two site types.  McCall & Pennings (2012) sampled *Uca* burrows at the

386    sampling sites described above.  Because they were sampling further back in the marsh, McCall &

387    Pennings (2012) reported greater numbers of burrows (approximately 20 m$^{-2}$, see Mouton & Felder 1996

388    above) and found a significantly lower density of burrows at the oiled sites 4 months after the spill and no

389    difference at 16 months after the spill.

390          Zengel *et al*. (2015) monitored *Uca* burrow densities in relation to shoreline treatment options

391    after the *DWH* spill (see above). Uca burrow densities in heavily-oiled and treated plots were similar to

392    reference at both 17 and 29 months; however, the heavily-oiled sites that were not treated were

393    significantly different at 17 months, but similar at 29 months.  Zengel *et al*. (2014) revisited the treatment

394    sites at 41 months after the spill (September 2013) and found significant differences between the oiled

395    control sites (no treatment) relative to the references sites. Maximum average burrow density was

396    approximately 10-15 burrows m$^{-2}$ similar to densities in our study. We also identified only *Uca longisignalis*

397    within our study sites.  The study at the treatment sites (Zengel *et al.*, 2014; Zengel *et al.*, 2015) identified

398    *U. spinicarpa* occurring at the heavily-oiled plots where there was reduced vegetation coupled with areas

399    of surface oil residue overlaid with thin algal mats and clay-like sediments.

400          Morris et al. (2015) conducted studies on adult male and female *Uca longisignalis* placing the

401    individuals in Total Polycyclic Aromatic Hydrocarbon (TPAH)50 concentrations from 0.07 (reference

402    sediment) to 26 mg/kg in the upper 2 cm of sediment. During the 10 day exposure period several females

403    became gravid. The gravid females were removed after 10 days and placed in clean water until the

404    embryos hatched. The zoea were collected, held in clean water, and exposed to varying levels of ambient

405    sunlight. They found substantial toxicity (calculated LC20 value of 0.62 mg/kg TPAH50 in the upper 2 cm

406    of sediment) to *Uca* zoea at relatively low concentrations of oil in the sediments.  Fig. 9 provides the total

407    petroleum hydrocarbon concentrations from 30 to 54 months after the spill at the 21 stations from this

408    study. It is difficult to compare the two hydrocarbon parameters and much of the aromatic portions of

409    the oil have weathered reducing the toxicity; however, the levels were found to be significant higher at

410    the HV sites compared to the RF and MD sites (Lin *et al.* unpublished data).

411    The negative correlations between *Uca* sp. burrow density and *S. alterniflora* stem density (all

412    oiling levels /all stations) and *L. irrorata* (MD stations 42 months after the spill) may be indicative of the

413    conflict between the burrowing and feeding activity of the fiddler crab in and on the marsh and the density

414    of *S. alterniflora*.  Mouton & Felder (1996) indicate that *U. longisignalis* burrow density increased

415    landward of the 4-m into the marsh where the vegetation became more open and scattered.  They report

416    the maximum density of burrows near the 8-m to 12-m marks into the marsh suggesting a potential

417    relationship between tidal flooding and the deposition of wrack contributing to food resources.

418    The extent of shoreline oiling within northern Barataria Bay (Zengel & Michel 2013) and the extent

419    and duration of oil in and on the water indicated in the Environmental Response Management Application

420    (ERMA) (http://gomex.erma.noaa.gov/erma) would indicate that the populations of *L. irrorata* and *Uca*

421    were affected in their ability to carry out their life cycle on the marsh (Morris *et al.* 2015) and in the water

422    column at all stations including the reference stations for some period following the entry of oil into the

423    region (Fodrie *et al.*, 2014; Pennings et al., unpublished data).  This study found that the dominant fiddler

424    crab within these marshes, *U. longisignalis*, recovered at all oil levels prior to sampling at 30 months after

425    the *DWH* spill.  There are indications that *L. irrorata* started recovery at the MD stations at 40 months

426    after the spill and population density is beginning to demonstrate seasonal cycling.  Recovery, however,

427    has not occurred at the HV stations.

428

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577

578     Fig. 1. Stations locations within Barataria Bay, LA.

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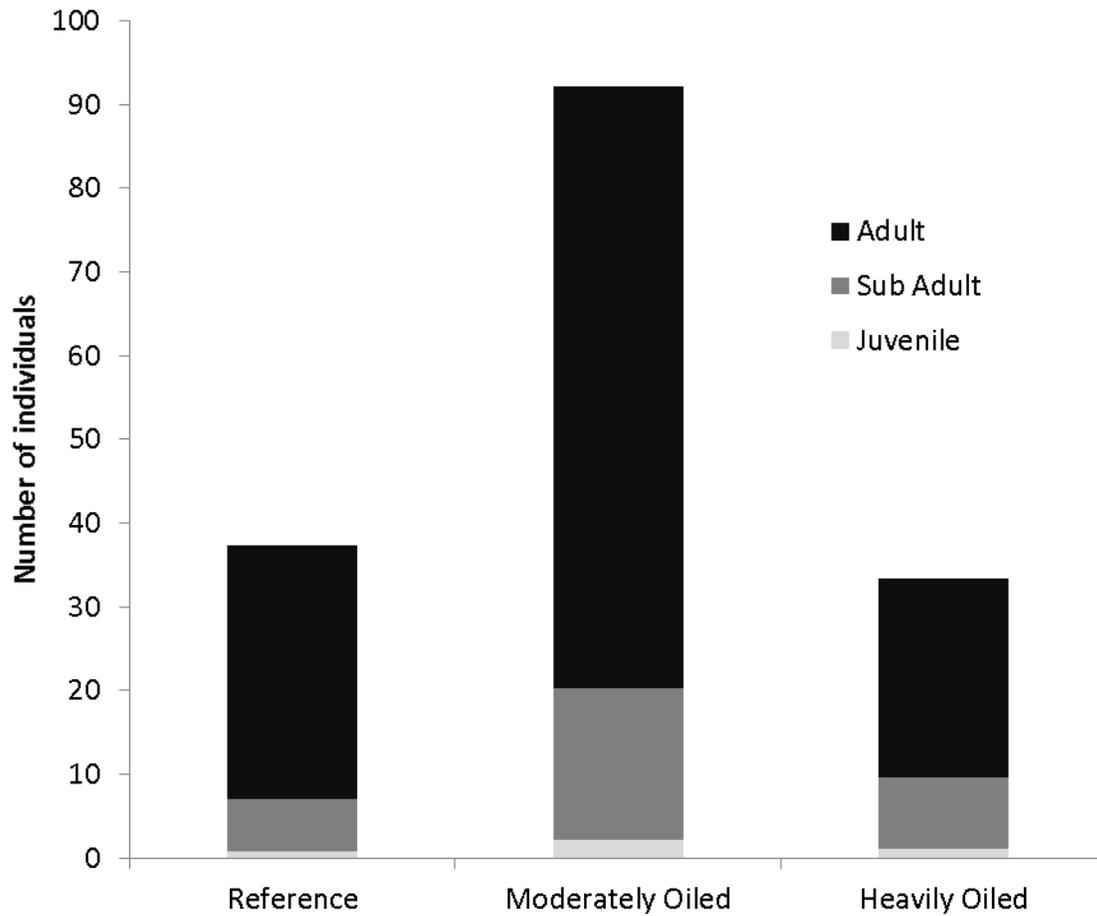
580

581   Fig. 2. Mean Littoraria abundance between oiling levels across all sampling times and within various size

582   classes. X-axis = site type, Y-axis = average density/m². Black = adults, grey = sub-adults, and light grey =

583   juveniles.

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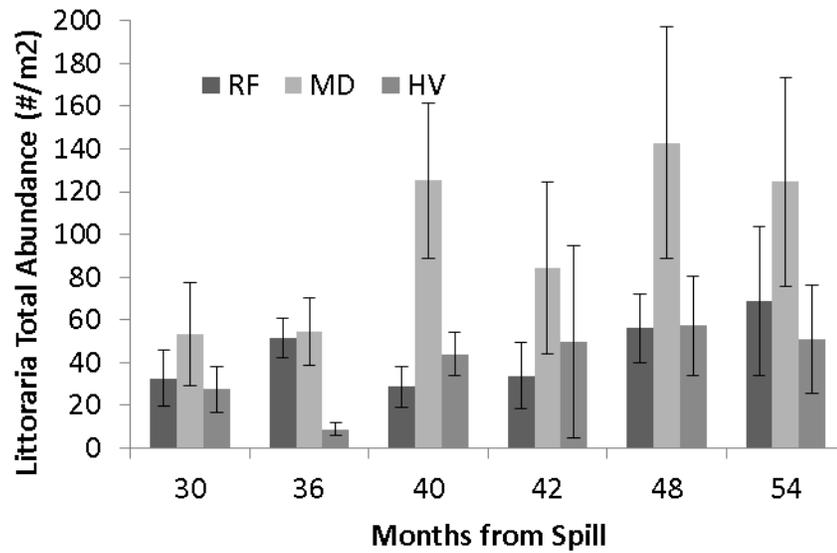
586

587    Fig. 3. Littoraria irrorata total density m$^{-2}$ 30 to 54 months after the *DWH* oil spill. Values are means (±SE;
588    n=7) for reference (RF), moderately-oiled (MD), and heavily-oiled (HV) shoreline marshes of northern
589    Barataria Bay, LA, USA.

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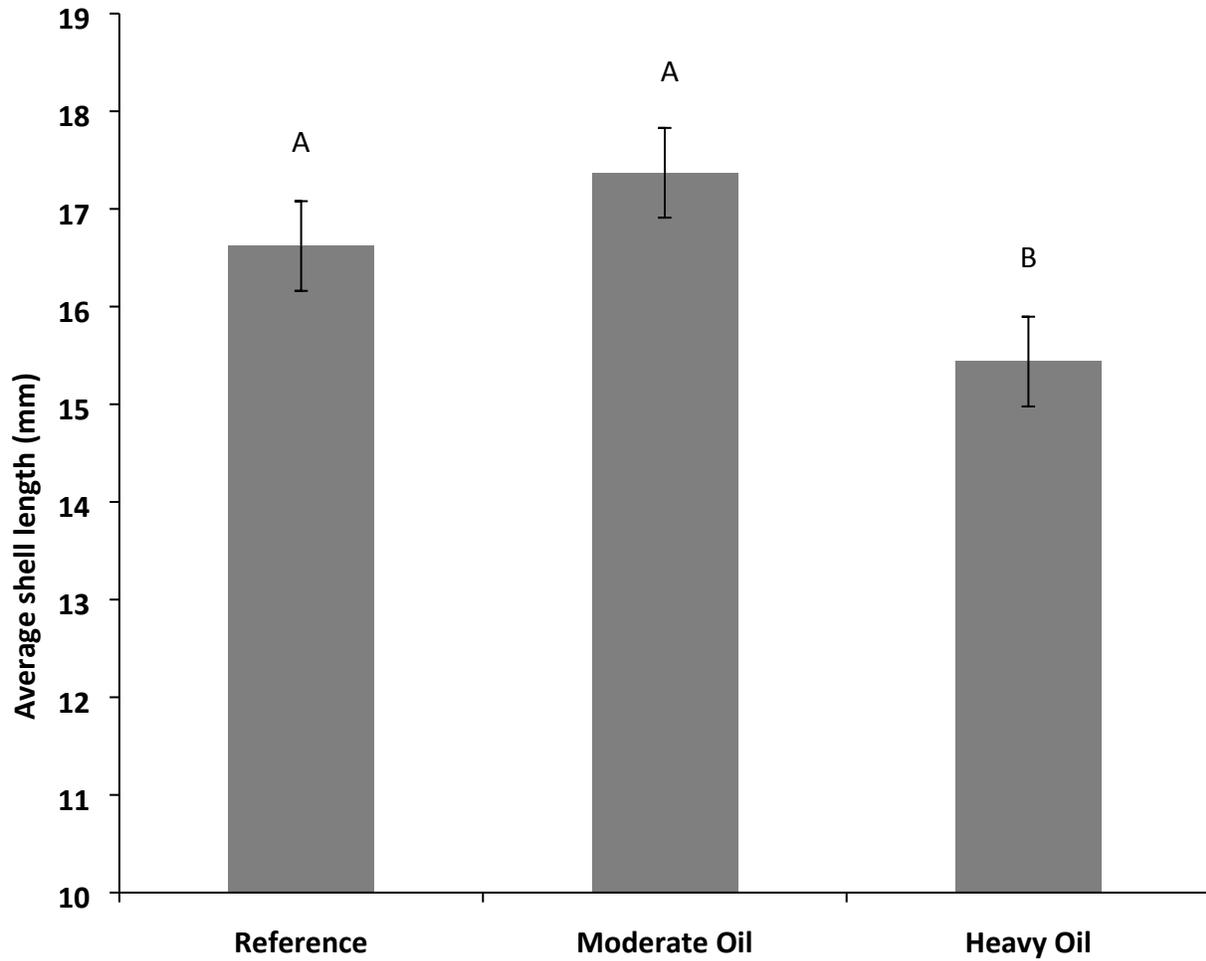
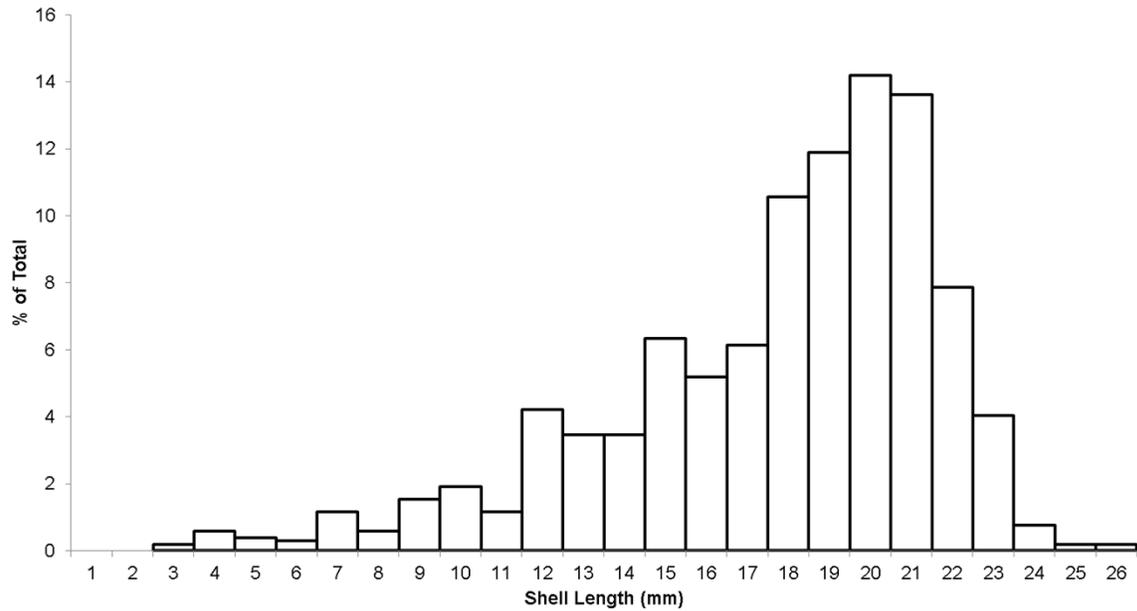


Fig. 4. Average Littoraria shell length by sampling site type across all sampling times. X-axis = site type, Y-
axis = average shell length (mm). Heavily-oiled (HV) sites had significantly smaller Littoraria than
reference (RF) or moderately-oiled (MD) sites.

595

596   Fig. 5. Reference (RF) sites Littoraria size distribution by percentage of total abundance. X-axis = shell
597   length in mm, Y-axis = percentage of total abundance represented by each specific shell length across all
598   sampling times.

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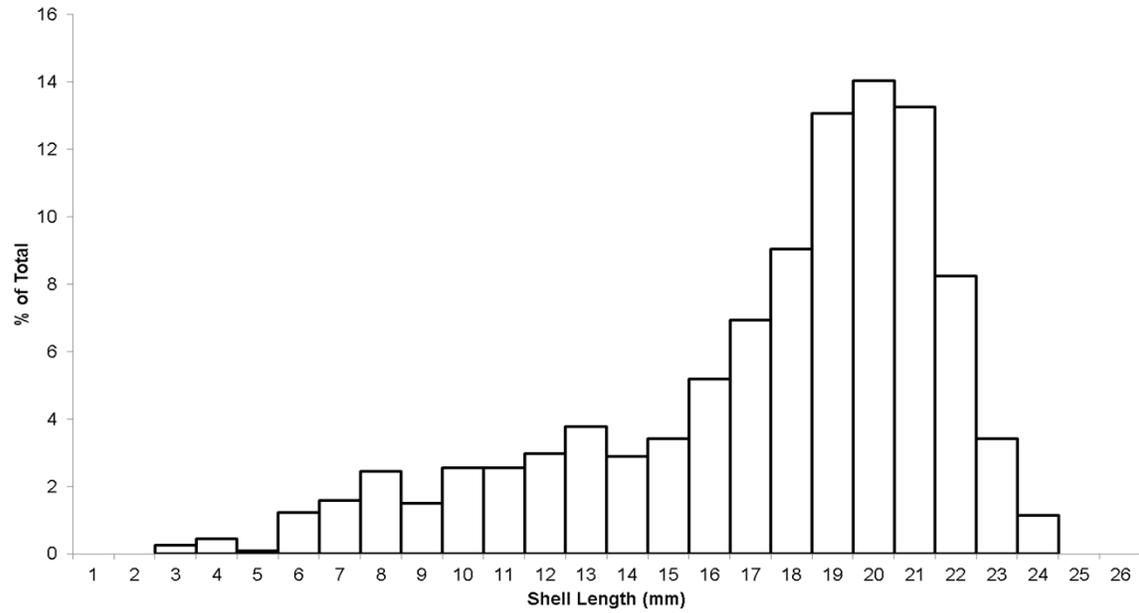
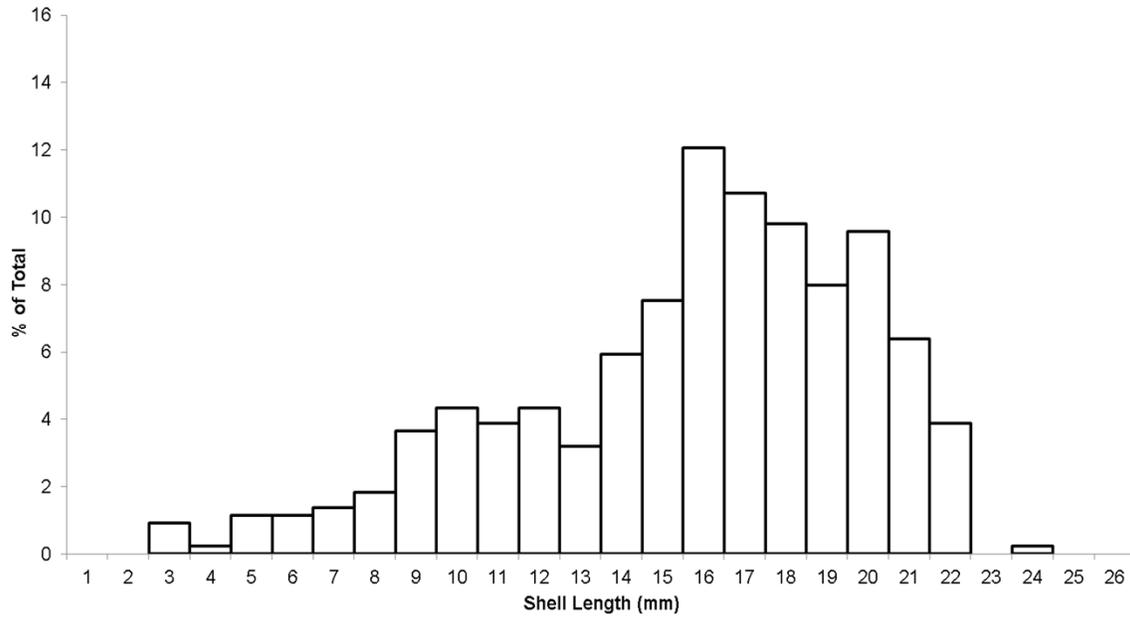
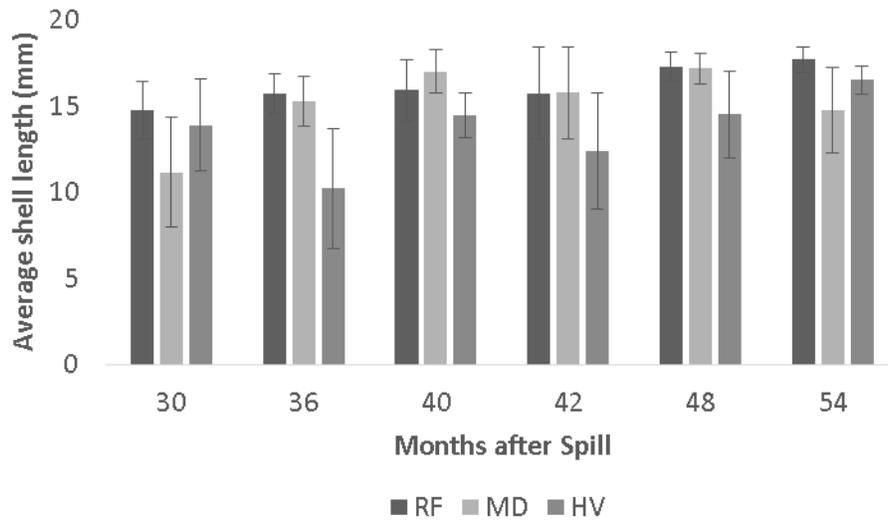
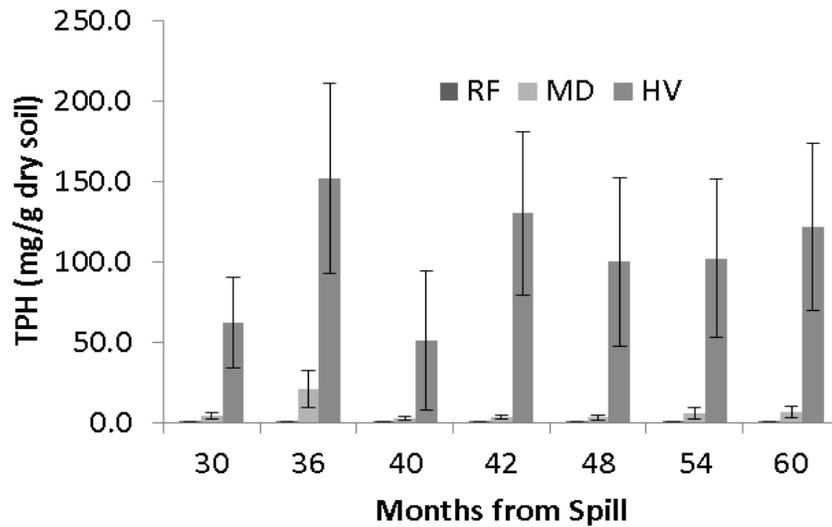

600

601   Fig. 6. Moderately-oiled (MD) sites Littoraria size distribution by percentage of total abundance. X-axis =
602   shell length in mm, Y-axis = percentage of total abundance represented by each specific shell length
603   across all sampling times.

604

605

606 Fig. 7. Heavily-oiled (HV) sites Littoraria size distribution as percentage of total abundance. X-axis = shell
607 length in mm, Y-axis = percentage of total abundance represented by each specific shell length across all
608 sampling times.

609

610

611     Figure 8. Average shell length (mm) for each oiling level within each sampling period after the spill.

612

613    Fig. 9. Surface soil (0-2 cm) total petroleum hydrocarbon concentrations 30 to 54 months after the *DWH*

614    oil spill. Values are means (±SE; n=7) for reference (RF), moderately-oiled (MD), and heavily-oiled (HV)

615    shoreline marshes of northern Barataria Bay, LA, USA (from Lin *et al.* unpublished data).

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