

1           **Macrobenthic community structure in the deep Gulf of Mexico one year after the**  
2                                       **Deepwater Horizon blowout**

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17   **Abstract**

18   The impacts of the 2010 Deepwater Horizon (DWH) disaster on deep-sea Gulf of Mexico  
19   benthic communities were analyzed one year after the blowout. Richness, diversity, and  
20   evenness were severely impaired within a radius of approximately 1 km around the DWH  
21   wellhead. However, lower diversity than background was observed in several stations up to 29  
22   km to the southwest of the wellhead. Compared to samples from 2010, abundance near the  
23   DWH wellhead increased in 2011 with some of the highest values found at stations within the 1

24 km radius. The increase was mostly caused by the high abundance of opportunistic polychaetes  
25 of the family Dorvilleidae, genus *Ophryotrocha*. At contaminated stations near the DWH  
26 wellhead, diversity did not change with increased sampling area, whereas a steep increase of  
27 diversity with increasing sampling area was observed at stations farther from the wellhead. The  
28 spatial extent of DWH impacts appeared to decrease from 2010 to 2011. Impacts on diversity  
29 near the wellhead were still observed; however, the large increase in abundance may indicate the  
30 initial stages of recovery, year-to-year variability, or an early stage of succession following a  
31 disturbance; and this can be resolved only with additional temporal sampling.

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33 **Keywords:** Benthos; Deep Sea; Diversity; Macrofauna; Oil Spill; PAH; Recovery; Succession;  
34 Temporal Variability

## 35 **1 Introduction**

36           The Deepwater Horizon (DWH) blowout on 20 April 2010 was an unprecedented  
37 accidental hydrocarbon release into the ocean, both in the quantity of oil released (3.19 million  
38 barrels, according to the United States District Court for the Eastern District of Louisiana (2015),  
39 but other credible estimates are as high as 6.2 million barrels (Griffith, 2012)) as well as the  
40 depth at which the blowout occurred (1525 m) (Griffiths, 2012). The release of large amounts of  
41 oil under great pressure caused the formation of a subsurface oil plume at around 1200 m and  
42 subsequent transportation of oil for several km (Peterson et al., 2012; Ryerson et al., 2012).  
43 Roughly 2.1 million gallons of dispersants were applied during the blowout and much of this was  
44 incorporated into the deep sea hydrocarbon plumes (Kujawinski et al., 2011).

45           Hydrocarbons in surface waters and the deep-sea plumes reached the seafloor by  
46 adsorption onto particles or incorporation in fecal pellets, which then rapidly sank to the bottom  
47 as marine snow (Passow, 2014). A large Marine Oil Snow Sedimentation and Flocculent  
48 Accumulation (MOSSFA) event occurred during the blowout due to phytoplankton and  
49 zooplankton production as well as microbial mucus formation, which was enhanced by the  
50 elevated discharge of the Mississippi River at the time (Daly et al., 2016). A microbial bloom  
51 occurred during the blowout, which may have increased extracellular polymeric substances  
52 created by microbes and exposed to dispersants, and therefore also increased oil deposition from  
53 flocculation (Fu et al., 2014; Valentine et al., 2014). Advective transport of the deep-sea plume  
54 and direct contact of the plume along the continental slope at depths between 1000 – 2000 m  
55 were also likely responsible for hydrocarbon deposition (Romero et al., 2015). Other possible  
56 mechanisms for deep-sea deposition include onshore-offshore transport, or sinking of heavy  
57 byproducts after burning (UAC, 2010).

58           The spatial extent of the impact on abundance and diversity of benthic meiofauna and  
59 macrofauna were conservatively estimated to be about 172 km<sup>2</sup> around the DWH wellhead  
60 during the 2010 sampling campaign (Montagna et al., 2013). Near the DWH wellhead  
61 meiofauna increased in abundance and had the highest nematode to copepod ratios. Meiofauna  
62 diversity and evenness increased with distance from the wellhead (Baguley et al., 2015).  
63 Abundance of macrofauna decreased near the DWH wellhead and attained the highest values at  
64 intermediate distances, while diversity and evenness increased with distance from the wellhead  
65 (Washburn et al., 2016).

66           Montagna et al. (2017) found no significant differences in contaminant concentrations  
67 (i.e., total petroleum hydrocarbons, total polycyclic aromatic hydrocarbons, and barium) between  
68 2010 and 2011. These trends indicate persistent contamination one year following the blowout,  
69 while persistent effects on the macrofaunal communities are evident by the approximately 23%  
70 and 36% reduction in taxa richness and diversity (Hill's N1) at impact stations compared to  
71 background stations (Montagna et al., 2017). While many of the observed impacts on benthic  
72 communities in 2011 were likely due to a lack of recovery from the 2010 spill, Kolian et al.  
73 (2015) found evidence of fresh oil from the DWH well as late as May 2012. This suggests a  
74 continued leakage of hydrocarbons into the deep-sea at least 20 months after the wellhead was  
75 capped and is further justification for the need to continue monitoring communities around the  
76 DWH wellhead.

77           Deep-sea benthic infauna are integral to decomposition, sediment bioturbation, nutrient  
78 regeneration, trophic transfer of pollutants and nutrients from the sediment to the water column,  
79 secondary production, and other ecosystem functions (Danovaro et al., 2003; Armstrong et al.,

80 2010). Changes in deep-sea benthic communities in contaminated areas likely resulted in  
81 alterations to these ecosystem functions.

82 In this paper we assessed the state of the deep-sea benthic ecosystem by examining the  
83 macrofauna communities of May and June 2011, approximately 14 months after the DWH  
84 blowout and 11 months after the wellhead was capped. Most of the 38 stations had also been  
85 sampled for macrofauna in September and October 2010, allowing for a comparison of the  
86 temporal dynamics and a potential recovery process of the communities. A study using  
87 biological, chemical, and environmental data from 32 of the 38 stations sampled in 2011 was  
88 performed by Montagna et al. (2017). While the study by Montagna et al. (2017) focused on  
89 longer-term (1 year later) impacts of the spill on the deep-sea Gulf of Mexico, the current study  
90 is a more detailed examination of benthic community structure. Macrofaunal community data  
91 were used to explore changes in taxonomic composition due to the spill and progression of time,  
92 changes in vertical zonation, sample size, and the relationships of certain taxa to contaminants.

## 93 **2. Methods**

### 94 2.1 Sample Collection and Processing

95 A total of 38 stations were sampled aboard the M/V Sarah Bordelon between 25 May and  
96 11 June 2011 (Figure 1, Table 1). At 34 of these stations macrobenthic communities were also  
97 sampled in September and October 2010 and analyzed by Washburn et al. (2016). Stations were  
98 located at depths ranging from 997 to 2389 m and at distances from 0.33 to 128 km from the  
99 DWH wellhead (Table 1).

100 Sediment samples were collected with an OSIL multicorer, which deployed twelve  
101 sediment cores simultaneously. Cores were 10 cm inner diameter and 60 cm long. Three cores

102 were used for benthic macrofauna analyses. The remaining cores were reserved for analyses of  
103 meiofauna, polycyclic aromatic hydrocarbons (PAH), heavy metals, sediment grain size, and  
104 sediment carbon and nitrogen content. Methods for chemistry and sediment analyses were  
105 described by Montagna et al. (2013). At each station, the multicorer was deployed three times,  
106 which are replicates, yielding a total of nine cores per station for macrofauna analyses.  
107 Macrofauna cores were extruded in three vertical sections (0-3, 3-5, and 5-10 cm) immediately  
108 following collection and preserved in 4% buffered formalin with Rose Bengal. At the  
109 laboratory, core sections were sieved on 0.3 mm mesh screens, and transferred to 70% ethanol.  
110 Polychaetes, crustaceans, and mollusks were identified to family; echinoderms and oligochaetes  
111 to class; and other taxa to phylum.

## 112 2.2 Univariate analyses of benthic macrofauna communities

113 Macrofaunal abundance at the sampling stations was determined by averaging  
114 abundances of all nine cores and extrapolating the counts to an area of 1 m<sup>2</sup> by multiplying the  
115 abundance by 125.5. Richness (S) was calculated by averaging the number of taxa per core.  
116 Macrofauna diversity and evenness were calculated using Hill's diversity number one (N1) (Hill,  
117 1973) and Pielou's evenness index (J') (Pielou, 1966), respectively. Hill's N1 is used because it  
118 is a measure of the number of dominant species. The diversity calculations were performed in  
119 two different ways. First, N1 was calculated for each of the nine cores per station individually  
120 and the values were averaged (core level, N1/78.2 cm<sup>2</sup>). Second, the macrofauna counts of all  
121 nine cores were summed (or pooled) together, and a single N1 value per station was calculated  
122 (station level, N1/704 cm<sup>2</sup>). The first method allows for comparison with earlier studies, and the  
123 second method was performed to determine if diversity increased with area sampled.

124 Stations were grouped into five diversity levels, based on gaps in the range of N1 values,  
125 and plotted in an ArcGIS map (Figure 1). N1 values below 8.0 were defined as very low  
126 diversity, 8.0-11.0 as low diversity, 11.0-14.0 as moderately diverse, 14.0-19.0 as diverse, and  
127 above 19.0 as very diverse. Stations were assigned a color based on diversity level: dark green  
128 for very high diversity, light green for high diversity, yellow for moderate diversity, orange for  
129 low diversity, and red for very low diversity (Figure 1).

130 To analyze the extent of change in the communities from the previous year, stations were  
131 divided into impact zones (IZ) based on the magnitude of hydrocarbon effects from 2010  
132 samples (Montagna et al., 2013), including: 1 = severely impacted, 2 = moderately impacted, 3 =  
133 uncertain impacts, 4 and 5 = background conditions. None of the stations collected in 2011 were  
134 located in zone 5. Four stations included in the present study (HIPRO, LBNL10, S36, and  
135 VK916) were not available for macrofauna analysis in 2010. These stations were assigned to an  
136 impact zone based on their geographic position. A k-dominance plot (Lambshead et al., 1983)  
137 was prepared to help visualize the diversity of benthic communities within the four impact zones.  
138 A nested analysis of variance (ANOVA) with stations nested within impact zones and Tukey's  
139 pairwise comparison tests among impact zones were performed to test for significant differences  
140 in abundance, richness, diversity, and evenness using PROC GLM in SAS 13.1 (SAS, 2013). A  
141 2-way partially hierarchical ANOVA model was used to test for differences among benthic  
142 community responses with changes in sediment depth (i.e., the 0 - 3, 3 - 5, and 5 - 10 cm vertical  
143 sections) and impact zone, with station nested within impact zone.

### 144 2.3 Multivariate analyses of benthic macrofauna communities

145 Macrofauna community structure of the different sampling stations was examined with  
146 non-metric multidimensional scaling (MDS) in Primer 6. For this analysis, we pooled the  
147 species lists at the station level, i.e., the second way to calculate diversity, as specified above.  
148 We then performed a logarithmic transformation of the count data and calculated a Bray-Curtis  
149 similarity matrix among samples. This matrix was plotted in an ordination plot, using MDS.

150 A two-way nested ANOSIM analysis was performed in Primer 6. The top-level factor  
151 that we tested for significant differences was impact zone. Individual stations represented the  
152 second factor. A one-way SIMPER analysis was performed in Primer 6 to investigate which of  
153 the taxa contributed to differences or similarities among the different impact zones.

### 154 2.4 Links between environmental variables and macrofauna communities

155 The gradient of environmental and chemical variables with distance from the DWH  
156 wellhead was explored. Cores from each drop were assigned for hydrocarbons (oxidation  
157 reduction potential, Dioctyl sodium sulfosuccinate, total extractable hydrocarbons (C9-C44), and  
158 polycyclic aromatic hydrocarbons (sum 44 toxic PAHs), heavy metals (Aluminum, Antimony,  
159 Arsenic, Barium, Beryllium, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead,  
160 Magnesium, Manganese, Mercury, Nickel, Potassium, Selenium, Silver, Sodium, Strontium,  
161 Thallium, Vanadium, and Zinc), pore-water chemistry (pH, oxidation reduction potential,  
162 sulfides, and ammonia), and other sediment properties (total carbon, total organic carbon, total  
163 nitrogen, and grain size). Data were downloaded from  
164 <https://www.diver.orr.noaa.gov/deepwater-horizon-nrda-data/> on 9 September 2016. Methods  
165 for the chemical analyses are described in the report found at



166 <http://www.nodc.noaa.gov/deepwaterhorizon/ship.html>. Pore-water was collected from the top 0  
167 – 5 cm of sediment. All other chemical and sediment measurements were made on vertical  
168 sections from the 0 - 1, 1 – 3, and 3- 5 cm of sediment. These vertical sediment fractions were  
169 pooled by sediment volume to compare the porewater, chemistry, sediment, and macrofauna to a  
170 sediment depth of 5 cm. The macrofauna were summed to a depth of 5 cm for comparison with  
171 sediment chemistry.

172 A principal components analysis was performed on the sediment chemical variables to  
173 explore gradients in these variables associated with distance from the DWH wellhead. A  
174 stepwise search for the best combination of environmental variables to explain the macrofauna  
175 distribution was performed in Primer 6 (BEST: BVStep). Regression analyses between PAH44  
176 concentrations and abundances of the common polychaete, crustacean, and bivalve families were  
177 performed, to find suitable taxa as indicators for hydrocarbon contamination.

### 178 **3. Results**

#### 179 3.1 Univariate analyses of macrobenthic communities

180 A total of 31,702 animals were collected and identified into 159 different taxa. The most  
181 abundant 20 taxa comprised 85% of the total abundance (Table 2). Annelida, Mollusca,  
182 Nemertea, and Crustacea were the dominant phyla. Organisms belonging to the polychaete  
183 families Maldanidae, Paraonidae, Capitellidae, Spionidae, Cirratulidae, Acrocirridae, and  
184 Syllidae, as well as the phylum Nemertea were found in at least one core at every station (Table  
185 2). Polychaetes accounted for 79% of all organisms found, but only for 27% of the taxa.  
186 Fourteen of the 20 most abundant taxa were polychaete families, and the 5 most abundant taxa,  
187 all polychaetes, accounted for over 50% of the total abundance. Crustaceans accounted for only

188 5% of all organisms but made up 41% of the taxa. Mollusks accounted for 8% of all organisms  
189 and made up 20% of the taxa. Echinoderms accounted for 3% of all organisms and made up 4%  
190 of the taxa while nemerteans also accounted for 3% of all organisms. The remaining taxa were  
191 comprised of 9 different phyla, which were not identified below phylum or class.

192 The highest abundance of polychaetes was found in impact zone 1, while abundances of  
193 crustaceans and mollusks were lowest in impact zone 1 and highest in the undisturbed impact  
194 zone 4. This corresponds to the dominance of polychaetes in impact zone 1, comprising 89% of  
195 the organisms. This dominance decreased to 69% in impact zone 2, and 39% and 57% in zones  
196 3 and 4, respectively. Molluscs comprised 7-8% of the total counts in impact zones 2-4,  
197 compared to 4% in zone 1. Crustaceans comprised 1% of the total in impact zone 1, 4 % in  
198 zones 2 and 3, and 10% in zone 4.

199 The average abundance of macrobenthic organisms over all sampling stations was 11,632  
200 n/m<sup>2</sup>. The lowest value (2,942 n/m<sup>2</sup>) was found at the deepest station D002S, the largest number  
201 (22,046 n/m<sup>2</sup>) at station D031S, close to the wellhead (Table 1). The high abundances found in  
202 some of the stations within a radius of approximately one km of the wellhead were largely  
203 caused by the high counts of polychaetes from the family Dorvilleidae. These polychaetes  
204 belong to an undescribed species of the genus *Ophryotrocha*. At station D031S, Dorvilleidae  
205 reached abundances of 17,110 n/m<sup>2</sup>, accounting for about 78% of the total macrofauna count.  
206 Macrofaunal abundances were significantly different among impact zones. Abundance was  
207 highest in impact zone 1 (13,818 n/m<sup>2</sup>), the area closest to the DWH wellhead and lowest in zone  
208 4 (9,949 n/m<sup>2</sup>), but abundance was not statistically significantly different among impact zones ( $P$   
209 = 0.07) (Table 3).

210           The station with the highest taxa richness, FFMT4, was the station farthest from the  
211 wellhead. The station with the lowest taxa richness, D038SW, was the station closest to the  
212 wellhead (Table 1). The nine stations within a one km radius of the wellhead had lower taxa  
213 counts than any other sampling locations, ranging from 21 to 44. The lowest number of taxa (47)  
214 outside this zone was found at the deepest station D002S, which also had the lowest abundance  
215 of macrofauna.

216           Diversity values of the 38 stations tended to increase with the size of the area sampled,  
217 but the effect of the sampling area did not affect all stations equally (Table 1). N1 values  
218 generally increased from core (average of nine cores) to station (sum of nine cores) areas.  
219 D002S, the deepest station by more than 500 m, had the highest increase with sampling area.  
220 Exceptions to this rule were the five least diverse stations near the DWH wellhead, whose N1  
221 values slightly decreased with increasing sampling area.

222           All five stations with very low diversity ( $N1=2.9 - 5.6$ ) were located within a radius of  
223 approximately one km of the wellhead (Figure 1, Table 1). Three of the stations with low  
224 diversity ( $8.3 - 10.6$ ) were also within 1 km of the wellhead. Among the eleven sampling  
225 stations within approximately 3-5 km from the wellhead, two to the southwest had moderate  
226 diversity levels ( $N1 = 12.2 - 13.2$ ), whereas the others had high diversity levels ( $N1 = 14.1 -$   
227  $19.5$ ). Stations that were located farther from the wellhead had mostly high diversity levels ( $N1$   
228  $= 14.2 - 22.9$ ). The exceptions were HIPRO with moderate diversity ( $N1 = 12.6$ ) located 29 km  
229 to the southwest of the wellhead, VK916 with low diversity ( $N1 = 10.3$ ), located 62 km northeast  
230 of the wellhead, and D002S with low diversity ( $N1 = 10.3$ ), located 63 km southeast of the  
231 wellhead.

232 In the k-dominance plot (Figure 3), the taxa accumulation curve of impact zone 1 started  
233 at about 51%, illustrating the strong dominance of the polychaete family Dorvilleidae. The curve  
234 is consistently and distinctively above the three other curves, with impact zone 2 above 3, and 3  
235 above 4. Overall, the trend indicates that impact zone 4 has the most evenly distributed benthic  
236 communities, closely followed by impact zones 3 and 2. The differences between impact zones  
237 2, 3, and 4 are small relative to impact zone 1.

238 Taxa richness, diversity, and evenness were significantly different among the four impact  
239 zones (Table 3). All three variables were significantly lower in impact zone 1 than in the other  
240 three zones. Evenness was at least 25% lower; richness at least 35% lower; and diversity at least  
241 55% lower in impact zone 1, when compared to the other zones. Impact zone 4 had the highest  
242 values for richness and diversity, but was not significantly different from zone 3 for any of the  
243 variables tested (Table 3).

244 The 2-way partially hierarchical ANOVA examining impact zone and vertical sediment  
245 sections found significant differences ( $\alpha < 0.05$ ) among zones for taxa richness, diversity, and  
246 evenness, but not abundance. There were significant differences among vertical sediment  
247 sections for all variables, and all interaction terms were significant (Table 4). The highest values  
248 for all variables were found in the surface 0 - 3 cm sections while the lowest values were always  
249 found in the deepest 5 - 10 cm sections. Abundances were eight times greater in the surface  
250 sediment compared to the 3-5 cm sections when all samples were examined together. Taxa  
251 richness was more than four times greater in surface sediments and diversity more than three  
252 times greater. Only evenness was not significantly different between surface and deeper  
253 sediments (Table 4). While total abundances were highest in cores from impact zone 1,  
254 abundances below the surface 0-3 cm of sediment were lowest in zone 1. Significant interactions

255 were caused by different patterns in vertical sediment distribution among zones. A larger  
256 proportion of total abundance is confined to 0 – 3 cm in zone 1 compared to other zones while  
257 richness, diversity and evenness are lower in surface sediments in zone 1 compared to other  
258 zones (Figure 2). Diversity, evenness, and richness were all significantly lower in the surface 0-  
259 3 cm and intermediate 3-5 cm of sediment of impact zone 1 compared to all other zones, which  
260 were not different.

### 261 3.2 Multivariate analyses of macrobenthic communities

262 In the MDS plot of our multivariate community analysis, a trend was observed where  
263 stations with the lowest diversity levels and the highest PAH44 values were placed on the left  
264 hand side. Towards the right side of the plot diversity was steadily increasing and hydrocarbon  
265 contamination steadily decreasing (Figure 4). The MDS plot visualizes the strong influence of  
266 impact zones on benthic community diversity.

267 The benthic assemblages in impact zones 1 and 2 were significantly different from each  
268 other and from impact zones 3 and 4 (ANOSIM, p-values between 0.0001 and 0.002). The  
269 macrobenthic communities of impact zones 3 and 4 were not significantly different from each  
270 other ( $p = 0.716$ ). The polychaete family Dorvilleidae contributed most to the distinctness of  
271 impact zone 1 (SIMPER analysis). The macrofauna communities of the other impact zones were  
272 mainly characterized by the polychaete families Maldanidae, Paraonidae, Capitellidae, and  
273 Spionidae.

### 274 3.3 Environmental variables and macrofauna

275 The first three principal components (PC1 – PC3) explained 56.6 % of the variability in  
276 the data set. PC1 explained 24.8%, PC2 18.6 %, and PC3 13.2 %. PC1 was heavily loaded for

277 the metals potassium, aluminum, beryllium, magnesium, vanadium, chromium, and cobalt. The  
278 only physical feature that loaded highly on PC1 was porewater content (Figure 5a). The metal  
279 barium and ammonium concentrations were negatively loaded on PC1. PC2 was heavily loaded  
280 for calcium, total carbon, and strontium content, and inversely loaded for arsenic content. PC1  
281 and PC2 appear to be driven by differences in the natural background. PC3 was positively  
282 loaded for polycyclic aromatic hydrocarbons, total hydrocarbons, barium, and antimony, and to a  
283 lesser degree to beryllium, total organic carbon, chromium, zinc, and dispersant (Figure 5b).  
284 Thus, PC3 represents contamination from the oil spill.

285         Neither the first nor second principal component (PC1 and PC2) for sediment chemistry  
286 correlated with distance from the DWH wellhead, the nearest hydrocarbon seep, or water depth,  
287 nor any benthic community metric (Table 5). The third component (PC3), however strongly  
288 correlated with distance from the wellhead, whereas there was no correlation between seep  
289 distance and depth. Thus, PC3 does represent chemical contaminants from the wellhead, and not  
290 natural seepage nor differences in depth zonation because as distance from the wellhead  
291 increases, the contaminants decrease. Whereas macrofauna abundance increases with PC3, all  
292 the diversity metrics decrease.

293         When comparing environmental variables to macrofaunal patterns in Primer, variables  
294 were log transformed because of their right-skewness and then normalized. Calcium was  
295 excluded from the analysis because it was strongly correlated with strontium ( $\rho > 0.95$ ). Of the  
296 remaining variables, the combination that explained the macrofauna distribution best included  
297 the concentrations of PAH44, barium, lead, manganese, sodium, strontium, and thallium. The  
298 correlation between the environmental variables and the macrofauna distribution was  $\rho = 0.450$   
299 (BEST analysis). The best indicator was the concentration of PAH44. Abundances of several

300 taxa were correlated with PAH concentrations in the regression analyses. The most distinct  
301 positive and negative correlations were found with dorvilleid polychaetes ( $\rho = 0.69$ ) and  
302 peracarid crustaceans ( $\rho = -0.31$ ), respectively (Figure 6).

#### 303 **4. Discussion**

##### 304 4.1 Macrobenthic communities one year after the blowout

305 The benthic macrofauna communities within one km of the DWH wellhead were  
306 impoverished, in terms of species richness, diversity, and evenness. Moderate diversity levels  
307 outside the one km radius were detectable southwest of the wellhead, one of the main trajectories  
308 of the subsurface oil plume (North et al., 2011).

309 The heavily impacted stations in impact zone 1, D031S, D034S, D038, D040S, and  
310 ALTNF001, were dominated by polychaetes from the genus *Ophryotrocha* (family  
311 Dorvilleidae). Because of the very high counts of dorvilleid polychaetes, macrofauna  
312 abundances at stations near the wellhead were relatively high. Dorvilleids are known to inhabit  
313 heavily polluted areas, such as harbors (Paavo et al., 2000) and aquaculture farms (Salvo et al.,  
314 2015), as well as reducing environments such as hydrothermal vents (Blake 1985) and  
315 hydrocarbon seeps (Hilbig & Fiege, 2001). Furthermore, dorvilleids dominate sediments that are  
316 saturated with petroleum hydrocarbon (Grassle, 1985; Petrecca & Grassle, 1990) and were found  
317 to be dominant at stations near the DWH wellhead immediately after the blowout (Washburn et  
318 al., 2016). The abundance of Dorvilleidae in this study was positively correlated with PAH44  
319 concentration (Figure 6). Near vents and seeps they feed on bacterial mats (Wiklund et al.,  
320 2009). The bacterial composition of the oiled sediment near the DWH wellhead resembled that  
321 of cold seeps (Liu & Liu, 2013), which might have acted as a cue for pelagic dorvilleid larvae to

322 settle. Rapid population turnover rates, found in some opportunistic dorvilleid species  
323 (Kingston, 1987), may explain the high population numbers in polluted areas where other  
324 organisms struggle to survive and to establish populations. We hypothesize that the dorvilleids  
325 in the northern Gulf of Mexico have a higher tolerance level for the toxic effects of the  
326 hydrocarbon contamination than most other benthic organisms there and are useful as indicators  
327 of oil pollution in the deep GoM.

328         Several taxa appeared to be disproportionately excluded from the heavily impacted zone  
329 1. Washburn et al., (2016) found over 20 taxa that had significantly higher abundances in  
330 background areas than in areas impacted by the DWH blowout. These taxa, such as the  
331 superorder Peracarida and the polychaete family Spionidae, appear to be negatively impacted by  
332 PAH concentrations (Figure 6). There are no cosmopolitan species or families that are  
333 bioindicators of impacted or background conditions. Because different regions are subject to  
334 countless biogeochemical factors, indicator taxa can only appropriately be used within the region  
335 of a specific study (Dean, 2008).

336         There were also stations that remained impacted by the DWH blowout, and were farther  
337 from the wellhead, than some stations that returned to background conditions. Station HIPRO,  
338 29 km from the wellhead, had low diversity and hydrocarbons from the DWH blowout may have  
339 accumulated there. One station in impact zone 1 (D034S) had a 261-fold increase in total PAHs  
340 from 2010 to 2011 illustrating the patchy nature of contaminants on small scales (Montagna et  
341 al., 2017). Another possible reason for the lower diversity at HIPRO may be that relatively high  
342 nutrient levels, caused by high surface primary production in the years 1998 – 2000 (mean  
343 chlorophyll concentrations 0.66 mg/m<sup>3</sup>) in this area (Rowe and Kennicutt, 2009), which favored  
344 a less diverse macrofauna community of opportunistic taxa. Low diversity levels at D002 were



345 caused by the low abundance at this, the deepest station (2388 m) sampled. Increasing the  
346 sampling area by pooling the species of all nine cores increased the diversity measurement  
347 dramatically, in contrast to the polluted stations where pooling did not increase diversity (Table  
348 1). Therefore, the low N1 values at station D002 are a result of the low abundance, rather than  
349 low diversity.

350 The station with the lowest diversity outside the one km radius of the DWH wellhead was  
351 VK 916, which is 62 km northeast of the wellhead. VK 916 was an oil and gas exploration site  
352 in the Viosca Knoll (VK) Block 916 where a single exploration well was drilled in 2001. No oil  
353 was produced at this site; however, drilling muds and cuttings were deposited around the  
354 exploration well, with layers up to 45 cm thick, covering an area of 13.37 ha (Continental Shelf  
355 Associates, Inc. 2006). Barium levels, an indicator of drilling activities, were very high (1273  
356 ppm, averaged across replicates), comparable to stations near the DWH wellhead. VK 916 was  
357 heavily dominated by polychaetes of the family Capitellidae, unlike stations near the DWH  
358 wellhead where Dorvilleidae prevailed. This corroborates the finding of a post-drill impact study  
359 in 2002, which found Capitellidae polychaetes to be dominating in this area (Continental Shelf  
360 Associates, Inc. 2006). Thus the environmental impact of the drilling muds and cuttings appear  
361 to remain nine years later, comparable to findings surrounding production sites in shallow water  
362 (Montagna and Harper, 1996). One important difference between oil spills and drilling activities  
363 is that drilling releases higher quantities of toxic metals that do not degrade (Peterson et al.  
364 1996).

365 One year following the DWH blowout, the macrobenthos around the wellhead appear to  
366 be exhibiting an enrichment effect, where heavily polluted communities have high abundances  
367 but are composed of fewer pollution-tolerant and opportunistic taxa, in this case primarily

368 dorvilleid polychaetes of the genus *Ophryotrocha*. There is also decreased diversity in the upper  
369 section of the sediment relative to the lower sections of the sediment (Figure 2). This pattern  
370 (high abundances of low diversity communities, restricted to surface sediments) resembles the  
371 benthic community response to organic enrichment (Pearson and Rosenberg, 1978; Berge, 1990)  
372 and physical disturbance (Rhoads et al., 1978) in shallow marine water ecosystems.

#### 373 4.2 Changes in the macrofauna communities between 2010 and 2011

374 There were many similarities in benthic community patterns among impact zones  
375 between samples collected in 2010 and 2011 as well as some differences. Richness and evenness  
376 were highest in impact zone 3 in 2010 (Washburn et al., 2016), but highest in impact zone 4 in  
377 2011. While year-to-year variability is possible, this change could be caused by a decrease in  
378 enrichment in impact zone 3 one year after the spill due to burial or uptake of organic matter. It  
379 may also have been caused by the fact that several of the deepest stations sampled in 2010 in  
380 impact zone 4 were not included in 2011, resulting in a lower average depth in the present study.  
381 Macrobenthic abundance often decreases with depth (Rex et al., 2006) while the effects of depth  
382 on diversity may differ between geographical regions and different taxa (Wilson, 2008; Carvalho  
383 et al., 2013).

384 Impact zone 1 had the lowest diversity, richness, and evenness immediately after the  
385 blowout in the fall of 2010, and all of the diversity metrics remained the lowest in impact zone 1  
386 in 2011. Abundances in 2010 were lowest in impact zone 1, but in 2011 they were highest in  
387 zone 1. Impact zone 2 was moderately impacted in 2010; however, abundance, richness and  
388 diversity were not different between zones 2 and 3 in 2011, indicating that some recovery may  
389 have occurred. The rapid sedimentation of material directly after the blowout (Brooks et al.,

390 2015) may have buried much of the organic material and toxic substances in zone 2 or it may  
391 have had a dilution effect on the sediment. Likewise, much of the hydrocarbons may have been  
392 biologically degraded between 2010 and 2011.

393         The similarity in abundances of macrobenthic organisms among impact zones may  
394 indicate that the toxic conditions have improved to a point where at least the most pollution  
395 tolerant taxa have successfully repopulated the benthos. Thus, it appears that opportunistic taxa  
396 were able to take advantage of spatial and nutritional resources in 2011. Montagna et al. (2017)  
397 found signs of recovery in areas impacted by the DWH blowout one year following the blowout.  
398 Meiofauna abundance and the nematode-to-copepod ratio (a commonly used variable for  
399 assessing impacts) were not significantly different between impacted and non-impacted areas.  
400 This is most likely an effect of the sediment depths where different sizes of benthic animals are  
401 found. Montagna et al. (2016) found that 87% of total meiofaunal abundance was located in the  
402 top 3 cm of sediment while 97% of the abundance of the more pollution sensitive harpacticoid  
403 copepods was located in the top 3 cm. In contrast, 97% of total macrofaunal abundance was  
404 located in the top 10 cm of sediment. Thus sedimentation will lead to burial of pollutants beyond  
405 the zone of meiofaunal communities much more quickly than macrofaunal communities.

406         In early organic enrichment studies (e.g. Pearson and Rosenberg, 1978; Roads et al.  
407 1978), macrofauna abundances are generally minimal at the source of enrichment then peak near  
408 or a short distance from the pollution source, and decrease further from the source due to  
409 decreased enrichment and r-selected species. Diversity generally increases with distance from  
410 the pollution source until it reaches a maximum at intermediate distances, within the area of  
411 enrichment but outside the heavily impacted area (Pearson and Rosenberg, 1978). Because  
412 abundances were highest closest to the DWH wellhead in 2011, the physical, chemical, and

413 toxicological conditions in impact zone 1 may have improved enough to allow the most pollution  
414 resistant organisms to repopulate the benthos. Berge (1990) found that benthic communities  
415 recover from oiling more quickly in areas experiencing more eutrophication before the event.  
416 The natural hydrocarbon seepage from the vast number of cold seeps in the GoM may have  
417 favored organisms that can adapt to oiled conditions, allowing for a rapid succession.

418         While it appears that there are differences in the response of macrobenthic communities  
419 to distance from the DWH wellhead between 2010 and 2011, the effects of natural temporal  
420 variability cannot be ignored. Samples were collected over the course of a few weeks in both  
421 2010 and 2011, effectively taking a snapshot of the deep-sea benthic communities. Moreover,  
422 samples were collected in different seasons during 2010 and 2011. Benthic communities in the  
423 deep GoM have been shown to greatly vary with season. Reuscher and Shirley (in review) found  
424 polychaete abundances doubled between fall 1983 and spring 1984 while decadal changes were  
425 not observed. In the current study samples were collected in 2010 during the fall and in 2011  
426 during late spring. Other studies have found changes in benthic abundances between different  
427 seasons or years due to changes in phytoplankton production and thus changes to the amount of  
428 organic matter reaching the deep sea (Galéron et al., 2001; Frid et al., 2008). Continued  
429 sampling and analysis in subsequent years are required to determine whether trends observed in  
430 2011 were due to recovery or natural variation.

431         The much impaired richness, diversity, and evenness in impact zone 1 show that the  
432 environmental impact of the oil spill was persistent as of 2011. The increased abundances near  
433 the wellhead in 2011 may be the result of succession, with abundances drastically increasing due  
434 to increased recruitment following the blowout. The lack of animals below the surface  
435 sediments near the wellhead (Figure 2) is indicative of a community in the early stages of

436 succession following a disturbance (Rhoads et al., 1978). Assuming the shallow water model of  
437 organic enrichment and disturbance can be applied to deep water, then the pattern observed  
438 within one km of the wellhead may actually not be caused by recovery but instead be an  
439 indicator of continued disturbance. That is, the increase in abundance of low diversity species in  
440 2011 is an indicator of a stage of disturbance succession.

441         The vertical distribution of benthic communities between shallow and deeper sediments  
442 also matches patterns expected from disturbance. In spatial or temporal proximity to a  
443 disturbance, animals are typically confined to surface sediments while deeper sediments become  
444 anaerobic. As time passes or distance increases from the disturbance, larger animals that live  
445 deeper in the sediment replace early colonizers. Bioturbation from these larger animals  
446 oxygenates deeper sediments, allowing smaller organisms to survive deeper in the sediment  
447 (Rhoads et al., 1978). In an experimental oil spill, several polychaete species shifted their  
448 burying activities to deeper sediment depths (Gilbert et al., 2015). In contrast, benthic organisms  
449 within close proximity to the DWH wellhead still appeared to be mostly restricted to surface  
450 sediments approximately one year after the blowout. With increasing distance from the wellhead  
451 macrofauna abundance in deeper sediments increased (Figure 2). The lack of differences among  
452 zones 2-4 in surface and intermediate sediments indicates that impacts from the DWH blowout  
453 may have a limited extent in surface sediments one year following the spill.

#### 454 4.3 How sampling area affects community analyses

455         Measurements of species richness and diversity depend on the size of the area sampled  
456 (Arrhenius 1921). Measures of diversity at most of our sampling stations were higher when the  
457 sampling area increased from the area of a core (nine cores averaged) to the area of a station (all

458 nine cores pooled). However, the increase of N1 with sampling area was not uniform across all  
459 stations. N1 values of stations in impact zone 1 were similar or slightly decreased when the  
460 sampling area increased, illustrating the very low variability in community structure in highly  
461 polluted areas (Figure 3, Table 1). In contrast, at stations furthest from the wellhead diversity  
462 measures were higher by over 50% when N1 was calculated from core sums as compared to  
463 from core averages. Highly polluted areas are often dominated by one or a few pollution-tolerant  
464 taxa (Pearson and Rosenberg, 1978; Rhoads et al. 1978). This leads us to believe that the benthic  
465 macrofauna of these stations were so impoverished that one single core may have been sufficient  
466 to represent the communities. The pooled data may be more representative of the community at  
467 a particular station, but they also greatly reduce the degrees of freedom available in the analysis.

#### 468 4.4 Conclusion

469 One year after the DWH blowout hydrocarbon contamination still affected deep-sea  
470 macrobenthic communities. However, the extent of impact appears to have decreased from 2010  
471 to 2011 and impacts close to the wellhead seem to have diminished. Impact zone 2 appeared to  
472 return to background conditions in 2011 and abundances were highest in 2011. In 2011  
473 abundances were still lowest near the wellhead for deeper sediments (< 3 cm) indicating that  
474 effects from the DWH blowout in 2011 may still be very similar to those observed in 2010, but  
475 the effects have been buried. Benthic communities found in surface sediments throughout the  
476 GoM during 2011 may be exhibiting intermediate disturbance because they were less exposed to  
477 the buried contaminants, than they were in 2010 when contaminants were at the surface. While  
478 the increase of diversity and evenness between 2010 and 2011 indicate some degree of recovery,  
479 they may also be caused by community succession under relatively constant impact from  
480 contamination. If no recovery took place between 2010 and 2011 macrobenthic communities

481 could still have changed due to the recruitment of r-selected taxa into polluted areas over this  
482 time period.

483 Continued monitoring of areas around the DWH wellhead is imperative if we hope to  
484 understand the long-term consequences of the 2010 blowout. While studies examining effects of  
485 the spill shortly after the event are key for determining the extent of possible damages, follow-up  
486 studies are needed to address how these damages will persist, change, or recover over time.  
487 Because of the relatively static environmental conditions and limited availability of organic  
488 matter, deep-sea organisms are often long lived and may be highly susceptible to disturbances.  
489 Slow deposition rates and low rates of metabolism in the deep sea may also lead to long  
490 residence times for pollutants (Gage and Tyler, 1992). However, the lack of knowledge on deep-  
491 sea biogeochemical processes prevents accurate predictions of how the deep sea will respond to  
492 human disturbances. Studies examining the DWH blowout effects years after the event will not  
493 only greatly increase our understanding of this blowout but also help improve policies and  
494 responses to future deep-sea drilling world-wide.

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517



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667

668 Table 1.  
669 Sampling stations sorted by distance from the wellhead (W). IZ is the impact zone as defined by  
670 Montagna et al. (2013). N is the abundance of macrobenthic organisms. S is the number of taxa  
671 per core. N1(C) represents the diversity averaged per core and N1(S) represents the diversity  
672 summed over nine cores.

Station	IZ	Latitude (N)	Longitude (W)	Depth (m)	W (km)	N (n/m <sup>2</sup> )	S	N1(C)	N1(S)
D038SW	1	28.740121	88.368133	1508	0.33	10,124	11.7	5.6	5.5
D034S	1	28.734340	88.362394	1561	0.52	14,488	11.8	3.8	3.4
ALTNF001	1	28.734550	88.370076	1554	0.58	12,578	14.6	5.4	5.0
D040S	1	28.742285	88.362701	1517	0.59	15,590	13.1	4.2	3.8
D042S	1	28.742302	88.370713	1499	0.66	10,723	16.1	8.3	9.5
NF006MOD	1	28.744784	88.359812	1517	1.00	14,935	19.0	10.6	13.0
D031S	1	28.731636	88.359198	1585	1.01	22,046	11.7	2.9	2.7
D044S	2	28.744524	88.374421	1491	1.11	13,652	20.4	11.8	16.8
LBNL1	1	28.732098	88.376777	1565	1.26	10,751	16.6	9.5	11.3
ALTNF015	2	28.709752	88.366436	1615	3	10,835	24.9	16.3	23.1
NF008	2	28.719989	88.388758	1591	3	8,827	20.8	13.2	19.0
NF009	1	28.738091	88.397459	1495	3	12,954	23.4	14.6	20.8
NF010	2	28.757251	88.522042	1440	3	12,913	29.4	19.5	27.2
NF011	1	28.765173	88.366656	1440	3	13,944	27.1	16.6	22.1
NF012	2	28.757857	88.344246	1528	3	12,954	26.2	16.1	22.3
NF013	2	28.738870	88.335635	1569	3	10,319	27.3	17.5	25.9
NF014	3	28.719982	88.344640	1588	3	9,399	24.9	16.0	24.1
LBNL14	2	28.730118	88.417347	1545	5	9,440	19.8	12.2	17.8
LBNL17	3	28.696626	88.385168	1616	5	9,301	25.0	16.1	23.5
LBNL3	2	28.705278	88.402037	1583	5	8,729	21.6	14.1	20.4
D050S	2	28.792493	88.348558	1440	6	14,377	26.9	17.1	22.7
D019S	4	28.672787	88.368802	1662	7	11,950	30.0	17.5	24.4
LBNL4	4	28.687908	88.418396	1465	8	16,887	25.8	16.0	24.5
2.21	3	28.784700	88.453846	1359	10	16,036	31.1	19.5	25.3
FF010	2	28.667819	88.430058	1357	10	11,253	24.4	15.3	21.4
LBNL7	2	28.639087	88.471193	1544	15	8,604	22.2	15.4	23.3
D024S	4	28.774613	88.167853	1709	20	8,673	26.0	19.4	30.1
FF005	3	28.803038	88.564810	1000	21	13,889	32.3	20.7	29.1
HIPRO	3	28.551521	88.579092	1578	29	12,425	23.2	12.6	16.6
LBNL9	3	28.514040	88.600484	1521	34	11,532	22.3	14.2	20.2
LBNL10	4	28.415556	88.704154	1408	48	10,096	25.2	16.7	23.9
D043S	4	28.988977	87.934540	1499	51	11,895	28.3	18.3	26.0
VK916	4	29.106744	87.888737	1132	62	16,664	20.9	10.3	12.1
D002S	4	28.556952	87.760436	2388	63	2,942	12.9	10.3	21.9
S36	4	28.918526	87.672135	1835	69	6,889	26.1	19.9	35.6
D062S	4	28.265489	88.923301	1313	76	8,841	24.2	16.7	24.9
FFMT3	4	28.218610	89.491843	998	125	8,646	31.4	22.9	38.0
FFMT4	4	27.828145	89.164753	1410	128	5,968	25.0	19.1	38.5



673 Table 2.

674 The 20 most abundant taxa collected during the spring 2011. Number of stations (n) where that  
675 taxon was found, average abundance (n/m<sup>2</sup>) per core, percent of total abundance, and cumulative  
676 percent abundance. Abbreviations: P = Polychaeta, N = Nemertea, M = Mollusca, E =  
677 Echinodermata.

Rank	Family	Stations (n)	Abundance (n/m <sup>2</sup> )	Percent	Cumulative Percent
1	Dorvilleidae (P)	36	2288	19.67	19.67
2	Maldanidae (P)	38	1178	10.13	29.79
3	Paraonidae (P)	38	1019	8.76	38.56
4	Capitellidae (P)	38	996	8.56	47.12
5	Spionidae (P)	38	737	6.33	53.45
6	Cirratulidae (P)	38	512	4.40	57.85
7	Acrocirridae (P)	38	462	3.97	61.83
8	Nemertea (N)	38	353	3.03	64.86
9	Thyasiridae (M)	34	328	2.82	67.68
10	Bivalvia (M)	35	272	2.34	70.02
11	Syllidae (P)	38	259	2.23	72.24
12	Echiura (E)	28	231	1.99	74.23
13	Sigalionidae (P)	35	212	1.82	76.05
14	Prochaetodermatidae (M)	34	189	1.62	77.68
15	Terebellidae (P)	37	187	1.61	79.29
16	Nereididae (P)	35	169	1.45	80.74
17	Lumbrineridae (P)	35	142	1.22	81.96
18	Hesionidae (P)	37	135	1.16	83.12
19	Ampharetidae (P)	37	115	0.99	84.11
20	Cossuridae (P)	33	98	0.84	84.95
139	Other Taxa		1750	15.05	100.00
	<b>Total</b>	<b>38</b>	<b>11632</b>	<b>100</b>	

678

679

680 Table 3.

681 Analyses of significant differences in abundance (n/m<sup>2</sup>), richness, diversity (N1), and evenness  
 682 (J') between different impact zones. A) Results for the nested ANOVAs. B) Tukey tests. Impact  
 683 zones are listed from highest to lowest, and underlined categories are not significantly different  
 684 at the 0.05 level. Abbreviations: df = degrees of freedom; IZ = impact zone.

A) ANOVA		P-Value for Macrofauna Trait			
Source	df	Abundance	Richness	N1	J'
Zone	3	0.0696	<0.0001	<0.0001	<0.0001
Station(Zone)	34	<0.0001	<0.0001	<0.0001	<0.0001
Error	304				

685

B) Tukey Tests					
Abundance	Zone	IZ 1	IZ 3	IZ 2	IZ 4
	Mean	110	96.4	88.3	79.3
Richness	Zone	IZ 4	IZ 3	IZ 2	IZ 1
	Mean	26.5	25.1	24.2	16.5
N1	Zone	IZ 4	IZ 3	IZ 2	IZ 1
	Mean	17	16.5	15.5	8.1
J'	Zone	IZ 4	IZ 2	IZ 3	IZ 1
	Mean	0.87	0.86	0.85	0.68

686

687

688 Table 4.

689 Analyses of significant differences in abundance (n/m<sup>2</sup>), richness, diversity (N1), and evenness  
 690 (J') among different vertical sediment depths and impact zones. A) Results for the 2-way  
 691 partially hierarchical ANOVAs. B) Tukey tests. Impact zones are listed from highest to lowest,  
 692 and underlined categories are not significantly different at the 0.05 level. Abbreviations: df =  
 693 degrees of freedom; IZ = impact zone.

ANOVA		P-Value for Macrofauna Trait			
Source	df	Abundance	Richness	N1	J'
Zone	3	0.0802	0.0004	<.0001	0.0037
Station(Zone)	34	<.0001	<.0001	<.0001	<.0001
Sec	2	<.0001	<.0001	<.0001	0.0033
Zone*Sec	6	<.0001	<.0001	<.0001	0.0035
Sec*Station(Zone)	38	<.0001	<.0001	<.0001	<.0001
Error	912				

694

B) Tukey Tests				
Abundance	Zone	0 - 3	3 - 5	5 - 10
	Mean	<u>77</u>	9.3	6.4
Richness	Zone	0 - 3	3 - 5	5 - 10
	Mean	<u>20.7</u>	4.7	3.2
N1	Zone	0 - 3	3 - 5	5 - 10
	Mean	<u>13.2</u>	3.9	2.8
J'	Zone	0 - 3	3 - 5	5 - 10
	Mean	<u>0.82</u>	0.80	0.76

695

696

697 Table 5. Spearman correlation coefficients (r) between principal component scores for sediment  
 698 chemistry and physical and biological metrics, and probability that H<sub>0</sub>: r = 0 for n = 38 stations.

699

Variable	PC1		PC2		PC3	
	r	P	r	P	r	P
Wellhead distance	-0.25	(0.1327)	0.01	(0.9402)	-0.73	(<0.0001)
Seep distance	-0.25	(0.1325)	0.12	(0.4914)	0.06	(0.7245)
Depth	0.18	(0.2900)	0.07	(0.6751)	-0.03	(0.8449)
Abundance	0.18	(0.2707)	-0.10	(0.5533)	0.52	(0.0007)
Richness	0.24	(0.1523)	0.02	(0.9055)	-0.55	(0.0003)
N1 Diversity	0.13	(0.4386)	0.11	(0.4934)	-0.65	(<0.0001)
H' Diversity	0.12	(0.4600)	0.12	(0.4591)	-0.65	(<0.0001)
J' Evenness	-0.07	(0.6699)	0.22	(0.1948)	-0.77	(<0.0001)

700

701

702 Figure captions

703

704 Figure 1. Location of sampling stations. A. All stations. B. zoomed view of stations within a 5  
705 km radius of the wellhead.

706

707 Figure 2. Mean abundance ( $n/m^2$ ) and diversity (N1) values for each vertical sediment section  
708 across impact zones.

709

710 Figure 3. K-dominance plot of the four impact zones. Abbreviation: IZ = impact zone.

711

712 Figure 4. MDS ordination plot of the 38 sampling stations. Abbreviation: IZ = impact zone.

713

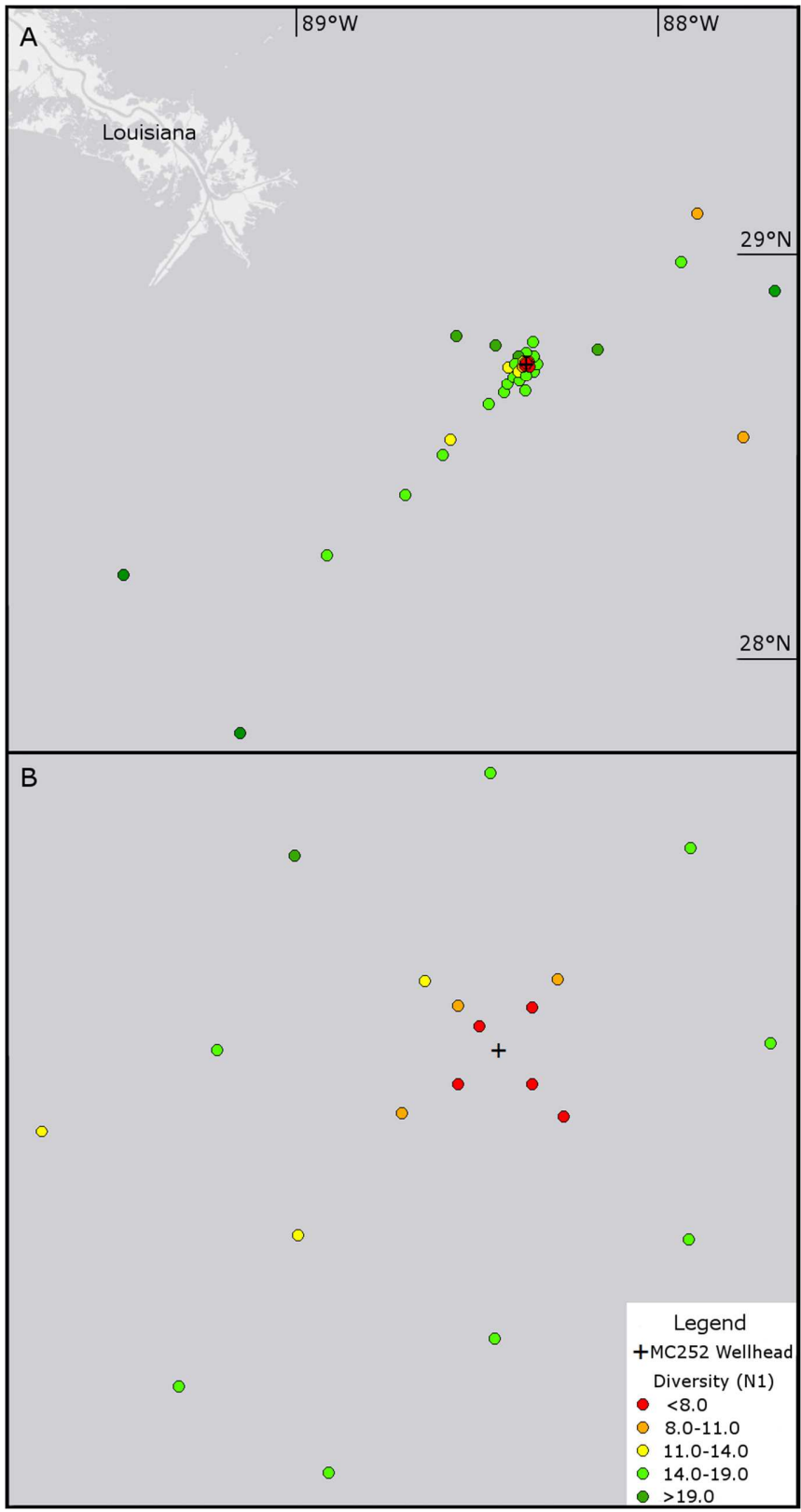
714 Figure 5. A) Sediment chemistry variable loads for the first (PC1) and second (PC2) principal  
715 components and B) Sediment chemistry variable loads for the second (PC2) and third (PC23)  
716 principal components. Axis label presents percent of variance explained by the principal  
717 component. Abbreviations as in Table S1.

718

719 Figure 6. Relationship between PAH44 concentration and abundances of A) Dorvilleidae and B)  
720 Peracarida. Blue lines represent the predicted values while red lines represent 90% confidence  
721 limits.

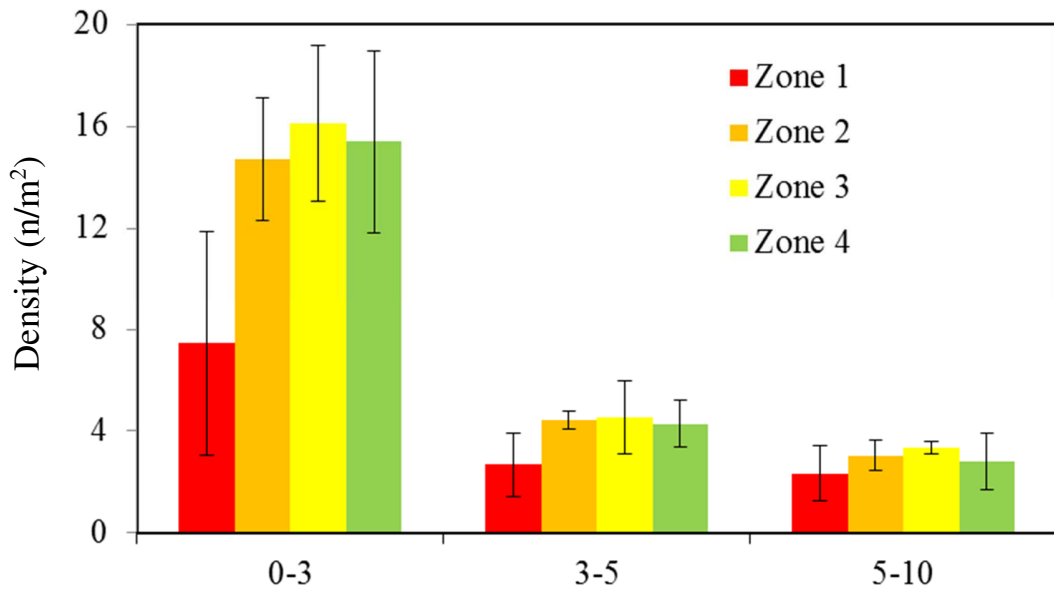
722

723 Figure 1



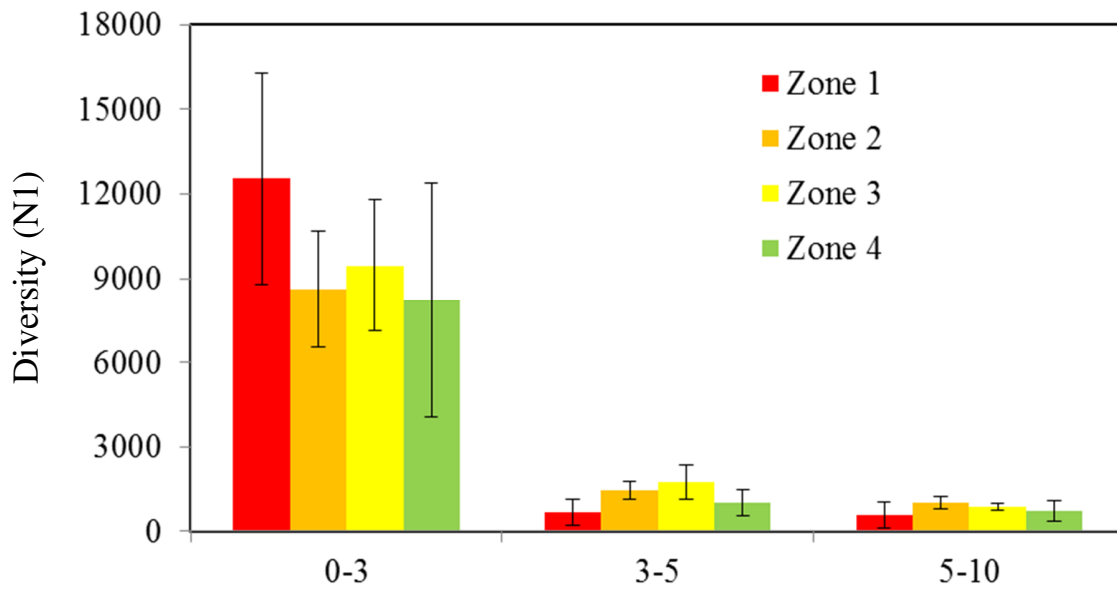
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725 Figure 2.



726

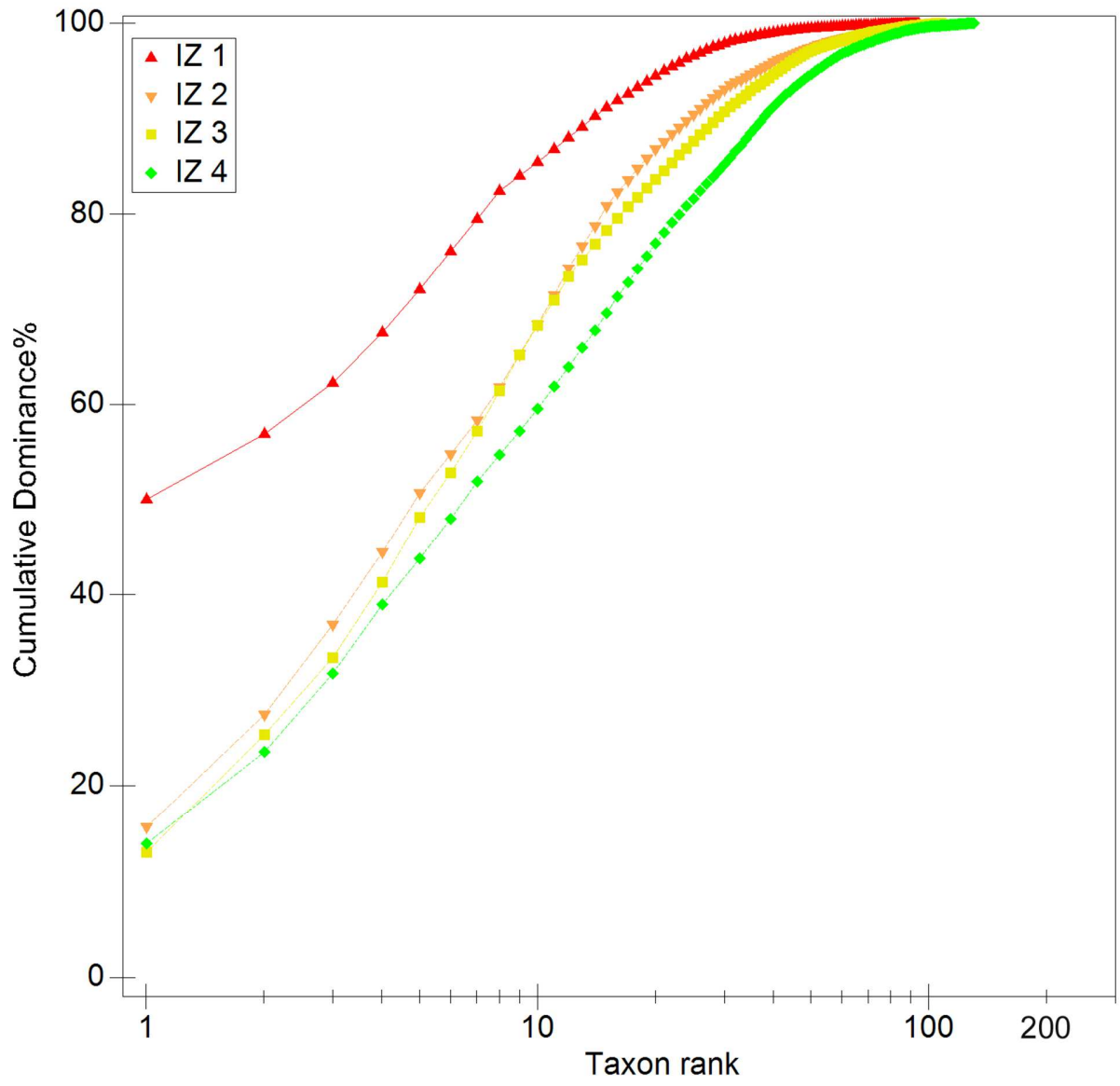
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728

729

730 Figure 3

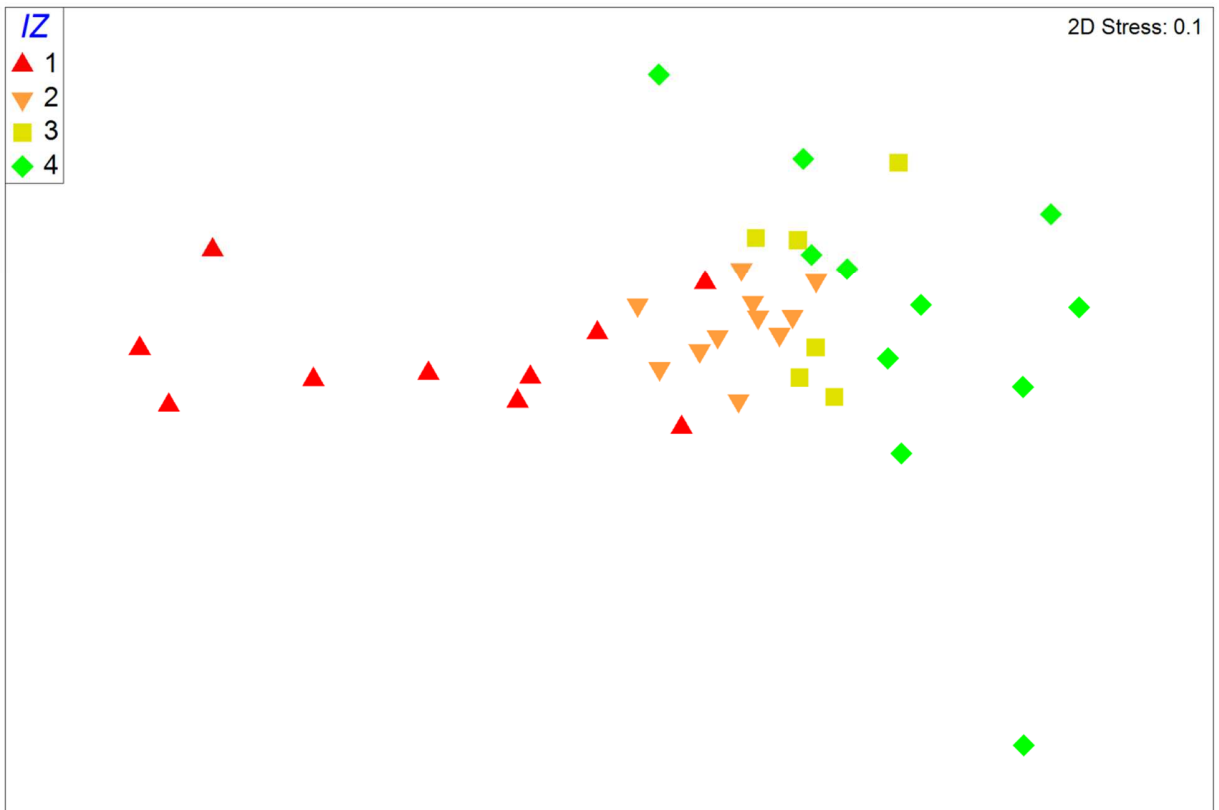


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732



733 Figure 4

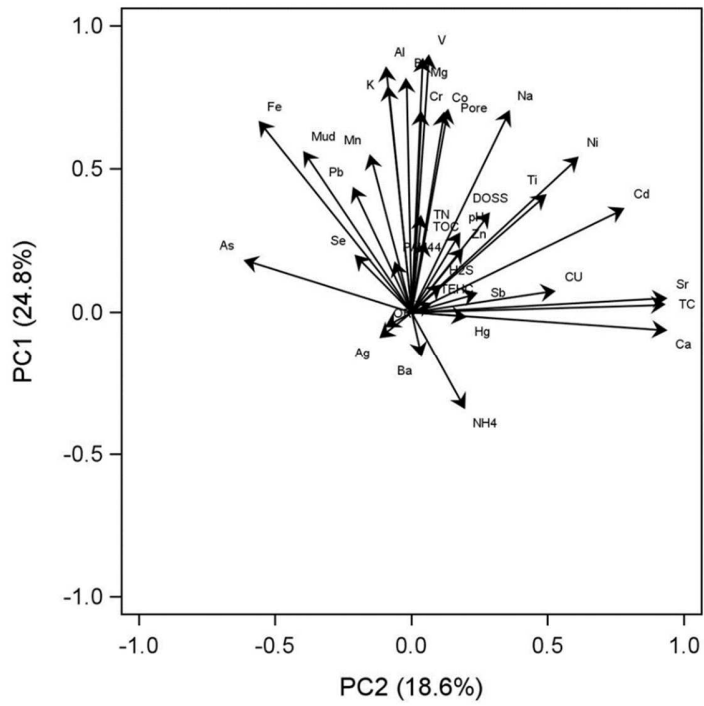


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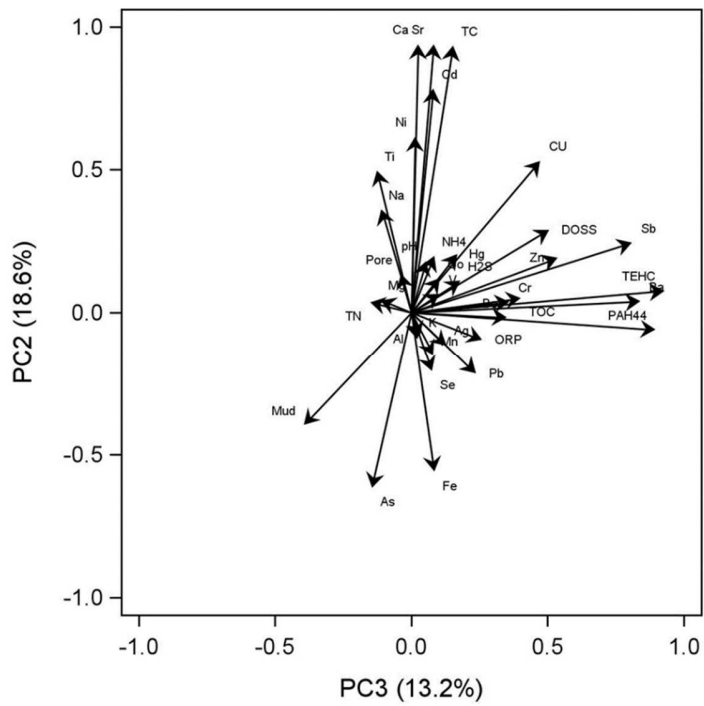
736 Figure 5.

737 A)



738

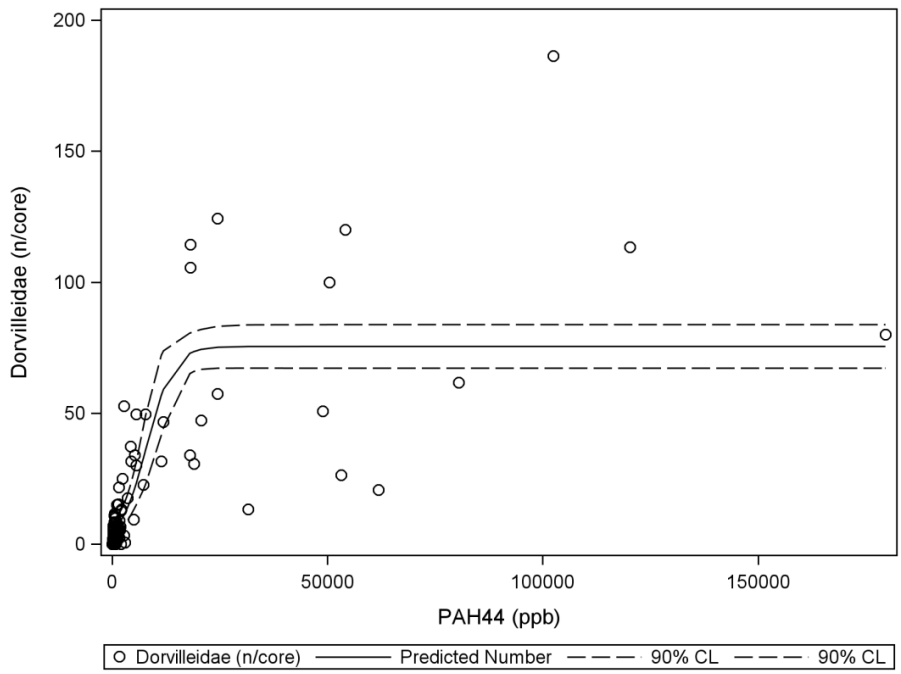
739 B)



740

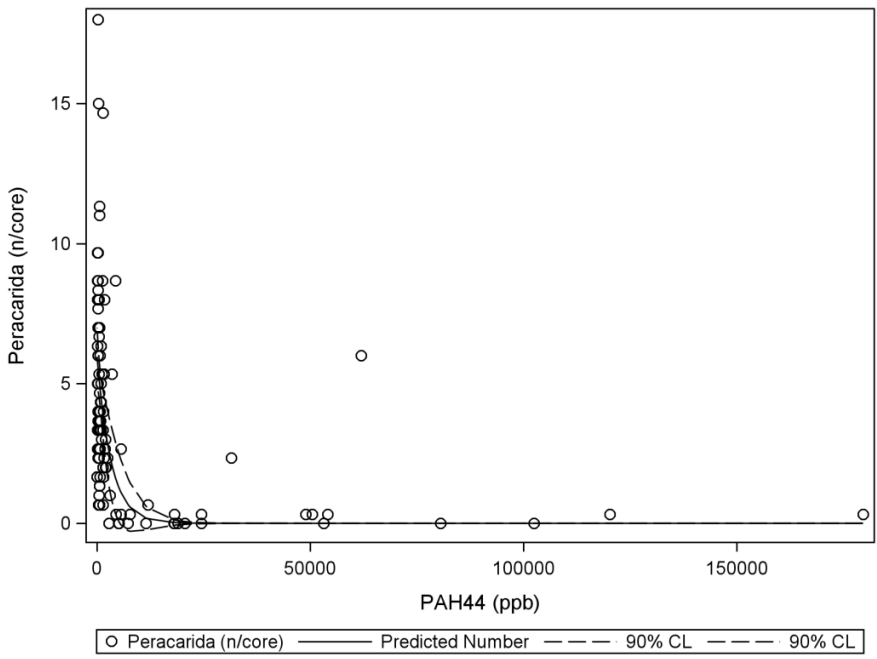
741 Figure 6

742



743 A)

744



745 B)